N/OFQ

► Orphanin FQ

N_2O

► Nitrous Oxide Antinociception and Opioid Receptors

Na+ Channel

Definition

Na^+ Channel is a voltage-dependent permeation pathway for sodium ions.

► Trafficking and Localization of Ion Channels

$Na^+K^+Cl^-$ Cotransporter

Definition

A transporter protein that mediates the transport of Na^+, K^+ and Cl^- into cells encoded by the gene slc12a2.

► GABA and Glycine in Spinal Nociceptive Processing

NAcc

► Nociceptive Processing in the Nucleus Accumbens, Neurophysiology and Behavioral Studies

nAChr

Synonym

Nicotinic receptors

Definition

Acetylcholine (ACh) is released from endothelial cells, keratinocytes and other cells following trauma and can activate nociceptors. In nociceptors, ACh can interact with either muscanic (mAChr) or nicotinic (nAChr) receptors. The nicotinic type can directly induce action potentials by the gating of ion channels. The muscarinic type acts via G-protein coupled receptors to either upregulate or down regulate nociceptor excitability.

► Nociceptors in the Orofacial Region (Skin/Mucosa)

Nadolol

Definition

Nadolol is a beta-blocker.

► Migraine, Preventive Therapy

Naloxone

Definition

Naloxone is an antagonist at the mu opioid receptor with a short duration of action (30–45 minutes). It can be administered for rapid reversal of opioid-induced sedation or respiratory depression, but its administration will also reverse opioid-induced analgesia and may precipitate withdrawal symptoms.

► Alternative Medicine in Neuropathic Pain
► Cancer Pain Management, Opioid Side Effects, Uncommon Side Effects

Narcolepsy

Definition

Narcolepsy is a condition marked by an uncontrollable desire for sleep, or by sudden attacks of sleep occurring at intervals.

► Hypothalamus and Nociceptive Pathways

Narcotic Bowel Syndrome

► Cancer Pain Management, Gastrointestinal Dysfunction as Opioid Side Effects

Narcotics, Major Analgesics

► Oral Opioids

Natural History

Definition

Natural course of a disease or a symptom.

► Placebo Analgesia and Descending Opioid Modulation

NCCP

Synonym

Non-cardiac chest pain

Definition

NCCP is a functional chest pain of esophageal origin without manifestation of pathology. The pain is very similar to cardiac angina.

► Visceral Pain Model: Esophageal Pain

Near-Infrared Spectroscopy

Synonym

NIRS

Definition

This is a relatively novel technique for measuring functional activation in infants, which is both non-invasive and localized. In order to measure a localized hemodynamic response within the brain in response to peripheral stimulation, paired near-infrared (NIR) light emitters and detectors ('optodes') are placed symmetrically on each side of the head over the somatosensory cortex during stimulation. NIR light at 4 wavelengths is conveyed through the head, and a controlling computer calculates the changes in optic absorption at each wavelength and converts these into changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin using known extinction coefficients. In studies on newborn infants, the presence and amplitude of the hemodynamic response is used to elucidate the maturation of cortical processing of stimuli. NIRS is ideally suited to the study of infants, because the infant skull is thinner than that of the adult so that the optical signal is cleaner and easier to detect.

► Infant Pain Mechanisms

Neck Pain

Definition

Localised neck or back pain is the symptom of a protruding disk frequently heralding a cervical or lumbosacral radiculopathy in spondyloarthritis. Non-specific neck pain is a prominent feature of polymyalgia rheumatica.

► Muscle Pain in Systemic Inflammation (Polymyalgia Rheumatica, Giant Cell Arteritis, Rheumatoid Arthritis)
► Radiculopathies

Necrosis

Definition

Necrosis is the localized death of living cells.

► Sacroiliac Joint Pain

Negative Affectivity

Definition

The stable disposition to experience negative affect and low mood (neuroticism).

► Hypervigilance and Attention to Pain

Negative Mucosal Potential

Definition

Electrical potential recorded from the respiratory epithelium that reflects the excitation of nociceptive nerve terminals.

► Nociception in Nose and Oral Mucosa

Negative or Punishing Responses

Definition

Negative or punishing responses (e.g. expressions of irritation, ignoring) represent a third category of responses to the expression of pain.

► Spouse, Role in Chronic Pain

Negative Reinforcement

Definition

Negative reinforcement is the removal of an aversive stimulus (tangible or non-tangible) following a behavior, with the goal of increasing future incidents of that behavior.

- ► Impact of Familial Factors on Children's Chronic Pain
- ► Operant Perspective of Pain

Negative Responding

- ► Spouse, Role in Chronic Pain

Negative Sensory Phenomenon

Definition

Negative sensory phenomenon is a clinical sign that is interpreted by the patient as less than when compared to normal bodily function and experiences.

- ► Hypoalgesia, Assessment
- ► Hypoesthesia, Assessment

Nematode

Definition

Nematode is a roundworm, a non-segmented worm phylum.

- ► Species Differences in Skin Nociception

Neocortical

Definition

Belonging to the top, approximately 2 mm thick layer of the two hemispheres of the brain.

- ► Prefrontal Cortex, Effects on Pain-Related Behavior

Neonatal Inflammation

- ► Visceral Pain Model, Irritable Bowel Syndrome Model

Neonatal Pain

- ► Visceral Pain Model, Irritable Bowel Syndrome Model

Neonate

- ► Newborn

Neospinothalamic Tract

Definition

Lateral and phylogenetically younger component of the spinothalamic tract, also known as the lateral spinothalamic tract. It is comprised of the axons nociceptive-specific and wide dynamic range neurons. It projects to the ventral posterolateral nucleus of the thalamus and is responsible for the discriminative aspects of pain (location, intensity, duration).

- ► Acute Pain Mechanisms
- ► Parafascicular Nucleus, Pain Modulation
- ► Somatic Pain

Nerve Blocks by Local Anesthetic Drugs

N

Definition

Nerve blocks by local anesthetic drugs stop nerve impulse conduction in nerve cells, inhibiting pain impulses from reaching the central nervous system (CNS). They will often also make the pain-free body part numb, with weak or paralyzed muscles.

- ► Cancer Pain Management, Anesthesiologic Interventions
- ► Epidural Steroid Injections for Chronic Back Pain
- ► Postoperative Pain, Acute Pain Management, Principles

Nerve Compression

Definition

Nerve compression or nerve entrapment is caused by mechanical obstruction. They usually involve mixed nerves so the symptoms are motor sensory. Compression of pure motor nerves, which carry muscle and joint afferents, may produce deep diffuse discomfort. Pain in the referred territory, numbness, exacerbated by movements are the main symptoms. Nerve compression is more acute than (chronic) nerve entrapment. The treatment of choice is decompression, either pharmacological (dexamethasone) or surgical. Nerve blocks are also useful.

- ► Cancer Pain

Nerve Conduction

Definition

Nerve conduction is a clinical test of named peripheral nerves, in which all axons are stimulated to threshold, and the responses of the largest cohort of myelinated axons are measured.

- ► Electrodiagnosis and EMG
- ► Hereditary Neuropathies

Nerve Growth Factor

Synonym

NGF

Definition

Nerve growth factor (NGF) belongs to a family of polypeptide growth factors. It consists of alpha, beta and gamma subunits. NGF is a target-derived factor and is essential for survival, differentiation, and maintenance of sympathetic and afferent neurons. In inflamed tissue, NGF biosynthesis is rapidly increased leading to elevated concentrations of NGF in inflamed tissues. It has been shown that NGF is a mediator of inflammatory hyperalgesia and also a modulator of immune cell function. An enhanced retrograde transport of NGF to the DRG leads to an increase in the production of brain-derived neurotrophic factor (BDNF) at the level of gene expression, mainly in trkA-expressing small- and medium-sized neurons. During embryonic and early postnatal stages, sensory neurons are dependent on NGF for survival. Although adult sensory neurons do not depend on NGF for survival, the functional properties of some nociceptive sensory neurons, such as responsiveness to capsaicin or noxious heat, are modulated by NGF. NGF can exert its actions either through the high-affinity trkA receptor or the low-affinity p75 neurotrophin receptor.

- ► Congenital Insensitivity to Pain with Anhidrosis
- ► ERK Regulation in Sensory Neurons during Inflammation
- ► IB4-Positive Neurons, Role in Inflammatory Pain
- ► Immunocytochemistry of Nociceptors
- ► Nerve Growth Factor, Sensitizing Action on Nociceptors
- ► Neutrophils in Inflammatory Pain
- ► Satellite Cells and Inflammatory Pain
- ► Spinal Cord Nociception, Neurotrophins
- ► TRPV1, Regulation by Nerve Growth Factor
- ► TRPV1, Regulation by Protons
- ► Wallerian Degeneration

Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain

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Synonyms

Transgenic Mice; NGF-OE mice

Definitions

NGF and Inflammatory Pain

In peripheral tissues, the level of nerve growth factor (NGF) expression is often elevated following inflammation or injury (Heumann et al. 1987; Weskamp and Otten 1987). Studies using rodents have shown that injection of NGF causes behavioral thermal and mechanical ► hyperalgesia (Lewin et al. 1993; Lewin et al. 1994). Increased NGF expression is also accompanied by elevation of other inflammatory mediators such as bradykinin, prostaglandins, serotonin, ATP and protons (Bennett 2001). These changes in the periphery are thought to collectively contribute to sensitization of sensory afferents and central pain processing pathways. The link between NGF and inflammatory pain signaling can be examined using a transgenic mouse model (see ► Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain) in which NGF is overexpressed in the skin, a major target of sensory afferents. In these mice (NGF-OE mice), NGF is overexpressed in basal keratinocytes of stratified, keratinizing tissues such as the skin and oral epithelium, using the human keratin K14 promoter and enhancer region to drive expression of the mouse NGF cDNA (Albers et al. 1994). As described below, the increase in NGF expression causes an increase in the developmental survival of neurons that project to K14-expressing epithelium, altering their physiological properties and the expression of genes related to nociceptive signaling.

Characteristics

Anatomical Characteristics of NGF-OE Transgenic Mice

Mice that overexpress NGF in the skin exhibit hypertrophy of both sensory and sympathetic neurons (Albers et al. 1994; Davis et al. 1994; Davis et al. 1997). NGF–OE mice have an approximate 2-fold increase in the number of trigeminal and dorsal root ganglion (DRG) sensory neurons, and a 2.5-fold increase in the number of sympathetic neurons in the superior cervical ganglia. In addition, preferential increases of unmyelinated and thinly myelinated fibers that project to the skin occur (Davis et al. 1997; Stucky et al. 1999), a finding consistent with the types of axons lost in ► ngf -/- mice (Crowley et al.

1994). Immunolabeling of skin and DRG have shown a preferential increase of peptidergic sensory neuron subtypes. For example, the percent of TrkA neurons is doubled, as is the percent of calcitonin gene related peptide-positive neurons (Goodness et al. 1997). The population of sensory neurons that bind the plant lectin IB4 is not increased, consistent with the finding that glial cell line-derived growth factor (GDNF) is a major contributor to the trophic support of these neurons (Molliver et al. 1997).

Electrophysiologic Properties of NGF-OE Cutaneous Afferents

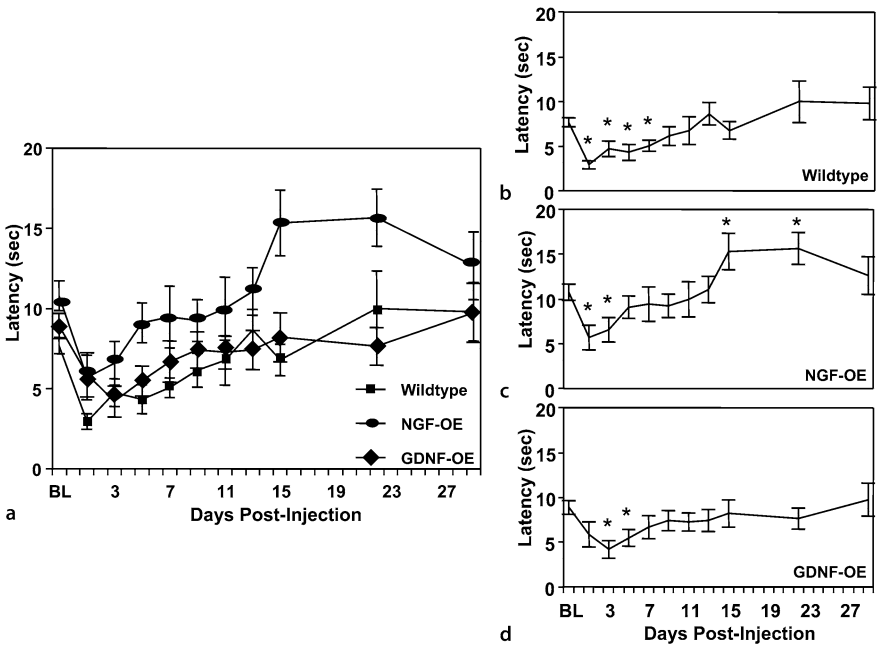
Electrophysiologic properties of cutaneous sensory afferents in the ► saphenous nerve of NGF-OE mice were analyzed using a skin-nerve preparation (Stucky et al. 1999). Large myelinated, low-threshold Aβ fibers showed no change in the proportion of slowly adapting (SA) or rapidly adapting (RA) fibers relative to wildtype animals. In addition, no significant difference in the mechanical stimulus-response properties, or conduction velocity, of SA or RA fibers of NGF-OE mice were found.

In contrast to Aβ fibers, both Aδ and C fiber nociceptors of NGF-OE mice had altered properties. The percent of Aδ mechanosensitive (AM) nociceptors was significantly increased from control values of 65% of all Aδ fibers analyzed to 97% in NGF-OE mice. Individual AM fibers also showed increased mechanical responsiveness, which was particularly evident at suprathreshold stimuli. A 100–300 mN sustained force evoked discharge rates in NGF-OE AM fibers double those of wildtypes. Though mechanically sensitized, AM fibers were unchanged with heat sensitivity. No significant difference was measured in the percent of AM fibers that respond to heat, the threshold for a response, or in the mean spikes per heat stimulus.

C fiber afferents showed a 50% increase in total number in the saphenous nerve of NGF-OE mice (Stucky et al. 1999). Nearly all C fibers (96%) responded to heat and showed a four-fold increase in the number of heat evoked action potentials per C fiber. In addition, C fibers in NGF-OE mice exhibited spontaneous activity that was much higher than C fibers of wildtype mice (60% versus 6.5%, respectively). This increase in sensitivity was not global however, since the response of C fibers to mechanical stimulation was half relative to control fibers. Thus, increased NGF in the skin regulates the receptive properties of cutaneous C and Aδ fibers in differential manners.

Behavioral Phenotype of Naïve NGF-OE Mice

To evaluate the response of NGF-OE mice to inflammatory stimuli, the behavioral response of NGF-OE mice to a focused heat source applied to the foot was measured. Two other types of animals were used for comparison in this analysis: littermate control mice (Blk6/C3H strain) and transgenic mice that overexpress GDNF in the skin (GDNF-OE mice). GDNF-OE mice have an enhancement of GDNF-dependent nociceptor neurons (Zwick et al. 2002). GDNF-dependent neurons are peptide poor neurons, which primarily project to lamina II of the spinal cord (with some overlap in lamina I) and bind the plant lectin IB4 (Vulchanova et al. 2001). During postnatal development, GDNF-dependent neurons switch dependence from NGF to GDNF, and express the



Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain, Figure 1 CFA injections did not cause increased hyperalgesia in NGF-OE and GDNF-OE mice. (a) Comparison of all three genotypes. (b) Wildtype, (c) NGF-OE and (d) GDNF-OE mice were injected with CFA and tested for behavioral heat hyperalgesia over a 1 month time period. Each mouse line exhibited significant hyperalgesia within 3 days of being injected. NGF-OE mice recovered first (by day 5), followed by GDNF-OE mice on day 7. Wildtype mice did not fully recover until day 9. NGF-OE mice also exhibited hypoalgesia on days 15 and 22 (relative to their pre-CFA baseline). ν=Wildype mice; μ=NGF-OE mice; u=GDNF-OE mice; BL=Baseline value.

tyrosine kinase receptor Ret and its coreceptor GFRα1 (Molliver et al. 1997). GDNF dependent neurons have been proposed to primarily modulate responses to ► neuropathic pain as opposed to inflammatory pain (Snider and McMahon 1998). To compare NGF and GDNF-dependent nociceptor populations, the behavioral response of NGF-OE, GDNF-OE and wildtype (WT) mice to heat was measured (Fig. 1). This analysis showed NGF-OE mice had slightly longer latencies (they were hypoalgesic) relative to WT and GDNF-OE mice, which had equivalent baselines (Zwick et al. 2003).

Response of NGF-OE and GDNF-OE Mice to Inflammatory Stimuli

The response of NGF-OE, GDNF-OE and WT mice to inflammatory pain was tested by injecting an emulsion of ► complete Freund's adjuvant (CFA) subcutaneously into the plantar skin of the hind paw (Zwick et al. 2003). Sets of 10 animals were tested for heat and mechanical hyperalgesia at various timepoints following CFA injection (Fig. 1). WT and NGF-OE mice showed decreased response times 1 day post-injection (Fig. 1b–d). On day 3, all three genotypes displayed hyperalgesic behavior compared to their respective baselines. All groups of animals showed recovery following the 3-day time-point, with WT mice recovering to normal by day 9, GDNF-OE mice recovering by day 7 and NGF-OE mice recovering by day 5. NGF-OE mice not only recovered faster than wildtype and GDNF-OE mice, they became hypoalgesic between days 15 and 22 relative to their starting baseline. Thus, the increased number of nociceptors in NGF-OE and GDNF-OE transgenic mice did not cause a hyperalgesic phenotype in the naïve or inflamed state.

The lack of enhanced behavioral hyperalgesia in NGF-OE and GDNF-OE mice suggested compensatory changes developed in each transgenic mouse line in response to the trophin-induced anatomical and physiological changes. To examine how these analgesic effects could be elicited, mRNA expression for selected genes thought to be involved in nociceptive signaling was analyzed in the L4/L5 dorsal horn and DRG of naïve mice (Tab. 1 and 2) (Zwick et al. 2003). ► Real time PCR analysis of reverse transcribed total RNA isolated from the dorsal horn of the spinal cord and lumbar DRG were done. No significant change for any of the genes examined was found in dorsal horn mRNA samples (Tab. 1).

However, in L4/L5 DRG, significant changes were measured for most of the gene products examined (Tab. 2). In NGF-OE DRG, changes were found for the opioid receptors MOR1, DOR1, KOR1 and NR1, NR2B, mGluR1 and the sodium channel Nav1.3. In GDNF-OE DRG, mRNAs encoding DOR1, KOR1 and mGluR1 were changed. Thus, opioid and glutamate signaling in the primary afferent may contribute to the compensatory changes evoked in transgenic OE animals in the naïve state.

How these selected genes changed on the transcriptional level, following CFA injection into the hind paw, was then examined (Molliver et al. 2005). Genes expressed in lumbar DRG of NGF-OE, GDNF-OE and WT animals were assayed using real time PCR (Fig. 2). Measures were done at 0 (baseline), 1-day, 4-day and 15-day time-points, post CFA treatment. These times coincide with the development, maximal expression and resolution of thermal hyperalgesia, as indicated by the behavioral measures (Fig. 1). This analysis showed that opioid receptor mRNA abundance is changed in DRG following CFA injection (Fig. 2). Following CFA injection in

Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain, Table 1 Change in mRNA abundance in mouse dorsal horn

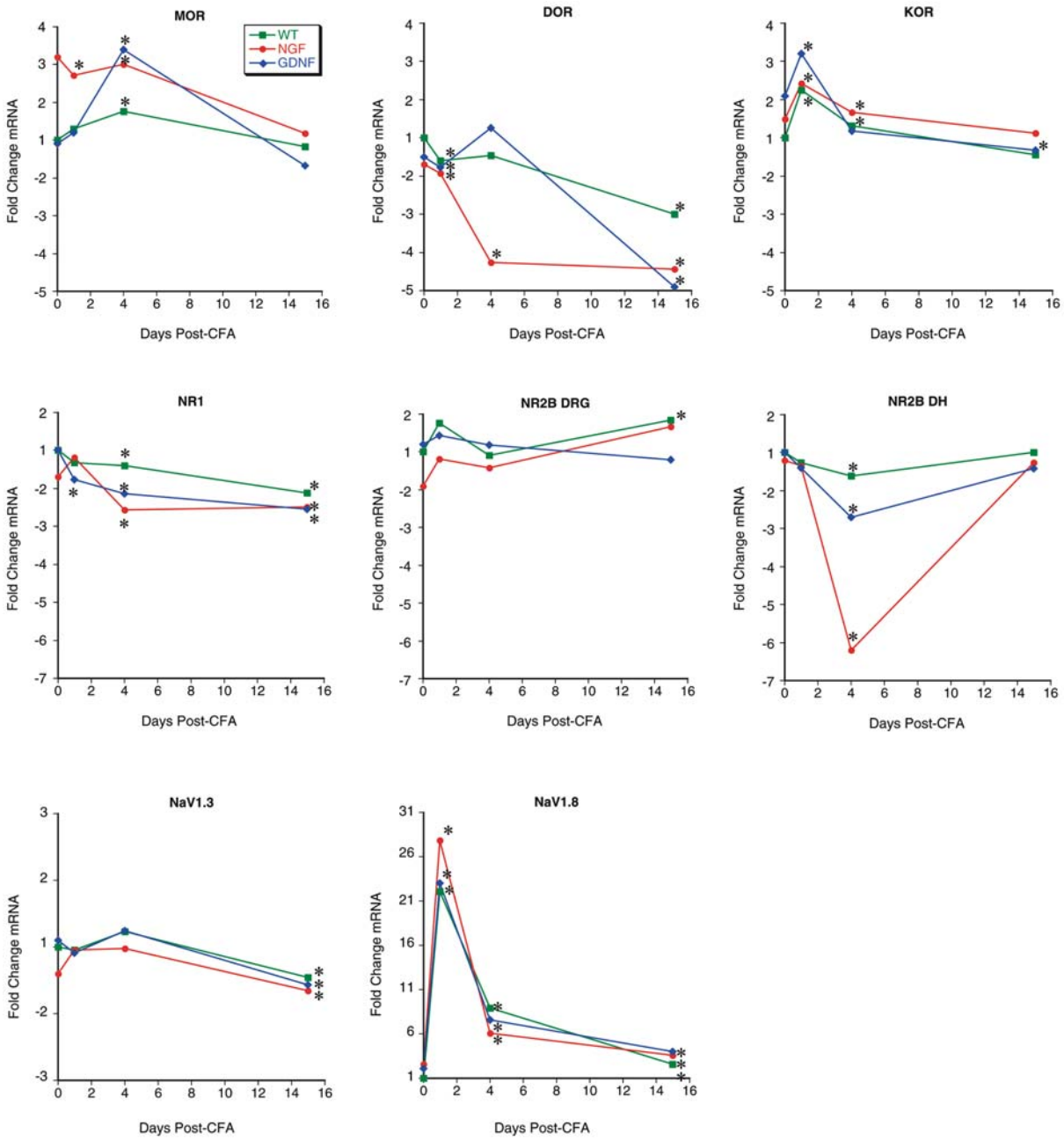
mRNA	WT vs. NGF-OE (fold change)	WT vs. GDNF-OE (fold change)
MOR1	1.0	+1.2
DOR1	1.0	+1.2
KOR1	+1.3	+1.1
NR1	-1.1	1.0
NR2B	+1.3	+1.3
mGluR1	+1.1	+1.1
DREAM	+1.1	+1.1

All values are reported as fold change relative to wildtype (WT) measurements. A value of "1" indicates no change. Negative values indicate a decrease. None of the observed changes were statistically significant

Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain, Table 2 Change in mRNA abundance in mouse L4-L5 dorsal root ganglia

mRNA	WT vs. NGF-OE (fold change)	WT vs. GDNF-OE (fold change)
MOR1	+3.2*	-1.1
DOR1	-1.6*	-1.5*
KOR1	+1.5*	+2.1*
NR1	-1.8*	1.0
NR2B	-1.8*	1.0
mGluR1	+3.1*	+3.7*
Nav 1.8	+2.6	+2.1
Nav 1.3	-1.4*	+1.1

All values are reported as fold change relative to wildtype (WT) measurements. Fold change equal to "1" indicates no change. Negative values indicate a decrease. Asterisk indicates $p < 0.05$



Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain, Figure 2 Comparison of the temporal change in mRNA levels of various genes related to nociception in DRG and dorsal horn of wildtype (green line), NGF-OE (red line) and GDNF-OE (blue line) mice following CFA injection in the hind paw. CFA injection was done at day 0 and the relative abundance of mRNAs for each gene determined using real time PCR assays. A significant change from the baseline value determined for each animal type is indicated by an asterisk.

WT mice, MOR mRNA levels were slightly elevated, in contrast to GDNF-OE mice, where a spike at 4 days occurred followed by a decline. Although the abundance of MOR mRNA was increased (3.2 fold) in NGF-OE mice at baseline, a steady decline in MOR levels was also measured in NGF-OE DRG. DOR mRNA was downregulated in WT mice following CFA injection, though an overall greater decline occurred in both OE lines. In all genotypes, KOR showed a peak rise at 1 day, followed by a decline back to near baseline levels.

For the NMDA receptor subunit NR1, a decrease for all genotypes occurred by the 4-day time-point and continued to the 15-day time-point. The decrease in NR1 in the transgenics is particularly profound, given the increased number of nociceptive neurons in these mice. Notably, both lines of transgenic mice recover from CFA-evoked

hyperalgesia early: NGF-OEs by day 5 and GDNF-OEs by day 7 (Fig. 1). The NR2B subunit showed no significant change in NGF-OE or GDNF-OE ganglia, and was only modestly elevated in WT ganglia at the 15-day timepoint. As NR2B may mediate central sensitization, mRNA abundance was assessed in the dorsal horn (DH) of the lumbar spinal cord. In WT mice, NR2B was slightly decreased in the dorsal horn at 4 days, with a return to baseline by 15 days. In the GDNF-OE and NGF-OE samples, this pattern of regulation was exaggerated, particularly for NGF-OE samples, which showed a near 7-fold decrease at 4 days. Similar to WT animals, both transgenic samples had a return to baseline by day 15. This suggests that NGF-OE mice compensate for the increased nociceptor input by downregulation of NR2B, which presumably restricts second messenger potentiation of NMDA currents, and inhibits sensitization of spinal synapses contributing to hyperalgesia.

The regulation of the sodium channel (Nav1.8 and Nav1.3) mRNA level in DRG of NGF-OE and GDNF-OE lines was very similar to the pattern of change in WT mice, i.e., a sharp rise in Nav1.8 is seen at 2 days post CFA, followed by a decline to near normal level by day 4. In contrast to the response in Nav1.8, Nav1.3 mRNA abundance showed a steady decline, which became significantly lower than baseline levels by day 15.

NGF overexpression in skin results in nociceptive primary sensory neurons that are hyperexcitable and present in substantially increased numbers. However, when tested behaviorally, these mice are resistant to inflammatory hyperalgesia and actually become hypoalgesic. Evidence suggests the resistance in each OE line to inflammatory pain is due to compensatory changes in nociceptive signaling, which act to reduce the impact of the increased nociceptive input. Understanding of how these compensatory changes develop and are regulated following injury will provide insight into the role of NGF in inflammatory pain processes. In particular, this model system has allowed identification of genes that are more susceptible to compensatory regulation. For instance, Nav1.8 is essentially the same in all genotypes after CFA, whereas in transgenic mice the opioid receptors and NMDA receptors exhibit striking alteration, relative to WT mice, in their patterns of transcriptional regulation following an inflammatory challenge. This suggests that specific elements in the transcriptional response to injury are particularly amenable to modulation and that their expression may determine the severity of the injury response, whereas other elements show a more fixed transcriptional response to injury. The NGF-OE mice provide a model system in which to examine this hypothesis. In addition, the OE system provides a means in which to determine how different subpopulations of nociceptive neurons respond to inflammatory stimuli. In this manner, a more global visualization of the role of growth factor expression in primary afferent sensitization following injury and their use as therapeutic targets can be constructed.

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Nerve Growth Factor, Sensitizing Action on Nociceptors

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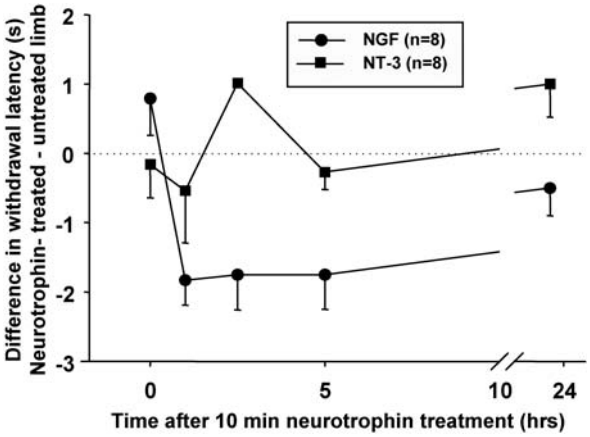
Definition

The response of the nociceptive system can be sensitized by exposure to a ► neurotrophin molecule called ► nerve growth factor (NGF). This sensitization has 2 components, one peripheral due to an enhanced response to nociceptive stimuli, and the other central due to increased action of nociceptive impulses in the dorsal horn.

Characteristics

Nerve Growth Factor is a member of a family of molecules called neurotrophins. Neurotrophins are best known for their function during development, specifically in promoting axonal growth and in assuring cell survival. Cells affected selectively by NGF express a specific receptor tyrosine kinase called ► trkA to which NGF binds. Nociceptors express trkA, which makes them sensitive to NGF during development (reviewed in Lewin and Mendell 1993; Mendell et al. 1999). Recently, however, a postnatal role for NGF has been established. Administration of NGF to an animal results in enhanced responsiveness to noxious stimulation (► hyperalgesia), which is partly due to direct sensitization of nociceptive afferents, i.e. peripheral sensitization. In addition, exposure of the receptive field of sensory neurons to NGF and other sensitizing agents, elicits changes in the cell body in the dorsal root ganglion that increase the central effect of sensory impulses, a phenomenon known as central sensitization. Several findings have established the involvement of endogenous NGF in sensitizing the subsequent response to nociceptive inputs after injury (reviewed in Lewin and Mendell 1993; Mendell et al. 1999). First, is the upregulation of NGF in skin and other peripheral tissues after inflammatory injury. A second is the demonstration that administration of exogenous NGF can elicit hyperalgesia. The third is the finding that inflammatory pain can be significantly reduced by interfering with endogenous NGF action, using either an antibody to NGF or an immunoadhesin (trkA-IgG) which sequesters endogenous NGF.

The time course of hyperalgesia elicited by systemically administered NGF (1 μg/g) has revealed 2 phases of the response, an initial thermal component beginning just a few minutes after NGF administration, and a later one beginning several hours after NGF administration that includes mechanical hyperalgesia (Lewin et al. 1994). The early response can also be elicited by local injections of NGF into the periphery (Fig. 1), suggesting that exogenous NGF directly sensitizes thermal nociceptive afferents but not high threshold mechanoreceptors (Shu et al. 1999). These confirm the results of previous recordings from individual nociceptors using a ► skin-nerve preparation. In these experiments, it has been found that the response to noxious heat is sensitized, measured as a decrease in threshold, whereas there is no systematic change in the threshold to mechanical stimulation (Rueff and Mendell 1996). This suggests that mechanical hyperalgesia is of central origin (Lewin et al. 1994; see below) although the possibility of e peripheral contribution by increased discharge of high threshold mechanoreceptors is not ruled out by currently available data.



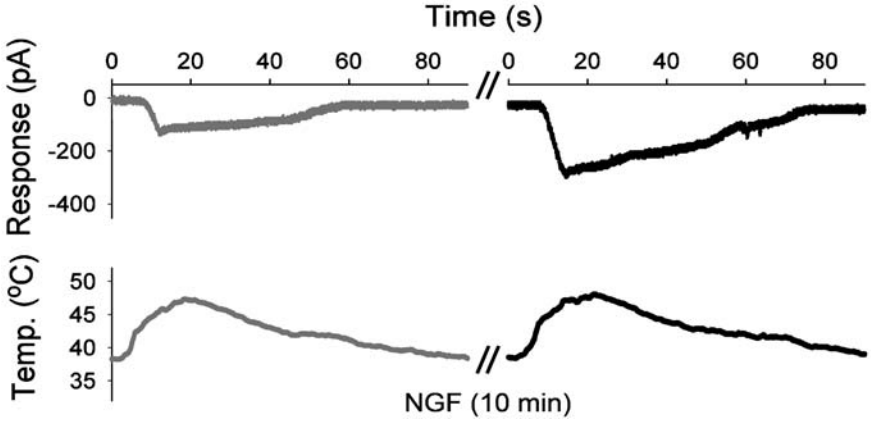
Nerve Growth Factor, Sensitizing Action on Nociceptors, Figure 1 Administration of NGF to the foot of the rat makes the affected paw hyperalgesic to noxious heat as measured by a reduced latency to withdrawal from a fixed thermal stimulus. The ordinate represents the mean difference in the latency of response of the affected limb compared to the contralateral limb (negative value implies that it took less time for the thermal stimulus to reach noxious threshold on the treated foot than on the untreated foot). NGF treatment gave a rapid and consistent thermal hyperalgesia lasting at least 1 day. NT-3 produced no change in response to noxious heat. (Adapted from Shu et al. 1999).

NGF has also been shown to operate as a sensitizing agent in visceral structures such as the bladder or the gut. As in skin, there is upregulation of NGF message and protein in painful inflammatory conditions, brought on by diseases such as interstitial cystitis or in an experimental model of ulcers (e.g. Lamb et al. 2004). Administration of NGF to the visceral periphery results in enhanced afferent activity. Experimental models of arthritis are also characterized by release of NGF into the synovial fluid, indicating a role in joint hyperalgesia (Manni et al. 2003).

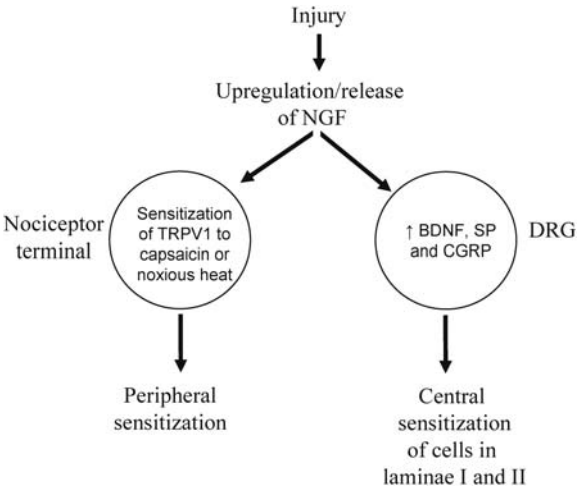
A difficulty in determining the mechanism of NGF action from these experiments arises from the multiplicity of cell types in the peripheral target tissues that express trkA (the high affinity receptor for NGF) or that release NGF. Many of these cells are non neural, and are believed to interact closely in the inflammatory cascade. For example, ► mast cells are known to express trkA

and to release NGF after injury, and ► keratinocytes have been shown to release NGF in response to histamine produced by mast cells (reviewed in Mendell et al. 1999). Degranulation of mast cells can diminish the sensitizing action of exogenous NGF and other inflammatory mediators (Lewin et al. 1994). In order to investigate the effect of NGF directly on nociceptors, small diameter cells acutely dissociated from DRG have been studied in culture. The assumption in carrying out such experiments is that the cell body in culture expresses the same receptors as the peripheral terminals *in situ*. A problem with this approach is that the original target (skin, muscle, viscera) can only be identified if it is prelabeled with a dye transported to the ganglion from the target tissue. However, this still leaves the identity of the receptor type (e.g. for skin: polymodal nociceptor, mechanical nociceptor, D-Hair, etc.) to be determined, since unique molecular identifiers are not yet available at this level of resolution. NGF is now recognized as an inflammatory mediator with a sensitizing action similar to that associated with other inflammatory mediators such as prostaglandin and bradykinin. The sensitizing effect of NGF has been examined most extensively on the response to capsaicin, which is now known to signal via the recently cloned ► TRPV1 receptor (also known as VR1). This receptor can also be activated by physiological stimuli, specifically noxious heat and low pH (rev. in Caterina and Julius 2001). Normally, the TRPV^1-mediated response studied in isolated cells is smaller to the second of 2 capsaicin or noxious heat stimuli (i.e., exhibits tachyphylaxis) that are separated by as much as 10 or 15 min (Galoyan et al. 2003; Shu and Mendell 1999). However, in the presence of NGF (100 ng/ml), tachyphylaxis does not occur in most cells; rather the second response is larger than the first, i.e. it is sensitized (Shu and Mendell 1999) (Fig. 2). These same studies have revealed that the initial response to noxious heat or capsaicin is larger on the average in the presence of NGF than in its absence. Sensitization by NGF is not accompanied by any systematic change in threshold temperature (Galoyan et al. 2003), unlike sensitization measured in the skin-nerve preparation (Rueff and Mendell 1996). Thus NGF-induced sensitization is not a property of nociceptors alone; other cells in the skin (keratinocytes, mast cells, etc.) are likely to contribute significantly. It is important to note that administration of NGF alone does not elicit any response from the cell; it merely sensitizes the response evoked by noxious heat or capsaicin. Immunohistochemical analysis of these cells reveals that the ability of NGF to sensitize these responses is strongly correlated with expression of trkA (Galoyan et al. 2003), indicating that sensitization to noxious heat by NGF involves an interaction between the ► trkA receptor and the TRPV1 receptor. Chuang et al. (2001) have demonstrated that activation of trkA disinhibits TRPV1 via action of phosholipase C (PLC) leading to a reduced level of PIP2 which, at normal levels, maintains a tonic level of inhibition of TRPV1. NGF also sensitizes the response of nociceptors by increasing their membrane gain, as determined by an enhanced action potential firing in response to an imposed current (Zhang et al. 2002). This occurs as a result of augmentation of a TTX-resistant Na^+ current known to be expressed in nociceptors. An additional factor underlying this enhanced response to depolarization is inhibition of a K^+ current. NGF mediates these actions on membrane gain by activating the ► p75 receptor, rather than trkA which is responsible for enhancing the inward current through TRPV1. The p75 receptor is coupled to the sphingomyelin signaling pathway, and exposure to ► ceramide, an independent intermediate of this signaling pathway, mimics the effect of NGF on membrane gain. Experiments with independent expression of p75 and TRPV1 in heterologous cells suggest that the p75 receptor is unlikely to be crucial for sensitization of the response of TRPV1 to capsaicin (Chuang et al. 2001). However, some modulatory effect of p75 on the response of trkA is not ruled out by these experiments.

Thus, NGF can sensitizes the response of nociceptors to noxious heat both by enhancing the response of the noxious heat sensitive receptor via trkA, and by amplifying the gain of the membrane via the p75 receptor, in effect sensitizing the response of the receptor as well as



Nerve Growth Factor, Sensitizing Action on Nociceptors, Figure 2 Response of small diameter DRG cell in acute cell culture to noxious heat stimulation. Note that the response to the second pulse of heat (bottom traces) measured 10 min after the initial response in the continuous presence of NGF (100 ng/ml) during the 10 min interval was a larger inward current (top traces) measured in perforated patch clamp mode. This sensitization is never observed under control conditions. (Adapted from Galoyan et al. 2003).



Nerve Growth Factor, Sensitizing Action on Nociceptors, Figure 3 Schematic diagram illustrating some effects of NGF in causing peripheral sensitization by direct action on nociceptive terminals and indirect central sensitization by upregulating peptides such as brain derived neurotrophic factor (BDNF), substance P (SP) and calcitonin gene related peptide (CGRP).

enhancing the gain of the impulse encoder. Longer term exposure to NGF also induces changes in the ► P2X3 Receptor composition of sensory neurons. Thus NGF can influence the response of these neurons to ATP which is released by non neural cells after damage or noxious stimuli (Scholz and Woolf 2002).

The central action of nociceptors is also sensitized by inflammatory stimuli including NGF (Scholz and Woolf 2002). NGF has not been shown to have any direct effect on spinal neurons in the superficial dorsal horn that are involved in transmitting nociceptive signals (Kerr et al. 1999). Rather, exposure of the peripheral terminals to NGF results in internalization of the NGF-trkA complex and transport to the cell body, where it stimulates upregulation of several peptides including substance P, CGRP and another neurotrophin, ► brain derived neurotrophic factor (BDNF). These peptides are released into the dorsal horn (e.g., Lever et al. 2001) where they can rapidly sensitize the response of dorsal horn neurons in lamina II to subsequent inputs (Garraway et al. 2003). They can also elicit changes in gene expression that are pronociceptive (long term central sensitization, Scholz and Woolf 2002).

Together, these studies indicate that the role of NGF in eliciting sensitization of nociceptors is complex with both direct peripheral and indirect central components (Fig. 3).

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Nerve Inflammation

► Inflammatory Neuritis

Nerve Injury

► Retrograde Cellular Changes after Nerve Injury

Nerve Lesion

Definition

Lesion to/damage of a peripheral nerve.

► Causalgia, Assessment

Nerve Ligation

► Retrograde Cellular Changes after Nerve Injury

Nerve Pain

► Peripheral Neuropathic Pain

Nerve Pain of Joint and Muscle Origin

► Neuropathic Pain, Joint and Muscle Origin

Nerve Stump Pain

► Neuroma Pain

Nerve Terminals

Definition

These axon endings are found in the dermis around the base of hair follicles and close to the surface of the skin (epidermis) where the hair emerges. These free endings contain specialized receptors that respond to changes in temperature and other events (pH) associated with tissue damage.

► Opioid Receptor Localization

Nerve Viral Infection

► Viral Neuropathies

Nervus Intermedius, Primary Otalgia

► Trigeminal, Glossopharyngeal, and Geniculate Neuralgias

Neural Blockade

► Cancer Pain Management, Anesthesiologic Interventions, Neural Blockade

Neural Compressive Syndrome

► Lower Back Pain, Physical Examination

Neural Foramen

Definition

Neural foramen is a foramen in the spinal canal which is bounded by the intervertebral disc, the pedicles and facet joints of the vertebrae above and below, and the posterior aspect of the vertebral bodies above and below. The nerve root exits through this foramen and the dorsal root ganglion is situated in the foramen.

► Dorsal Root Ganglionectomy and Dorsal Rhizotomy

Neural Plasticity

Definition

The ability of the brain and/or certain parts of the nervous system to change in order to adapt to new conditions such as an injury, and can include changes in synaptic connectivity and strength between cells.

► Cytokines, Effects on Nociceptors

Neuralgia

Definition

Neuralgia is pain that occurs along the distribution of a nerve or nerves initiated or caused by a primary lesion or dysfunction in the nervous system. Common usage often implies a paroxysmal quality, but neuralgia should not be reserved for paroxysmal pains.

► CRPS, Evidence-Based Treatment
► Opioids in Geriatric Application
► Orofacial Pain, Taxonomy/Classification

Neuralgia, Assessment

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Definition

Neuralgia is defined as a pain in the distribution of a nerve or nerves (IASP Pain Terminology 1994) (Merskey and Bogduk 1994). It is mostly associated with neuropathic pain states that occur after nerve lesion. It is a pure descriptive term that does not imply the etiology of the pain generation, nor the underlying pathophysiological mechanism, nor the characteristic of the pain. According to this definition, neuralgia pain may be located superficially in the skin or also in deep somatic structures, it

may be of constant spontaneous type, shooting type or of evoked type ► (hyperalgesia, ► allodynia).
Note: Common usage, especially in Europe, often implies a paroxysmal quality, but neuralgia should not be reserved for paroxysmal pains.
Although the definition clearly states the fact that the pain of neuralgia occurs within the innervation territories, the symptoms may in individual cases spread to some degree beyond the innervation territories. This is particular true for allodynic pain in for example ► postherpetic neuralgia or posttraumatic neuralgia that sometime occurs in formerly unaffected dermatomes or peripheral nerve territories. Thus the symptoms, signs and their distribution can lead to confusion with regard to the diagnosis.
According to the underlying etiology several different terms for neuralgias are commonly used in pain medicine. The following comprises examples of some syndromes without being complete.

Characteristics

Post-traumatic Neuralgia (PTN)

Traumatic mechanical partial injury to a peripheral nerve may lead to PTN. The cardinal symptoms are spontaneous burning pain, shooting pain and hyperalgesia and mechanical and, in some cases severe, cold allodynia. These sensory symptoms are confined to the territory of the affected peripheral nerve, although allodynia may extend beyond the border of nerve territories to a certain degree (Wahren and Torebjörk 1992; Wahren et al. 1991; Wahren et al. 1995).
Special forms of ► post-traumatic neuralgias are chronic compression injuries to peripheral nerves, e.g. spermatic neuralgia and ► meralgia paresthetica.

Postherpetic Neuralgia (PHN)

PHN is one of the most common types of neuropathic pain. Its etiology is well known; the recrudescence of the varicella zoster virus (VZV) with inflammation and damage to dorsal root ganglion cells. If the pain lasts more than 3–6 months after the acute shingles, the criteria for PHN are fulfilled. It typically occurs in elderly but otherwise healthy individuals with no previous history of chronic pain. The diagnosis is straightforward, based on the history of a dermatomal rash and the dermatomal distribution of the pain. The incidence of PHN in zoster-affected patients of all age groups is about 15%. The pain of PHN appears as the acute viral infection subsides and persists, often indefinitely. The severity is frequently sufficient to completely disrupt the lives of otherwise healthy individuals. Patients with PHN report one or more of the following: a steady, deep aching pain that often has an abnormal quality, a lancinating pain that is brief, intense and often described in terms reminiscent of ► trigeminal neuralgia and finally, dynamic mechanical allodynia, which is the induction of a sharp pain by light, moving, cutaneous stimuli. In individual patients, the most unpleasant aspect of their pain may be either a continuous deep aching pain, lancinating pain or allodynia (Dworkin and Portenoy 1994; Fields et al. 1998).

Cranial Nerve Neuralgias (Burchiel 2003; Kapur et al. 2003)

Trigeminal Neuralgia (TN)

Trigeminal neuralgia (tic douloureux) is a disorder of the fifth cranial (trigeminal) nerve that causes episodes of intense, stabbing, electric shock-like pain in the areas of the face where the branches of the nerve are distributed – lips, eyes, nose, scalp, forehead, upper jaw and lower jaw. The disorder is more common in women than in men and rarely affects anyone younger than 50. The attacks of pain, which generally last several seconds and may be repeated one after the other, may be triggered by talking, brushing teeth, touching the face, chewing or swallowing. The attacks may come and go throughout the day and last for days, weeks or months at a time, and then disappear for months or years. Trigeminal neuralgia is not fatal, but it is universally considered to be the most painful affliction known to medical practice (Fields 1996).

Glossopharyngeus Neuralgia (GN)

► Glossopharyngeal neuralgia is described as sharp, jabbing, electric or shock-like pain located deep in the throat on one side. It is generally located near the tonsil, although the pain may extend deep into the ear. It is usually triggered by swallowing or chewing.

Facial (Geniculate) Ganglion Neuralgia (FN)

Pain paroxysms are felt in the depth of the ear, lasting for seconds or minutes or intermittently. A trigger zone is present in the posterior wall of the auditory canal. Disorders of lacrimation, salivation and taste sometimes accompany the pain. There is a common association with herpes zoster.

Post-sympathectomy Neuralgia (PSN)

► Post-sympathectomy neuralgia is a pain syndrome associated with a lesion at the sympathetic nervous system. 1 to 2 weeks after lumbar or cervicothoracic sympathectomy, up to 35% of the patients develop a deep, boring pain. The pain of PSN characteristically has a proximal location within the innervation territory of the sympathectomized nerves. PNS patients describe a variable degree of deep somatic tenderness in the area of pain, which typically responds to oral cyclooxygenase inhibitors. PSN is often nocturnal and typically remits in a few weeks without specific treatment (Baron et al. 1999).

Meralgia Paresthetica

Meralgia paresthetica, a painful mononeuropathy of the lateral femoral cutaneous nerve, is commonly due to focal entrapment of this nerve as it passes through the in-

guinal ligament. It is a purely sensory nerve and has no motor component. Pain associated with paresthesias and numbness in the area of the anterolateral thigh are common symptoms. Rarely, it has other etiologies such as direct trauma, stretch injury or ischemia. It typically occurs in isolation. The clinical history and examination is usually sufficient for making the diagnosis. However, the diagnosis can be confirmed by nerve conduction studies. Treatment is usually supportive.

Differential Diagnoses

Atypical Facial Neuralgia and Post-traumatic Facial Pain

The typical symptom is a continuous, unilateral, deep, aching pain, sometimes with a burning component within the face, most commonly in the area of the second trigeminal branch. More than half the patients with nondescript facial pain report its onset after trauma to the face, often surgical trauma. Orbital enucleations, sinus procedures and complicated dental extractions are the most common procedures that antedate the appearance of pain. Fortunately, for the large majority of the patients, their pain problem is self-limited; within 1–5 years it subsides whether symptomatic treatment is effective or not. The mechanism underlying this disorder presumably involves activation or central pain transmission pathways; how and why this occurs remains to be elucidated (Burchiel 2003; Kapur et al. 2003).

Complex Regional Pain Syndrome type II (CRPS II, Causalgia)

Injury of a peripheral nerve may lead to CRPS II. In contrast to patients with post-traumatic neuralgias, CRPS II patients exhibit a more complex clinical picture. They show marked swelling and a tendency for progressive spread of symptoms in the entire distal extremity. Spontaneous and evoked pains are felt superficially as well as deep inside the extremity and the intensity of both is dependent on the position of the extremity (Baron et al. 2002; Janig and Baron 2003; Wasner et al. 1998).

Assessment of Neuralgia

Since neuralgia is a pure descriptive term defined as a pain that occurs within the innervation territory of a peripheral nerve or a nerve root, there are no objective diagnostic procedures. However, in addition to the pain history and the clinical symptoms clinical signs that are characteristic for neuropathic pain states are also helpful and should be analyzed with quantitative sensory testing to aid the diagnosis of neuropathy (e.g. in postherpetic neuralgia, posttraumatic neuralgia, meralgia paresthetica) (see below) (Baron 2000). However, it should be recognized that in several forms of neuralgia (e.g. idiopathic trigeminal neuralgia) sensory testing does not reveal any abnormalities.

Symptom-based Classification of Neuropathic Pain. I. General Definitions

Negative sensory symptoms

- Loss of sensory quality
- Due to system involved: hypoesthesia, hypoalgesia, thermhypoesthesia, pallhypoesthesia etc...
- Bothering, but not painful

Positive sensory symptoms

- Paresthesias
- Dysesthesias
- Spontaneous pain (burning ongoing pain, shock-like pain)
- Evoked pain (see below)
  - Allodynia: a normally non-painful stimulus evokes pain
  - Hyperalgesia: a painful stimulus evokes pain of higher intensity

Symptom-based Classification of Neuropathic Pain. II. Definition of Different Evoked Pains

- Static mechanical allodynia
  - Gentle static pressure stimuli at the skin evokes pain
  - Present in the area of affected (damaged or sensitized) primary afferent nerve endings (primary zone)
- Punctate mechanical allodynia
  - Normally stinging but not painful stimuli (stiff von Frey hair) evoke pain
  - Present in the primary affected zone and spread beyond into unaffected skin areas (secondary zone)
- Dynamic mechanical allodynia
  - Gentle moving stimuli at the skin (brush) evoke pain
  - Present in the primary affected zone and spread beyond into unaffected skin areas (secondary zone)
- Warm allodynia, heat hyperalgesia
  - Warm or heat stimuli at the skin evoke pain
  - Present in the area of affected (damaged or sensitized) primary afferent nerve endings (primary zone)
- Cold allodynia
  - Cold stimuli at the skin evoke pain
  - Characteristic of post-traumatic neuralgia and some polyneuropathies

- Temporal summation
  - Repetitive application of identical single noxious stimuli (interval <3 s) is perceived as increasing pain sensation

Quantitative Sensory Testing (QST) in Neuralgia

A bedside testing should be part of the physical examination to confirm e.g. loss of afferent function, as well as evoked pain symptoms (e.g. allodynia and hyperalgesia), i.e. dynamic mechanical allodynia (cotton swab). Additionally standardized psychophysical tests (von Frey hairs, thermotest) should be used to detect impairment and changes in warm and cold sensation as well as heat and cold pain thresholds. By these means the function of small myelinated and unmyelinated afferent fibers is assessed.
So far, no characteristic sensoric pattern of patients with neuralgia has been identified. However, the analysis is useful to determine and quantify the individual signs of each patient and to document successful response to treatment.

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Neuralgia, Diagnosis

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Synonyms

Neurodynia

Definition

Pain in the distribution of a nerve, ostensibly due to an intrinsic disorder of that nerve (Merskey and Bogduk 1994).

Characteristics

The taxonomical distinction between ► neuralgia and ► neuropathic pain is contentious. The distinction is largely historical, in that the classical neuralgias were named before the entity of neuropathic pain was popularised. Nevertheless, certain semantic, anatomic, and pathologic distinctions apply.
The term – neuralgia – explicitly means pain along a nerve. It neither identifies the aetiology, pathophysiology nor any specific feature e.g. pain quality. Neuropathic pain implies that the affected nerve has a disease, and typically it is associated with features of abnormal nerve function, such as numbness, hyperaesthesia, or allodynia. These latter features are characteristically absent in neuralgias, or are subtle and minor.
Neuralgia should also be distinguished from ► radicular pain. Although similar to neuralgia in some respects clinically, radicular pain has certain distinguishing features clinically, and with respect to aetiology and mechanisms (see ► radicular pain).

Archetypical Conditions

There are two archetypical conditions that are different from one another in many respects; and each is representative of other, less common conditions. These are: ► trigeminal neuralgia, and its relatives glossopharyngeal and vagal neuralgia; ► post-herpetic neuralgia, which is probably a unique condition, and which may exemplify other dorsal root ganglionopathies, such as tabes dorsalis, and possibly Guillan-Barre syndrome.

Site of Lesion

In trigeminal neuralgia the lesion is in the sensory root. It is not in the ganglion or in the peripheral nerve.
In post-herpetic neuralgia the lesion is largely in the dorsal root ganglion, but may also extend into the peripheral and central nervous systems.

Histopathology

Trigeminal neuralgia is a focal disorder of the cell membrane of the sensory root of the nerve. Focal demyelination is the primary pathology, located between the ganglion and the dorsal root entry zone of the nerve into the brainstem (Kerr 1967). The remainder of the root is normal (Rappaport et al. 1997). In the majority of cases demyelination is due to irritation of the sensory root by an aberrant blood vessel, typically, the superior cerebellar artery (Loeser 2001). Other causes include: aberrant veins, angiomas, and tumours of the posterior cranial fossa. Intrinsic demyelination may occur in patients with multiple sclerosis.

In post-herpetic neuralgia the fundamental pathology is intrinsic inflammation of the affected dorsal root ganglion. In time, inflammation is replaced by axonal degeneration. Demyelination, degeneration, and fibrosis occur in the dorsal root ganglion, associated with atrophy of the dorsal horn and the dorsal root, which may extend distally into the peripheral nerve, with loss of axons and a lymphocytic response (Watson et al. 1991).

Pathophysiology

In trigeminal neuralgia, areas of demyelination act as a site for generation of ectopic impulses ("ectogenesis") and/or abnormal impulse traffic. The ignition hypothesis (Devor et al. 2002) calls for ectopic generation of action potentials. The Calvin model only requires abnormal refractory periods and reflection of normally generated impulses (Calvin et al. 1977). In both models the location of the lesion proximal to the ganglion seems critical. This allows impulses to reflect between the lesion and ganglion, which becomes the basis for the characteristic reverberation of the pain.

In post-herpetic neuralgia, inflammation of the affected nerve may cause pain on the basis of neuritis, but progressive necrosis of peripheral axons and the cell bodies in the dorsal root ganglion may result in deafferentation and disinhibition of dorsal horn neurons. Thereby, post-herpetic neuralgia converts from a peripheral neuropathic pain to a ► central pain.

Epidemiology

The incidence of trigeminal neuralgia is estimated to be four per 100,000 persons per year (Katusic et al. 1990). Risk factors include multiple sclerosis and hypertension (Katusic et al. 1990). There is a weak association with multiple sclerosis, but multiple sclerosis could be an incidental finding given that vascular compression of the trigeminal root has been reported in individuals as young as seventeen years (Katusic et al. 1991).

Whilst few children develop post-herpetic neuralgia, the risk of contracting this disorder and the intractability of pain increases with age. This susceptibility is attributed to a selective decline in cellular immunity to varicella virus; and recurrences are strongly related to immunosuppressive conditions such as HIV and SLE (Head and Campbell 1900).

Clinical Features

Trigeminal neuralgia is characterised by electric shock-like brief stabbing pains, with pain–free intervals between attacks, during which the patient is completely asymptomatic. Onset is usually abrupt. Pain is restricted to the trigeminal nerve distribution, and idiopathic trigeminal neuralgia is not associated with sensory loss. Non-nociceptive triggering of the pain (light touch, hair movement, chewing, speech, wind puffs) is almost ipsilateral to the pain, and usually from the peri-oral region. Intra-oral trigger zones may be accompanied by a decline in general health.

Patients with PHN present with a constellation of painful sensations including burning, dysaesthesia, aching, itching, or severe paroxysmal of stabbing pain. Allodynia or hyperpathia may occur, and the sensitivity to touch is the most distressing feature to patients. These various features are consistent with loss of nerve fibres, of all types, and ultimately with central disinhibition.

Treatment

Although trigeminal neuralgia and post-herpetic neuralgia are both called neuralgias, they differ in pathology and pathophysiology. Consequently, they respond differently to treatment. There is no treatment universally applicable to all neuralgias. To be effective treatment should target the known pathomechanisms.

Since trigeminal neuralgia is a membrane disease, membrane-stabilisers (anti-convulsants) are the treatment of first choice. Classical agents include: phenytoin, clonazepam, and carbamazepine. Of these, only carbamazepine has been vindicated in placebo-controlled trials. Baclofen may be used an adjunct, if required. For resistant cases, gabapentin, and lamotrigine are reputedly effective, as is intravenous lignocaine (Sindrup and Jensen 2002).

When the condition is refractory to pharmaceuticals, various surgical interventions are known to be effective. The choice lies with the preference of the operator. They include ganglionolysis, radiofrequency neurotomy, balloon compression, or injection of glycerine or alcohol; and microvascular decompression (see ► Dorsal root ganglionectomy and dorsal rhizotomy).

For post-herpetic neuralgia, amitriptyline is the drug of first choice (Watson and Evans 1985). It is the only agent for which there is consistent and strong evidence of efficacy, from controlled trials. However, only about 60% of patients obtain reasonable benefit. For resistant cases, a large variety of interventions have been recommended, but few with evidence of efficacy (Kingery 1997). Gabapentin and opioids appear to be effective for resistant cases (Watson 2000). In contrast to their efficacy for trigeminal neuralgia, baclofen,

Neuralgia, Diagnosis, Table 1 Similarities and differences between trigeminal neuralgia, post-herpetic neuralgia, and radicular pain

DOMAIN	TRIGEMINAL NEURALGIA	POST-HERPETIC NEURALGIA	RADICULAR PAIN
Anatomical site	sensory root	**dorsal root ganglion**	**dorsal root ganglion**
Key pathology	demyelination	**inflammation**	**inflammation**
Aetiology	**extrinsic**	intrinsic	extrinsic
Mechanism	reverberating impulses	disinhibition	ectopic discharge
Site	pre-central	central	peripheral
Pain	paroxysmal	constant,	intermittent,
		burning	lancinating
Associated	trigger point	allodynia	nil
Neurological	normal	**sensory loss**	**sensory loss**
Treatment	**decompression**		**decompression**
	neuro-ablation		
	anticonvulsants	tricyclics	steroids

Bold text indicates features shared by two of the conditions, otherwise features are unique and distinctive

phenytoin, and carbamazepine are usually not helpful in post-herpetic neuralgia (Kingery 1997).
Topical applications of local anaesthetic or capsaicin can be used to palliate the cutaneous sensory symptoms, but these interventions do not target the fundamental mechanism of post-herpetic neuralgia. Topical capsaicin has been vindicated in a placebo-controlled trial (Watson et al. 1993). In extreme cases, surgical interventions can be undertaken (see ► Dorsal root ganglionectomy and dorsal rhizotomy).

Comparison

Whilst the ► neuralgias share certain features, they also share some features with radicular pain, but are distinct in others (Table 1). The similarities invite some practitioners to assume that the conditions belong to the same class, and should respond to the same treatments. However, the differences in pathology and mechanisms predicate distinctly different responses to treatment. Appreciating these differences is pivotal to successful management. Treatments that work for one type of neuralgia, will not work for another. Nor do treatments commonly used for neuralgias work for radicular pain.

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Neuralgia of Cranial Nerve V

► Tic and Cranial Neuralgias

Neuralgia of Cranial Nerve VII

► Tic and Cranial Neuralgias

Neuralgia of Cranial Nerve IX with or without Cranial Nerve X

► Tic and Cranial Neuralgias

Neuraxial Blocks

Definition

Neuraxial blocks or central neural blockade comprise of intrathecal (spinal) and epidural (cervical, thoracic, lumbar and caudal) blocks. They are the most widely used regional blocks. The blocks have a well-defined end-point and can be reliably produced with a single injection. Most neuraxial blocks are performed in the lumbar region. The arachnoid membrane is a delicate, nonvascular membrane that is closely attached to the outermost layer, the dura mater. Deep to the arachnoid membrane and between the arachnoid mater and the pia mater lies the intrathecal or subarachnoid space. It contains cerebrospinal fluid, the spinal nerve roots, a trabecular network between the two membranes, blood vessels that supply the spinal cord, and the lateral extensions of the pia mater, the dentate ligaments. The epidural space surrounds the dural mater sac. Anteriorly, it is bound by the posterior longitudinal ligament; posteriorly by the ligamenta flava and the periosteum of the laminae; and laterally by the pedicles and the intervertebral foramina with their neural roots. Cranially, the epidural space is closed at the foramen magnum where the spinal dura attaches with the endosteal dura of the cranium. Caudally, the epidural space ends at the sacral hiatus that is closed by the sacrococcygeal ligament. The epidural space contains loose areolar connective tissue, fat, lymphatics, arteries, a plexus of veins, and the spinal nerve roots as they leave the dural sac and pass through the intervertebral foramina. The epidural space communicates freely with the paravertebral space through the intervertebral foramina.

► Multimodal Analgesia in Postoperative Pain

Neuraxial Infusion

Definition

Neuraxial infusion is the delivery of medications via a catheter inserted into the epidural or intrathecal space.

► Cancer Pain Management, Anesthesiologic Interventions, Spinal Cord Stimulation, and Neuraxial Infusion

Neuraxial Morphine

Definition

Morphine administered into the cerebrospinal fluid (or epidurally) to reach the spinal cord directly, causing profound analgesia.

► Postoperative Pain, Acute Pain Team

Neuraxis

Definition

Neuraxis is the term that refers to the entire nervous system, from receptors in periphery to spinal cord and to the subcortical structures and cortex of the brain.

► Hypoalgesia, Assessment
► Hypoesthesia, Assessment

Neurectomy

Definition

Neurectomy is the removal of part of a nerve, implying surgery. This usually refers to the distal, or nerve portion farthest from the brain.

► Cancer Pain Management, Neurosurgical Interventions
► Trigeminal Neuralgia, Diagnosis and Treatment

Neuritis

► Inflammatory Neuritis

Neuroablation

Definition

Neuroablation is an irreversible surgical technique that permanently blocks nerve pathways to the brain by destroying nerves and tissues at the source of the pain. This may be caused by various means, such as thermal or chemical, and occur in various places, such as in peripheral nerve or the brain.

► Dorsal Root Ganglionectomy and Dorsal Rhizotomy
► Trigeminal Neuralgia, Diagnosis and Treatment

Neuroactive Substance

Definition

A substance that can activates cells of the nervous system.

► Cytokines, Regulation in Inflammation

Neuroaxial

Definition

The epidural and intrathecal (spinal) spaces. Neuroaxial analgesic/anesthetic techniques involve the administration of agents into these spaces.

► Postoperative Pain, Appropriate Management

Neuro-Behçet

Definition

Behçet's disease with neurologic manifestations.

► Headache Due to Arteritis

Neurobehavioral Scores

Definition

Neurobehavior testing is a means of assessing neurologic status of the neonate. The United States Food and Drug Administration has mandated the use of a neurobehavior test to assess the neonatal effects of medications on the newborn. The neurologic and adaptive capacity score (NACS) was developed for this purpose and is commonly used in research studies by anesthesiologists.

► Analgesia During Labor and Delivery

Neurochemical Markers

► Immunocytochemistry of Nociceptors

Neurochemistry

► Immunocytochemistry of Nociceptors

Neurodegeneration

Definition

Neurodegeneration is the continuous and progressive dying of neurons.

► Viral Neuropathies

Neurodynia

► Neuralgia, Diagnosis

Neurofilament Protein NF200

Definition

Neurofilaments are a class of intermediate filaments that are found in neurons. They form the structure of the cytoskeleton and are particularly abundant in axons. DRG neurons express low (68 kD), medium (155 kD) and high (200 kD) molecular weight neurofilament proteins. Large light DRG neurons are rich in expression of neurofilaments, especially the high molecular weight (200 kD) protein, whereas the small dark neurons are poor in expression of neurofilaments. The phosphorylated form, the 200 kD neurofilament, is localized specifically to the large light cell population, and therefore, the presence of immunoreactivity for this protein can be used to distinguish large light cells from small dark cells. Since a low level of non-phosphorylated 200 kD neurofilament is found in small and large neurons, an antibody against the non-phosphorylated form of the 200 kD subunit does not distinguish well between the large light and small dark neurons.

► Immunocytochemistry of Nociceptors

Neurogenic Claudication

► Lower Back Pain, Physical Examination

Neurogenic Inflammation

Definition

A subset of nociceptive Aδ and C afferents contain pro-inflammatory neuropeptides (mainly "sleeping" nociceptors). If a peptidergic C fiber is activated, it releases neuropeptides from all the nerve terminals, which belong to this C fiber (axonal tree). Since always more than one C fiber will be activated by noxious stimulation, a homogenous area of neurogenic inflammation in the vicinity of the painful stimulus occurs.Release of

these neuropeptides upon nociceptive activation causes inflammatory responses consisting of protein ► plasma extravasation (edema; mainly induced by substance P and neurokinin A) and vasodilatation (mainly mediated by CGRP). Endothelial activation and secretion, degranulation of perivascular mast cells and the attraction of leucocytes has additionally been observed in some tissues like the dura mater. This reaction was first described as the axon reflex by Thomas Lewis, and underlies the flare and wheal response often seen surrounding local tissue damage.

► Arthritis Model, Adjuvant-Induced Arthritis
► Cytokines, Effects on Nociceptors
► Formalin Test
► Freezing Model of Cutaneous Hyperalgesia
► Functional Imaging of Cutaneous Pain
► Inflammation, Modulation by Peripheral Cannabinoid Receptors
► Mechano-Insensitive C-Fibres, Biophysics
► Neurogenic Inflammation and Sympathetic Nervous System
► Nociceptor, Axonal Branching
► Nociceptors in the Orofacial Region (Meningeal/Cerebrovascular)
► Quantitative Thermal Sensory Testing of Inflamed Skin
► Substance P Regulation in Inflammation
► Sympathetically Maintained Pain and Inflammation, Human Experimentation

Neurogenic Inflammation and Sympathetic Nervous System

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Synonyms

Neurogenic inflammation, sympathetic nervous system

Definition

There is evidence that ► neurogenic inflammation may be influenced by the sympathetic nervous system. This evidence is based on experiments, mainly on animals, in which neurogenic inflammation was elicited by chemical activation of nociceptors, and the extent of either ► plasma extravasation (neurogenic edema) or vasodilation (flare) was measured before and after interventions on the sympathetic nervous system. Pharmacological experiments indicate that the interaction between sympathetic neurons and primary afferent neurons, which may be responsible for sympathetic modulation of neurogenic inflammation, can take place proximal and/or distal to the neurovascular junction, i.e. prejunctionally on the nociceptor terminal, and/or postjunctionally at the level of the blood vessels. Only a prejunctional interaction can specifically modulate ► neuropeptide release from small-diameter afferent terminals, whereas at the postjunctional level, the interaction would occur non-specifically and indirectly via changes in blood flow. However, both modes of interaction may be difficult to distinguish in experiments. Here only sympathetic modulation of neurogenic inflammation is considered likely to occur at the prejunctional level.

Characteristics

Capsaicin-Induced Neurogenic Inflammation in Skin

In healthy humans, after topical application of ► capsaicin to forearm skin, the area of flare was significantly decreased during whole-body cooling, which enhances sympathetic vasoconstrictor activity to the skin (Baron et al. 1999). However, capsaicin-induced pain and ► mechanical hyperalgesia were not changed, indicating an inhibitory effect of sympathetic activity on the signs of neurogenic inflammation, but not on the corresponding pain symptoms.

In contrast, in animal experiments, the flare response after intradermal capsaicin was found to be partially dependent on the sympathetic nervous system. It was reported that capsaicin injected intradermally into the hind paw of rats elicits dorsal root reflexes in peptidergic afferents, which antidromically evoke vasodilation outside the axon reflex area, as far as 20 mm remote from the capsaicin injection site. This part of the flare response was almost abolished by surgical ► sympathectomy, unaffected by decentralization of the postganglionic sympathetic neurons supplying the hind paw, and depended on an α_1-adrenoceptor-mediated mechanism (Lin et al. 2003). The sympathetic co-transmitter ► neuropeptide Y (NPY) via Y2 receptors also seemed to contribute to the flare (Lin et al. 2004). Vasopressin had no effects on the flare response, ruling out the possibility that sympathetic effects on the flare were indirectly due to the evoked cutaneous vasoconstriction. These findings suggest that sympathetic neurons contribute to neurogenic inflammation, and that the integrity of the sympathetic terminals, but not the ongoing activity of postganglionic neurons, may be the crucial factor. Interestingly, an almost identical sympathetic dependence was reported for mechanical hyperalgesia induced by intradermal capsaicin in the same rat model. Intradermal capsaicin injection led to mechanical hyperalgesia at the injection site (► primary hyperalgesia) and in areas remote from the injection site (► secondary hyperalgesia). Capsaicin-induced secondary hyperalgesia was blocked by the α_1-adrenoceptor antagonist prazosin but not by the α_2-adrenoceptor antagonist yohimbine. Surgical sympathectomy before capsaicin injection prevented secondary hyperalgesia (Kinnman and Levine 1995), but decentralization of sympathetic

postganglionic neurons did not affect mechanical hyperalgesia.

Neurogenic Inflammation of the Rat Knee Joint

Capsaicin injection into the knee joint of rats produces intraarticular plasma extravasation lasting for 30–40 min, which was found to be reduced after chemical or surgical sympathectomy (Coderre et al. 1989). This indicates that small diameter afferent-evoked plasma extravasation in the synovia is in part dependent on the sympathetic nervous system. However, release of norepinephrine from postganglionic sympathetic terminals by joint perfusion with 6-hydroxy-dopamine led to a much larger and prolonged plasma extravasation, which was almost abolished after surgical sympathectomy and after pretreatment with indomethacin, restored in the presence of prostaglandin E_2 but unchanged in rats treated with capsaicin neonatally. Similar results were obtained when bradykinin, instead of 6-hydroxy-dopamine, was injected into the knee joint. Decentralization of postganglionic sympathetic neurons supplying the hind limb left bradykinin-induced plasma extravasation unchanged (Miao et al. 1996). Indirect effects due to changes in blood flow resulting from interventions on the sympathetic nervous system were excluded. These observations led to the concept that neurogenic inflammation in the rat knee joint has two components: a relatively small component depending on primary afferent terminals and in part dependent on sympathetic neurons, and a second larger component depending on the presence of postganglionic sympathetic terminals but not on on-going sympathetic activity nor on capsaicin-sensitive afferents. Inflammatory mediators such as ► bradykinin are thought to release prostaglandins, and possibly other mediators from postganglionic terminals, to elicit this so-called sympathetically dependent neurogenic inflammation (Green et al. 1998).

In contrast, knee joint inflammation in rats induced by kaolin and carrageenan was found to depend, in part, on primary afferents, but was unaffected by sympathectomy (Sluka et al. 1994).

Prejunctional Control of Neuropeptide Release from Nociceptive Afferents by Sympathetic Transmitters

It is well established that in the superficial dorsal horn, synaptic release of glutamate from small-diameter afferents is inhibited by α_2-adrenoceptor agonists (e.g. Pan et al. 2002), indicating the presence of presynaptic α_2-adrenoceptors. Presynaptic inhibition of nociceptors via these receptors is thought to be one mechanism by which descending monoaminergic pathways control input from nociceptors at the level of the spinal cord. The evidence that the release of neuropeptides, such as substance P and CGRP, is inhibited in parallel with that of glutamate at spinal synapses is scarce. However, *in vitro* pharmacological studies on animals indicate that α_2-adrenoceptors are also present on the peripheral terminals of capsaicin-sensitive afferents (prejunctional receptors), and inhibit stimulation-induced release of neuropeptides.

In the guinea-pig lower airways, capsaicin-sensitive afferents elicit neurogenic inflammation by liberating ► substance P, ► neurokinin A and ► CGRP. Both CGRP release and neurokinin-evoked bronchoconstriction after a low dose of capsaicin or low frequency (1 Hz) antidromic stimulation of the vagus nerve were attenuated by α_2-adrenoceptor agonists (Lou et al. 1992). These inhibitory effects were small when high doses of capsaicin or high frequency (10 Hz) electrical stimulation of afferents were used. Similar experiments provided evidence for the presence of inhibitory prejunctional NPY (Y2) receptors on small-diameter afferents (see Lundberg 1996).The effects of both prejunctional α_2-adrenoceptors and Y2 receptors are likely to be mediated by the opening of large conductance Ca^{++}-activated K^+ channels (Stretton et al. 1992). As these observations were made on vagal rather than spinal afferents, it may be questioned whether these results can be generalized to all peptidergic small-diameter afferents.

However, results obtained in other animal models are similar. In the perfused mesenteric vascular bed of the rat, *in vitro* perivascular nerve stimulation elicits vasodilation, which depends on CGRP released from capsaicin-sensitive afferents (Kawasaki et al. 1988) that are mainly of spinal origin. Pharmacological experiments in this model indicate that norepinephrine, released from sympathetic postganglionic terminals, can suppress CGRP release from perivascular afferents by activation of prejunctional α_2-adrenoceptors, and that NPY also inhibits CGRP release via prejunctional Y receptors (Kawasaki 2002). Thus, results identical to those obtained in the lower airways of the guinea-pig were found in the rat mesentery, and these were confirmed in a number of *in vitro* studies on the control of isolated autonomic targets by neuropeptides originating from capsaicin-sensitive afferents.

Taken together, evidence indicates that the release of neuropeptides, which can elicit neurogenic inflammation from the peripheral terminals of capsaicin-sensitive afferents, is inhibited via prejunctional receptors for sympathetic transmitters. However, it remains to be shown that these prejunctional receptors play any functional role in the intact organism under the conditions of physiological or pathophysiological regulation. Rates of on-going activity in sympathetic neurons are normally low, and in particular, the release of NPY from sympathetic terminals requires a high rate of on-going activity that may rarely occur *in vivo*.

In conclusion, there is evidence that neurogenic inflammation, induced by the liberation of neurokinins and CGRP from peripheral terminals of capsaicin-sensitive afferents, may be influenced by sympathetic

postganglionic neurons by a direct action on peripheral afferent terminals. However, the evidence is not yet conclusive. While in some studies capsaicin-induced neurogenic inflammation in the skin and knee joint of the rat was found to depend, in part, on sympathetic neurons, other studies indicate that there are prejunctional α_2-adrenoceptors and NPY receptors on afferent terminals that may inhibit neurogenic inflammation.

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Neurogenic Inflammation, Vascular Regulation

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Definition

► Neuropeptides, such as ► neurokinins (► substance P, neurokinin A) and CGRP, are the mediators of ► neurogenic inflammation. Their primary targets are blood vessels within the microvasculature where they elicit vasodilation and, by increasing the leakiness of the blood-tissue-barrier, ► plasma extravasation. Neurokinin effects may, in part, be mediated indirectly by the activation of mast cells and endothelial cells. At the neurovascular junction, neurokinins and ► CGRP interact with sympathetic neurotransmitters, vasoactive hormones and autacoids produced by the endothelium that are involved in the on-going regulation of the vasculature.

Characteristics

Vascular Effects of Neurokinins and CGRP

Both neurokinins and CGRP act primarily on blood vessels, but their efficacy in evoking vasodilation and plasma extravasation is different. The neurokinins substance P and neurokinin A are the main mediators of neurogenic plasma extravasation. They act on neurokinin 1 (NK1) receptors on postcapillary venues, and within seconds lead to the opening of circular gaps of about 1.5 μm diameter between endothelial cells, exposing the basement membrane and permitting the leakage of plasma proteins into the interstitial space (McDonald 1998). In addition, substance P also degranulates mast cells, which enhances plasma extravasation by an indirect mechanism involving histamine. CGRP alone does not elicit plasma extravasation. However, it seems to cooperate with neurokinins, since it potentiates neurokinin-induced plasma extravasation, possibly resulting from its vasodilator action or from its inhibitory effect on substance P degradation (Gamse and Saria 1985, Escott and Brain 1993). Generally, neurogenic edema requires longer-lasting and higher frequency stimulation of small-diameter afferents than vasodilation, probably because neurokinin effects are short-lived and a higher amount of neuropeptides may be necessary.

The main mediator of vasodilation in neurogenic inflammation is CGRP, acting via CGRP 1 receptors. CGRP is one of the most potent vasodilators known, and upon brief stimulation of small-diameter afferents, elicits long-lasting vasodilation by relaxing small arteries, arterioles and precapillary sphincters. Most of the vasodilation, in particular during the later phase, can be blocked by the CGRP 1 receptor antagonist $CGRP_{8\text{-}37}$, but part of the vasodilation remains, indicating that CGRP is not the only vasodilator involved. Substance P and neurokinin A applied exogenously also evoke strong but short-lasting vasodilation. Their role in the vasodilation elicited by adequate or electrical stimulation of small-diameter afferents has been disputed, because in animal models NK1 receptor an-

tagonists can block vasodilation evoked by exogenous substance P and neurokinin A, but no clear effects of NK1 receptor antagonists on the vasodilation evoked by stimulation of small-diameter afferents was seen (Rinder and Lundberg 1996; Delay-Goyet et al. 1992). However, in the hairy and hairless skin of the rat, it has been possible to demonstrate that an NK1 receptor antagonist can delay antidromic vasodilation elicited by electrical stimulation of small-diameter afferents by several seconds. Furthermore, the NK1 receptor antagonist potentiated the reduction of the amplitude of antidromic vasodilation by $CGRP_{8\text{-}37}$, implying that substance P and/or neurokinin A play a role in the early phase of antidromic vasodilation (Häbler et al. 1999).

Endothelium Dependence of Vascular Neurokinin and CGRP Effects

The effects of substance P and neurokinin A, when applied exogenously, on vasodilation and plasma extravasation, are probably mediated indirectly, at least in part, via NK1 receptors located on vascular endothelium leading to the production of ► nitric oxide (NO), because these effects can be reduced by blocking NO synthesis (e.g. Whittle et al. 1989). It is, however, unclear whether the effects of neurokinins released upon adequate or antidromic electrical stimulation of small-diameter afferents are endothelium-dependent and involve NO. As perivascular nerves contact vascular smooth muscle on the adventitial side, it is an open question whether neuropeptides released at the neurovascular junction can penetrate the vascular wall to act on receptors located on the endothelium. Experimental studies addressing this issue are scarce. A study on neurogenic edema elicited by antidromic nerve stimulation in the rat found an involvement of NO in the response, but NO was generated by the neuronal isoform rather than the endothelial isoform of NO synthase (Kajekar et al. 1995). In another study on rats, the NK1 receptor dependent component of antidromic vasodilation was unaffected by blocking NO synthesis (Häbler et al. unpublished).

In contrast to the neurokinins, CGRP, applied exogenously or released from small-diameter afferents, exerts its vascular effects in a manner independent of the endothelium in most vascular beds, including that of the skin. Exceptions are the rat aorta and the gastric microcirculation of the rat, where the vasodilator effects of CGRP are partially inhibited by blocking NO synthesis (Holzer et al. 1995).

Interaction of Sympathetic Efferents and Small-Diameter Afferents in Vascular Regulation

As the vascular bed of most organs, including skin, is regulated under physiological conditions by low-frequency on-going activity in sympathetic vasoconstrictor fibers, the question arises, how this activity interferes with small-diameter afferent-induced vasodilation. In human skin, antidromic vasodilation evoked by transcutaneous electrical stimulation was decreased under the conditions of body cooling, which raises sympathetic vasoconstrictor activity to skin. This effect was abolished by an anesthetic block of the proximal nerves supplying the skin territory. Other stimuli that are known to increase sympathetic vasoconstrictor activity to skin, such as deep breaths or emotional stress, also transiently reduced antidromic vasodilation (Hornyak et al. 1990). In rat hairless skin, antidromic vasodilation elicited by brief stimulation of the corresponding dorsal root was able to override the vasoconstriction evoked by electrical stimulation of the sympathetic chain, up to a frequency of 3 Hz. Higher sympathetic frequencies suppressed antidromic vasodilation, but this suppression could be overcome by longer-lasting stimulation of the afferents at high frequency (Häbler et al. 1997). These studies show that the vasodilation elicited by thin afferents is likely to dominate over sympathetic vasoconstriction under almost all conditions of normal regulation, and may be reduced only when sympathetic vasoconstrictor activity is exceptionally high. Pharmacological experiments indicate that the interaction of both neural vasomotor systems occurs mainly at the postjunctional level, but inhibitory prejunctional α_2-adrenoceptors on peripheral terminals of small-diameter afferents may also be involved.

Implication of Neuropeptides Derived from Small-Diameter Afferents in Systemic Vascular Regulation

Neuropeptides are released from small-diameter afferents, not only in the context of noxious stimulation and local neurogenic inflammation, but they also appear in the systemic circulation, where they may be of importance for vascular regulation under physiological and pathophysiological conditions. Without any overt noxious stimulation, CGRP is present in the plasma of humans, and CGRP levels increase during exercise (Lind et al. 1996) and in patients with sepsis (Shimizu et al. 2003) and severe hypertension (Edvinsson et al. 1992). Studies on spontaneously hypertensive rats suggest that CGRP release from perivascular nerves may be impaired, implying a role for peptidergic small-diameter afferents in the long-term control of systemic vascular resistance and blood pressure (Kawasaki 2002).

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Neurogenic Pain

Definition

A pain syndrome arising after damage to the somatosensory pathways, from peripheral nerves and dorsal roots (peripheral neurogenic pain) to the spinal cord, brainstem, thalamus and cortex as well as the fibers in-between (central neurogenic pain). The denominations deafferentation pain, dysesthetic pain, neuropathic pain (for peripheral type) and central pain are also used. Neurogenic pain is characterized by the following clinical descriptors: 1) pain localization in and around the deafferented body part, 2) pain qualities (pins and needles, electrical discharges, burning, tearing and compressive), and 3) timing of the pain: continuous, intermittent in attacks (lasting a fraction to a few seconds) or in episodes lasting more than a minute. The history and the neurological examination often reveal the evidence and signs of somatosensory damage (hypoesthesia and hypoalgesia). The examination may, however, be normal in some patients if the deficits have been compensated over time. Neurogenic pain responds specifically to antiepileptics and antidepressants, and represents the most frequent indication for pain surgery in the case of chronicity and resistance to non-invasive therapies.

► Thalamotomy for Human Pain Relief

Neurogenic Pain of Joint and Muscle Origin

► Neuropathic Pain, Joint and Muscle Origin

Neurogenic Pain, Painful Neuropathy

► Neuropathic Pain, Diagnosis, Pathology and Management

Neurogenic Vasodilation

► Nociceptor, Axonal Branching

Neuroglial Cells

► Satellite Cells and Inflammatory Pain

Neuroimaging

Definition

Neuroimaging is the production of images of the brain and/or spinal cord. It can include Computerized Tomography (CT) scanning, Magnetic Resonance Imaging (MRI), Photon Emission Computerized Tomography (SPECT) and Positron Emission Tomography (PET).

► Amygdala, Pain Processing and Behavior in Animals

Neuroimmune Activation

Definition

Neuroimmune activation is the adaptive, specific activation of endothelial cells, microglia, and astrocytes leading to the production of cytokines, chemokines, and the expression of surface antigens (Deleo 2001).

► Viral Neuropathies

Neuroimmune Interaction

Definition

Interactions between the immune system and the nervous system.

► Cytokines, Regulation in Inflammation

Neuroinflammation

Definition

Following an immune challenge or an injury in the nervous system, immune cells invade from the vascular system. T-lymphocytes enter the central nervous system where the microglia are activated and express major histocompatibility complexes, particularly class II (MHC II). Blood derived macrophages also become activated and encroach the perivascular space. In the dorsal root ganglia and sympathetic ganglia, where the vasculature is leakier and microglia are absent, the inflammatory response involves activation of endogenous macrophages and invasion of hematogenous ones and T-cells. The role of the immune cells is controversial. The macrophages/activated microglia are phagocytic if neuronal death occurs, but T-cells may be neuroprotective.

► Viral Neuropathies

Neurokinin

Definition

The tachykinins are a family of small biologically active peptides whose principle mammalian members are substance P (11 amino acids) and neurokinin (NK) A and B (10 amino acids). These peptides are derived from precursor proteins, the preprotachykinins, which are encoded by two different genes. Three receptors for tachykinins, the so-called neurokinin receptors NK1, NK2, NK3, have been cloned and characterized to have seven transmembrane spanning segments, to be coupled to G proteins and to be linked to the phosphoinosite signaling pathway. Although NK1 receptors are considered to be substance P-preferring, NK2 receptors NKA-preferring, and NK3 receptors NKB-preferring, substance P, NKA and B are full agonists at all three tachykinin receptors.

► Cancer Pain Management, Gastrointestinal Dysfunction as Opioid Side Effects
► Neuropeptide Release in Inflammation
► Neuropeptide Release in the Skin
► Substance P Regulation in Inflammation

Neurological Deficit

Definition

Neurological deficit refers to loss of function related to the nervous system.

► Hypoalgesia, Assessment
► Hypoesthesia, Assessment

Neurolytic Drugs

Definition

Neurolytic drugs (usually 50-96% ethanol or 5-7% phenol) destroy nerve cells and stop pain-impulse transmission for days to months.

► Cancer Pain Management, Anesthesiologic Interventions, Neural Blockade
► Trigeminal Neuralgia, Etiology, Pathogenesis and Management

Neuroma

Definition

When a nerve is cut, the fibers in the nerve distal to the cut die, while the fibers in the nerve that lie closer to the brain survive, and after some time may begin to heal. When both the nerve and its insulation have been cut and the nerve is not fixed, the growing nerve fibers may grow into a ball at the end of the cut, forming a nerve scar or neuroma. A neuroma can be painful and cause an electrical sensation when tapped (Tinel's sign). If the nerve injury was partial such that the insulation was not cut, new fibers may grow down the empty cover of the tissue until reaching a muscle or sensory receptor.

► Ectopia, Spontaneous
► Sympathetically Maintained Pain in CRPS II, Human Experimentation

Neuroma Endbulb

Definition

Severed axons form swollen terminal endbulbs. This usually occurs as a prelude to sprouting, but endbulbs may persist in the absence of sprouting.

► Neuroma Pain

Neuroma Model of Neuropathic Pain

► Anesthesia Dolorosa Model, Autotomy

Neuroma Pain

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Synonyms

Nerve Stump Pain; Pain Associated with Traumatic Nerve Injury; Traumatic Nerve Endbulb Pain

Definition

The peripheral nerve consists of nerve fibers, supporting ► Schwann cells and associated elements such as blood vessels, extracellular matrix molecules and the nerve sheaths. The cell body of motor fibers (somatic and sympathetic) is in the anterior or intermedio-lateral part of the gray matter of the spinal cord, whereas the cell body of sensory fibers is contained in the paraspinal, dorsal root ganglion. When the peripheral nerve is cut, the injured fibers form terminal endbulbs and outgrowing sprouts (Fawcett and Keynes 1990; Fried et al. 1991). The nerve fibers distal to the cut are not supported by the cell body and as a result they undergo (Wallerian) degeneration. The Schwann cells survive and begin to divide, a process apparently triggered by denervation. When the denervated Schwann cells are encountered by the sprouting fibers, after nerve repair for example, regeneration proceeds in an orderly fashion. When Schwann cells guides cannot be accessed by the outgrowing sprouts, as for example happens in the event of amputation or if there is a large gap between the two ends of the severed nerve, the fibers entangle into an often bulbous mass at the proximal cut end of the nerve. This nerve end structure is known as a neuroma (Fawcett and Keynes 1990; Sunderland 1978).

Characteristics

Neuromas go through a life-cycle as the nerve fibers continue to grow out into the adjoining tissue. The ultimate appearance of the neuroma depends on milieu, how the nerve was injured and the amount of time that has passed (Campbell 2001). When a nerve is caught by a suture, as may happen inadvertently in surgery, a swollen bulb tends to form at the site, as the outgrowing sprouts are contained by the epineurium and scar tissue. When the nerve is cut and otherwise left alone in a healthy bed of tissue, the neuroma tends to be less discrete, as the outgrowing nerve fibers may advance and spread widely through the host tissue. Variations between full regeneration and a swollen ► neuroma endbulb abound, however. These variations may reflect the age of the patient, the nature and point of injury, the nature of the surrounding tissue, vascularization and genetic factors. All of these parameters can affect the fraction of severed nerve fibers that emit sprouts, the number that regenerate successfully, the number that fan out in local tissues at the nerve end and the number that become trapped within the swollen endbulb (Sunderland 1978). They *may* also affect whether the neuroma will be a source of pain. It is likely that most neuromas are not a source of pain.

Most neuromas, painful and non-painful, are due to nerve trauma caused by penetrating injuries, amputations, burns, bone fractures and surgery. Tumors and vascular insufficiency are also common causes. The location of some nerves, and perhaps their intrinsic biological properties, make them particularly prone to generating a (painful) neuroma. Entrapment may cause a neuroma if severe enough to actually sever axons. In these cases, the clinician observes a significant swelling of the nerve just proximal to the entrapment. After the entrapment is relieved the nerve fibers may regenerate. Morton's "neuroma" actually in most cases represents an entrapment neuropathy and may or may involve neuroma formation. Pain is associated with neuromas of cutaneous nerves and of nerves that serve muscles. It is uncertain how frequently pain originating in other deep tissues and viscera is related to neuroma formation.

Causes of Neuroma Pain

Surgeons in the past have followed the logic:

- Nerve injuries lead to neuromas.
- Nerve injuries are painful.
- *Ergo*, neuromas cause pain.

The exploration of this hypothesis has led to a richly complicated understanding of the basis of neuropathic pain. In short what we can say as of now is that yes, the neuroma is an important element in the genesis and perpetuation of pain, but that the biology of this pain goes well beyond the neuroma. A considerable amount of information has been obtained from observations on humans and on animal models, but these studies still leave unanswered questions. Most informative are electrophysiological recordings from injured nerves, from which we can say the following:

- Ectopic impulse activity in C-fibers, presumably nociceptive afferents, arises from the neuroma. Neuroma A-fibers also become abnormally active (Devor 2005).
- Abnormal spontaneous activity also arises in the dorsal root ganglion, though the prevailing data indicate that this activity is primarily in large cells that presumably give rise to A-beta (non-nociceptive) fibers (Devor 2005).
- The nociceptors that share the innervation territory of the injured nerve also become spontaneously active (Ali et al. 1999; Wu et al. 2001).

- Central abnormalities develop (e.g. in the dorsal horn) following nerve injury. It remains unclear to what extent discharges in nociceptive transmission pathways in the spinal cord and brain are dependent on peripheral inputs (Ji et al. 2003).

These considerations leave open the question of how important the neuroma itself is in generating pain. A very simple human experiment can go a long way towards resolving this issue. One may merely anesthetize the nerve injury site and determine whether the pain goes away for the duration of the block. If the pain goes away, then the neuroma generated the relevant neural signals that led to pain. If the pain does not go away, then the pain generator is elsewhere. Amazingly, there remains no comprehensive study of this question. For sure, there are cases where simply anesthetizing the neuroma gives rise to partial if not complete relief of pain. In other cases, however, pain persists (Campbell 2001).

In the instance where the neuroma does indeed directly play a role in generating pain, we still have a question of major clinical significance to consider. The neuroma may induce pain simply by virtue of (Burchiel et al. 1993) inherent spontaneous activity that arises in the nociceptors and/or it may induce pain when activated by virtue of its location. Neuromas have *mechanosensitivity* (Devor 2005; Koschorke et al. 1991; Koschorke et al. 1994). This means that mechanical stimuli applied to the neuroma produce neural activity and pain (the ► Tinel sign). Mechanical stimuli can arise in different ways. For example, the neuroma at the end of a stump of an amputated limb may be stimulated by the prosthesis. The neuroma infiltrating muscles or tendons in the hand or adhered to these by scar tissue may be stimulated every time the patient opens and closes his/her hand.

Treatment Approaches

In the clinical situation, where applying a temporary local anesthetic to the neuroma convincingly removes pain, what should be done? One possibility is to simply remove the neuroma. However, neuroma resection is a misguided surgical mission. As long as the sensory nerve fibers in the proximal nerve end are connected to the dorsal root ganglion, the neuroma reforms. Thus, neuroma resection is in fact *neuroma relocation* to the position of the new nerve cut. Will neuroma relocation work? This depends. If the dominant mechanism of pain production from the neuroma is inherent spontaneous activity, relocating the neuroma would not be expected to be effective. If however, the dominant mechanism is ► ectopic mechanosensitivity, then neuroma relocation does indeed make sense, if it is moved to a new location less prone to mechanical stimulation. There are numerous results in the literature about neuroma relocation surgery. Reports of efficacy range from 30 to 100% (Burchiel et al. 1993; Dellon et al. 1995).

If a nerve is only partially severed, intact conducting nerve fibers and severed neuroma sprouts may intermingle forming what may be termed a "► neuroma-in-continuity". Resection in this case may relieve the neuroma pain, but at the cost of residual nerve function. Nerve grafting may be feasible.

Part of the variability of neuroma pain may be due to unexpected peculiarities of the anatomy associated with the nerve's attempts to regenerate. For example, neuromas may form on the wrong (distal) side of an injured nerve (Belzberg and Campbell 1998). Nerves routinely have nerve branches going back and forth to one another. For example, in the forearm the superficial radial nerve has nerve branches to the lateral antebrachial cutaneous nerve. A nerve branch that makes its way to the distal end of another nerve that has been severed upstream encounters denervated Schwann cells. These denervated Schwann cells appear to attract outgrowing sprouts from the intact nerve. Over time the growing fibers could make their way back upstream along the distal portion of the severed nerve to reach the distal end of the cut nerve. The surgeon would do well to consider the possibility of such scenarios in planning neuroma relocation surgery.

Neuromas may be surgically treated in other ways as well. When a major nerve is involved, the primary approach is to repair the nerve. Clinical experience and some data from experimental animals (Lancelotta et al. 2003) suggest that pain is less when the nerve successfully regenerates. This raises an interesting clinical issue. In the instance where motor recovery is not feasible, for example in the event of proximal lesions involving the lower brachial plexus or sciatic nerve, should nerve repair be considered as a means to relieve pain? The answer is a qualified yes: "qualified" because little data are available to answer this question; "yes" because the rationale is compelling and the other options are less attractive. Painful cutaneous neuromas should be treated with proximal resection (*neuroma relocation surgery*), particularly when diagnostic block indicates that the origin of pain is in the neuroma. This is because the morbidity of the surgical procedure is low, regardless of the fact that efficacy may be as low as 30% (Burchiel et al. 1993; Sunderland 1978).

Other surgical options exist. Surgical sympathectomy may be considered in cases of sympathetically maintained pain. In the case of spinal nerve injury, it might make sense to consider dorsal root rhizotomy or dorsal root ganglionectomy, though these options are notorious for late recurrence of pain. Spinal cord or direct nerve stimulation may have striking palliative benefits. Pain might subside with time. Finally standard pharmacological approaches to neuropathic pain may be useful.

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Neuroma-in-Continuity

Definition

A bulbous swelling in the nerve formed by sprouting axons that are intermixed with nerve fibers that are in continuity. The nerve appears intact to gross inspection but is swollen at the point of pathology.

- ► Neuroma Pain
- ► Neuropathic Pain Model, Chronic Constriction Injury

Neuromatrix

Definition

Neuromatrix is the term used by Ronald Melzack to describe a proposed cortical/subcortical network of interconnections, including the limbic system, responsible for pain. See Ronald Melzack, "Gate Control Theory: On the Evolution of Pain Concepts," Pain Forum 5 (1996): 128-38.

- ► Ethics of Pain, Culture and Ethnicity

Neuromodulation

Definition

The delivery of an electric current (neurostimulation) or drugs (intrathecal drug delivery systems) directly to targeted nerve fibers to treat pain.

- ► Cancer Pain Management, Anesthesiologic Interventions, Spinal Cord Stimulation, and Neuraxial Infusion
- ► Dorsal Root Ganglionectomy and Dorsal Rhizotomy
- ► Stimulation Treatments of Central Pain

Neuromodulator(s)

Definition

Neuromodulators are signaling molecules that play a role in the alteration of baseline neural activity. These neural effector molecules can increase or decrease baseline membrane activation. Examples are substance P, dynorphin, enkephalin, galanin, cholecystokinin, and bombesin.

- ► Nociceptive Neurotransmission in the Thalamus
- ► Placebo Analgesia and Descending Opioid Modulation
- ► Spinothalamic Tract Neurons, Peptidergic Input
- ► Thalamic Neurotransmitters and Neuromodulators

Neuron

Definition

Peripheral nociceptive neurons are afferent nerve fibers (i.e. A-delta - and C fibers) that transfer nociceptive impulses from the periphery to the dorsal horn. In the central nervous system, nociceptive neurons only respond to stimuli that are noxious or painful.

- ► Allodynia (Clinical, Experimental)
- ► Drugs Targeting Voltage-Gated Sodium and Calcium Channels
- ► Hypoalgesia, Assessment
- ► Lateral Thalamic Pain-Related Cells in Humans
- ► Postherpetic Neuralgia, Pharmacological and Non-Pharmacological Treatment Options

Neuron Restrictive Silencer Factor

Synonyms

NRSF

Definition

Is a repressor that is predominantly expressed in non-neuronal cells. NRSF silences neuronal genes in non-neuronal cells by binding to the NRSE (neuron restrictive silencer element) motif.

► Substance P Regulation in Inflammation

Neuronal Architecture

► Spinothalamic Tract Neurons, Morphology

Neuronal Dysfunction

Definition

Neuronal dysfunction is a state in which neurons display abnormal properties compared to those observed in normal development. For example, neuronal injuries or various neurodegenerative diseases can change normal neuronal conduction velocity or activation threshold by altering receptor distribution and properties, protein synthesis, activation of secondary messengers, etc.

► Dietary Variables in Neuropathic Pain

Neuronal Hyperexcitability

Definition

Increased responsiveness of central neurons; may include increased activity in response to stimulation, reduced threshold, increased afterdischarge, and expansion of receptive field size.

► Central Pain, Pharmacological Treatments
► Post-Seizure Headache

Neuronal Release

► Opioid-Induced Release of CCK

Neuronal Structure

► Spinothalamic Tract Neurons, Morphology

Neuronavigation

Definition

A stereotaxic system used for locating internal structures in 3D space without the need for fixing the patient in a stereotaxic frame.

► Motor Cortex (M1)
► Secondary Somatosensory Cortex (S2) and Insula, Effect on Pain Related Behavior in Animals and Humans

Neuropathic Pain

Definition

Neuropathic pain is pain initiated or caused by a primary lesion or dysfunction in the nervous system. Although neuropathic pain includes a number of different conditions some characteristics are shared: pain is often described as stabbing or burning, sensory abnormalities are common and treatment is difficult. Neuropathic (neurogenic pain) has been described in about 1% of the population. It is caused by functional abnormalities or structural lesions in the peripheral or central nervous system and occurs without peripheral nociceptor stimulation. It is caused by heterogeneous conditions unexplained by a single etiology or anatomic lesion. There are many different causes of neuropathic pain. Neuropathic pain may arise from infection/inflammation (postherpetic neuralgia, HIV-associated neuralgia, postpoliomyelitis, leprosy, interstitial cystitis, spinal arachnoiditis, acute inflammatory polyradiculopathy); non-infectious illness (multiple sclerosis, diabetic neuropathy, thalamic pain syndrome, essential vulvodynia) and pain associated with pressure/entrapment (neoplasia, trigeminal and glossopharyngeal neuralgia, carpal tunnel) and injury/trauma (surgery, complex regional pain syndrome, spinal cord injury). The etiology may be classified as localized (ischemic neuropathy, Complex Regional Pain Syndrome, phantom limb) or diffuse (toxins, AIDS, alcohol). Damage can affect the peripheral nerves, the cranial nerves, the posterior nerve roots, the spinal cord and certain regions within the brain. A variety of pain-related phenomena (mechanisms) may be operative in an individual patient necessitating mechanistic-based treatment. Patients with chronic NP are over-represented amongst those who are refractory to classic analgesic including opioid therapy. Although NP is not always opioid insensitive, the treatment of choice are tricyclic antidepressants (e.g. amitriptyline) and antiepileptic drugs (e.g. gabapentin).

► Allodynia (Clinical, Experimental)
► Alpha(α) 2-Adrenergic Agonists in Pain Treatment
► Amygdala, Pain Processing and Behavior in Animals
► Cancer Pain
► Cancer Pain Management, Overall Strategy

- ► Cancer Pain Management, Treatment of Neuropathic Components
- ► Complex Chronic Pain in Children, Interdisciplinary Treatment
- ► COX-1 and COX-2 in Pain
- ► CRPS, Evidence-Based Treatment
- ► Deep Brain Stimulation
- ► Diabetic Neuropathy, Treatment
- ► Dorsal Root Ganglionectomy and Dorsal Rhizotomy
- ► DREZ Procedures
- ► Drugs Targeting Voltage-Gated Sodium and Calcium Channels
- ► GABA and Glycine in Spinal Nociceptive Processing
- ► Guillain-Barré Syndrome
- ► Human Thalamic Response to Experimental Pain (Neuroimaging)
- ► Hyperalgesia
- ► Hyperpathia
- ► Hypoesthesia, Assessment
- ► Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain
- ► Neuropathic Pain, Diagnosis, Pathology and Management
- ► Neuropathic Pain Model, Diabetic Neuropathy Model
- ► Nociceptive Circuitry in the Spinal Cord
- ► Opioids and Reflexes
- ► Opioids in Geriatric Application
- ► Opioids in the Periphery and Analgesia
- ► Opioid Modulation of Nociceptive Afferents In Vivo
- ► Opioid Responsiveness in Cancer Pain Management
- ► Pain Modulatory Systems, History of Discovery
- ► Pain Treatment, Intracranial Ablative Procedures
- ► PET and fMRI Imaging in Parietal Cortex (SI, SII, Inferior Parietal Cortex BA40)
- ► Postherpetic Neuralgia, Pharmacological and Non-Pharmacological Treatment Options
- ► Postoperative Pain, Appropriate Management
- ► Postoperative Pain, Persistent Acute Pain
- ► Postoperative Pain, Postamputation Pain, Treatment and Prevention
- ► Rest and Movement Pain
- ► Retrograde Cellular Changes after Nerve Injury
- ► Spinal Cord Injury Pain

Neuropathic Pain, Diagnosis, Pathology and Management

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Synonyms

Neurogenic Pain, Painful Neuropathy

Definition

► Neuropathic pain is pain initiated or caused by a primary lesion or dysfunction in the nervous system (Merskey and Bogduk 1994).

This definition allows neuropathic pain to encompass disorders of either the peripheral nervous system or the central nervous system. In the present context, neuropathic pain refers to pain associated with diseases or injuries of peripheral nerves. Pain associated with disorders of the central nervous system is more specifically referred to as ► central pain, and covered separately (see ► central pain).

Characteristics

Peripheral neuropathic pain occurs in a variety of diseases of peripheral nerves. These include: painful peripheral neuropathies, such as diabetic neuropathy, alcoholic neuropathy, and postherpetic neuralgia. It can also occur after injuries to peripheral nerves, such as avulsions, stretching or crush injury, or nerve transection.

Diagnosis

The diagnosis of neuropathic pain is suggested by the presence of certain features revealed by the ► medical history and neurological examination (see ► Diagnosis of Pain, Neurological Examination). The history reveals certain features about the nature of the pain, and may provide a cause. The examination reveals features of loss of neurological function or exaggerated neurological function.

Neuropathic pain is commonly worse at night. It is often described as shooting, stabbing, lancinating, burning, or searing. It can be continuous, but often presents as paroxysms of pain in the absence of any identifiable stimulus. In some conditions, such as nerve entrapment syndromes, the pain follows a nerve distribution, whereas in others such as neuropathies and poststroke pain, the distribution is more diffuse and may affect more than one body region. Pain experienced in a numb or insensate site (anaesthesia dolorosa) is highly suggestive of the diagnosis.

Cardinal to the diagnosis are features of disturbed neurological function. These may be in the form of loss of function, such as numbness, which indicates nerve damage directly; or they may be in the form of exaggerated function, which suggest loss of inhibition, and imply nerve damage. The latter features include: hyperalgesia (increased sensitivity to noxious stimuli), hyperpathia (increase response to minimal noxious stimulation), hyperaesthesia (increased sensitivity to touch), and allodynia (touch or brush is perceived as painful). Sometimes, neuropathic pain can be accompanied by the features of ► complex regional pain syndrome, such as temperature changes in the skin, swelling, skin colour changes, and increased or decreased sweating. Patients suffering neuropathic pain commonly suffer insomnia,

anxiety, depression, and a significant deterioration in their quality of life.
The diagnosis is essentially made on the basis of these clinical features. The diagnosis is consolidated if the patient has an obvious reason to have pain, such as a nerve injury or a metabolic disorder known to cause peripheral neuropathy. Investigations, such as ► electrodiagnostic studies, are not required, but may help to establish the exact aetiology in cases of peripheral neuropathy.

Pathology

Nociceptive pain is caused by noxious stimulation of Aδ fibre mechanothermal and C fibre polymodal nociceptors by algogenic substances. In contrast, neuropathic pain results from damage to, or pathological changes in, the axons of peripheral nerves.
The nature of the pathological changes leading to pain is poorly understood. Much of our understanding is based on animal models of neuropathic pain. However, the relevance of such models to the human experience of neuropathic pain, has been questioned.
The physiological and structural changes following nerve injuries have been summarised in several review articles (Siddall and Cousins 1998; Woolf and Mannion 1999). Following peripheral nerve injury, changes include:

1. Hyperexcitability and spontaneous action potential discharges from the damaged primary afferent fibres proximal to the injury, and near the dorsal root ganglion, due to an accumulation of voltage-sensitive sodium channels at the injury site and along the damaged axons;
2. Formation of neuromas where a nerve has been cut, or microneuromas where a nerve has been partially injured, which generates spontaneous action potentials in nociceptive afferents;
3. Sympathetic neo-innervation of the dorsal root ganglia of the damaged primary afferents which may lead to sympathetic activity initiating activity in sensory nerve fibres;
4. Changes in the morphology and physiological properties of dorsal horn neurones;
5. Decreased inhibitory mechanisms within the spinal cord due to several mechanisms, including a reduction of the inhibitory transmitter – GABA, and upregulation of the expression of CCK: an inhibitor of opioid receptors, and downregulation of GABA and opioid receptors;
6. Sprouting of the central terminals of damaged Aβ touch fibres from Lamina IV of the dorsal horn to lamina II, where normally only nociceptors terminate, and formation of contacts with pain transmission neurones projecting to the brain,
7. Spontaneous firing of pain transmission neurones that project to the brain but have lost their normal peripheral afferent input;
8. Death of interneurons in lamina II of the dorsal horn.

Some combination of these changes are thought to account for at least some of the clinical features of neuropathic pain conditions.

Prevalence

Neuropathic pain has been estimated to affect at least 0.6% of the United States population (Bennett 1998). However, this is most likely to be an under–estimation, as it does not include sufferers of chronic musculoskeletal conditions such as neck and back pain, including post-surgical patients, who often present with symptoms and signs highly suggestive of neuropathic pain. The true prevalence is thought to be closer to 2%.

Management

The management of neuropathic pain conditions is dependent on the diagnosis. For certain conditions such as nerve entrapment syndromes, management of the underlying cause of pain, whether it be malignant or non-malignant, often result in rapid improvement in symptoms. With varying degrees of success, neuromas can be resected, buried, or ligated, using a variety of neurosurgical techniques (see ► Dorsal root ganglionectomy and dorsal rhizotomy).
For most other neuropathic pain conditions, management is not as rewarding, and a multimodal approach is required.
Psychological approaches are geared at challenging and improving patterns of negative thoughts and dysfunctional attitudes, promoting healthy thoughts and emotions and encouraging reactivation, as well as educating the patient regarding their illness and in non-medical pain management strategies such as ► relaxation, imagery and hypnotic techniques.
Physical therapists have a role in maximising function in deactivated patients and in assessing the potential benefit of splints and other aids.
Medical approaches often form the mainstay of treatment, and includes the use of pharmacological agents, ► nerve blocks, ► intravenous infusions, ► TENS, ► acupuncture and ► spinal cord stimulation.
Pharmacological agents can be administered topically or systemically. Several of the agents presently available for the management of neuropathic conditions are believed to act as blockers of neuronal sodium channels, or by their action on noradrenaline and serotonin re-uptake.
For most neuropathic pain conditions, ► tricyclic antidepressants including amitriptyline and imipramine, and the anticonvulsant – gabapentin, are considered first-line agents. Their effectiveness has been confirmed for postherpetic neuralgia and for painful diabetic neuropathy (Bakonja et al. 1998; Graff-Radford et al. 2000; Max et al. 1987; Rowbotham et al. 1998; Sindrup et al. 1989)

There is increasing evidence for the effectiveness of topical lignocaine in relieving the pain of postherpetic neuralgia and other neuropathic pain conditions (Galer 2001; Sawynok 2003). In view of its good safety profile, this preparation is being increasingly used and gaining acceptance as a first-line agent.
Other pharmacological agents commonly used in the management of neuropathic pain include topical capsaicin, mexiletine, carbamazepine, sodium valproate, tramadol, slow release opioids, intravenous and intranasal ketamine, and subcutaneous lignocaine.
For patients with intractable pain, a programmable intrathecal drug pump allowing continuous delivery of active agents, or spinal cord stimulation are options.

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Neuropathic Pain, Joint and Muscle Origin

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Synonyms

Neurogenic Pain of Joint and Muscle Origin; Nerve Pain of Joint and Muscle Origin

Definition

Pain caused by a lesion or dysfunction of a dorsal root, spinal nerve or peripheral nerve trunk, that is reported by the patient to be originated in muscle or joint tissue (projected neuropathic pain).
Pain caused by a lesion or dysfunction of nerve fibers in consequence of disease processes affecting primarily non-neuronal joint or muscle tissues.

Characteristics

A typical example of a proximal nerve lesion associated with pain felt in deep somatic tissues is lumbar radiculopathy (sciatica) evoked by herniation of an intervertebral disc. The lesion results from mechanical nerve compression in concert with localized inflammation. Depending on the lumbar root affected, the pain is predominantly felt in the low back, anterior or posterior thigh, knee or lower leg. Many patients describe their pain as aching and cramping, which are characteristics of pain arising from muscle. Others describe their pain as sharp and shooting (Dubuisson 1999). Other injuries affect nerves that supply solely muscle tissue; here the patient's pain is definitely projected neuropathic muscle pain. Examples are iatrogenic injury of a spinal accessory nerve, which occurs as a complication of radical neck dissection or cervical lymph node biopsy (London et al. 1996), and entrapment of a part of the brachial plexus, the subscapular nerve, which is a known cause of the thoracic outlet syndrome (Huang and Zager 2004). Sciatica is painful because the nerve lesion generates extra action potentials in sensory neurons. The magnitude and pattern of the evoked impulse barrage, along with the innervation territory of the affected nerve fibers, determine the quality of the pain sensation and the tissue domain where the patient feels the pain. Neuronal activity may originate in sensory neurons that are directly affected by the nerve lesion. For example, following axotomy, impulse generation occurs in the neuroma and ► dorsal root ganglia (DRGs) containing axotomized neurons. Mechanisms of ► ectopic activity generation in lesioned neurons are outlined elsewhere in this volume.
Preclinical work indicates a specific form of projected neuropathic pain of muscle origin. Following surgically induced nerve lesions in rats, muscle afferents proved to be particularly prone to developing ongoing ectopic discharges originating in DRGs, whereas cutaneous afferents did not (Michaelis et al. 2000). Muscle afferents started to produce this abnormal activity even when they were not directly injured. Lesion of neighboring nerves was sufficient, and the more neighboring afferents were lesioned, the higher were the frequency of the ectopic discharges. Some firing muscle afferents probably had nociceptive function. Ongoing activity in these afferents may therefore generate ongoing muscle pain. Ongoing activity in muscle afferents may in addition contribute

to the development of central sensitization. Intriguingly, input through small diameter muscle afferents appears to produce central sensitization of longer duration than a similar input through cutaneous C fibers, which may be of clinical relevance beyond neuropathic pain (Wall and Woolf 1984). Furthermore, nerve lesion induces increase in brain-derived neurotrophic factor (BDNF) expression in large-diameter DRG neurons (Michael et al. 1999), which may be another key factor for development of central sensitization after nerve lesion (Thompson et al. 1999).

Neuropathic pain that is exclusively projected to a joint is very unlikely, because relatively few sensory neurons supply joints compared to muscle and the likelihood that a process like disc herniation predominantly affects joint afferents is accordingly low. However, projected neuropathic pain often includes joints.

Traumatic nerve lesions are well known triggers for the generation of neuropathic pain. The final treatment option for patients with knee or hip osteoarthritis is surgical joint replacement, which inevitably destroys the distal parts of many nerve fibers, particularly those that had supplied subchondral bone of the removed joint. Intriguingly, the vast majority of patients is completely pain free or report substantial pain reduction soon after replacement surgery (McLain and Weinstein 1999). Thus, joint replacement surgery very probably does not induce neuropathic pain.

The question as to whether or not in the course of chronic painful diseases that affect primarily non-neuronal joint or muscle tissues, a neuropathic component is developing is hard to answer with certainty. This is mainly because the clinical diagnosis of neuropathic pain is difficult when an overt neurological disease (e.g. postherpetic neuralgia or traumatic lesion of a major peripheral nerve) has been ruled out. In a recent study, Rasmussen et al (2004) asked whether signs and symptoms cluster differentially in groups of patients with increasing evidence of neuropathic pain. Patients were first categorized to have definite, possible or unlikely neuropathic pain. Subsequently, patients were examined using pain descriptors, intensity of five categories of pain, questionnaires and sensory tests. This resulted in a considerable overlap concerning signs and symptoms; single pain descriptors could not distinguish between the three categories of patients (Rasmussen et al. 2004). In addition, disease markers for neuropathic pain (e.g. biochemical or functional imaging) are not available yet. Regardless, patients with painful osteoarthritis, by far the most common joint disease, or rheumatoid arthritis are traditionally said to have nociceptive or inflammatory pain, not neuropathic pain (Backonja 2003; Rasmussen et al. 2004).

What can we learn from animal models? Recently, two rat models of osteoarthritis with well-characterized and human OA-like joint histopathology have been analyzed for pain related behavior (Combe et al. 2004; Fernihough et al. 2004). Surprisingly, at the same time that osteoarthritic joint pathology developed, a marked tactile allodynia was detected. Pharmacological investigations in one of the models, OA induced by intraarticular injection of iodoacetate, revealed that morphine and gabapentin, which are effective in the treatment of neuropathic pain, but not diclofenac were able to diminish the tactile allodynia (Combe et al. 2004; Fernihough et al. 2004). Since tactile allodynia is not a hallmark of OA symptomatology, the relevance of this preclinical result for human OA pain is unclear.

In conclusion, it is not known yet whether neuropathic pain can be caused by a lesion or dysfunction of nerve fibers that innervate joint or muscle tissues.

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Neuropathic Pain Model, Anesthesia Dolorosa Model

► Anesthesia Dolorosa Model, Autotomy

Neuropathic Pain Model, Chronic Constriction Injury

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Synonyms

Bennett Model; chronic constrictive injury; CCI

Definition

The ► chronic constriction injury (CCI) is a partial nerve injury, mostly used in rodents, which is produced by tying several ligatures around a nerve, such that these ligatures slightly constrict the nerve. This induces an incomplete nerve injury, which entails behavioral signs of ► hyperalgesia in the animals.

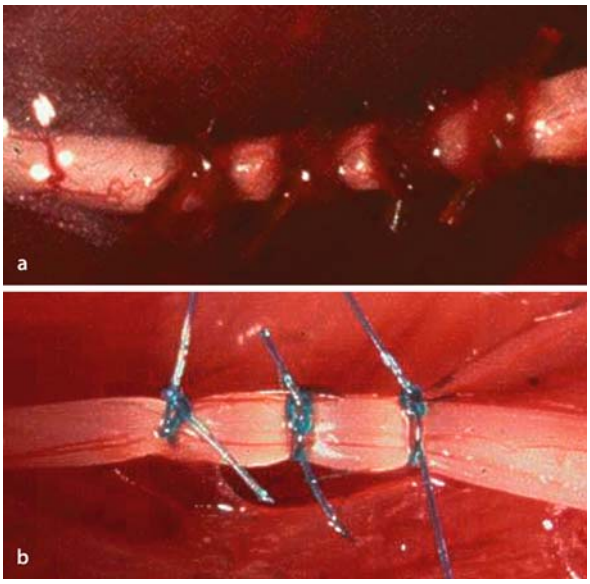
Characteristics

Animals, Technique, and Behavioral Data

Originally, the model was established in rats (Bennett and Xie 1988), but it has since been successfully used in mice (Sommer et al. 1997). Most often, the sciatic nerve is constricted, other nerves that have been used are the infraorbial nerve (Vos et al. 1994) and the median nerve (Day et al. 2001). The nerve is exposed by blunt dissection of the overlying muscles and several ligatures are tied around it such that they slightly constrict the nerve. Originally, four 4.0 chromic gut ligatures were used to constrict the sciatic nerve, which were tied around the nerve above the trifurcation with a spacing of 1 mm (Fig. 1a).

Many investigators have since used modifications of the model, either varying the number of ligatures (between two and four) or the type of ligatures (e.g. silk or vicryl). In mice, prolene (7.0 to 10.0) has been used (Fig. 1b).

In spite of minor differences, with most variations it has been possible to produce a reliable model of neuropathic pain. The degree of constriction may be more important than the type of suture in determining the degree and duration of behavioral signs, concomitantly with the degree of nerve injury and the time needed for regeneration (Myers et al. 1993; Lindenlaub and Sommer 2000). The measure of constriction is defined as to 'just barely constrict' the nerve and to retard, but not arrest circulation through the epineurial vessels (Bennett and Xie 1988). Typically, this type of constriction is associated with a short twitch of the respective limb of the animal. In the original description, paw withdrawal latencies to heat returned to normal between 60 and 90 days after the injury (Bennett and Xie 1988). In another early study, guarding behavior, increased responses to heat, cold and pressure returned to normal between six and eight weeks after the injury (Attal et al. 1990), which is very much what most investigators report. Some investigators report mechanical allodynia tested with von Frey hairs to occur less reliably in CCI compared to other models (Kim et al. 1997), however, this is in contrast to our own experience.



Neuropathic Pain Model, Chronic Constriction Injury, Figure 1 (a) Rat sciatic nerve. Four chromic gut sutures are tied around the nerve to produce a CCI. (b) Example of CCI in mouse sciatic nerve using three prolene 8.0 ligatures.

Neuropathology

Obviously, the amount of nerve damage depends upon the degree of nerve constriction by the ligatures, which makes the model quite variable between laboratories. Even if the initial constriction is only slight, the nerve develops edema, self-strangulates between the ligatures and thus develops additional damage. The temporal course of neuropathology of rat sciatic nerve with CCI has been extensively investigated (Munger et al. 1992; Coggeshall et al. 1993; Guilbaud et al. 1993; Sommer et al. 1993; Sommer et al. 1995). In summary, CCI induces the typical process of ► Wallerian degeneration. Most myelinated fibers, and about two thirds of the unmyelinated fibers degenerate, with maximum nerve damage during the first two weeks and a slow regeneration which is still incomplete three months after the injury. Remaining long-term changes include the formation of minifascicles and ► neuroma-in-continuity. Whereas endoneurial macrophages reflect axonal degeneration and phagocytosis, epineurial macrophages are the result of the foreign body reaction induced by the ligatures, which is one aspect in which CCI differs from models not using foreign material, like crush or partial sciatic nerve transection (Lindenlaub and Sommer 2000). Whether this epineurial inflammation plays a major role in the development of hyperalgesia is still a matter of debate.

CCI induces a large number of anatomical and neurochemical changes in the ► DRG, in the spinal cord, and possibly in the CNS. These include sprouting of sympathetic fibers in the DRG, changes in gene expression in DRG neurons, activation of spinal cord glia, changes in cerebral blood flow and many others. Expounding upon these is beyond the scope of this chapter. Correlations between any of these changes and hyperalgesia have to be considered with caution, since these may be general reactions to a nerve injury, irrespective of whether neuropathic pain develops or not.

Neurophysiology

After CCI, spontaneous activity develops in all fiber types, but more frequently in myelinated fibers, and can originate from the DRG or directly from the injury site (Xie and Xiao 1990; Kajander and Bennett 1992; Tal and Eliav 1996). Furthermore, spontaneous activity can be recorded in the L5/6 dorsal horn of the spinal cord (Sotgiu and Biella 2000). In addition, DRG-neurons from nerves with CCI are more excited by inflammatory mediators (Song et al. 2003). Thus, CCI, like other incomplete nerve injuries, induces a hyperexcitable state in the nerve fibers, which may be underlying the pain related behavior observed in the animals.

The qualities most often investigated in experimental animals with CCI are the withdrawal latencies to heat ('► thermal hyperalgesia') and the withdrawal thresholds to von-Frey-hairs ('► mechanical allodynia'). Several investigators have tried to determine which qualities are transmitted by which fiber type (A or C fibers), and whether peripheral or central changes are responsible for the respective quality in CCI. At present, the questions are not entirely solved. In summary, exaggerated responses to heat and cold are believed to be mediated by C-fibers, mechanical allodynia and hyperalgesia by Aβ and A^{TM}-fibers (Field et al. 1999).

Human Correlates

CCI has been claimed to be a model for different human pathological conditions that are associated with neuropathic pain. At present, no single disease is exactly modeled by CCI. One condition sharing some symptoms and many pathological features is ► carpal tunnel syndrome. Here, the median nerve is constricted, which leads to edema of the nerve, initially associated with hyperexcitability and pain only, later with overt nerve damage and neurologic deficits. Recovery is possible if decompression is performed in time. The difference to CCI is the abrupt onset in the animal model as opposed to the chronic course in the human disease, and, of course, the presence of foreign material in CCI.

Some parallels have been found with ► CRPS type II (Daemen et al. 1998; Suyama et al. 2002), specifically the presence of ► neurogenic inflammation and of osteoporosis. Importantly, CCI entails an incomplete injury of a (usually) mixed peripheral nerve, such that injured and uninjured fibers run in close vicinity in the nerve and that uninjured fibers are exposed to the altered endoneurial microenvironment induced by Wallerian degeneration. This situation is also present in incomplete nerve injuries in humans. One has to keep in mind that, in spite of the ligatures hindering regeneration, peripheral nerve regeneration in a rodent is considerably faster than in humans and that this difference in time course may account for some CCI-specific findings that may not be reflected by human disease.

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Neuropathic Pain Model, Diabetic Neuropathy Model

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Synonyms

Diabetic neuropathy model; Experimental Diabetic Neuropathy

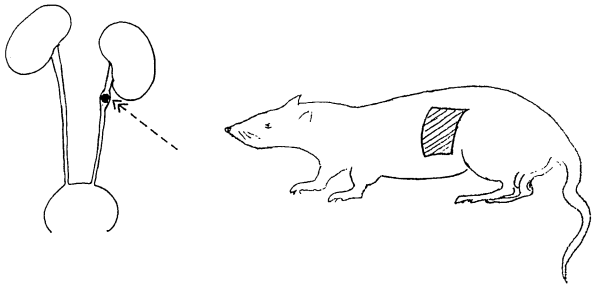
Definition

Diabetes mellitus is characterized by elevated blood glucose levels. This may be a consequence of either insulin deficiency (Insulin-Dependent Diabetes Mellitus or Type 1 diabetes) or impaired action (Non-Insulin Dependent Diabetes Mellitus or Type 2 diabetes). The term neuropathy implies physical degeneration of the nervous system, although it has been applied more broadly to encompass any neurochemical, physiologic or structural disorders of nerves. Diabetes can affect any combination of the sensory, motor or autonomic components of the peripheral nervous systems, and the central nervous system may also be vulnerable. Classifications of diabetic neuropathy reflect the distribution of nerves showing clinical evidence of dysfunction and the presumed etiology of the disorder (Llewelyn 2003). The most common form of diabetic neuropathy is a distal symmetrical polyneuropathy, which presents as sensory loss and/or pain in the distal extremities and progresses proximally towards the trunk.

Characteristics

Humans

Peripheral nerves obtained by biopsy or autopsy show pathologic changes in most cell types. Schwann cells exhibit reactive and degenerative changes that are presumed to represent early disorders, and precede overt pathologic features such as widening of the nodes of Ranvier and segmental demyelination. Axons undergo ► Wallerian degeneration, which is followed by inefficient regeneration and subsequent fiber loss. The ► vasa nervorum display endothelial cell hyperplasia and hypertrophy, which can reduce lumen size, and there is reduplication of the basal lamina. In final stages of diabetic neuropathy there can be almost complete loss of axons, Schwann cells and blood vessels, with the vacated endoneurial space filled by collagen. The physiologic consequences of this progressive degenerative neuropathy include nerve ischemic hypoxia, conduction slowing, progressive loss of sensation, muscle weakness and autonomic dysfunction. Some diabetic patients also describe the occurrence of inappropriate sensations such as pain or paresthesias, usually starting in the feet. Where painful diabetic neuropathy is present, the pain may be spontaneous or evoked by light touch, persistent or sporadic, and is frequently described as having a burning or lancinating quality.



Neuropathic Pain Model, Diabetic Neuropathy Model, Figure 1

Animal Models of Diabetes

Diabetes occurs spontaneously in a range of species including domestic companion animals and rodents. The latter have been bred to provide such models as the BB Wistar rat and NOD and db/db mice. Insulin-deficiency can also be induced by injecting animals with the β cell toxins ► streptozotocin or alloxan to produce models of severe type1 diabetes, while hyperglycemia in the presence of normal insulin levels can be induced by dietary overfeeding of sugars such as galactose or fructose. The feature common to all of these models is hyperglycemia, and this is assumed to be a primary pathogenic mechanism underlying diabetic neuropathy.

The streptozotocin-diabetic rat is the most commonly studied animal model of diabetic neuropathy, having the advantages of being cheap, easy to induce and allowing initiation of hyperglycemia at a defined stage of maturity. Disadvantages of the model include the extreme hyperglycemia (20–50 mmol/l) relative to diabetic patients (8–12 mmol/l) that can produce an unrepresentative catabolic dominance and ► cachexia. If not treated with insulin, these animals also have a life-span of only a few months. Concerns that nerve disorders in these animals are either unrepresentative of the human condition

or do not have the time to develop, may be addressed by using low level insulin replacement therapy to maintain muscle mass and general health of the animals whilst retaining hyperglycemia.

The relatively short life span of diabetic rodents may help explain why features of overt degenerative neuropathy are rarely reported. There is no significant loss of axons in the major nerve trunks of diabetic rodents within the time frame commonly studied (1–4 months), although diabetic mice develop loss of epidermal innervation (Christianson et al. 2003). Subtle ultrastructural changes may be found in some models and resemble early changes seen in diabetic patients (Kalichman et al. 1998), but in general, diabetic rodents are not faithful models of the pathology of diabetic neuropathy. In contrast, diabetic rodents do show physiologic disorders similar to those seen in diabetic patients. Most prominently, they exhibit slowing of nerve conduction velocities (NCV) by large myelinated motor and sensory fibers within weeks of the onset of hyperglycemia, and this has been used to justify these models as reflecting early stages of diabetic neuropathy. In the absence of the obvious axonal loss, nodal widening or segmental demyelination that is seen in human diabetic neuropathy, NCV slowing in diabetic rodents has been assumed to have a neurochemical or ultrastructural origin. While NCV slowing in diabetic humans and rodents may not necessarily have the same etiology, this defect has been studied extensively, to reveal mechanisms linking it to hyperglycemia and also to screen potential therapeutic agents. Other physiologic and neurochemical disorders that develop within weeks of the onset of hyperglycemia in rodents include increased glucose metabolism by enzymes of the ► polyol pathway, reduced nerve blood flow, increased ► oxidative stress, resistance to ischemic conduction block, decreased slow ► axonal transport and altered synthesis, transport and release of neuropeptides secondary to impaired ► neurotrophic support. Discovery of these disorders has inspired many hypotheses regarding the pathogenesis of diabetic neuropathy and prompted development of a number of therapeutic strategies, although none as yet have translated successfully to clinical use.

Diabetic rodents have also been used to model states of sensory loss or pain. This carries all of the caveats associated with animal models of ► neuropathic pain, with the additional concern that behavioral indices might also be modified by the parallel effects of hyperglycemia on motor nerve function or on non-neural systems. For example, diabetic rodents exhibit reduced locomotor activity and, when allowed to progress to a state of extreme untreated hyperglycemia, show a general behavioral depression. Nevertheless, there is accumulating evidence to suggest these models display indices of sensory dysfunction that reflect both the sensory loss and allodynia or hyperalgesia seen in diabetic patients.

Diabetic rats react to light touch of the plantar surface of the hind paws, indicating tactile allodynia that is of a magnitude similar to other models of neuropathic pain, such as spinal nerve ligation (Calcutt et al. 1996). This allodynia is frequently used in screening tests for agents designed to alleviate neuropathic pain (reviewed in Calcutt 2002). The etiology of tactile allodynia has not yet been identified, but unlike the majority of other nerve disorders in diabetic rodents, does not appear to be related to glucose metabolism by the polyol pathway (Calcutt et al. 1996).

Limb withdrawal from a more substantial mechanical stimulus is also altered in diabetic rodents, with both the tail pinch and paw pressure tests indicating an increased sensitivity, either by a faster withdrawal from a fixed pressure or a response to lower amounts of pressure. As with tactile allodynia, mechanical hyperalgesia in diabetic rodents has been widely used to evaluate the potential of drugs designed to alleviate pain (see Calcutt 2002), although whether the disorder has a direct correlate in the human condition is not clear.

Thermal hyperalgesia has been described in some diabetic patients, although in most cases there is a progression to thermal hypoalgesia, which is assumed to be associated with loss of epidermal thermal nociceptors (Kennedy et al. 1996). Studies in diabetic rodents have reported thermal hypoalgesia, thermal hyperalgesia or no change relative to controls. This lack of agreement between studies may reflect variations in experimental protocols such as differences in the species, sex, strain, limb tested, heating method and degree or duration of diabetes. We have found that when exposing the hindpaw plantar surface of diabetic rats to a temperature ramp, rising from 30° C at a rate of 1° C per second over 20 seconds, they exhibit a transient thermal hyperalgesia after 4 weeks of hyperglycemia that progresses to thermal hypoalgesia in 2–3 months. Concerns that the progression to hypoalgesia reflects general behavioral depression of ailing animals may be assuaged, by the observation that it can be prevented, by blocking glucose metabolism by the polyol pathway without affecting systemic indices of insulin-deficient diabetes (Calcutt 2002). The etiology of thermal hyperalgesia is unclear, and the hypothesis that the progression to thermal hypoalgesia reflects loss of epidermal thermal nociceptors remains to be confirmed.

Diabetes also modifies other behavioral tests that were developed to study mechanisms of hyperalgesia, particularly those that highlight the role of spinal nociceptive processing (Yaksh 1999). The transient thermal hyperalgesia induced by direct application of substance P to the spinal cord of normal rats is prolonged in diabetic animals (Calcutt et al. 2000a). Injection of dilute formalin into the hindpaw induces a biphasic pattern of defined flinching behavior, in which the second phase incorporates spinal amplification of primary afferent input. In diabetic rats there is more flinching during both phase 2

and the intervening quiescent phase (Calcutt at al 1996). Interestingly, diabetic mice show a loss of responses to formalin (Kamei et al. 1993).

The description of a range of behavioral disorders that are associated with sensory dysfunction in diabetic rats has stimulated a search for underlying electrophysiologic or neurochemical changes in these animals. There is accumulating evidence that both peripheral and spinal sites of nociceptive processing may be involved, while the effects of diabetes on higher processing centers has been poorly studied to date. In the periphery, changes in threshold or firing response to stimuli have been explored (Ahlgren et al. 1992), and while the literature has been divided regarding the occurrence of spontaneous activity in sensory fibers of diabetic rats, some recent reports support this view (Khan et al. 2002). One impediment to the concept of hyperalgesia in diabetic rats being driven by exaggerated peripheral primary afferent activity is that the synthesis, axonal transport and stimulus-evoked release of excitatory neurotransmitters is decreased in diabetic rats, despite concurrent hyperalgesic behavior (Calcutt et al. 2000b). This has prompted consideration of the role of spinal cord hypersensitivity in behavioral allodynia and hyperalgesia (Calcutt 2000a), and increased spontaneous activity has been recorded (Pertovaara et al. 2001; Chen and Pan 2002) along with increased local production of inflammatory mediators (Freshwater et al. 2002).

Diabetic rodents are probably best viewed as modeling the early biochemical and physiologic disorders associated with diabetic neuropathy. Behavioral studies have shown that they exhibit tactile allodynia, mechanical and chemical hyperalgesia and changes in thermal discrimination. The validity of studying the etiology of these disorders, will only be supported when mechanisms suggested in the animal models are identified in patients with diabetic neuropathy, and when drugs screened using these behavioral assays successfully translate to clinical use.

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Neuropathic Pain Model, Neuritis/Inflammatory Neuropathy

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Synonyms

Sciatic Inflammatory Neuropathy; SIN; Sciatic Inflammatory Neuritis

Definition

Neuropathic pain can arise from frank nerve trauma and/or inflammation of peripheral nerves (Bourque et al. 1985; Said and Hontebeyrie-Joskowicsz 1992). In addition, pathological pain can arise from: a) tissues innervated by neighboring healthy nerves, called extraterritorial momenpain (Willis 1993) and b) healthy areas contralateral to the site of tissue damage, referred to as mirror pain (Koltzenburg 1999). Animal models have focused on examining mechanisms underlying neuropathic pain associated with nerve damage (Bennett and Xie 1988; Seltzer et al. 1990; Kim and Chung 1992; DeLeo et al. 1994). The sciatic inflammatory neuropathy (► SIN) model was recently developed to

examine how inflammatory, extraterritorial and mirror image pain are created without frank nerve trauma (Chacur et al. 2001; Gazda et al. 2001; Milligan et al. 2003; Spataro et al. 2003).

Prior procedures that create inflammation of the sciatic nerve were used as a foundation to develop the SIN model (Eliav et al. 1999; Eliav et al. 2001). Modifications were made to of these prior procedures in order to allow the full time course of ipsilateral and mirror image allodynias to be productively studied, even at their earliest onset, after induction of peri-sciatic immune activation. Further, these modifications allow peri-sciatic immune activation to be induced in freely moving rats without the use of anesthetics, as these drugs can grossly alter immune responses (Lockwood et al. 1993; Sato et al. 1995). Lastly, SIN can be used to examine the responses of peri-sciatic immune cells and spinal cord neurochemical function underlying acute (less than 24 hrs) and extended (up to 14 days) allodynias. This chapter will describe detailed surgical methods for chronic peri-sciatic catheterization, and a brief protocol for induction of inflammation to create SIN.

Characteristics

Tools and Supplies for Preparing Peri-Sciatic Catheters, Surgery, Catheter Injectors and Catheter Cleaners

Peri-sciatic catheters are constructed from:

1. Sterile gelfoam sheets (Approximately 2×6 cm; NDC#0009-0315-03; Upjohn, Kalamazoo, MI). Each sheet will produce 4 gelfoam pieces
2. Silastic tubing (1.57 mm inner diameter, 2.41 mm outer diameter; Helix Medical Inc., Carpinteria, CA)
3. Sterile silk suture (4-0 & 3-0) with attached needle (cutting FS-2 and FS-1, respectively; Ethicon, Somerville, NJ)
4. Sterile polyethylene tubing (PE-50, Becton Dickinson, Sparks, MD)
5. #11 scalpel blade
6. Sterile metal metric ruler (15 cm)

Tools used for Chronic Peri-Sciatic Catheter Surgery are:

1. 2 pairs of micro-forceps
2. 1 pair toothed forceps
3. 1 pair blunt dissection scissors
4. suture hemostats with scissors
5. #11 or #10 scalpel blades
6. 1 scalpel handle
7. 2 small towel clips
8. 4–8 glass Pasteur pipettes with the tips previously melted into small hooks
9. 1 hot glass-bead sterilizer (World Precision Instruments, Sarasota, FL) to sterilize tools between use
10. 1 shaver
11. 70% alcohol
12. Exidine-2 surgical scrub solution (undiluted, Baxter Healthcare, Deerfield, IL)
13. Sterile gauze (4×4 inches) to create a drape around the surgical site
14. ~800 ml of both an antibacterial water mixture and water for cleansing hands between surgeries
15. Sterile autoclave paper (12×12 cm) to provide a clean surface for placing sterilized tools
16. 1 sleeve/rat that protects the exteriorized portion of the peri-sciatic catheter (cc-sleeve) described in detail previously (Milligan et al. 1999)

The catheter injector is made from:

1. A sterile 23-gauge, ^{1}-inch hypodermic needle (Becton Dickinson, Franklin Lakes, NJ)
2. PE-50 tubing (Becton Dickinson, Sparks, MD)
3. An autoclaved 100 ul Hamilton glass syringe (Fisher Scientific, Houston, TX)
4. A black permanent fine-tip marker
5. Sterile metal metric ruler (15 cm)
6. Dental probe with a 45° angel (microprobe; Fisher Scientific, Houston, TX)

The catheter cleaners are made from:

1. A sterile 3-cc syringe
2. A 23-gauge needle
3. PE-50 tubing (Becton Dickinson, Sparks, MD)

Chronic Peri-Sciatic Catheter Construction, Surgery, Injection and Cleaning Procedures

The procedures detailed here are for:

- The construction of supplies required for chronic peri-sciatic catheters
- Chronic peri-sciatic catheter surgeries
- Cleaning and injecting drugs around the sciatic nerve

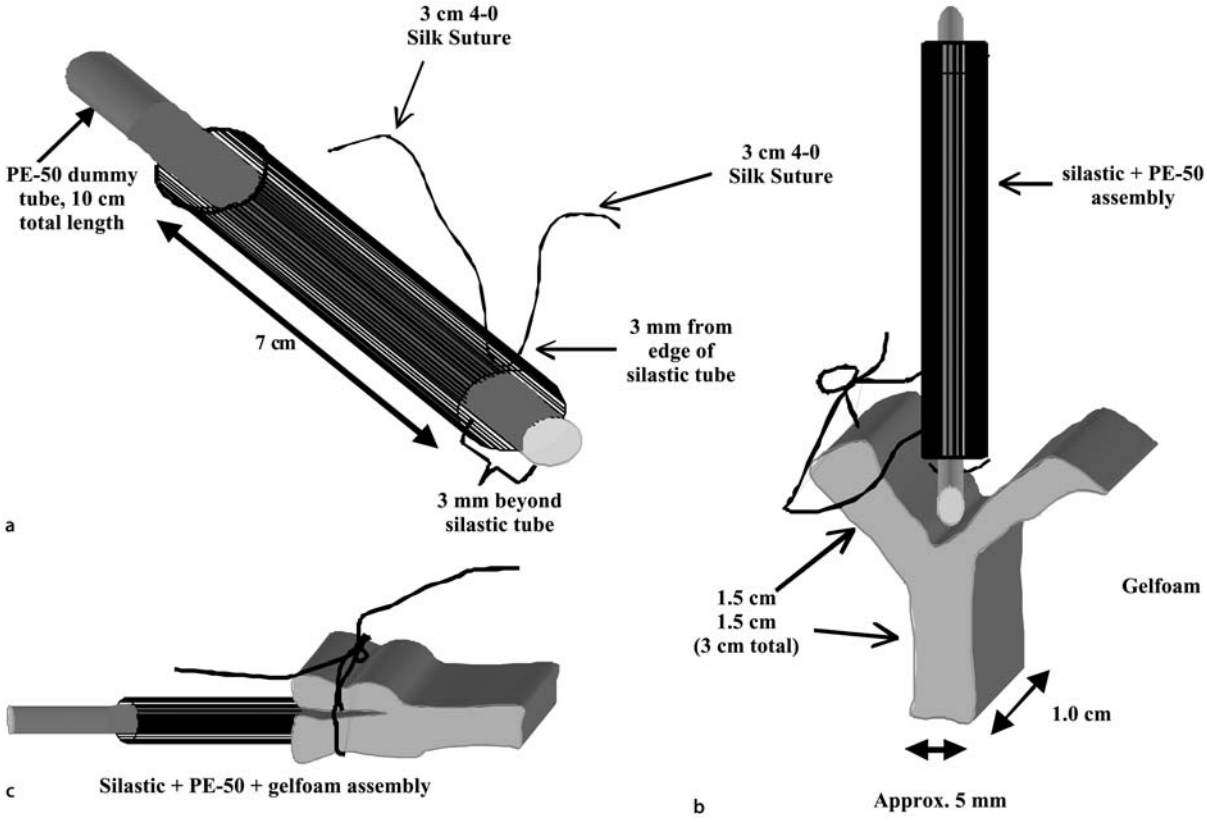
Importantly, all procedures are conducted aseptically. All instruments (forceps, scissors, and scalpels) used to handle or make the supplies are sterile. It is imperative that the catheters for these surgeries are endotoxin free as well as sterile since: (a) endotoxin is not destroyed by autoclaving or gas sterilization and (b) endotoxin and bacterial contamination activates immune cells.

All instruments are sterilized prior to conducting surgery on each animal using a glass bead mini-sterilizer. Hands are washed with antibacterial soap between animals. The skin surrounding the open wound is draped with sterilized gauze or autoclave paper.

Constructing Chronic Peri-Sciatic Catheters

All of the following steps should be performed under a sterile cell-culture grade hood.

Step 1: A 7 cm sterile silastic tube is threaded with 10 cm PE-50 tubing (Fig. 1a). One end of the PE-50 tubing remains flush with one end of the silastic tubing. This 10 cm PE-50 tubing serves as a place-holder, to ensure that the silastic tube does not become blocked either by silk su-



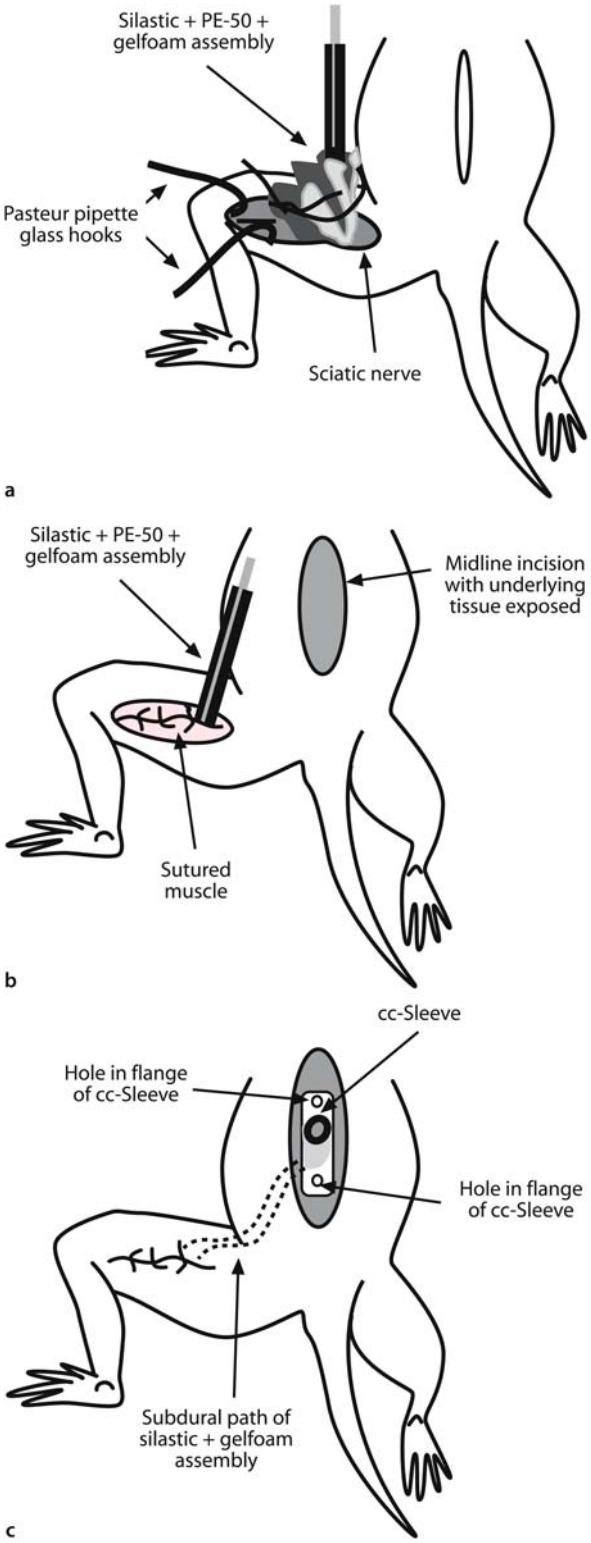
Neuropathic Pain Model, Neuritis/Inflammatory Neuropathy, Figure 1 (a) The silastic+Pe-50 gelfoam assembly. This is the assembly immediately prior to attaching it to the gelfoam. (b) Attachment of the silastic+PE-50 gelfoam assembly to the gelfoam. Note that this is the size of the gelfoam after it has been cut from the gelfoam sheet. (c) Attachment of the silastic+PE-50 gelfoam assembly to the gelfoam. Note that this is the size of the gelfoam after it has been cut from the gelfoam sheet. Reprinted with permission (Milligan et al. in press).

tures used for making the silastic tube+gelfoam assembly or by the surgical anchoring step (described below).
Step 2: Sterile 4-0 silk suture with needle attached is hooked through a small portion of the silastic tube 3–4 mm from the end, where the PE-50 place-holder is flush with the silastic tube (Fig. 1a).
Use a sterile metal ruler for accuracy. It is critical that the 4-0 silk suture does not pierce the indwelling PE-50 place-holder tubing. The final length of the silk-suture is cut to approximately 6 cm, which is needed to easily tie gelfoam (described below) to the silastic+PE-50 assembly, as well as to tie the gelfoam ends together after enwrapping the sciatic nerve (described below). Store this unit in a sterile 50 ml conical tube (Fisher Scientific, Houston, TX) until needed.
Step 3: The silastic+PE-50 assemblies are stored in a sterile, dry 50 ml conical tube that is tightly capped until the prepared gelfoam is ready to be attached. The peri-sciatic gelfoam is constructed from sterile gelfoam sheets (6 (L) × 0.5 (W) ×2 (H) cm) cut, using a sterile #11 scalpel blade on a sterile metal metric ruler, into 4 equal parts with approximate dimensions of 3 (L) ×0.5 (W) ×1 (H) cm strips. Use a sterile metal ruler for accuracy. One end of the gelfoam strip is bisected (0.25 cm W) to a depth of 1.5 cm, creating 2 gelfoam flaps.
Step 4: The 4-0 silk suture end of the silastic+PE-50 assembly is inserted between the gelfoam flaps to a depth of approximately 1.5 cm (Fig. 1b).
The gelfoam flaps are tightly closed together around the silastic+PE-50 assembly by tying the 4-0 silk-suture around one gelfoam flap, and again around the opposing flap (Fig. 1c).
The silastic+PE-50+gelfoam assembly is stored in a sterile, dry 50 ml conical tube (which can store up to 6 silastic+PE-50+gelfoam assemblies) until the time of surgery.

Chronic Peri-Sciatic Catheter Surgery

Step 1: Surgery is conducted under isofluorane anesthesia (Halocarbon Laboratories, River Edge, NJ), 2.5 volume% in oxygen, which is chosen because it has minimal effects on immune cell function compared to other commonly used anesthetics (Lockwood et al. 1993, Sato et al. 1995).
Step 2: The dorsal aspect of the rat faces up, with the nose facing away from the surgeon, the left and right hind legs are positioned laterally to the left of the surgeon or

splayed laterally to the left and right of the surgeon. The fur is shaved from the left leg and lower back.
Step 3: The exposed skin is cleaned with 70% alcohol-soaked gauze. A small amount of concentrated Exidine-2 surgical scrub solution applied to fresh gauze is then used to further clean the surgical area.
Step 4: A midline incision at the lower back area is then made with either a #10 or #11 surgical scalpel blade and the skin around the cut is separated from its underlying loose connective tissue using blunt dissection scissors. This is done to provide space for subcutaneous implantation of the cc-sleeve (described below).
Step 5: A second incision is made along the lateral aspect of the left thigh and separation of the skin from underlying connective tissue extends beyond the incision site to the midline incision (in Step 4), where the exterior portion of the catheter will be encased by the cc-sleeve.
Step 6: The shaved and cleaned skin surrounding the wound is lightly retracted using small-toothed towel clips and then draped with sterile gauze.
Step 7: Exposure of the sciatic nerve is achieved by blunt dissection, and connective tissue is gently teased apart using glass Pasteur pipette hooks, sterilized in the glass-bead mini-sterilizer before each use.
Step 8: At approximately mid-thigh level, a portion of the sciatic nerve is slightly lifted using a sterile glass Pasteur pipette hook, followed by adding a couple of drops of sterile isotonic, endotoxin-free saline to keep the nerve moist.
Step 9: The gelfoam of the silastic+PE-50+gelfoam assembly is then gently threaded around the sciatic nerve starting from the quadriceps side to maintain a clear view of the implant site (Fig. 2a). The surrounding muscle walls are maneuvered to support the silastic+PE-50+gelfoam assembly upright.
Step 10: The 4-0 silk-suture that is part of the silastic+PE-50+gelfoam assembly (as previously described Chronic Peri-Sciatic Catheter Construction) is used to tie together the proximal and distal ends of the gelfoam once it forms a U-shape around the sciatic nerve. Precise control is best when using 2 pairs of microforceps. The surrounding muscle walls are then closed around the gelfoam-enwrapped sciatic nerve, leaving the silastic+PE-50 portion exteriorized (Fig. 2b).
Step 11: The silastic tube is anchored to the muscle by threading a sterile 4-0 silk suture with attached suture needle through the muscle at the most proximal portion of the dissection site, followed by threading the suture through the silastic tube, avoiding the internal PE-50 place-holder tube, and then through the opposing muscle. The remaining overlying muscle is closed with one or two more sutures through the muscle.
Step 12: The exposed portion of the silastic catheter is tunneled subcutaneously to exit through the lower back incision (Fig. 2c).



► **Neuropathic Pain Model, Neuritis/Inflammatory Neuropathy, Figure 2,** (a) The process of enwrapping the gelfoam portion of silastic+PE-50+gelfoam assembly around the sciatic nerve. (b) Illustrating the exteriorized portion of the silastic+PE-50 catheter after the gelfoam has been implanted and muscle walls sutured closed. (c) cc-Sleeve attachment to the lower back area after the silastic+PE-50+ gelfoam assembly has been implanted. Reprinted with permission (Milligan et al. in press).

Step 13: The skin overlaying the sutured muscle is closed with wound clips.
Step 14: The PE-50 dummy catheter is carefully removed while holding the silastic catheter in place, to ensure that the gelfoam does not become torn or displaced.
Step 15: The exposed portion of the silastic catheter is threaded through the cc-sleeve. The reader is referred to the methods paper that describes in detail the construction and use of this cc-sleeve (Milligan et al. 1999). The cc-sleeve is then anchored to the muscle overlaying the lumbosacral area, by threading one or two 3-0 silk sutures with attached suture needle through each flange on the cc-sleeve. The overlying skin is then sutured closed with 3-0 silk.
Step 16: The remaining exteriorized portion of the silastic tube is folded into the cc-sleeve and an air-dried concave plug (part of the cc-sleeve) (Milligan et al. 1999) is inserted inside the tip of the sleeve. A small amount of silastic silicon sealant is coated over the end of the plug and cc-sleeve with a moistened Q-tip.
Step 17: The wound area around the hind leg and lower back are lightly cleaned with 0.9% saline. Total surgical time is typically 15–20 minutes.
Step 18: Beginning 4–5 days following surgery, when catheters are used to induce chronic allodynia for extended periods of time, (the wound areas of the hind leg and lower back are cleaned with 0.9% saline every 2 days for as long as 2 weeks). This decreases the amount of inflammation, such as redness, slight bleeding and scabbing of the skin around the surgical sites. The cc-sleeve and the indwelling peri-sciatic silastic catheter are also cleaned with separate single-use peri-sciatic catheter cleaners.

Peri-Sciatic Catheter Injectors

Peri-Sciatic Catheter Injectors are made using the following steps:
Step 1: The beveled end of a sterile 23-gauge, 1-inch hypodermic needle is inserted into one end of a 30 cm PE-50 tube.
Step 2: A mark is made 7.3 cm from the opposite end, using a black permanent fine-tip marker. The mark must line up with the exterior end of the peri-sciatic silastic catheter upon PE-50 tubing insertion. This alignment assures that the interior end of the PE-50 tubing is 3 mm beyond the indwelling silastic catheter within the gelfoam.
Step 3: Prepared peri-sciatic catheter injectors are stored in a sterile, dry place (typically, an autoclavable box) until the time of injections.

Peri-Sciatic Catheter Injection Procedures

Peri-sciatic catheter injections are conducted using the following steps:
Step 1: The 23-gauge needle is attached to a sterile Hamilton 100l micro-syringe.
Step 2: The sterile glass Hamilton micro-syringe and the peri-sciatic catheter injector are flushed with sterile, endotoxin-free water and tightly connected, making the syringe and injector airtight.
Step 3: An air bubble is then created in the 30 cm PE-50 tubing of the peri-sciatic catheter injector by drawing up 1 of air followed by the drug. The length of the injection catheter will vary depending on the volume of drug injection. A 1.0 um volume occupies approximately 0.41 cm of PE-50 tubing.
Step 4: Animals are gently placed in crumpled soft cotton towels and allowed to move freely underneath the towels.
Step 5: The cc-sleeve area is exposed, the indwelling concave rubber plug is removed with a dental probe, and the folded portion of the silastic catheter is exteriorized. The reader is referred to the methods paper that describes in detail the construction and use of this cc-sleeve (Milligan et al. 1999).
Step 6: Fluid that had accumulated in the indwelling silastic catheter is suctioned off with the peri-sciatic catheter cleaner (described below) and discarded.
Step 7: Drug injection is completed using the prepared PE-50 injectors (described above). The PE-50 tubing from the peri-sciatic catheter injector is inserted into the silastic catheter until the 7.3 cm mark on the PE-50 tubing of the peri-sciatic catheter injector is flush with the edge of the silastic catheter. Chronic allodynia is maintained for over 2 weeks by repeated injections every 2 days, which are conducted in steps identical to that described immediately above (steps 1–7).

Peri-Sciatic Catheter Cleaning Procedures

In chronic allodynia, an additional step of cleaning the inside of the cc-sleeve with a separate peri-sciatic catheter cleaner is done, to decrease local inflammation/infection around this foreign body. Peri-sciatic catheter and cc-sleeve cleaning followed by drug injections are done every two days to maintain chronic allodynia. Using this paradigm, unilateral and bilateral allodynia remain stable during the entire testing period, in terms of both pattern (i.e., unilateral does not change to bilateral nor the reverse) and magnitude. Peri-sciatic Catheter Cleaners are used to suction out fluid accumulation within the indwelling silastic catheter starting 4–5 days after surgery and prior to drug injections.
Step 1: The catheter cleaners are made from the same supplies as the injectors, except the Hamilton 100μl micro-syringe is replaced with a sterile 3-cc syringe.
Step 2: The cleaners are constructed in the same way as the injectors (described above) except that the 3-cc syringe is attached to the 23-gauge needle. Prepared peri-sciatic catheter cleaners are stored in a sterile, dry place (typically, an autoclavable box) until the time of injections.

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Neuropathic Pain Model, Partial Sciatic Nerve Ligation Model

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Synonyms

PSL model; Seltzer Model

Definition

The ► partial sciatic nerve ligation (PSL) model of neuropathic pain refers to a rodent ► neuropathic pain model that is produced by tightly ligating the dorsal third to half of the common sciatic nerve at the upper-thigh level (Seltzer et al. 1990).

Characteristics

Methods for Producing the PSL Model

Animals

Young adult male rats of various strains are commonly used. Like most other behavioral tests, it is helpful for rats to be acclimated for about a week in the laboratory holding facility with free access to food and water before performing any experiments. It is also convenient to keep rats in a room with a reversed light-dark cycle, allowing behavioral tests to be conducted during their active period.

N

Surgical Operation

Rats are deeply anesthetized with general anesthetics and placed in the prone position. Under a dissection microscope, the dorsum of the sciatic nerve is freed from surrounding connective tissues at a proximal site just distal to the point where the posterior biceps semitendinosus nerve branches off. An 8-0 silk suture is inserted into the nerve with a 3/8 curved needle, trapping the dorsal third or half of the nerve. The trapped portion of the nerve dorsal to the suture is tightly ligated. The wound is sutured closed. The original developers of the model (Seltzer et al. 1990) emphasized the importance of the site of ligation, which needs to be proximal enough so that the sciatic nerve is not yet fasciculated into individual nerves. Therefore, sciatic injury is made at a site between the branch point of the posterior biceps semitendinosus and a little fat pad (which is located just distally) since the sciatic nerve becomes fasciculated into branches just distal to the fat pad (Schmalbruch 1986; Seltzer et al. 1990).

Extent of Injury and Behavioral Outcome

The number of fibers injured by this procedure varies between animals (Seltzer et al. 1990). It is possible that some fibers in the unligated portion of the nerve may undergo degeneration due to secondary events such as: 1) disruption of the perineurium, 2) interference of local blood flow, 3) focal edema and 4) reaction to the ligature, etc. Therefore, variability in the number of injured fibers after partial sciatic nerve ligation comes from the fact that a variable number of fibers are being ligated and

that presumably variable numbers of unligated fibers are being injured by the above secondary causes.

Operated animals normally do not show severe motor deficits, except for the two lateral toes, which are flexed (Seltzer et al. 1990). At rest, the operated rats guard their operated limb somewhat when placing the limb on the floor; yet they show excessive abnormal grooming behaviors on the operated limb, such as repeated licking of the paw. However, none of operated rats show self-mutilating behavior due to the partially deafferented hind paw. During walking, they do not show obvious limping or any other severe locomotive abnormalities.

Practically all operated rats show various behavioral signs of neuropathic pain such as ► ongoing pain, ► heat hyperalgesia, and mechanical as well as ► cold allodynia (Seltzer et al. 1990; Kim et al. 1997). Behaviors believed to represent ► mechanical allodynia can be quantified either by measuring foot withdrawal frequency to mechanical stimuli applied to the paw with von Frey filaments (Kim et al. 1997) or by determining the mechanical threshold (Seltzer et al. 1990). Heat hyperalgesia can also be measured by determining the heat threshold of the paw for foot withdrawals or duration of responses to suprathreshold heat stimuli (Seltzer et al. 1990). In addition, behaviors reflecting spontaneous pain have been shown by measuring the duration of foot withdrawals (guarding behaviors) in the absence of any obvious stimuli. Behavior thought to represent cold allodynia was assessed by measuring frequency of foot withdrawals to cold stimuli (e.g. acetone droplet application) applied to the paw (Kim et al. 1997).

Kim et al. (1997) made a comparison between the chronic constriction injury (CCI), spinal nerve ligation (SNL), and PSL models. When magnitudes of behaviors were compared with the CCI and SNL models, the PSL model fell in between the two for mechanical allodynia and spontaneous pain, but the magnitude of cold allodynic behavior was similar in all three models. Behaviors in PSL are partially reversed by sympathectomy (Shir and Seltzer 1991; Seltzer and Shir 1991) suggesting that sympathetic abnormality is involved in producing pain behaviors.

Factors Influencing Variability

Many factors influence neuropathic pain behavior in the PSL model as in other models. As shown in the SNL model (Mogil et al. 1999a; Mogil et al. 1999b; Yoon et al. 1999), genetic factors influence pain behaviors in the PSL model (Shir et al. 2001). Another important factor is diet. The effect of diet on neuropathic pain behavior has been studied in detail using the PSL model (Shir et al. 1998; Shir et al. 2002). Although the mechanisms are not clear, a diet with high soybean content reduces the expression of neuropathic pain behaviors. Finding such factors is important not only in terms of future studies on underlying mechanisms but also for potential therapeutic implications.

Advantages and Disadvantages of the PSL Model Compared to Others

The PSL model has several advantages over other neuropathic pain models. Tight ligation models such as the PSL provide information about the timing of injury better than loose ligation models. The most attractive feature of the PSL model is that it most closely resembles the original description of causalgia patients with injuries produced by high velocity missile impact (Mitchell 1872).

Acknowledgments

This work was supported by NIH Grants NS 31860 and NS 11255.

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Neuropathic Pain Model, Spared Nerve Injury

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Synonyms

Spared Nerve Injury Model; SNI Model; Sural Spared Nerve Injury Model; sSNI Model

Definition

The rodent spared nerve injury model (SNI) consists of the selective injury of two of the three terminal branches of the sciatic nerve (the tibial and common peroneal nerve), leaving the third branch, the sural nerve, intact (Decosterd and Woolf 2000). Rapid and robust pain hypersensitivity to mechanical and thermal external stimuli is produced in the sural nerve skin territory, similar to stimulus-evoked pain observed in clinical ► neuropathic pain syndromes.

Characteristics

Description of the Model

Since the original description of ► anesthesia dolorosa by Wall (Wall et al. 1979), several models of transection/ligation-related injury to peripheral nerves have been described, allowing evaluation of the response to an applied external innocuous or nociceptive stimulus (stimulus-evoked pain) (Mosconi and Kruger 1996; Seltzer et al. 1990; Kim and Chung 1992; Bennett and Xie 1988; Vos et al. 1994). Unlike complete denervation of the paw after a sciatic nerve transection, these models enable assessment of pain sensitivity of the spared intact nerve fibers.

However, in the partial sciatic nerve injury (Seltzer et al. 1990) and the chronic constriction injury (Bennett and Xie 1988), the degree of nerve damage is difficult to reproduce, leading to variability within and between laboratories. Developed by Kim and Chung, the tight ligature of L5 and L6 spinal nerves ostensibly leaves all L4 afferents intact, but the surgery itself may damage or produce an inflammatory reaction to the intact L4 spinal nerve. The SNI model has the advantage of a simple surgical procedure and a high degree of reproducibility (Decosterd and Woolf 2000).

Two of the three terminal branches of the sciatic nerve are completely transected, i.e. the tibial and common peroneal nerves, and the sural nerve remains intact. If the surgical procedure is well executed, variability depends only on anatomical variation. Pain hypersensitivity is recorded in the skin territory of the spared sural nerve, preferentially on the plantar surface of the paw. Withdrawal threshold modifications are also present in the dorsal hairy sural nerve skin territory, and to a less extent in the saphenous nerve skin territory. The onset of allodynia- and hyperalgesia-like behavior is rapid (within 3 days post injury), and sensitivity changes last for months (up to 6 months). No change in the contralateral paw is observable when compared to same age sham-injured animal recordings. A crush injury to the tibial and common peroneal nerves induces pain hypersensitivity, but within nine weeks withdrawal thresholds return to baseline value. The original model was described in rats.

Despite technical advantages or disadvantages of each model, a distinct pattern should be distinguished in order to better understand specific mechanisms and possible differences between models. The emerging concept that non-injured fibers may participate in the generation and maintenance of neuropathic pain symptoms, as well as chemical cross talk and non-neuronal cell signaling, makes it important to distinguish models in relation to the co-mingling of injured primary sensory neurons/afferent fibers and uninjured neurons/fibers (see Table 1).

Surgery

Under general anesthesia, the sciatic nerve portion at thigh level is exposed using a longitudinal section through the biceps femoris muscle. The three terminal branches are easily located. Common peroneal and tibial nerves are delicately dissected in order to separate them from the surrounding tissue. The tip of a fine curved forceps is placed under the common peroneal nerve, avoiding any lift up. Five centimeters of 5.0 silk

Neuropathic Pain Model, Spared Nerve Injury, Table 1 Anatomically-related specific patterns of intermingling of primary sensory neuron cell bodies and axons distal to the peripheral nerve injury in animal models of neuropathic pain

Animal model	Sensory ganglia	Peripheral axons distal to injury
Complete sciatic nerve transection (associated or not with femoral nerve transection)[1]	Co-mingling of injured &non-injured neurons in L4 and L5 DRGs	*Injured axons only*
Chronic constrictive injury of the sciatic nerve or the infra-orbital nerve [2]	Co-mingling of injured &non-injured neurons in L4, L5 DRGs or in trigeminal ganglion	Intermingling of injured A-fiber and intact C-fiber axons
Partial sciatic nerve ligation (PSN) [3]	Co-mingling of injured &non-injured neurons in L4, L5 DRGs	Intermingling of injured and intact nerve fibers
Spinal nerve ligation (SNL) [4]	Non-injured neurons in L4 DRG &injured neurons only in L5 and L6 DRGs, no co-mingling	Intermingling of injured and intact axons
Spared nerve injury (SNI) [5]	Co-mingling of injured &non-injured neurons in L4 and L5 DRG	No intermingling of injured and intact axons

[1] Wall et al. 1979. [2] Bennett and Xie 1988; Vos et al. 1994, Mosconi and Kruger 1996. [3] Kim and Chung 1992. [4] Wall et al. 1979. [5]Decosterd and Woolf 2000.

suture is placed into the forceps' tip and slipped under the nerve. Two tight knots are made and the nerve is distally transected, including the removal of a portion of 2–4 mm. The same procedure is repeated for the tibial nerve. Crush injury is performed as above, except that injured nerves are only crushed for 30 seconds by a pair of small arterial clamps (with smooth protective pads). Special care needs to be taken to prevent any lesion to the spared nerve, and especially to avoid lifting up, touching or stretching the spared sural nerve during the surgical procedure.

Experimental Conditions

Genetic and environmental factors influence the course of pain behavioral studies (Chesler et al. 2002), and efforts are needed to standardize study conditions when using the SNI model. We try in our laboratory as much as possible to minimize the major variables:

- The investigator: the same investigator conduct a specific study, and he or she must be blind to the treatment/genotype applied
- Young adult male Sprague-Dawley rats (weighting initially 180–200 g, Charles River Inc) are very docile and all animals develop the neuropathy-related behavior. The same vendor and vendor's strain is recommended for each study
- Behavioral assessment of treated and control animals is performed during the same testing session in order to maintain the same experimental environment in both groups
- The behavioral testing is always performed at the same time of the day, the same order of testing is respected in between testing sessions
- The animals are housed and tested in a room of constant temperature and light cycle, and they have free access to food (same diet) and water. Animal transportation is avoided immediately before the testing session. Recordings are performed in a room devoted only to behavioral testing

Behavioral Assessment

The animals are daily habituated to the environment, testing material and investigator for at least two weeks before the first recordings. Behavioral assessment is performed as described originally (Decosterd and Woolf 2000) before and after SNI surgery. For plantar application, rats are enclosed in a home made transparent Plexiglas observation chamber (22 x 13 x 13 cm) atop a wire mesh floor (mesh of 0.25 cm^2). For dorsal application, rats are placed on a plane neutral floor. The investigator gently holds the animal and has direct access to the dorsal side of the hind paw.

During a study, mechanical and cold sensitivities were tested consecutively, with a 30 minute time interval between modalities tested. Heat assay are performed the next day and the animals are placed in the same observation chambers, but onto a transparent glass floor (Ugo Basile, Comerio, Italy).

► Mechanical allodynia-like behavior: the threshold is determined at the lowest force that evokes a brisk withdrawal response to one of five stimuli. Von Frey monofilaments are applied perpendicularly to the skin, in ascending force order, in the lateral area of the hind paw. Five stimuli cover the area, and contact with footpad or hairs are carefully avoided. The paw withdrawal threshold is recorded, and decreases significantly after surgery in both hairy and glabrous skin territory of the sural nerve (Fig. 1a and Decosterd and Woolf 2000). There is no contralateral effect of SNI.

► Cold allodynia-like behavior: a drop of acetone solution (99.6 %) is delicately placed under the plantar lateral side of the paw using a blunt needle. Acetone evaporates and produces a cold sensation. In the affected side, acetone induces long-lasting paw withdrawal as well as paw shaking and licking (Fig. 1b and Decosterd and Woolf 2000). The total duration of paw withdrawal is recorded, with a minimal time at 0.5 s for brief response and a cut-off at 60 s.

► Mechanical hyperalgesia-like behavior: a pinprick test is performed in the same skin area, using a safety pin. A single prick was given at a force such that the skin dimpled but was not penetrated. The duration of paw withdrawal was recorded, with a minimal time at 0.5 s for brief response and a maximal cut-off at 60 s. Response duration is increased for the injured paw, but to a lesser extent than after acetone stimulation (Fig. 1b and Decosterd and Woolf 2000).

► Heat hyperalgesia-like behavior: a movable radiant infrared heat source enables stimulation of the lateral part of the hind paw (Hargreaves et al. 1988)). Withdrawal reflex latency and duration due to heat stimulation are recorded with a 0.5 s minimal and a 60 s cut-off. Latency of stimulation is not modified by SNI, but the duration of the abnormal response is significantly increased (Fig. 1c and Decosterd and Woolf 2000). Although possible, plantar heat stimulation through transparent plane hard floor is difficult for the SNI lesioned paw. The animals refrained from weight bearing on the affected paw, and the foot is everted due to both pain hypersensitivity and neuro-muscular defects. This may lead to loose contact between the floor surface and the paw, altering the transmission of the thermal stimulus.

In summary, the spared sural nerve SNI model is an easily reproducible model of neuropathic pain. Mechanical and cold pain-hypersensitivity are assessable behaviorally, and, therefore, may provide functional information on mechanisms responsible for stimulus-evoked pain in neuropathic pain conditions.

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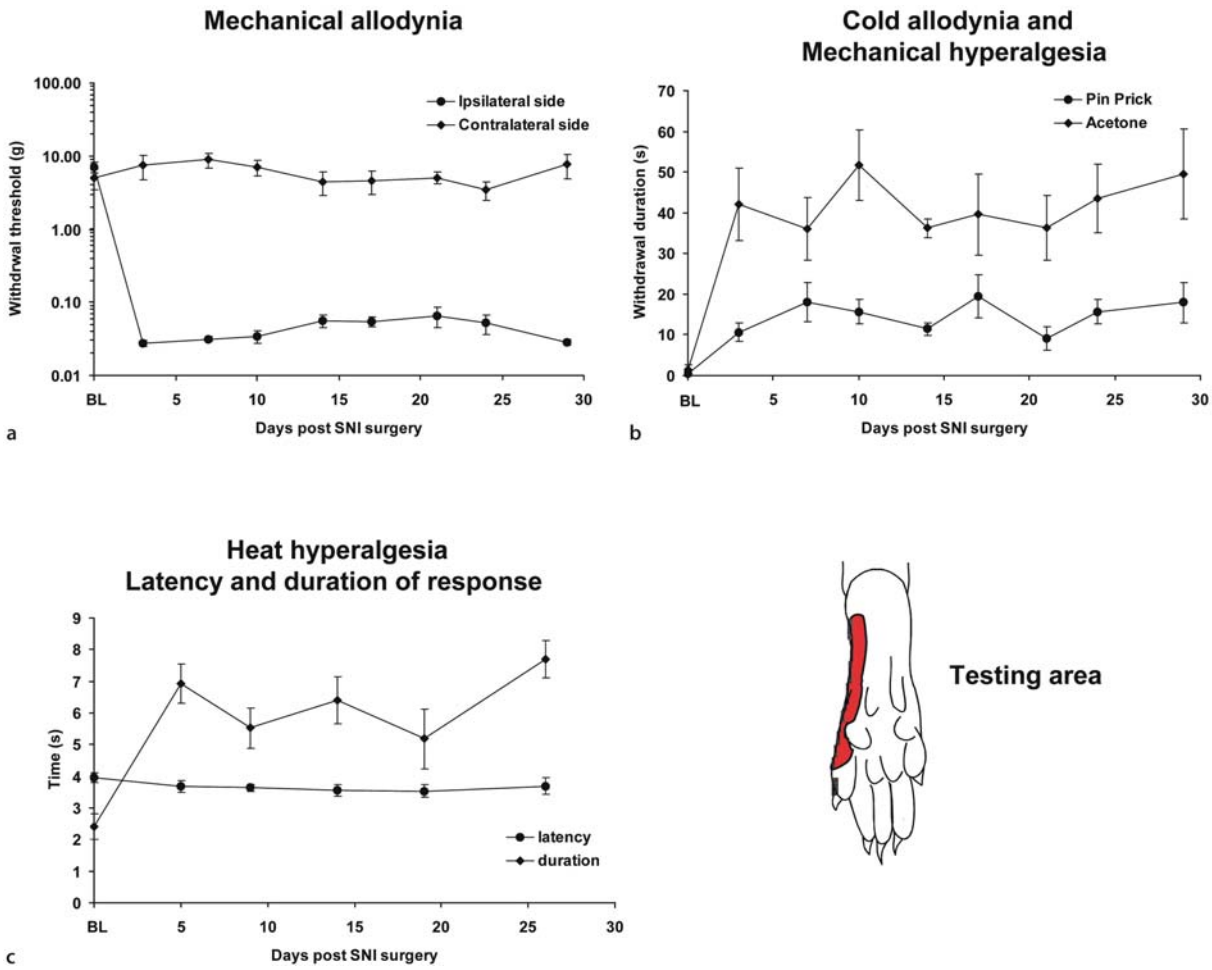

Neuropathic Pain Model, Spared Nerve Injury, Figure 1 Mechanical and thermal hypersensitivity recorded in the spared sural nerve territory after SNI. (a) Withdrawal threshold in g determined after application of ascending series of von Frey monofilaments. (b) Withdrawal duration in s after application of acetone or pin prick stimulation. (c) Heat sensitivity (withdrawal latency and duration in s). BL, baseline. Representative series of 12 animals tested before (BL) and after SNI surgery. Results are displayed as the mean value ∓SEM.]

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Neuropathic Pain Model, Spinal Nerve Ligation Model

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Synonyms

Spinal Nerve Ligation Model; SNL Model; Chung model

Definition

The spinal nerve ligation (SNL) model of ► neuropathic pain, refers to a rodent neuropathic pain model that is produced by tightly ligating the lumbar segmental spinal nerve (L5 alone or both L5 and L6) (Kim and Chung 1992). The lumbar segmental spinal nerve refers to a short length of the peripheral nerve distal to the dorsal

root ganglion before it joins with other segmental nerves to form the lumbar plexsus. The lumbar segmental spinal nerve divides into a small dorsal ramus and a large ventral ramus, and the SNL model usually ligates the ventral ramus only since the dorsal ramus is denervated during the surgery.

Characteristics

Methods to Produce the SNL Model

Animals: Most commonly, young adult male rats of various strains are used (see Factors influencing variability). After purchase, rats are normally acclimated for about a week in the Institutional Animal Care Center, with free access to food and water in a room with a reversed light-dark cycle (dark: 8 A.M.-8 P.M.; light: 8 P.M.-8 A.M.) before experimental manipulation.

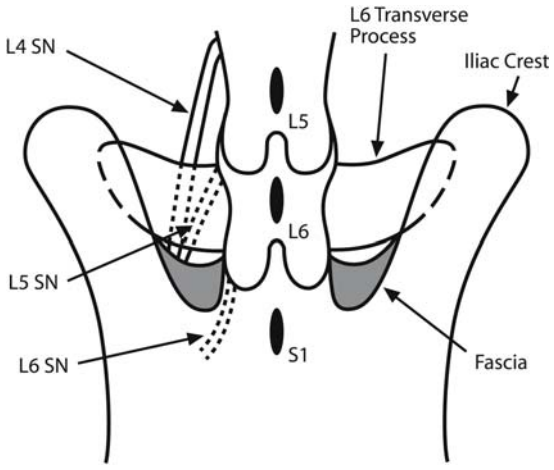
Surgical Operation: Figure 1 shows the anatomy of the lumbosacral paraspinal region. Rats are anesthetized with either inhalation gas or intraperitoneal injection of sodium pentobarbital and placed in the prone position. Under sterile conditions, a longitudinal incision is made at the lower lumbar/upper sacral level, exposing the paraspinal muscles on the left. Using a pair of small scissors with blunt tips, the paraspinal muscles are isolated and removed from the level of the L5 spinous process to the S1. This opens up the space ventrolateral to the articular processes, dorsal to the L6 transverse process, and medial to the ileum. Connective tissues and remaining muscles are removed with a small scraper. Under a dissecting microscope, the L6 transverse process, which covers the ventral rami of the L4 and L5 spinal nerves, is removed using a small rongeur. Access to the L5 spinal nerve is easier when the transverse process is removed very close to the body of the vertebrae. One can normally visualize the ventral rami of the L4 and L5 spinal nerves (a thin sheet of connective tissue may cover them in some animals) once the L6 transverse process is carefully removed. The L4 spinal nerve usually runs more laterally (or ventrally in some animals) than the L5, and these two nerves join distally in a common epineurial sheet, however, there is a great deal of individual variability where these two nerves join. Thus, the L4 and L5 spinal nerves need to be separated in some animals to make the L5 spinal nerve accessible for ligation. It is very important not to damage the L4 nerve during this process, because we find that even slight damage to the L4 spinal nerve invariably results in a greatly reduced mechanical sensitivity of the foot. Damage to the L4 spinal nerve can occur with a seemingly mild mechanical trauma (excessive touch, gentle stretch, or slight entrapment within the epineurial sheet). Once enough length of the L5 spinal nerve is freed from the adjacent structure, a piece of 6-0 silk thread is placed around the L5 spinal nerve and the nerve is tightly ligated to interrupt all axons in the nerve. Another option would be to cut the spinal nerve just distal to the ligation to make sure all fibers are interrupted.

The L6 spinal nerve can also be ligated if so desired. The L6 spinal nerve runs underneath the sacrum and is not visible without chipping away a part of the sacrum. Since the sacrum bleeds a lot when chipped, it would be better to approach the L6 spinal nerve blindly without chipping the sacrum. After carefully removing the fascia joining the sacrum to the ileum, one can place a small glass hook underneath the sacrum and gently pull the L6 spinal nerve out into the paravertebral space and ligate it tightly with 6-0 silk thread.

Upon completion of the operation, which normally takes about 10–15 minutes (after some practice), hemostasis is confirmed and the muscles are sutured in layers using silk thread and the skin is closed with metal clips, anesthesia is then discontinued. Animals are then kept in a cage with warm bedding until they completely recover from anesthesia.

Behavioral Outcome of Surgery: Successfully operated animals normally do not show any motor deficits beyond a mild inversion of the foot with slightly ventroflexed toes. The most common and obvious motor deficit of unsuccessfully operated animals is dragging the hind limb of the operated side, a sign of paralyzed proximal muscles. This invariably indicates damage to the L4 spinal nerve, since this nerve innervates many proximal muscles of the hind limb.

A successfully operated rat shows various behavioral signs of neuropathic pain such as ongoing pain, heat ► hyperalgesia, and mechanical as well as cold ► allodynia. Since the SNL model shows a particularly robust sign of mechanical allodynia, one can use the degree of hypersensitivity to gauge the success of the operation. Mechanical sensitivity is quantified either by measuring response frequency to mechanical



Neuropathic Pain Model, Spinal Nerve Ligation Model, Figure 1 Schematic diagram showing the dorsal view of the bony structures and spinal nerves at the lumbosacral level after removal of paraspinal muscles.

stimuli applied with ▶ von Frey filaments (Kim and Chung 1991; Kim and Chung 1992) or by determining the mechanical threshold (Chaplan et al. 1994). A successful surgical operation will result in a clear sign of mechanical allodynia demonstrated by either: 1) lowering the foot withdrawal threshold below the normal nociceptor activation threshold [below 1.4 g (Leem et al. 1993)], 2) frequent foot withdrawals to mechanical stimulation at a strength below the normal nociceptor activation threshold, or 3) frequent foot withdrawals to obviously innocuous stimulations. On the other hand, sham-operation should not produce any significant changes in mechanical sensitivity, except a mild transient effect lasting one or two days. A significant and long lasting hyperalgesia following sham-operation invariably indicates that the surgery induced damage and/or inflammation to the nerve and is thus an unsuccessful operation.

Factors Influencing Variability

Multiple factors seem to influence the behavioral outcome after SNL and hence contribute to variability of data. The strain of rats is an important variable. Not only do different strains of rats show different levels of neuropathic pain behaviors, but also different levels of pain behaviors can also be seen in different substrains of Sprague-Dawley rats obtained from different suppliers (Yoon et al. 1999). Another important factor that influences the sign of mechanical allodynia is the exact testing spot on the paw where the mechanical stimulation is applied. To represent the most intensely painful area of a human patient, one must measure the threshold at the most sensitive area of the rat. The most sensitive area of the paw after ligation of the L5 or both the L5 and L6 spinal nerves is the base of the 3^{rd} or 4^{th} toe (Xie et al. 1995). The most sensitive spot of the paw after spinal nerve ligation is confined to a small area and does not vary much between rats, presumably due to stereotyped denervation of the foot by the surgical procedure. When measuring in the most sensitive area, the threshold is usually well below the 1 g range, whereas the threshold ranges from 2 to 3 g if one measures it by stimulating the mid-plantar area (Chung et al. 2004).

Advantages and Disadvantages of the SNL Model Compared to Others

The SNL model has several advantages over other models. These include that: 1) the injury is stereotyped, 2) it has successfully adapted to multiple species of animals, and 3) the injured and uninjured afferents are segregated to different spinal segments. Tight ligation is advantageous over loose ligation in terms of knowing the timing of injury, as well as the population of fibers being injured. In addition, a tight ligation of a specific set of nerves in every animal will reduce the variability among animals. Many neuropathic pain models, including the SNL model, which was originally developed using the rat, are now successfully adapted to the mouse (Mogil et al. 1999). The SNL model has also been successfully applied to the monkey (Carlton et al. 1994). The uniqueness of the SNL model, however, is that spinal inputs of injured and uninjured afferents are segregated at separate spinal segments. This feature allows the investigation of the contribution of injured and uninjured afferent fibers to neuropathic pain.

There are also some disadvantages to the SNL model. These include that: 1) the surgical procedure is invasive, and 2) the model is highly artificial. Because the spinal nerves are located deep, the surgery to expose them requires some level of skill and, hence, may be a source of variability. A particularly technically difficult aspect is to preserve the L4 spinal nerve completely undamaged while ligating the L5 spinal nerve since they are located in close proximity. Since it is rare to find a patient with discrete spinal nerve injury, this model is highly artificial.

Different models tend to produce different behavioral outcomes. A previous study compared the behaviors of 3 different models [SNL, chronic constriction injury (CCI), and partial sciatic nerve ligation (PSL) models] (Kim et al. 1997). The CCI model produced the most robust ongoing pain behaviors, whereas mechanical allodynic behaviors were most prominent in the SNL model.

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Neuropathic Pain Model, Tail Nerve Transection Model

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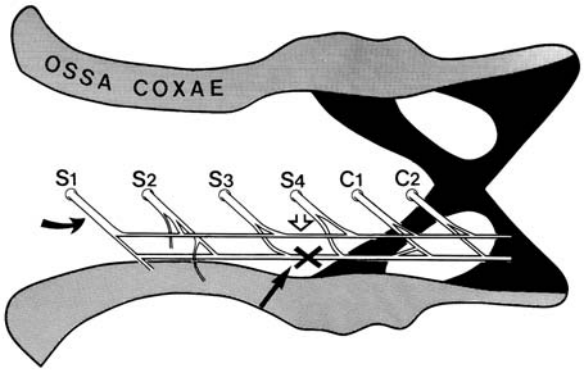
Definition

The tail nerve transection model is produced by the incomplete injury of the nerves (i.e. the inferior and/or superior caudal trunks) innervating the tail. The model displays chronic neuropathic signs like ► mechanical allodynia, ► cold allodynia and ► warm allodynia in the tail skin.

Characteristics

The tail nerve transection model is one of the ► peripheral neuropathic pain animal models. Peripheral nerve injury sometimes results in neuropathic pain. This type of pain is characterized by spontaneous burning pain accompanied by ► hyperalgesia and ► allodynia lasting variable times. Several experimental animal models for neuropathic pain, produced by a partial injury of the nerves supplying the rat hind paw, were developed by Bennett & Xie (1988), Seltzer et al. (1990) and Kim & Chung (1992), respectively. Although these models display clear signs of neuropathic pain, there are some inherent problems in performing behavioral tests due to foot deformity. To avoid these problems, the tail nerve transection model, produced by transaction of interior caudal trunk at the level between the S3 and S4 spinal nerve, was developed (Na et al. 1994; Kim et al. 1995). This model shows neuropathic signs without tail deformity. In addition, modified methods, such as the transection of superior caudal trunk (Back et al. 2002) or both trunks (Kim et al. 2001) also showed similar neuropathic changes.

The tail skin is innervated by the inferior and superior caudal trunks, which are located in the ventral and dorsal parts of the pelvic bone, respectively. The two trunks are composed of the dorsal and ventral divisions, respectively, of the four sacral and the first two caudal spinal nerves. To induce neuropathic pain in the tail skin, the inferior and/or superior caudal trunk(s) is (are) exposed carefully from the surrounding tissues and transected at the level between the S3 and S4 spinal nerves. This surgery eliminates the S^{1}-S3 spinal nerve innervation of the tail *via* the trunk(s). Figure 1 illustrates schematically how these trunks are composed and the level of the transection of the inferior caudal trunk.



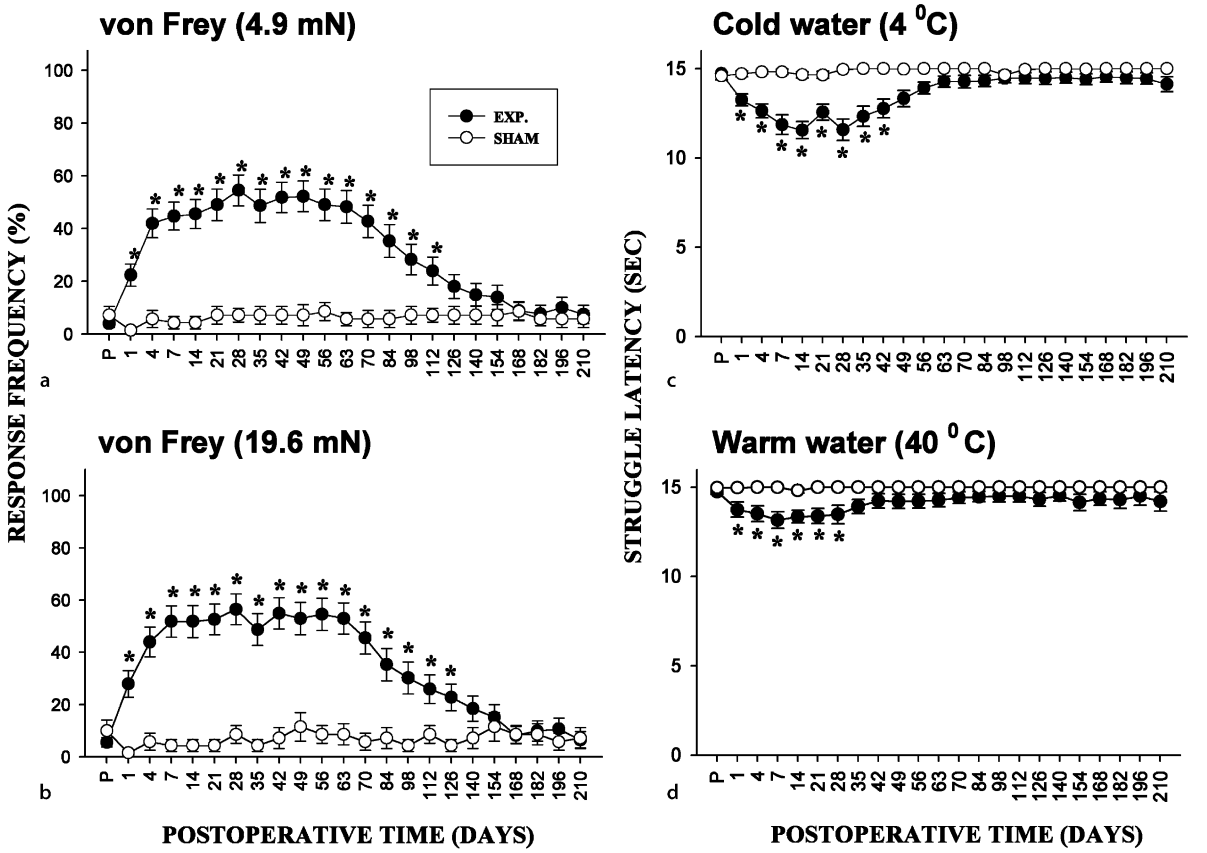
Neuropathic Pain Model, Tail Nerve Transection Model, Figure 1 A schematic diagram (dorsal view) illustrating how the inferior (black arrow) and superior (open arrow) caudal trunks are composed and the level of transection (×) of the inferior caudal trunk. The curved arrow indicates the S1 spinal nerve.

The signs indicative of mechanical allodynia can be sought by applying normally innocuous mechanical stimuli to the tail using ► von Frey hairs. For convenient application of the stimuli, the animal is restrained in a transparent plastic tube and the tail is laid on a plate. The most mechanically sensitive spot of the tail is first determined by rubbing various areas of the tail with the shank of the von Frey hair, and then, this area is poked systematically with the von Frey hair to locate the most sensitive spot. An abrupt tail movement of about 0.5–20 cm in response to the von Frey hair stimulation is considered to be an abnormal response, indicative of mechanical allodynia. During repeated trials, the test stimuli are delivered to the same spot without difficulty, since the tail is usually stationary.

Figure 2a and 2b show the data obtained with the von Frey hairs (4.9 mN and 19.6 mN). Prior to the neuropathic surgery, the frequency of the abnormal tail response to von Frey hair stimulation is near 0 percent. However, after the neuropathic surgery, the frequency increases dramatically from 1 day PO (postoperatively) and lasts for at least 4 months, unlike the frequency in sham-operated animals. These results suggest that the partial injury of the nerves innervating the tail leads to mechanical allodynia in the tail.

The signs indicative of cold and warm allodynia can be sought by immersing the tail in 4°C and 40°C water, respectively. The rat is restrained in a plastic tube, and the tail is drooped for convenient application of the thermal stimuli. Following the tail immersion, the investigator measures the latency of the tail withdrawal response with a cut-off time of 15 s. A tail withdrawal response with a latency shorter than the cut-off time is considered to be an abnormal tail response indicative of thermal allodynia. The tail immersion test is repeated 5 times at 5 min intervals to obtain the average tail response latency.

Figure 2c and 2d show the data obtained with the cold (4°C) and warm (40°C) water, respectively. Prior to the



Neuropathic Pain Model, Tail Nerve Transection Model, Figure 2 Tail responses to mechanical (a, b), cold (c) and warm (d) stimuli. The mean (± SEM) response frequency in the case of mechanical stimulation with von Frey hairs (4.9 mN, 19.6 mN) and the mean (± SEM) response latency in the case of cold (4°C) and warm (40°C) stimulation of experimental (Exp, n=44) and sham (n=14) groups are plotted against the experimental days (P: 1 day before nerve injury). Asterisks indicate the scores significantly different from the preoperative value ($P<0.05$ by the Friedman test followed by a pairwise post-hoc test).

neuropathic surgery, most rats did not show abnormal tail responses to the cold or warm water stimuli. However, after the neuropathic surgery the tail response latency significantly decreased from 1 day PO and lasted for 5–7 weeks. These results suggest that the partial injury of the nerves innervating the tail leads to cold and warm allodynia in the tail. The possibility that the abnormal tail responses to the 4°C or 40°C water immersion are due to the mechanical contact of the tail with the water instead of thermal stimulation is essentially ruled out, since 1) in a vast majority of the cases, 30°C water does not induce any abnormal tail responses and 2) the 4°C or 40°C water-induced responses have latencies greater than at least a few seconds, unlike the von Frey hair-evoked responses which had virtually no latencies. The tail nerve transection model, like the previously developed ones, shows chronic neuropathic signs like mechanical and thermal (cold and warm) allodynia. Furthermore, the model offers several advantages in surgical approach and performing the behavioral tests. First, surgical procedures for the model are so simple that even neonatal rats or mice can be used (Back et al. 2002). In fact, although rat models for neuropathic pain have been applied to the mouse (Malmberg and Basbaum 1998; Mansikka et al. 2000; Mogil et al. 1999), there are some problems from the invasiveness of these approaches. Second, since the inferior and superior caudal trunks are composed of the four sacral and the two caudal spinal nerves, the number of injured fibers or the spinal level of injury can be changed according to the transection site. This advantage was helpful to elucidate of the fact that the extent of ► sympathetic fiber sprouting in the ► dorsal root ganglion (DRG) was related to the number of injured nerve fibers (Kim et al. 2001) and the distance between the DRG and injury site (Kim et al. 1996). Third, application of both mechanical and thermal stimuli to the partially denervated area (i.e. the tail) is straightforward. For example, tail immersions into cold or warm water make all thermal receptors in the tail receive the same thermal stimulation simultaneously. In addition, since there is no deformity in the tail after the nerve injury, the mechanically sensitive spot is easily located and blind behavioral tests are available.

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Neuropathic Pain Models, CRPS-I Neuropathy Model

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Synonyms

Reflex Sympathetic Dystrophy; Algodystrohy; Sudeck's Atrophy; CRPS-I neuropathy model

Definition

Complex regional pain syndrome (CRPS) is a neuropathic pain disorder that usually develops after a noxious event. Pain is frequently described as burning and continuous and exacerbated by movement, continuous stimulation or stress. The syndrome includes spontaneous pain and/or stimulus evoked pain (► allodynia and ► hyperalgesia), exceeding in both magnitude and duration the clinical course expected to follow the inciting event. Regardless of the site of injury, the symptoms begin and remain most intense in the distal extremity and are not limited to the distribution of a single peripheral nerve. At some point in time, pain may be associated with edema, changes in skin blood flow and abnormal sudomotor activity in the same area, often resulting in significant impairment of motor function and showing variable progression over time. Two forms of CRPS have been identified. CRPS-I usually develops following trauma with only minor nerve damage or without any demonstrable nerve lesion whereas CRPS-II is associated with a clear nerve injury that can be characterized by abnormal clinical and/or electrodiagnostic findings (Stanton-Hicks et al. 1995). The tetanized sciatic neuropathy (TSN) model is a preparation in rodents that results in allodynia, hyperalgesia and vasomotor disturbances that mimic CRPS-I. This model is produced by activating unmyelinated afferents (C-fibers) at '► wind up' (Mendell 1966) parameters, using a 10 min electrical stimulation (i.e. tetanization) of an intact sciatic nerve.

Characteristics

Harden und Bruehl (2005) recently proposed the following diagnostic algorithm to diagnose CRPS-I in humans. This algorithm is based on presentation of at least one sign in two out of the following four categories: (a) sensory abnormalities; allodynia and/or hyperalgesia, (b) vasomotor abnormalities; temperature asymmetry and/or skin color changes and/or asymmetry, (c) sudomotor abnormalities or edema; swelling and/or altered sweating and/or sweating asymmetry, (d) motor abnormalities or dystrophy; decreased range of motion and/or motor dysfunction (weakness and/or tremor and/or dystonia) and/or trophic changes in hair and/or skin and/or nails. Edema and autonomic dysregulation are usually seen in the early stage of the disease, while movement disorders and trophic changes are more apparent in the later stage. For many decades, sympathetic blockade was the method of choice for the diagnosis and treatment of CRPS-I. Pain relief in response to this procedure has been used as a criterion for diagnosis, hence the term reflex sympathetic dystrophy (RSD). However, it now appears that not all patients may have sympathetically maintained pain in CRPS (Stanton-Hicks et al. 1995).
Several animal models have been developed to study the mechanisms underlying neuropathic pain mimicking CRPS-II, including the chronic constriction injury (CCI), partial sciatic ligation (PSL) and spinal nerve ligation (SNL) models. Some of these are described elsewhere in this section. The common denominator of these models is that abnormal sensory responses to stimuli and spontaneous behavior, indicative of neuropathic pain, are produced by partial denervation of a paw, tail or the face. The typical spontaneous pain behaviors include guarding behavior, repeated flicking

of the partially denervated paw, excessive licking and holding the paw in the mouth, claw pulling, elevated paw position and antalgic gait, as well as vocalization, reduced appetite, weight loss and self-mutilation (autotomy). The abnormal sensory responses to stimuli include a reduced withdrawal threshold to a stimulus that is normally non-noxious (allodynia), and exaggerated responses to a stimulus that is normally noxious (hyperalgesia). The latter is manifested as increased duration and robustness of nocifensive responses. A wealth of data on the mechanisms that trigger and underlie the pain in CRPS-II has been gathered from these animal models, since they are all produced by some type of nerve injury that partially denervates a limb. However, since in CRPS-I there is no evidence for such a nerve injury, these models may not be relevant to the study of its mechanisms (Jänig and Baron 2001).

The signals that trigger CRPS-I as well as the mechanisms maintaining the abnormalities that characterize this syndrome are still enigmatic. Since the clinical signs of CRPS-I and CRPS–II are similar, they may be produced by the same triggering input. When sensory fibers are injured, 25–33% of transected axons emit a barrage of impulses termed ► injury discharge (ID). This is the first neural message to notify the CNS that an injury has occurred (Wall et al. 1974). This message comprises a burst of high frequency discharge in A-fibers and of low frequency firing in C-fibers. This burst decays in most A- and C-fibers within minutes after the injury. However, about 10% of injured fibers continue to fire for many hours and may not stop for days (Baik-Han et al. 1990; Blenk et al. 1996; Sackstein et al. 1996). Muscular afferents emit a clearly more robust ID than cutaneous afferents. ID is a distinct signal in the 'alphabet' of these fibers, unlike their normal response to natural stimuli, since the peak discharge of ID is 2–6-fold higher than the response to maximal normal stimulation (Sackstein et al. 1996). ID is an important signal in triggering neuropathic pain disorders in some animal models and possibly in humans as well (reviewed by Kissin, 2000).

The TSN model has been developed on the basis of the working hypothesis that CRPS I may be caused by a bodily injury that does not result in frank nerve injury but produces a massive nociceptive input that is similar to ID. The following description provides methodological details of the TSN model.

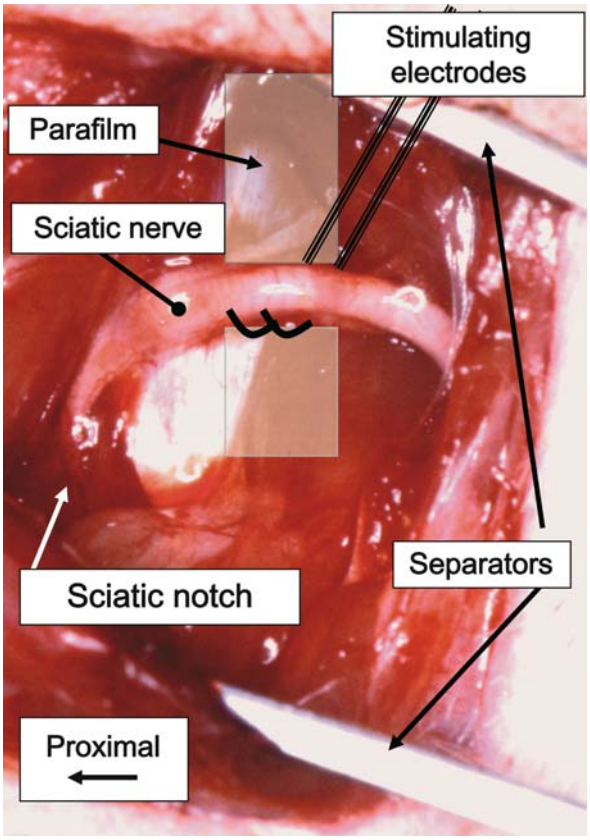
Animals

The original model was developed in the rat but it can easily be adapted to the mouse.

Preparation of the TSN Model

Surgery

Under inhalation anesthesia and aseptic conditions, the sciatic nerve on both sides is exposed at midthigh level and the surgical field is kept widely open with separators, taking care not to pull the posterior biceps semitendinosus nerve or other thigh nerves. The sciatic nerve is then carefully separated from neighboring tissues (Fig. 1) and a sheet of parafilm is placed under the nerve. A pair of stainless steel stimulating electrode hooks is inserted under the nerve on both sides. The exposed nerves are covered with mineral oil (37°C) to prevent damage by drying. The side that receives the tetanic stimulation should alternate between individual animals, to minimize the bias of the experimenter when testing.



Neuropathic Pain Models, CRPS-I Neuropathy Model, Figure 1 The sciatic nerve is exposed at midthigh level and the surgical field is kept widely open with separators. A sheet of parafilm separates the nerve from neighboring tissues. A pair of stainless steel stimulating electrode hooks is inserted under the nerve.

Tetanization

Electrical stimulation of intact nerves activates leg jerking that may pull the nerves by the hook electrodes. To prevent the injury, both hind paws, the pelvis and the tail are taped to the surgical board. The tetanization includes a train supramaximally activating C-fibers at a wind-up frequency, in addition to A-fibers (shock duration = 0.5 msec, frequency = 0.5 Hz, intensity = 5 mA, train duration = 10 min, n = 300 shocks). It is noteworthy that no sensory disorders were detected when activating A-fibers only (Vatine et al. 1998). Directly observing the surgical field with a dissecting microscope during the tetanization, the experimenter should verify

that the nerves are not pulled by the electrodes. The contralateral sciatic nerve receives a sham stimulation at the same time as the ipsilateral nerve is tetanized. A separate control group of bilaterally sham-tetanized animals should optimally be included in every experiment.

Determination of Sensory Disorders

Tactile Allodynia

The animal is placed on top of a metal mesh floor, and covered with an opaque plastic cage. This enables the experimenter to introduce the filaments from underneath, preventing the animal from observing the stimulus approaching. Allodynia is assessed with a set of von Frey hairs. These hairs are nylon monofilaments of different diameters that exert defined levels of force when pressed against the plantar skin with sufficient force to cause the hair to bend. Each hair is indented 5 times at a frequency of about 2 Hz. The testing process begins by using the lowest hair in the set, ascending in the series until the animal responds at threshold by lifting the paw, withdrawing from the filament. The set typically ranges from 0.05 to 25 g and needs to be calibrated weekly using a top load balance. Other methods can be used (Bennett et al. 2002). Figure 2a shows that the average group withdrawal threshold (in g) of TSN rats is significantly decreased for a period of about 40 days, compared to sham tetanized rats.

Heat Hyperalgesia

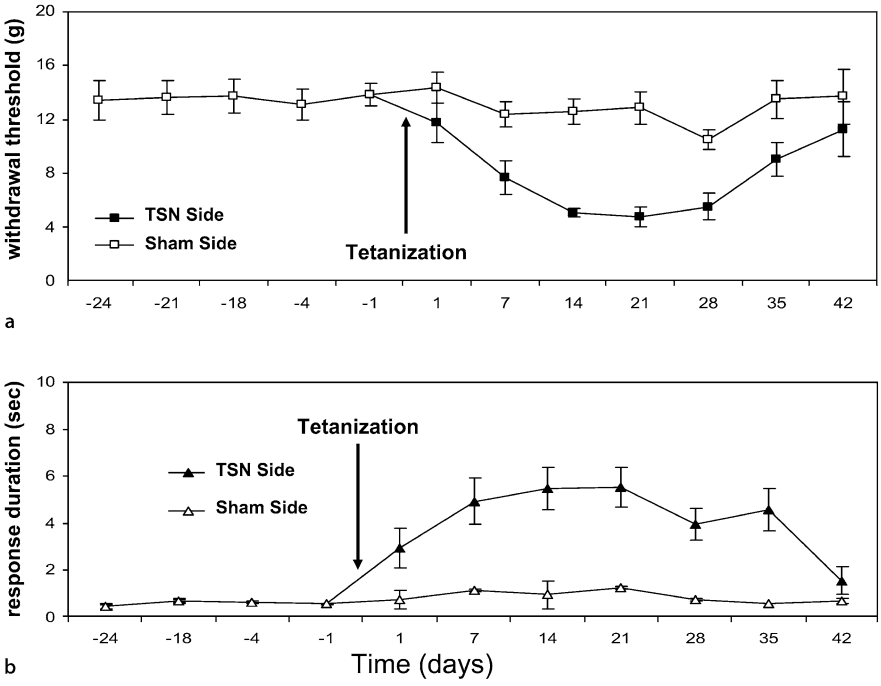
Several methods can be used, including the Hargreaves instruments or a laser. For the latter method, a painful pulse of infrared energy is beamed from a CO_2 laser (120 msec, 5 W, 150 mCal and 1.5 mm in diameter) to the midplantar area of the hind paw from underneath, targeted by the visual aid of a He/Ne laser beam. This intensity causes sharp stinging pain to humans. Sham tetanized rats respond by a momentary paw flick or paw lift lasting less than a second. When stimulated at the TSN side, rats typically respond by immediate withdrawal followed by prolonged licking, paw lifting and claw pulling lasting on average up to 10 sec, depending on the post tetanization day and genetic and environmental variables. Figure 2b shows that the average group response duration (in sec) to stimulation on the TSN side significantly increased for a period of about 40 days, compared to the sham tetanized side.

Cold Hyperalgesia

A drop of acetone from a syringe is smeared on the plantar surface of the paw through the mesh floor of the testing chamber. As a control stimulus, a drop of tap water at room temperature is likewise applied, alternating between the acetone and water. The response time to each stimulus is recorded, subtracting the water from the acetone and the net result averaged for the group for each stimulated side. Increased response duration indicates cold hyperalgesia.

Mechanical Hyperalgesia

An increased response duration to pinprick applied from underneath to the midplantar area of the hind paw reflects hyperalgesia.



Neuropathic Pain Models, CRPS-I Neuropathy Model, Figure 2 (a) *Tactile allodynia.* Baseline tactile sensitivity was tested with a set of von Frey hairs. The withdrawal thresholds (g) to repetitive touch on the plantar side of both hind paws were tested on 5 sessions prior to nerve stimulation (days -24, -21, -18, -4 and -1). On day 0 the rats underwent unilateral tetanic stimulation of the sciatic nerve for 10 min. Tactile allodynia developed ipsilaterally, but not on the sham side. (b) *Heat hyperalgesia.* Baseline sensitivity to a noxious heat pulse from a CO_2 laser was tested on days -24, -18, -4 and -1 prior to nerve tetanization in intact rats. On day 0, the rats underwent unilateral tetanic stimulation of the sciatic nerve for 10 min. The significantly increased response duration (in sec) to the noxious stimulus, denotes heat hyperalgesia developed ipsilaterally on the tetanized sciatic side but not on the sham side.

Determination of Vasomotor Disorders

Since the temperature of the plantar skin area of conscious animals fluctuates, the paw temperature is measured 2 min after the rat is lightly anesthetized with an inhalation gas and 30 sec after the temperature stabilizes. Plantar hind paw temperature is recorded bilaterally using a remote infrared sensing thermometer, while the rat is lying on its ventral side in a room with an ambient temperature maintained at 21.0±0.5˚C. Unilateral tetanic stimulation of the sciatic nerve causes a relative cooling of the tetanized hind paw.

Variables Potentially Affecting the TSN Model

Genetic Considerations

Work done in other animal models of chronic pain showed that the choice of animals might have a critical effect on the outcome, since genetic variation plays an important role. Some lines may develop robust neuropathic pain, while others may develop very weak, short lasting or even undetectable abnormalities. The original experiments on the TSN model were carried out on male Wistar rats (Vatine et al. 1998) and HA and LA rats (Vatine et al. 2001). The latter lines were selected from a stock of out bred rats (Sabra strain) based on contrasting levels of autotomy behavior following hind paw denervation by sciatic and saphenous transection (Devor and Raber 1990). Strain/line-specific differences in levels of allodynia, hyperalgesia and paw temperature abnormalities were noted. Previous reports showed differences in pain levels using the same model on animals from different vendors but also within vendors over time.

Environmental Considerations

The following environmental variables may dramatically affect the levels of neuropathic pain in animal models, including the TSN model. These include organismic variables like age, sex, hormonal status, prior experience with pain or drugs and seizures and variables relating to husbandry, like litter size and sex ratio, age at weaning, caging system, housing density, relation of cage mates, male-male fighting, handling frequency, bedding material, colony health status, prenatal (maternal) stress, maternal deprivation, ambient noise, ambient temperature and illumination. Also important are the circadian phase, circannual phase, meteorological factors, temperature, humidity, barometric pressure, experimenter, testing apparatus, restraint and drug injection method. Variables related to the stimulus can be no less important, including type of noxious stimulus, intensity, location, testing apparatus particularities, dependent measure, repeated testing, data transformation and experimenter proneness to bias. Investigators should exercise extreme care to control these as much as possible.

The TSN model shows similar types and durations of sensory disorders to those produced by intended nerve injury as in the CCI, PSL, SNL and PNI models. But lack of an overt nerve injury in the TSN model, combined with the appearance of mechanical allodynia , mechanical and thermal hyperalgesia and some vasomotor disturbances resembles the abnormalities of CRPS-I in humans (Stanton-Hicks et al. 1995; Baron et al. 1996), support the suggestion that this preparation can be used to study the mechanisms underlying CRPS-I.

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Neuropathic Syndrome

► Lower Back Pain, Physical Examination

Neuropathic Pain of Central Origin

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The recognition that disease or injury to the central nervous system (CNS) leads to conditions of chronic pain can be traced back to the 1800s. One of the first descriptions of symptoms, including pain, of a condition later to be called Wallenberg's syndrome was reported by Marchet (1811). A number of later reports further documented severe spontaneous pain associated with vascular lesions of the brainstem and ► thalamus. Shortly after the turn of the century Dejerine and colleagues presented their classic papers defining the term thalamic syndrome, which included disturbances of superficial and deep sensibility combined with severe, persistent, paroxysmal, often intolerable pain (Dejerine and Egger 1903; Dejerine and Roussy 1906).
Throughout the early 1900s, reports continued to document the condition of pain following injury or disease in the CNS. Head and Holmes (1911) described spontaneous pain associated with lesions of the spinal cord and brainstem. Several years later the term ► central pain was used by Holmes (1919). Although the terms "pain of central origin" and "pain due to lesions of the ► central nervous system" were used by a number of authors, it wasn't until 1938 that the definition of central pain was firmly established (Riddoch et al. 1938). By the late 1940s, the concept of central pain was firmly entrenched in the medical literature and was characterized by the presence of ► spontaneous pain, ► hyperpathia, ► hyperalgesia and exaggerated motor and autonomic reactions. Interestingly, many of these symptoms are commonly associated with pain following injury to peripheral nerves. Although comparisons have been made, there are sufficient differences pertaining to incidence, prevalence, time of onset and response to therapy to easily justify separate categories for pain with peripheral-central mechanisms (► neuropathic pain) *versus* pain associated solely with disease or injury in the CNS (central pain) (Bonica 1999).

Definition

The term central pain was initially considered synonymous with thalamic pain and for this reason most descriptions have placed both in the same category. Although thalamic lesions are considered to be one of the most common causes of central pain, it is also recognized that central pain can result from lesions anywhere along the neuraxis from the spinal cord to the cerebral cortex (Boivie 1989; Cassinari and Pagni 1969; White and Sweet 1969). In 1994 the International Association for the Study of Pain defined central pain as pain initiated or caused by a primary lesion or dysfunction within the CNS.

Epidemiology

Post-Stroke Pain

The incidence of central poststroke pain (CPSP) was estimated in 1991 to be approximately 750,000–1,000,000 patients worldwide (Bonica 1991). This calculation was based on a figure for post-stroke pain of 1–2% as described by Bowsher (1993). Recent reports however, describe this condition as more frequent than previously thought. Andersen et al. (1995) reported that central pain affected 8% of 207 central post-stroke patients with 5% having moderate to severe pain. Kumral et al. (1995) described 9% of patients with thalamic hemorrhage experiencing central pain and CPSP was found in 25% of patients following brainstem infarcts with 49% having somatosensory deficits (MacGowan et al. 1997).

Spinal Cord Injury Pain

The prevalence of pain following ► spinal cord injury ranged from 34–94% (mean 69%) in ten studies published between 1947 and 1988 (Bonica 1991) and 36–96% (mean 66%) in studies from 1975–1991 (Yezierski 1996). In recent years the use of more comprehensive pain assessment strategies has raised the overall prevalence of SCI pain to 70–80% (Rintala al. 1998; Widerstrm-Noga et al. 1999). In a recent study Siddall and colleagues (1999) reported 91% of subjects with pain of any type 2 weeks after injury. The percentage decreased to 64% 6 months after injury.

Surgical Lesions

Surgical lesions involving spinal or supraspinal levels of the neuraxis intended to relieve pain can result in the onset of pain. Although Cassinari and Pagni (1969) reported that the incidence of ► dysesthesia and central pain following functional neurosurgery was 10–60%, more conservative numbers have been reported following cordotomy (3–5%) (Lipton 1989; White and Sweet 1969). The incidence of pain after medullary tractotomies was described by Bonica (1991) as 30%, with 70–100% of patients experiencing pain after open mesencephalic tractotomy and 5–10% after stereotaxic mesencephalic tractotomy (Bonica 1991).

Other Neurological Disorders

Other central neurological conditions are known to be associated with chronic pain. For example, mul-

tiple sclerosis (see ► Central Pain in Multiple Sclerosis) causes central pain in 29–75% of patients (Bonica 1991). Although systematic epidemiological studies for pain associated with other types of neurological disorders have not been carried out, other conditions associated with central pain include ► epilepsy (Young and Blume 1983), ► Parkinson's disease (Koller 1984) and Huntington's disease (Albin and Young 1988).

Clinical Characteristics of Central Pain

Spontaneous, persistent (usually burning), diffuse and / or intermittent, shooting, ice-like, aching, lancinating (with or without evoked elements) sensations, i.e. ► hyperesthesia, hyperalgesia, ► allodynia and hyperpathia, are characteristics commonly associated with central pain. Dysesthesias, hypersensitivity to somatic stimuli, enhancement of pain by emotion, radiation of sensation, summation of repeated stimuli and prolonged aftersensations have also been described as components of central pain (Pagni 1998).

Central pain can develop explosively and usually continues long after stimulation. Pain intensity can vary during the day, often due to external (e.g. touch, vibration, cold) and frequently emotional factors. Spontaneous pain occurs in a large number of cases, varying from uncomfortable ► paresthesias to aching, shooting, burning pain of great intensity. As a rule, spontaneous pains frequently vary in position and may change in character and are aggravated by somatic or visceral stimulation as well as by stress and emotion, especially anxiety. Auditory, visual, olfactory and visceral stimuli can provoke or exacerbate spontaneous pain.

► Quantitative sensory testing in a region where pain is localized generally shows a paradoxical lowering of sensitivity to painful stimuli, i.e. ► hypoalgesia. Within this hypoesthetic zone, a painful region is most closely correlated with a zone of decreased sensitivity to thermal stimuli (especially cold) with the intensity of pain being proportional to the loss of thermal sensibility. Studies indicate that there may be two recognizable subclasses of central pain, one signaled by loss of cold, warmth and sharpness sensibilities in which burning pain is experienced and one in which the ongoing pain is described as pricking, shooting and aching where tactile allodynia may predominate.

Temporal Profile

Central pain can start at any time after insult, although it usually begins within the first 3 months. The time of onset does not appear to depend on the location of the lesion and there are no definite correlations between the time of onset and associated pathology. In general, central pain following stroke develops gradually as sensory impairment and weakness improve. In cases involving ► ischemia or hematomyelia, pain has been reported to appear suddenly after insult. Shieff and Nashold (1987) described patients with pain from the time of initial insult as well as intervals varying up to 2 years. Andersen et al. (1995) described cases with pain 1 month after stroke, at 1–6 months and more than 6 months. In Tasker's series of patients (1991) central pain of brain origin was found to have a delayed onset in two-thirds of the cases, being less than 1 year in 50% of patients. Leijon described patients where pain began within 1 day after stroke, during the first month, after 3–12 months, or after 2–3 years (Leijon et al. 1989). Following spinal injury Siddall and colleagues (1999) reported ► at-level neuropathic pain 2 weeks after injury for 53% of subjects and reported ► below-level neuropathic pain for 41% of subjects within this same time period. Twenty-four percent of subjects reported neuropathic below level pain 3 months after injury while 18% reported this type of pain 6 months after injury.

Location of Pain

The distribution of central pain is nearly always related to the somatotopic organization of the brain structure damaged by trauma, disease or vascular insult. Because of this, it is possible to identify the location of the lesion in cases of dorsal horn and bulbar lesions, whereas it is difficult to distinguish between cortical, subcortical and thalamic lesions. In the majority of cases, central pain coincides with all or part of the territory in which sensory loss is clinically observed or revealed by quantitative sensory testing. Central pain is generally described as having a diffuse distribution; however, it can involve only one extremity or a portion of an extremity, e.g. hand or side of the face, and is therefore more accurately described as extensive rather than diffuse (Boivie 1994).

Bilateral girdle pain is found in cases of intramedullary tumors or ► syringomyelia. Spinal injury to the anterolateral quadrant is often referred to the opposite side of the body below the lesion. Dysesthesias from injury to the posterior column or dorsal column nuclei are typically located on the same side, below the lesion and may be unilateral or bilateral. Pain and dysesthesia due to vascular pontomedullary lesions usually have an alternating distribution, face on the lesion side and limbs and trunk contralateral to the lesion. This distribution is largely due to the fact that bulbar pain syndromes commonly result from the involvement of the posterior inferior communicating artery. Bulbar lesions can give rise to bilateral facial pain when the lesion impinges on the descending root of the trigeminal nerve on one side and on the crossed trigeminothalamic fibers coming from the other side. With pontine lesions, pain in the face is most often on the side opposite the lesion as is pain experienced in the limbs and trunk. Follow-

ing mesencephalopontine lesions, pain occurs on the side of the body contralateral to the lesion, typically with a hemiplegic distribution. Pain and dysesthesia due to thalamic lesions also have a hemiplegic distribution and affect the side of the body contralateral to the injured thalamus. Finally, cortical or subcortical lesions result in pain referred to the contralateral distal parts of the body (regions with the most extensive cortical representation).

Central Pain Syndromes

Thalamic Syndrome

The major features of thalamic lesions include severe, often intolerable, persistent or paroxysmal pain on the side opposite the lesion (Dejerine and Roussy 1906). This syndrome is characterized by slight hemiparesis, persistent superficial ► hemianesthesia, mild hemiataxia and astereognosis. While spontaneous pain may be absent in thalamic syndrome, excessive reaction to stimulation of affected body parts is consistent. Pain or discomfort can be evoked by almost any stimulus capable of arousing a sensation and is commonly characterized as intensely disagreeable and unbearable. Aside from spontaneous variations in pain intensity, fluctuations in pain are often exacerbated by environmental changes (especially cold), emotional stress (sudden fear, joy), strong taste or smell, loud noises, bright lights, movements, light touch, smoking and intellectual concentration. Pain is typically prolonged after stimulation and stimuli that normally have no obvious affective qualities may elicit a reaction in patients with thalamic syndrome. Patients with thalamic pain often have signs of autonomic impairment, e.g. vasoconstriction, abnormal sweating, edema (Bowsher et al. 1989).

Post-Stroke Pain

The most common cause of central pain is vascular abnormalities, e.g. ischemic lesions, with an etiology usually including supratentorial thrombotic stroke. Infarcts are not the only vascular disorders causing pain however, as subarachnoid hemorrhage is also associated with the onset of chronic pain (Bowsher et al. 1989; Tasker et al. 1991). The condition associated with thalamic lesions resulting from stroke was redefined by Leijon et al. (1989) as central poststroke pain (CPSP) and pain originating from extrathalamic lesions was referred to as pseudothalamic pain (Boivie 1994). CPSP is characterized by sensory deficits involving cold and warm stimuli, pinprick and to a lesser extent vibration, touch and 2-point discrimination (Verstergaard et al. 1995). There may be spontaneous or evoked sensory disturbances such as paresthesia, dysesthesias, hyperpathia and allodynia to cold.

The majority of patients with post-stroke pain also have more than one kind of pain which can be described as aching, pricking, shooting, stabbing, throbbing, squeezing, stinging, lancinating or lacerating. The pain may be superficial or deep and is typically constant, although it is not uncommon for patients to have intermittent pain and / or pain-free periods lasting a few hours. While there is evidence that a spinothalamic deficit is a necessary condition for post-stroke pain (Andersen et al. 1995; Dejerine and Roussy 1906), it is not a sufficient condition, since spinothalamic deficits are seen in more that 50% of stroke patients who show no signs of pain. However, there is evidence that the development of sensory loss and hyperalgesia in a body part deafferented by stroke is a necessary and sufficient condition for the development of central pain.

Pain Following Spinal Lesions

Painful sensations are a frequent and troublesome sequela of paraplegia and quadriplegia following partial or complete lesions of the spinal cord. Perhaps the most comprehensive classification of spinal injury pain was proposed by Donovon and colleagues who described five pain syndromes based not only on damage to the cord, but also secondary pathological changes, e.g. spinal nerve damage, overuse of muscles and compromised visceral function, that contribute to the onset of various post-injury pain syndromes (Donovon et al. 1982). This list was amended by Davidoff and Roth (1991) with the addition of lesional pain, reflex sympathetic dystrophy and limb pain secondary to compressive mononeuropathies. Recognizing the need for a simpler classification of different SCI pain syndromes Siddall et al. (2002) proposed a taxonomy consisting of two broad categories (a) nociceptive and (b) neuropathic; with subcategories of (1) musculoskeletal and (2) visceral in the nociceptive category and (1) above-level (2) at-level and (3) below-level in the neuropathic category.

Although there is no question concerning the diversity of different pain states associated with spinal injury, of greater importance is the practical impact of SCI pain on a patient's quality of life. Widerstrm-Noga et al. (1999) described 37% of patients rating SCI pain as very hard to deal with (rating of 7–10 on a scale of 0–10). In this study, a cluster analysis of different consequences of injury showed a strong interrelationship among ratings for pain, spasticity, abnormal sensations and sadness further supporting the negative impact of pain on quality of life following injury.

Imaging Central Pain

Central pain patients can be studied with neurometabolic techniques such as ► single photon emission computed tomography (SPECT), ► positron emission tomography (PET), ► functional magnetic resonance imaging (fMRI) and magnetic resonance

spectroscopy (MRS), which together with pharmacologic dissection can be helpful in classifying patients according to the pathophysiological mechanism(s) responsible for producing central pain. Unfortunately, there are only a few neurometabolic studies demonstrating the involvement of thalamic and / or cortical hyperactivity associated with central pain (Cesaro et al. 1991; Pagni and Canavero 1995). In PET studies patients with chronic pain show a decrease in thalamic activity (Di Piero et al. 1994). These findings may be compatible with a decrease in thalamic neuronal activity between bursts observed in patients with central pain secondary to spinal injury. Cesaro et al. (1991) in a SPECT study using an amphetamine tracer found hyperactivity in the thalamus contralateral to the pain. In another SPECT study, Canavero et al. (1995) observed hypoactivity in the parietal cortex of a patient with central pain, suggesting that under normal conditions the cortex exerts an inhibitory control over thalamic structures. Consistent with this, four patients with central poststroke pain, two with hyperpathia, showed hyperactivity in the thalamus contralateral to the hyperpathic side. Defining the potential neural and biochemical changes associated with central pain is important in determining the mechanism underlying the onset and maintenance of injury induced pain. Pattany and colleagues (2002) used proton magnetic spectroscopy to study alterations in metabolites resulting from injury induced functional changes in thalamic nuclei following SCI. In patients with pain the concentrations of N-acetyl- and myo-inositol were different compared to those without pain, suggesting anatomical and functional changes in the region of thalamus.

Lesions Causing Central Pain

Central pain can be caused by any lesion of the nervous system that affects either completely, incompletely or subclinically the spinothalamocortical pathway. Based on an extensive review it was concluded that central pain can be due to lesions localized anywhere along this afferent sensory projection system, irrespective of whether cells or fibers are destroyed (Cassinari and Pagni 1969). Lesions leading to central pain are generally slow developing and the highest prevalence of central pain is reported in cases of lesions in the spinal cord, lower brainstem and ventroposterior part of the thalamus (Boivie 1992; Bonica 1991; Tasker 1991).

The most severe injury to the spinal cord is a complete spinal transection following which patients can experience phantom limbs and complain of uncomfortable sensations such as tightness or pain. Severe pain may follow hemisection, but remote pains are rare, usually transient, lasting only a few days and are generally referred to the paralyzed, non-analgesic side of the body, but may be bilateral. Holmes attributed the spontaneous pain in these patients to local irritative effects of the lesion. Other lesions of the spinal cord causing central pain include (a) ► anterolateral cordotomy (b) dorsal root entry zone coagulation (see also ► Junctional DREZ Coagulation) and (c) cordectomy (Pagni 1998). Spinal contusion (see also ► Spinal Cord Injury Pain Model, Contusion Injury Model) is the most common cause of spinal injury pain. Spinal tumors can also lead to local pain in the case of extramedullary neoplasms. Local segmental pain with intramedullary tumors is infrequent, but does occur in some cases especially when the tumor arises in the posterior gray matter. One of the most pathologically destructive conditions giving rise to central pain is syringomyelia (Madsen et al. 1994). More than half of patients with delayed onset of central pain following SCI have syringomyelia and it appears that the syrinx rather than the original injury is responsible for the pain (Tasker et al. 1991).

The most common brainstem site for the development of central pain is the medulla. Central pain follows thrombosis of the posterior inferior cerebellar artery (PICA), described as Wallenberg's syndrome and includes analgesia in the trigeminal area on the side of the lesion, which results from damage to the descending nucleus of the fifth nerve and the crossed ascending fibers in the anterolateral system. Garcin (1968) described 56 cases of pain of bulbar origin *versus* 28 of pontine origin. In this analysis the order of frequency of different bulbar lesions included (a) vascular, especially thrombosis of PICA (b) syringobulbia (c) disseminated sclerosis and (d) pontobulbar tumors.

Bulbar spinothalamic tractotomy and bulbar trigeminal tractotomy (Sjoqvist's operation) are also associated with central pain. In general, pain from pontobulbar lesions whether spontaneous or evoked has the same general characteristics as pain of thalamic origin. Pontobulbar pain is aggravated by emotional disturbances and whether facial or remote is often chronic and resistant to ► pharmacotherapy. The striking fact that central pain of mesencephalic origin is uncommon may well be due to the absence of sensory nuclei in this region. Except for cases of pontomesencephalic tumors, central pain associated with pure midbrain lesions has not been reported, although surgical lesions following spinothalamic tractotomy at mesencephalic levels have been associated with central pain (Pagni 1998).

Within the thalamus three regions have been implicated in the onset of central pain (a) the ventroposterior part including the posterior and interior nuclei bordering this region (b) the medial-intralaminar region and (c) the reticular nucleus. Damage to the reticular nucleus is thought to release the medial and intralaminar nuclei from their normal control, thereby leading to pain and hypersensitivity (Mauguiere and Desmedt 1988).

Leijon et al. (1989) described nine patients with lesions in the ventroposterior thalamus that were associated with central pain. These reports are consistent with the contention that the posterior inferior part of the ventroposterior region is a critical location for lesions causing central pain. Thalamic pain is usually caused by ischemic and hemorrhagic vascular lesions, less frequently by tumors (Tovi et al. 1961), trauma (Riddoch 1938) or A-V malformations (Waltz and Ehni 1966). Lesions restricted to motor thalamus, medial thalamus and pulvinar do not appear to cause the onset of central pain.

Cortical lesions causing central pain are located primarily in the parietal cortex and perhaps the second sensory cortex where the ► spinothalamocortical projections are known to terminate. In general pain is rare after cerebral trauma (Marshall 1951), brain tumors, craniotomies or thalamotomies for movement disorders. Whether cortical lesions alone can cause central pain remains controversial, as in most reported cases there is damage to subcortical white matter (Breuer et al. 1981; Sandyk 1985). As a rule pain and hyperpathia occur when both sensory cortex and subcortical white matter are damaged, possibly due to the destruction of inhibitory corticothalamic fibers. Several reports have described patients with combined subcortical and cortical lesions leading to central pain, particularly with lesions in the insular region (Schmahmann and Leifer 1992). These lesions include those caused by infarcts, hematomas, meningiomas and trauma.

Pathophysiology of Central Pain

A number of theories have been proposed to explain central pain (a) irritation of spinothalamic and lemniscal pathways (Dejerine and Roussy 1906) (b) loss of inhibitory mechanisms controlling pain pathways (Head and Holmes 1911; Jeanmonod et al. 1994) (c) switching of importance from primary to secondary pain pathways (Cassinari and Pagni 1969; Tasker et al. 1980) (d) the emergence of abnormal spontaneous and hyperexcitable cells (secondary to ► deafferentation) at spinal and / or supraspinal levels of the neuraxis (Pagni 1989) and (e) irritation of the ► sympathetic nervous system. Since most central pain patients have abnormal temperature and pain sensibility, but near normal thresholds to touch, vibration and joint movement (Boivie et al. 1989), it was concluded that central pain occurs only after lesions of projections to the ventroposterior thalamic region (Pagni 1998). The fact that thalamic involvement is believed to be at the center of the mechanism responsible for the emergence of pain is underscored by the fact that anatomical and functional abnormalities are found at the termination site of pathways in this region of the brain. For this reason, central pain is thought to result primarily from surgical or spontaneous lesions that invariably affect afferent sensory pathways. Therefore, it seems reasonable to conclude that lesions sparing fibers and cells of the spinothalamic and dorsal column system are unlikely to give rise to central pain. Cassinari and Pagni (1969) concluded that lesions of the spinothalamic system may give rise to dysesthesias, pain and hyperpathia, while lesions of the dorsal column system give rise to dysesthesias only and not pain. A critical question regarding the mechanism of central pain concerns the location of neurons responsible for this condition. Neurons within the ventroposterior nuclei have been shown to have increased spontaneous activity characterized by bursts of action potentials in the region of the nucleus representing the painful area of the body (Lenz et al. 1989). Bursting is believed to be a fundamental characteristic of central pain and is found in both lateral and medial thalamus. Whether this abnormal burst activity is due to loss of excitatory afferent drive on postsynaptic receptors or increased activity at ► NMDA receptors is not known. In patients with thalamic pain, spontaneous neuronal hyperactivity is also found in the mediodorsal, central lateral, central median and parafascicular nuclei (Rinaldi et al. 1991). In patients with central pain secondary to spinal transection, cells without receptive fields due to loss of sensory input show increased bursting, but decreased firing rates between bursts. These findings support the hypothesis that loss of STT input leads to hyperpolarization of these cells with resulting increased burst firing. Since some of these cells are involved in pain signaling pathways, this bursting activity may signal the sensation of pain. Although the hypothesis that abnormal neuronal activity in the ventroposterior thalamic region is important for the onset of central pain, one must reconcile the fact that in some patients this region is completely silent due to existing pathology. In fact some authors contend that this region is precisely where a thalamic lesion must be located in order to precipitate central pain (Leijon et al. 1989). Some thalamic lesions are thought to remove the inhibitory influence exerted by the reticular thalamic nucleus on medial and intralaminar nuclei, thereby releasing abnormal activity leading to pain and hypersensitivity (Cesaro et al. 1991).

The pathophysiology of central pain states may also involve the irritation of cells and fibers of sensory pathways and nuclei that develops at the lesion site. The resulting disruption of normal function is thought to lead to the development of an irritant focus (Dejerine and Roussy 1906; Livingston 1943). This hypothesis however, does not explain pain onset following complete destruction of sensory pathways and nuclei or pain due to section of fiber tracts. One explanation for the sudden disappearance of central pain after focal strokes in

the subparietal white matter suggests that central pain is generated by a disturbance in the normal oscillatory mechanisms between cortex and thalamus (Canavero 1994).

Another proposed mechanism of central pain is based on the ► disinhibition hypothesis of Head and Holmes (Craig 2002). This thermosensory disinhibition theory proposes that central pain results from the disinhibition of pain resulting from imbalanced sensory integration caused by the loss of temperature sensation. This theory suggests that central pain is a thermoregulatory disorder that produces a thermal distress signal that is modulated by homoeostatic processing. At the heart of this proposal is the fact that loss of input from the lateral spinothalamic tract unmasks a homeostatic spinobulbothalamic pathway to the medial thalamus that is responsible for the development of central pain. This theory proposes that loss of activity in the thermosensory cortex in the dorsolateral mid / posterior insula disinhibits polymodal activation of the medial dorsal nucleus and anterior cingulate cortex, which produces burning pain.

Sympathetic Mechanisms

Sympathetic dysfunction is thought to play a role in central pain because signs of abnormal sympathetic activity, e.g. edema, decreased sweating, lowered skin temperature, changes in skin color and trophic skin changes have been described in many patients (Riddoch 1938). Unfortunately sympathetic blockade, which if effective would support a role of sympathetic mechanisms has shown contradictory results with only a small proportion of patients showing pain relief (Loh et al. 1981).

Spinal Injury: A Model of Central Pain

A major problem with the study of the pathophysiology and central mechanisms of central pain has been the lack of appropriate experimental models. In recent years this has been addressed with regard to the study of central pain following spinal injury with the development of models with pathological and behavioral characteristics consistent with the clinical profile of SCI (Christensen et al. 1996; Siddall et al. 1995; Vierck and Light 1999; Xu et al. 1992; Yezierski et al. 1998). One of the similarities between spinal cord injury and peripheral nerve and / or tissue damage is that both result in an increase in spinal levels of ► excitatory amino acids (EAAs). With this in mind, it is easy to envision a scenario whereby the physiological changes associated with SCI are linked to the same central injury cascade initiated by peripheral injury (Yezierski 1996). For example, the hypersensitivity of dorsal horn ► wide dynamic range neurons (WDR) described after ischemic and excitotoxic injury of the spinal cord reflects changes similar to those described following peripheral injury. The fact that these effects are blocked by the non-competitive NMDA receptor antagonist MK-801 implicates glutamate in these changes in functional properties. The abnormal bursting patterns and evoked responses of thalamic neurons in patients with SCI supports the hypothesis that the functional changes after spinal injury are not limited to the spinal cord, but as with peripheral injury can also be found at supraspinal sites.

An important factor contributing to changes in functional state of sensory neurons following SCI is believed to be the loss of spinal inhibitory mechanisms (Wiesenfeld-Hallin et al. 1994). Consistent with this is the reversal of the hypersensitivity of WDR neurons after transient spinal cord ischemia with the $GABA_b$ agonist baclofen (Hao et al. 1992). Spinal cord injury may therefore have multiple factors contributing to increased neuronal excitability (a) loss of inhibitory tone due to the loss of inhibitory interneurons and (b) changes in membrane properties due to prolonged periods of depolarization (central sensitization). Not to be ignored in this discussion are the physiological effects of deafferentation, which provide yet another factor capable of influencing the functional state of spinal and especially supraspinal neurons following SCI. Attempts to develop models of experimental thalamic pain have included placing electrolytic lesions in different thalamic nuclei (LaBuda et al. 2000; Saade et al. 1999) or excitotoxic lesions in the lateral thalamus (LaBuda et al. 2000) or giving cortical injections of picrotoxin (Oliveras and Montagne-Clavel 1996). All of these models produce heightened responses to peripheral stimuli, thereby providing support for their use in the study of central pain states.

Treatment of Central Pain

At present a long-term effective treatment for central pain is not available and for this reason the strategy for treatment is to try all available treatment modalities in order to systematically determine the best approach for an individual patient. The realistic goal of central pain treatment is to reduce the intensity of pain intensity to a tolerable level. With this in mind, it is commonly believed that opiate narcotics are totally ineffective in the treatment of central pain, although more systematic studies are needed. Central pain also responds poorly to most conventional analgesics, better to antidepressants, temporarily to sodium thiopental and propofol and may respond to i.v. pentothal. Agents that enhance norepinephrine and dopamine neurotransmission and anticonvulsants have some therapeutic efficacy. A review of controlled studies related to the efficacy of pharmacological treat-

ments of neuropathic pain is recommended for additional reading (Sindrup and Jensen 1999). In addition to pharmacotherapy a number of other strategies to treat central pain have been used. These include (1) ► peripheral nerve blocks (2) peripheral neurectomy and ► rhizotomy (3) ► sympathectomy and sympathetic blocks (4) ► spinal block and (5) stimulation and ablative procedures.

Conclusion

Chronic pain associated with injury or disease of the central nervous system represents a long-standing enigma that presents a significant challenge to the scientific and health care communities. As a condition that seems to depend on damage to the very substrate required for pain perception, it is one that has defied effective therapeutic intervention and continues to baffle those searching for an underlying mechanism. In spite of efforts to understand the pathophysiology and underlying etiology, there remain many unanswered questions. Parallels between chronic pain states resulting from injury or disease of peripheral and central substrates offers hope for the future. The fact that there are spontaneous and evoked components of central pain suggests multiple mechanisms underlying these and other divergent clinical findings as well as the varied temporal profile and location of pain in patients with different central lesions. The role of disinhibition, sensitization, denervation and other plastic changes in central sensory pathways remain to be addressed. Continued efforts to develop experimental models and strategies to study the human condition will hopefully lead to new insights into the progression of anatomical, chemical and functional changes from the site of injury to higher levels of the neuraxis along with the development of novel treatments.

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Neuropathy

Definition

Neuropathy is a disturbance of function or pathological change in a nerve (Merskey and Bogduk 1994). Mononeuropathy refers to affection of a single nerve, mononeuritis multiplex to several nerves, and polyneuropathy to diffuse involvement of the peripheral nerves.

► Descending Modulation and Persistent Pain
► Guillain-Barré Syndrome

- ► Hereditary Neuropathies
- ► Toxic Neuropathies

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Neuropathy Due to HAART

- ► Pain in Human Immunodeficiency Virus Infection and Acquired Immune Deficiency Syndrome

Neuropeptide Release in Inflammation

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Synonyms

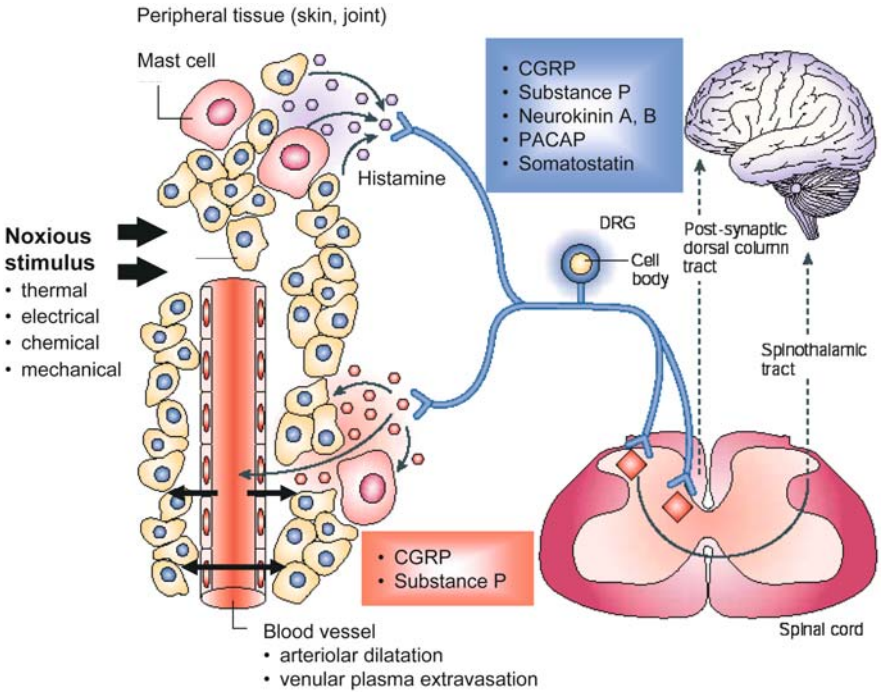
Inflammation, Neuropeptide Release

Definition

► Neuropeptides are a group of small peptides with 4 to more than 40 amino acids found in the central and peripheral nervous system. This essay focuses on neuropeptides that are released into peripheral tissues upon neuronal activation; that is the case, for example, during an ► inflammation. The neuropeptides are synthesized in the cell body of primary afferent (sensory) neurons located in the ► dorsal root ganglia and are transported through the axons into preferentially the peripheral nerve endings. The best-known neuropeptides in primary afferent neurons are ► substance P and ► calcitonin gene-related peptide.

Characteristics

► Nociceptors are primary afferent neurons many of which are characterized by their ability to release neuropeptides such as substance P (SP, 11 amino acids), calcitonin gene-related peptide (CGRP, 37 amino acids) and ► neurokinins (10 amino acids) from their peripheral terminals upon noxious stimulation (Maggi et al. 1995). In isolated tissue preparations (e.g. skin and dura), the release of SP and CGRP was shown to be induced by noxious stimulation with ► inflammatory mediators (► bradykinin, ► serotonin, ► histamine, ► prostaglandins and protons) (Averbeck and Reeh 2001; Ebersberger et al. 1999). In addition, electrical stimulation of cutaneous nociceptors was demonstrated to cause neuropeptide release from the skin (Kress et al. 1999). The release of SP and CGRP leads to arteriolar vasodilatation, which becomes visible as a flare surrounding a site of injury, and to plasma extravasation from post-capillary venules, which may become apparent as a wheal at the site of injury. Since these inflammatory signs depend on the function and integrity of the peripheral sensory nervous system, the response has been termed ► neurogenic inflammation. By means of this mechanism, neuropeptides are able to induce the release of secondary mediators like prostaglandins, cytokines and histamine from endothelial and inflammatory cells (monocytes, mast cells) thereby maintaining inflammation. In addition, neuropeptides show immunomodulatory and trophic effects and thus play a role in tissue repair. A direct exciting effect on nociceptors could not be found for neuropeptides, whereas sensitizing effects have been described in the literature. SP was shown to sensitize nociceptors to mechanical stimulation in the knee joint of anesthetized cats (Herbert and Schmidt 2001) and preliminary data point to CGRP sensitizing nociceptors to heat stimulation in the isolated rat skin. The biological actions of neuropeptides are limited by degradation caused by neutral endopeptidases located in many tissues surrounding the primary afferent nerve fibers.
Inflammation is a critical protective reaction to irritation, injury or infection, characterized by redness (*rubor*), heat (*calor*) swelling (*tumor*), loss of function (*funtio lasea*) and pain (*dolor*). During inflammation, inflammatory mediators reach the nerve terminals, causing excitation and sensitization of nociceptors, which results in ► hyperalgesia and pain. Due to the fact that inflammatory mediators induce neuropeptide release by acting directly on nociceptors, inflammation is linked to an increased neuropeptide release from inflamed tissue. In addition, inflammation may result in an up-regulation of neuropeptide levels in primary afferent nerves, due to an enhanced neuropeptide biosynthesis in the dorsal root ganglion cells. In rats with ► adjuvant-induced arthritis, the levels of CGRP were found to be increased in the dorsal root ganglia and in the sciatic nerves, particularly in those fibers that innervate the inflamed area (Kuraishi et al. 1989). In contrast, the synovia of the arthritic joints of these animals showed less immunostaining for SP and CGRP, pointing to an increased neuropeptide release from the nerve terminals into the synovial fluid (Konttinen et al. 1990). The phenomenon of low neuropeptide immunostaining in the synovia and high neuropeptide levels in the synovial fluid was also found in patients with rheumatoid arthritis (Menkes et al. 1993). In experimental ► colitis in rats, a significant reduction of CGRP- and SP-immunoreactive nerve fibers was observed in the mucosa, again pointing to an enhanced neuronal neuropeptide release in inflammation (Miampamba and Sharkey 1998). The observation that the intensity and density of neuropeptide containing nerve fibers increased in the circular muscle 7 days after the induction of colitis suggests their possible involvement in tissue repair (Miampamba and Sharkey 1998).



Neuropeptide Release in Inflammation, Figure 1 Neuropeptides in inflammation. Neurogenic inflammation. Painful stimuli on peripheral tissues are detected by primary afferent neurons (nociceptors), the cell bodies of which lie in the dorsal root ganglion (DRG). The painful signal is then transmitted to neurons in the spinal cord and further on to higher centers of the brain. Nociceptor activation results in the release of neuropeptides, which are synthesized in the nociceptors' cell bodies and transported to the central and peripheral nerve endings. The release of neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), from the peripheral nerve endings causes arteriolar vasodilatation and plasma extravasation from post-capillary venules, seen as typical signs of neurogenic inflammation: local edema, hyperemia and an erythema which extends beyond the site of stimulation (so-called flare response). The neuropeptides released maintain inflammation by releasing secondary mediators, e.g. histamine by mast cell degranulation. (Modified from Mantyh et al. 2002; reprinted by permission from Nat Rev Cancer 2: 201-209 copyright 2002 Macmillan Magazines Ltd).

In inflammatory skin diseases, certain neuropeptides have been found to be enhanced, for example SP and CGRP in ► urticaria, and SP and pituitary adenylate cyclase activating polypeptide (PACAP) in ► psoriasis (Steinhoff et al. 2003). Thus, the increased number of mast cells and their characteristic degranulation observed in early psoriasis might be due to pathological neuropeptide release.

Blocking the neuropeptide response by blocking the neuropeptide binding sites may have anti-inflammatory and analgesic effects. A CGRP antagonist being clinically tested in ► migraine headache revealed an analgesic effect (Olesen et al. 2004). Thus, blocking CGRP effects postjunctionally (e.g. on blood vessels) seems to be an effective antinociceptive mechanism, at least in migraine. Another analgesic principle would be the use of anti-inflammatory neuropeptides. Somatostatin (SOM, 14 amino acids) which is synthesized in primary afferent neurons, but also in most major peripheral organs, revealed anti-inflammatory effects in inflammatory pain models (Heppelmann and Pawlak 1997). SOM, like SP and CGRP, is released upon nerve fiber activation and in inflammation. In rats, SOM was demonstrated to be released into the blood circulation upon electrical stimulation of nociceptors, revealing a systemic anti-inflammatory action (Szolcsànyi et al. 1998). As the clinical use of SOM is limited by its rapid degradation after systemic injection and its neurotoxicity when applied intrathecally, a new analgesic strategy for inflammatory pain involving the modulation of localized release of SOM is under discussion.

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Neuropeptide Release in the Skin

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Definition

Cutaneous nerves release small peptides into the skin that are important mediators in inflammation, peripheral hyperalgesia and immune reactions. Nociceptive afferent nerve endings constitute the largest neuronal source of such peptides.

Characteristics

Neuropeptide Release from Sensory Nerve Endings

The skin is densely innervated by nociceptive nerve fibres that can be regarded as the first line of defence against potentially damaging stimuli. Large proportions of these afferents have unmyelinated (► C-fibre) axons, and terminate as free nerve endings in all layers of the skin. Many belong to the group of polymodal nociceptors, which are activated by thermal as well as mechanical and chemical stimuli of high intensity. While their obvious function is the afferent transmission of nociceptive information via the spinal and trigeminal ganglia to the central nervous system, they have a second, efferent role in mediating local defence reactions in the skin. The latter effects are mediated by ► neuropeptides released from the peripheral endings of these nociceptive afferents. An overview of the functions of sensory neuropeptides in the skin is given in Table 1.

In 1927, T. Lewis had already described the "triple response" (Brain 1996) as when a noxious stimulus is applied to the skin, instantaneous erythema and wheal develop at the site of injury followed by a flare that spreads beyond the injured area. Much later it was shown that this response depends on the integrity of polymodal nociceptors and the release of neuropeptides, mainly ► substance P and ► calcitonin gene-related peptide (CGRP) (Holzer 1997). The current concept holds that noxious stimulation of polymodal nociceptors or heat nociceptors leads to an axon reflex, which is depicted in Figure 1. The evoked action potential travels not only centrally, but also invades – in a retrograde direction – intracutaneous axon collaterals where substance P and CGRP are released. Substance P binds to specific ► tachykinin NK_1 receptors on nearby arterioles, to induce rapid vasodilatation mediated by production of ► nitric oxide (NO) in endothelial cells. Action on venules leads to extravasation of plasma proteins into the perivascular tissue with consecutive development of a local oedema, the wheal. Furthermore, substance P causes mast cells to degranulate and to release a cocktail of inflammatory mediators, which can also contribute to the formation of oedema. The slower spreading flare reaction, however, is attributed to CGRP. This neuropeptide is a potent vasodilator with a much longer duration of action than substance P. Moreover, CGRP can potentiate the effects of substance P, because it inhibits the latter's degradation by peptidases.

In order to elicit this combination of reactions, also called "neurogenic inflammation", it is sufficient to stimulate a single afferent C-fibre unit. The evoked flare relies on nerve conduction and does not spread beyond the boundaries of the unit's receptive field (Lynn 1996). The axon reflex is a good explanation for the underlying anatomical arrangement. However, recently Lin et al. (1999) showed that in some cases the reflex arc includes the spinal cord.

Mediation of the triple response is not the only action of sensory neuropeptides released by noxious stimuli. These peptides influence most cell types occurring in the skin (Table 1). Endothelial cells are stimulated to produce adhesion molecules, thereby leading to accumulation of granulocytes with consecutive metabolism of arachidonic acid to prostaglandins, leukotrienes and thromboxanes as well as production of proinflammatory cytokines. Similarly, keratinocytes are induced to synthesize proinflammatory cytokines. These are the first steps of the pathophysiologic mechanisms leading to long-term inflammation of the skin. Furthermore, fibroblasts and keratinocytes start to proliferate in response to substance P or ► vasoactive intestinal polypeptide (VIP), which is an important component

Neuropeptide Release in the Skin, Table 1 Neuropeptides released from sensory nerves in the skin and their effects on cutaneous structures (Wallengren et al. 1987; Brain 1996; Holzer 1997; Scholzen et al. 1998; Steinhoff et al. 2003)

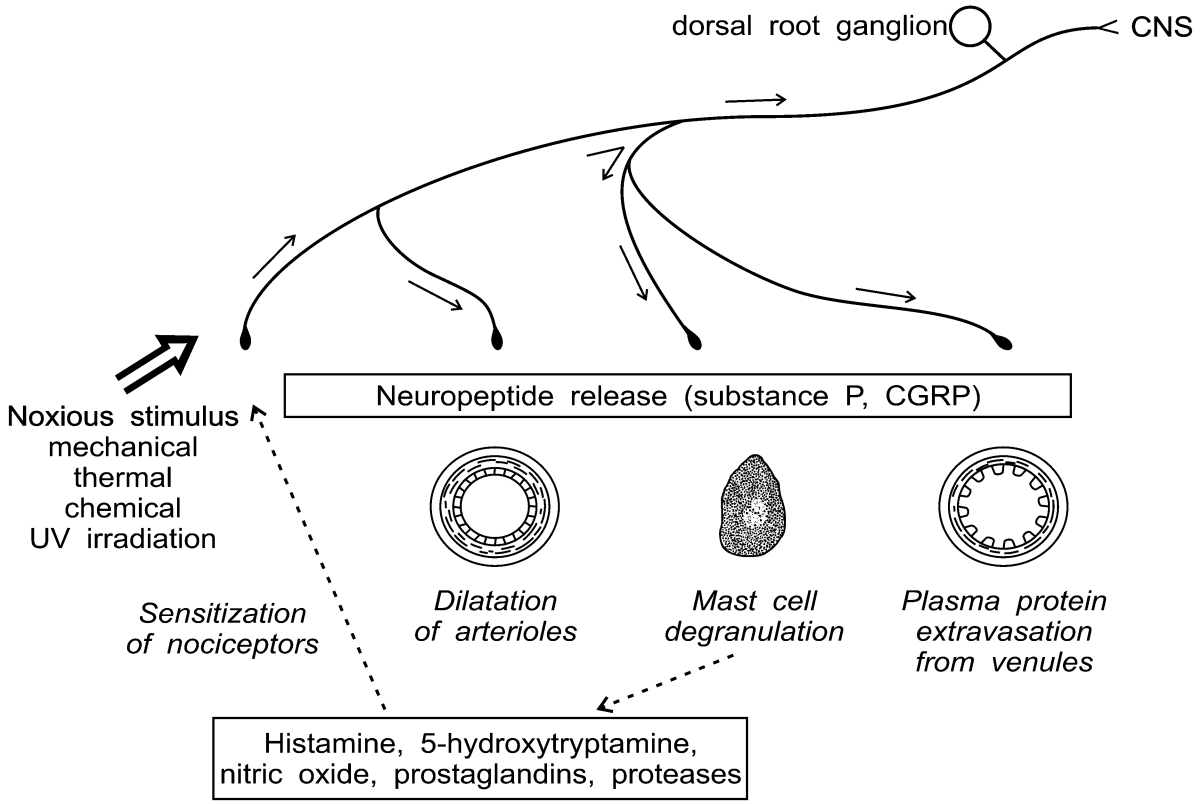
Neuropeptide	Target in skin	Effect
Substance P	arterioles	endothelium-mediated vasodilatation, proliferation of endothelial cells, upregulation of adhesion molecules, accumulation of granulocytes with release of cytokines, prostaglandins, leukotrienes, thromboxanes, NO, opioid peptides[1]
	venules	plasma protein extravasation
	mast cells	degranulation with release of histamine, 5-hydroxytryptamine, prostaglandins, leukotrienes, tumour necrosis factor α, proteases, NO
	monocytes	chemotaxis, release of interleukin-1, tumour necrosis factor α, prostaglandins, leukotrienes, thromboxanes
	keratinocytes	proliferation, upregulation of adhesion molecules, production of proinflammatory cytokines
	fibroblasts	chemotaxis, enhancement of cytokine-induced proliferation
► Neurokinin A	venules	plasma protein extravasation
	keratinocytes	production of proinflammatory cytokines, upregulation of NGF expression
Calcitonin gene-related peptide (CGRP)	arterioles	vasodilatation (relaxation of smooth muscle), proliferation of endothelial cells, leukocyte adhesion
	leukocytes	antagonism of substance P-induced intravascular accumulation
	T-lymphocytes (human) (mouse)	chemotaxis inhibition of proliferation and production of interleukin–2
	keratinocytes	proliferation, cytokine production
	Langerhans cells	inhibition of antigen presentation
Somatostatin[2]	mast cells	degranulation
	nociceptive afferent endings	presynaptic inhibition of substance P/CGRP release
Vasoactive intestinal polypeptide (VIP)	arterioles	vasodilatation
	endothelial cells	expression of adhesion molecules, neutrophil accumulation
	mast cells	degranulation
	macrophages	suppression of phagocytosis
	keratinocytes	proliferation, migration
PACAP	arterioles	vasodilatation
	mast cells	histamine release
	T-lymphocytes, macrophages	suppression of cytokine production
Galanin	nociceptive afferent endings	presynaptic inhibition of neuropeptide release

[1] in inflamed tissue

[2] in humans it is most likely only in skin disease, not in healthy skin (Wallengren et al.. 1987)

of wound healing. In fact, the impaired wound healing and development of spontaneous skin ulcers in patients with diabetic neuropathy are attributed to the loss of peptidergic afferent nerve fibres (Gibran et al. 2002). However, sensory neuropeptides are not generally proinflammatory agents. CGRP, for example, reduces intravascular accumulation of granulocytes and inhibits antigen presentation by ► Langerhans cells.

Injury to, or inflammation of, the skin is usually accompanied by local hyperalgesia. This is caused by a feedback mechanism of various mediators released from the target cells of neuropeptides. Nociceptive nerve endings are equipped with specific receptors for neuropeptides, prostaglandins, 5-hydroxytryptamine, cytokines, growth factors, ► vanilloids, protons, as well as with ► proteinase-activated receptor-1 and -2.



Neuropeptide Release in the Skin, Figure 1 Schematic representation of the axon reflex as the basis for neurogenic inflammation.

During an inflammatory reaction, agonists for these receptors are released by mast cells or leukocytes, while ► nerve growth factor (NGF) is produced by stimulated keratinocytes. All of them can sensitize the nociceptive nerve endings by reducing their stimulation threshold, or even induce further neuropeptide release (Handwerker and Reeh 1992; Richardson and Vasko 2002). Moreover, growth factors produced in inflamed skin increase the production and release of sensory neuropeptides. Other sensory neuropeptides, such as ► somatostatin and ► galanin, act on presynaptic receptors to inhibit further release of substance P and CGRP (Szolcsányi et al. 1998; Xu et al. 1991).

Neuropeptide Release from Autonomic Efferents

Neuropeptides occur not only in afferent nerves but also in autonomic efferents. A painful stimulus also activates the sympathetic nervous system. In sympathetic efferents, ► neuropeptide Y (NPY) coexists with noradrenaline and is responsible for the long-lasting vasoconstriction seen after peripheral nerve stimulation. Recently, NPY Y1 receptors have been localized to CGRP-containing sensory nerves in rat skin, where NPY is likely to facilitate the release of substance P and CGRP (Brumovsky et al. 2002). Moreover, NPY influences a variety of immune mechanisms (Bedoui et al. 2003). The sympathetic cholinergic fibres innervating the sweat glands contain VIP as a co-transmitter.

In the parasympathetic neurons, VIP and ► PACAP coexist with acetylcholine. These neuropeptides, however, are also localized in sensory neurons, and so far no data exists about a differential action depending on their release from sensory or autonomic cutaneous nerves.

Release of Opioid Peptides from Non-Neuronal Sources in the Skin

While in healthy skin less than a third of unmyelinated axons express functional μ-opioid receptors, all three types of opioid receptors are expressed on the peripheral endings of sensory nerves in inflamed tissue (Stein et al. 2003). Opioid peptides, such as β ► -endorphin, enkephalins and ► dynorphin, are released from immune cells, activated by, e.g. interleukin-1, and mediate local analgesia by reducing the sensitivity of the nociceptors.

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Neuropeptide Y

Synonym

NPY

Definition

Neuropeptide Y (NPY) is a 36-amino acid peptide containing many tyrosine (Y) residues. NPY is a co-transmitter of noradrenaline in sympathetic neurons, with long-lasting vasoconstrictor effects and potent influences on the immune system. It is not contained in sensory afferents. It is the most abundant neuropeptide known in the brain. NPY modulates pain sensation as well as other neuronal processes, and is involved in the regulation of food intake and energy expenditure, in the central control of reproductive hormones, and in anxiolytic and antiepileptic control circuits.

► Neuropeptide Release in the Skin
► Peptides in Neuropathic Pain States

Neuropeptides

Definition

Neuropeptides are bioactive molecules built up of a varying number of amino acids (2 to > 40), synthesized (mostly, but not exclusively) in and released from neurons. The final, pharmacologically active peptides are cleaved from large precursor peptides. Over the past two decades, more than 40 biologically active polypeptides, termed neuropeptides, have been found in the central and peripheral nervous system. Neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin (NK) A and B (10 amino acids), somatostatin (SOM), vasoactive intestinal peptide (VIP), neuropeptide Y and cholecystokinin (CCK) are synthesized in the cell body of autonomic and sensory neurons located in the autonomic or sensory (dorsal root) ganglia. As they are released upon neuronal activation, neuropeptides act centrally as neurotransmitters or neuromodulators. In the periphery, neuropeptides have various functions like vasodilatation, plasma extravasation, sweat gland stimulation, mast cell degranulation, immunomodulation and trophic effects, thereby playing an important role in inflammation. Neuropeptides are metabolically more stable than amine transmitters, therefore they have a longer duration of action and can diffuse over longer distances. Neurons may contain several neuropeptides coexisting with small molecule transmitters.

► Cytokines, Effects on Nociceptors
► Descending Circuitry, Transmitters and Receptors
► Neuropeptide Release in Inflammation
► Neuropeptide Release in the Skin
► Nociceptive Neurotransmission in the Thalamus
► Nociceptor Generator Potential
► Opioid Modulation of Nociceptive Afferents In Vivo
► Retrograde Cellular Changes after Nerve Injury
► Sensitization of Muscular and Articular Nociceptors
► Spinothalamic Tract Neurons, Peptidergic Input
► Thalamic Neurotransmitters and Neuromodulators

Neuropeptides in Neuropathic Pain States

► Peptides in Neuropathic Pain States

Neuropile

Definition

Neuropile is the complex network of axonal, dendritic, and glial arborisations in nuclei and laminae of the CNS containing the cell bodies

► Deafferentation Pain

Neuroplasticity

Definition

Neuroplasticity is a general term referring to persistent changes in neural activity or function. A neuroplastic change is caused by frequent usage of a neuron or a neuronal connection, which stays for a longer period of time after the end of the neuronal activity that started the change. Memory processes and the transition from acute to chronic pain are examples of neuroplastic changes.

- ► Amygdala, Pain Processing and Behavior in Animals
- ► Arthritis Model, Kaolin-Carrageenan Induced Arthritis (Knee)
- ► Metabotropic Glutamate Receptors in Spinal Nociceptive Processing
- ► Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology
- ► Sensitization of Muscular and Articular Nociceptors
- ► Transition from Acute to Chronic Pain
- ► Trigeminal Brainstem Nuclear Complex, Immunohistochemistry and Neurochemistry

Neurosecretion

Definition

Neurosecretion is the active process of releasing signaling molecules from nerve terminals by exocytosis.

- ► Inflammation, Modulation by Peripheral Cannabinoid Receptors

Neurosensory Testing

Synonyms

NST

Definition

This is a form of Quantitative Sensory Testing that refers to the use of the Pressure-Specified Sensory Device to measure the cutaneous pressure threshold of the large myelinated fibers for both moving (quickly-adapting fibers) and static (slowly-adapting fibers) touch. The pressure required to discriminate one from two static touch stimuli is the first to become abnormal in the patient with a chronic nerve compression, like carpal or tarsal tunnel syndrome. All QST, by definition, is subjective, requiring cognitive input from the person being tested to identify the end-point of the test.

- ► Carpal Tunnel Syndrome

Neurosteroids

Definition

Steroid hormones with neurotransmitter-like actions produced in the central nervous system via metabolism of parent steroids or by local synthesis from cholesterol.

- ► Premenstrual Syndrome

Neurostimulation

- ► Stimulation Treatments of Central Pain

Neurosurgery for Pain

Definition

Is a part of so-called "Functional Neurosurgery". Functional Neurosurgery was defined in 1956 by P. Wertheimer from LYON as follows : "Functional Neurosurgery aims at correcting the functional disorders which cannot be normalized by direct cure of the causative lesion. Operations are based on neurophysiological information. Procedures consist of removing irritative foci or interrupting excitatory pathways to compensate failing inhibitory systems" (Pierre Wertheimer 1956).

- ► Brachial Plexus Avulsion and Dorsal Root Entry Zone

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Neurosurgery for Pain in the DREZ

- ► Brachial Plexus Avulsion and Dorsal Root Entry Zone

Neurotomy

Definition

The dissection, or anatomy, of the nervous system.

- ► Facet Joint Pain

Neurosurgical Treatment of Pain

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Introduction

Since the 1970s there have been significant changes in neurosurgical approaches to the treatment of pain. Specifically, there has been a substantial decrease in the numbers of ablative procedures and an increase in the numbers of stimulation or augmentative procedures for treatment of chronic pain. The decrease in ablative procedures is in part due to the recognition of the occurrence of ► neuropathic pain following surgical injuries to the nervous system (Boivie 1999; Tasker 1984). Ablative procedures, which have survived have often been associated with a clearer understanding of the rationale and indications for the surgery. This is so in the case of sympathetically maintained pain treated by ► sympathectomy. Other current ablative procedures are associated with a high degree of efficacy are ► cordotomy for cancer pain or surgical treatments for tic douloureux. This review is derived, in part, from the topical essays included in this section

Ablative Neurosurgical Procedures with Improved Rationales

The practice of surgery on the sympathetic nervous system has been altered by advances in the understanding of the ► sympathetically maintained pain (SMP), a subset of patients with ► complex regional pain syndrome (CRPS). CRPS typically occurs after trauma without (CRPS type 1) or with concomitant nerve injury (CRPS type 2). In SMP the distal extremities, areas rich in sympathetic innervation, can be affected with edema, hyperalgesia and cool and sweaty skin. The dramatic relief of pain that occurs with selective blockage of the sympathetic nervous system defines SMP.

The mechanism of SMP depends upon α_1-adrenergic receptors as demonstrated by the relief of hyperalgesia produced by application of an α_2 agonist to the painful skin in patients with SMP (Davis et al. 1991). Binding at the α_2-adrenergic receptors, located on sympathetic terminals blocks norepinephrine release. When phenylephrine, a selective α_1-adrenergic agonist was applied to the clonidine treated area, pain was rekindled. The density of α_1-adrenoceptors in the epidermis of the hyperalgesic skin of patients with CRPS is increased (Drummond et al. 1996). Thus, the data suggest that the α_1-adrenergic receptor plays a pivotal role in SMP. Sympathetic mechanisms may also play a role in other types of pain. For example, injection of noradrenaline around stump neuromas or skin in patients with postherpetic neuralgia induces an increase in spontaneous pain (Chabal et al. 1992; Choi and Rowbotham 1997; Raja et al. 1998).

The gold standard for diagnosing SMP has been determination of the response to blockade of the appropriate level of the sympathetic chain (► sympathetic block) with local anesthetic. An intravenous infusion of phentolamine, an α-adrenergic antagonist, (► phentolamine test) given systemically has proven to be a safe, specific test for SMP. Skin temperature is monitored. If the skin temperature does not rise with phentolamine, then a higher dose of phentolamine may have to be given. A positive result is the finding that phentolamine relieves pain, which result identifies patients with SMP (Arner 1991; Raja et al. 1991).

By definition, SMP is relieved by performance of a sympathetic block. The pain relief of sympathetic block often outlives the pharmacological action of the block. Similar, long lasting pain relief has also been reported following systemic phentolamine infusion (Galer et al. 1992). In cases where sympathetic blockade provides only transient pain relief, surgical ► sympathectomy may offer lasting pain relief (Singh et al. 2003). Thus, recent research findings have advanced our understanding of the basis of SMP and rationalized surgical therapy, which has led to a resurgence of this surgical treatment.

Ablative Neurosurgical Procedures with High Efficacy

► Percutaneous cordotomy produces relief of pain by interrupting the transmission of signals in the ► spinothalamic tract (STT) from below the level of intervention and caused by cancer. It is not helpful for the steady burning element of neuropathic pain. It may, however, be useful for the relief of allodynia, hyperpathia and neuralgic pain associated with ► neuropathic pain syndromes (Tasker and Dostrovsky 1989; Tasker et al. 1992). It is usually unsuccessful if done in the lower cervical area in relieving pain above the C5 dermatome.

Published data suggests a 63–77% range of complete, 68–96% significant, contralateral pain relief (Tasker 1988). Complete pain relief was found in 90% of patients immediately post operatively, 84% after 3 months, 61% at 1 year, 43% between 1 and 5 years and 37% between 5 and 10 years (Tasker 1988). If the expected rate of immediate significant pain relief with unilateral cordotomy is 80% then that after bilateral surgery is 80% × 80% or 64%. If the first procedure interfered with automatic respiration ipsilaterally, it is unwise to proceed on the other side. The efficacy of this technique in the face of intense, refractory pain due to cancer makes it a viable procedure in patients with a limited survival.

The surgery of ► tic douloureux has been revolutionized by a number of 'minimally destructive procedures' such as stereotactic radiosurgery, percutaneous ganglion level procedures including RF thermocoagulation, glycerolysis and balloon compression. The surgery of tic douloureux also includes ► microvascular decompression (MVD) procedures. Some of these latter procedures, such as MVD, for treatment of intracranial neuralgias were reinvented in the 1960s and found to be effective and safe procedures, although they are not without significant risks (McLaughlin et al. 1999; Patel et al. 2002).

These procedures are strikingly effective for the treatment of typical pain of tic douloureux. Ganglion level procedures had high initial levels of initial pain relief (91–99%) with subsequent recurrence rates of 10–25% over the various study times (25% at 14 years) (Peters and Nurmikko 2002). Stereotactic radiosurgery has recently been promoted and found to produce complete pain relief without medication in 57% at 1 year, and 55% at 3 years in a group of whom 60% had previously been operated on. The long-term effects of radiation therapy close to the brainstem are unknown. The first line of treatment for young healthy patients is MVD and glycerol rhizotomy procedures (Peters and Nurmikko 2002; Pollock et al. 2002). The striking effectiveness of these procedures in the treatment of the medically intractable pain of typical tic douloureux has supported the present high rates of performance of these procedures.

Ablative Neurosurgical Procedures with Tighter Indications

Spinal and ► nucleus caudalis DREZ lesioning procedures can be an effective means of treating deafferentation pain syndromes in carefully selected patients. Traumatic ► brachial plexus avulsions frequently result in a characteristic pain syndrome, which must be confirmed clinically, as peripheral nerve injury pain does not respond to DREZ lesions. This is the best indication for the ► DREZ procedure. In spinal cord injury, radicular or segmental pain syndromes occurring in the partially deafferented levels adjacent to the level of injury also respond well to DREZ procedures. Diffuse pain occurring below the level of injury, especially constant burning pain in the sacral dermatomes does not respond to DREZ procedures. In the case of injuries to the conus medullaris and cauda equina at the T12 to L1 levels, the best results are observed in patients with incomplete neurological deficits and those with 'electric' pain. Finally, patients suffering phantom pain after limb amputation respond well to DREZ procedures, significantly better than those with stump pain.

Overall, larger series report long-term pain relief in over 60–90% (Dreval 1993; Rath et al. 1997; Sindou et al. 2001; Thomas and Kitchen 1994). Variations in results can be attributed to differences between criteria for patient selection, outcome measures, times of follow-up and techniques (Dreval 1993; Friedman et al. 1988; Iskandar and Nashold 1998; Nashold and El-Naggar 1992; Sampson et al. 1995; Sindou 2002; Spaic et al. 2002; Thomas and Kitchen 1994). Careful selection of patients is critical since complication rates can be significant, especially following DREZ lesions for brachial plexus avulsion pain. Of this group, 41% experienced objective sensory deficits and 41% objective motor deficits (Friedman et al. 1988). Overall, the better defined indications for DREZ procedures has led to better outcomes and a resurgence of interest in these procedures.

Selection of the proper patients for intracranial ablative procedures is focused on patients with pain involving the head and / or neck, upper extremities or pain that is widespread throughout the body. The etiology of the pain must be clearly defined and the severity of the pain must be consistent with the etiology (Gildenberg 1973; Gildenberg and DeVaul 1985). Ordinarily, such surgery is reserved for persistent pain accompanying serious conditions, such as head or neck cancer. Patients must be free of significant psychological issues.

Intracranial procedures include lesions in the spinothalamic tract by stereotactic ► mesencephalotomy (Gildenberg 1974) and lesions of the mesencephalic central grey (Nashold et al. 1969). The spinoreticulothalamic pathways may be lesioned in the intralaminar and centromedian nuclei and posteriorly adjacent nuclei (Gybels and Sweet 1989; Jeanmonod et al. 1993). A common target for psychosurgery of the limbic system is the anterior portion of the cingulate bundle as it wraps around the anterior end of the corpus callosum. Ablation of that same area has been successful in alleviating severe persistent pain, particularly that of cancer and particularly in patients with severe emotional distress (Hassenbusch 1998). Thus the improved indications for surgery have led to an improved rationale for these uncommon procedures.

Stimulation Procedures – Improved Indications and Demonstrated Efficacy

Since the 1970s stimulation of the nervous system has largely supplanted lesioning of the nervous system for control of pain (Gybels and Sweet 1989; White and Sweet 1969). The increase in the numbers of augmentative or stimulation procedures for the treatment of chronic pain arises from their effectiveness and reversibility. The term augmentative refers to the characteristics of procedures that are not destructive, as in the case of implanted nervous system stimulators and drug

pumps. Some of these procedures are characterized by the use of clinical or pharmacologic criteria to identify patients who are candidates for these surgeries. For example, successful pain relief by ► motor cortex stimulation may be predicted if patients have significant pain relief in response to infusions of intravenous thiamylal (Yamamoto et al. 1997). Additionally an intact motor system, but not an intact somatosensory system is required.

The most commonly used stimulation modality is ► spinal cord stimulation (SCS), which is indicated only when pharmacological or surgical treatment options for chronic pain have been exhausted. In general, good results, defined as >50% reduction in chronic pain are reported by 60–70% of the patients (Meyerson and Linderoth 2000; Simpson 1994). Large numbers of retrospective analyses have demonstrated reduction in chronic pain as demonstrated by reduction in analgesic medication and by patient satisfaction.

The most common indication is ► lumbo-sacral rhizopathy, a diagnosis that often represents a mix of nociceptive, neuropathic and inflammatory pain located in the lumbar region. This "low back pain" is less likely to respond to SCS than is the "radiating leg pain" that is amenable to SCS (North et al. 1993). The second common indication is pain following peripheral nerve injury or disease. Of the many forms of neuropathy due to metabolic disease, ► diabetic polyneuropathy is the most common and it is likely to respond to SCS. Pain due to peripheral nerve injury, which sometimes presents as ► complex regional pain syndrome (CPRS) is also considered to be a good indication (Kumar et al. 1997). A recent trend is the treatment of the pain of ► peripheral vascular disease or ► angina pectoris by SCS; this is performed at a small number of centers, mostly in Europe.

Stimulation of the thalamus or midbrain for treatment of chronic pain has a 50-year history. Patient selection for placement of DBS is an important part of current treatment using this modality (Hosobuchi 1986; Levy et al. 1987; Young and Rinaldi 1997). In many published studies, patients have been assessed by intravenous morphine infusion tests, based on the hypothesis that nociceptive but not neuropathic pain responds to opioids. Then ► nociceptive pain is treated by ► periaqueductal gray (PAG) stimulation and ► neuropathic pain is treated with thalamic stimulation.

There are a number of large studies demonstrating that DBS can be effective for both neuropathic and nociceptive pain (Hosobuchi 1986; Levy et al. 1987; Young and Rinaldi 1997). A meta-analysis of 13 studies (1114 patients) evaluating DBS for the treatment of chronic pain reported that 50% of all patients experienced long-term pain relief. Patients with nociceptive pain experienced a 60% long-term relief from pain with PAG stimulation, while those with neuropathic pain experienced a 56% long-term success rate with Vc stimulation.

Stimulation of motor cortex for relief of neuropathic pain of the head, neck or upper extremity has recently emerged as an option for patients with chronic pain. The first series, based on studies in animals was carried out by Tsubokawa who reviewed a series of 11 patients with central pain after putaminal or thalamic hemorrhage treated with ► motor cortex stimulation for 2 years with significant (> 80%) pain relief, sustained in 45% of patients (Tsubokawa et al. 1993). As in the stimulation modalities described above, there are well-described protocols for selection of patients to be treated by motor cortical stimulation. For example, Yamamoto and coworkers (Yamamoto et al. 1997) noted that successful pain relief by motor cortex stimulation could be predicted if patients responded by at least 40% pain relief to incremental infusions of intravenous thiamylal to a maximum dose of 250 mg, but not to morphine in doses of up to 18 gm given over 5 hours (Yamamoto et al. 1997). Additionally, motor cortex stimulation requires an intact motor system to be effective, but not an intact somatosensory system. Thus current stimulation procedures are based upon improved indications and demonstrated efficacy, as in the case of current ablative procedures.

It seems likely that the future will reflect the fact that the conditions we are now treating surgically are all ultimately dependent upon the chemical mechanisms. Surgical treatment of these conditions will be elaborations of the currently available drug pump technology. These therapies will involve selective intrathecal administration to of a drug or drugs (Rainov et al. 2001) specific to the condition being treated (Penn 2003; Weiss et al. 2003). Examples of such tailored drug administration are found in the case of patients with pain due to spasticity (Middleton et al. 1996) or with pain following spinal cord injury or of patients in opiate withdrawal (Lorenz et al. 2002). The possibility of anatomic as well as chemical approaches to surgical targets within the forebrain will shortly be a possibility. Intra-axial administration is becoming practical for delivery of drugs to anatomically or physiologically defined structures. The feasibility of this approach for selectively lesioning neurons but not axons by convection delivery through an intracerebral catheter has been demonstrated in primate models of Parkinson's disease (Lieberman et al. 1999). The intracerebral delivery of neurotransmitters or proteins, such as growth factors or neurotransmitters, into defined structures can also be accomplished by stereotactically placed catheters or by implantation of other novel drug delivery systems (Gouhier et al. 2002; Pappas et al. 1997).

These technologies promise to revolutionize the neurosurgical treatment of pain in the future.

Acknowledgement

Supported by grants to FAL from the NIH: NS39498 and NS40059.

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Neurotransmitter

Definition

A neurotransmitter is a chemical released from a neuron into the synaptic cleft, which can trigger a response in the adjacent neuron on the opposite side of the cleft. Neurotransmitters may excite, inhibit, or otherwise influence the activity of cells.

► Cell Therapy in the Treatment of Central Pain
► Pain Treatment, Implantable Pumps for Drug Delivery
► Somatic Pain

Neurotransmitter Receptors

Definition

Neurotransmitter receptors are membrane proteins to which synaptic transmitters bind, leading to a physiological response in the postsynaptic cell. Neurotransmitter receptors can be ionotropic and cause a change in membrane conductance by an action on membrane channels or they can be metabotropic, causing activation of intracellular second messenger systems. Metabotropic receptors are often coupled to metabolic pathways through G proteins.

► Spinothalamic Tract Neurons, Role of Nitric Oxide

Neurotrophic Factors

Definition

Molecules by which tissues or cells affect nerve cell survival and/or phenotype.

► Wallerian Degeneration

Neurotrophic Support

Definition

Both developing and mature neurons require the ongoing delivery of a range of factors such as cytokines and neurotrophic factors to facilitate survival and maintain the phenotype of the neuron. This neurotrophic support may be supplied directly by cells of the target organ for the neuron, other cells adjacent to the axon or cell body or via the blood supply. Changes in neurotrophic support can induce changes in phenotype such as altered patterns of neurotransmitter synthesis or potential death of the neuron. Loss of neurotrophic support has been implicated in the etiology of diabetic neuropathy.

► Neuropathic Pain Model, Diabetic Neuropathy Model

Neurotrophin

Definition

Neurotrophins are dimeric growth factors that regulate development and maintenance of central and peripheral nervous systems. Members of this protein family include nerve growth factor (NGF), neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and neurotrophin-4/5 (NT-4/5). They regulate growth, survival, differentiation of neurons, and many other neuroectoderm tissues. All bind with low affinity to the p75 receptor and with high affinity to three structurally related receptor tyrosine kinases, named Trk receptors. NGF specifically activates TrkA, whereas BDNF and NT-4/5 specifically activate TrkB. NT-3 primarily activates TrkC, but recognizes both TrkA and TrkB to a lesser extent. Trk receptors consist of an extracellular ligand-binding domain, a single transmembrane region and an intracellular tyrosine kinase (TK) domain. Ligand binding to Trk receptors results in dimerization of

receptor molecules followed by autophosphorylation of their cytoplasmic tyrosine residues.

- ► Cell Therapy in the Treatment of Central Pain
- ► Congenital Insensitivity to Pain with Anhidrosis
- ► Nerve Growth Factor, Sensitizing Action on Nociceptors
- ► NGF, Regulation during Inflammation
- ► Spinal Cord Nociception, Neurotrophins
- ► Trigeminal Brainstem Nuclear Complex, Immunohistochemistry and Neurochemistry

Neurotrophin Receptors

Definition

Two types of neurotrophin receptors are involved in retrograde neurotrophin signaling, the low affinity p75 (NTR) receptor, also referred to as Nerve Growth Factor Receptor (NGFR), which binds with all the above neurotrophins, and the high affinity tyrosine kinase (Trk) receptors. The tropomyosin receptor kinase TrkA (NTRK1) is the signaling receptor for NGF, TrkB (NTRK2) is the signaling receptor for BDNF and NT-4/-5, TrkC (NTRK3) is the primary receptor for NT-3, although NT-3 also binds to TrkA and TrkB, yet with lower affinity.

- ► Congenital Insensitivity to Pain with Anhidrosis

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Neurotrophins in Spinal Cord Nociception

- ► Spinal Cord Nociception, Neurotrophins

Neutral Cell (RVM)

Definition

Third class of RVM neurons, defined by the lack of reflex-related activity. Neutral cells do not respond to mu-opioid agonists. RVM serotonergic neurons behave as neutral cells, although some authors prefer to consider serotonergic cells as a fourth class of RVM neurons, distinct from neutral cells. Neutral cells as defined *in vivo* are likely be a subset of primary cells defined *in vitro*.

- ► Opiates, Rostral Ventromedial Medulla and Descending Control

Neutral Medical Examination

- ► Independent Medical Examinations

Neutrophils in Inflammatory Pain

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Definition

The initial stages of the inflammatory response are characterized by the vascular reaction and the local biosynthesis of mediators that sensitize primary afferent neurons. Thereafter, immune cells are attracted to the site of injury, initiating processes that lead to either tissue repair or destruction. Immigration of polymorphonuclear neutrophil granulocytes (PMN), already in the early cellular phase of inflammation, serves to destroy infectious agents and/or cellular debris. In addition, PMNs are sources of various mediators that can affect, directly or indirectly, the sensitivity of primary afferent neurons.

Characteristics

Tissue injury triggers the release of a large number of mediators from neuronal and non-neuronal resident cells, resulting in microvascular changes and increased afferent neuronal sensitivity, effects that provide the basis of key symptoms of inflammation, redness, edema, and pain. Arachidonic acid metabolites are central in this initial response. Conversion of arachidonic acid through the cyclooxygenase pathway leads to enhanced biosynthesis of ► prostanoids, which increase tissue perfusion and sensitize primary afferent neurons. Pharmacological inhibition of cyclooxygenase activity by ► NSAIDs, Survey (NSAIDs) is, therefore, effectively used to alleviate inflammatory pain and edema.

In addition to mediators that serve the initial vascular and neuronal defense reaction, compounds with chemotactic activity are produced by injured tissue. This leads to immigration of PMN to the site of injury, a process that involves a cascade of events, primarily consisting of leukocyte rolling along the endothelial layer, adherence to, and transmigration through the endothelium and vascular wall. This process is under control of various

► cytokines, ► chemoattractants, and ► chemokines (Wagner and Roth 2000).

PMNs can produce a wide range of mediators (e.g. arachidonic acid metabolites, various cytokines, proteases, reactive oxygen intermediaries, and nitric oxide) (Sampson 2000) that serve to initiate the immune response and destroy infectious agents and/or cellular debris. It is evident that, in chronic inflammatory disease, the severity of the cellular inflammatory response and accompanying tissue destruction correlates with inflammatory pain. This provides a rationale for the use of ► disease modifying anti-rheumatic drugs (DMARDs), which primarily target immune cell function, in diseases such as rheumatoid arthritis. However, there are reasons to assume that PMNs are involved in the development of inflammatory pain at earlier stages of inflammation, a concept that is primarily based on a number of studies by Levine and associates (Levine et al. 1984; Levine et al. 1986).

With regard to the role of PMN in inflammatory pain, one chemoattractant is of particular interest, ► leukotriene (LT) B_4, which has been shown to be present in inflammatory exudates in human inflammatory diseases such as ► rheumatoid arthritis (Davidson et al. 1983). LTB_4 is a product of the 5-lipoxygenase pathway of arachidonic acid metabolism; it is a potent chemoattractant for neutrophils, which, in turn, are also major sources of LTB_4. In experimental inflammation, it has been shown that LTB_4 produces ► hyperalgesia, which is not prevented by ► Cyclooxygenases inhibitors (Levine et al. 1984). In the presence of LTB_4, PMNs release a 15-lipoxygenase product of arachidonic acid, (8R, 15S)-Dihydroxyeicosa-(5E-9,11,13Z)-tetraenoic acid [8R,15S)-diHETE], which directly sensitizes ► primary afferent neurons (Levine et al. 1986). Since it has been shown that the hyperalgesic activity of exogenous LTB_4 is dependent on the presence of PMN (Levine et al. 1984), it seems likely that the final mediator of LTB_4-induced hyperalgesia is a product of PMN 15-lipoxygenase. According to this concept, PMNs would promote inflammatory hyperalgesia in a dual fashion, as sources and as targets of LTB_4.

In view of these experimental data, inhibition of LTB_4 biosynthesis would be expected to show analgesic effects. In fact, there are studies showing analgesic effects of 5-lipoxygenase inhibition in some models of experimental inflammation. Thus, it has been shown in rodents, that ► nerve growth factor (NGF) elicits ► thermal hyperalgesia, which is dependent on PMNs and is blocked by inhibition of the 5-lipoxygenase (Amann et al. 1996, Bennett et al. 1998, Schuligoi 1988). The demonstration of PMN-dependent hyperalgesia induced by NGF may be important, because NGF has been shown to contribute to inflammatory hyperalgesia and bronchial hyperreflexia (Renz 2001). However, the studies mentioned above were conducted using exogenous NGF, and, therefore, did not provide direct evidence for the presence of a PMN-dependent, 5-lipoxygenase mediated mechanism in inflammatory pain.

In a more recent study, Cunha et al. (2003) have shown that mechanical hypersensitivity, induced by antigen challenge in rats immunized with ovalbumin, is suppressed by inhibition of 5-lipoxygenase and a LTB_4 receptor antagonist. Although in this study the participation of PMNs as sources of LTB_4 was not addressed, the observation that, at later stages of the inflammatory response, LTB_4 was the principal mediator of hyperalgesia points to the involvement of PMNs.

In contrast to these experimental studies in rodents, which are suggestive of involvement of PMN 5-lipoxygenase in inflammatory hyperalgesia, there is no firm evidence that 5-lipoxygenase inhibition can attenuate inflammatory pain in patients, although selective inhibitors of 5-lipoxygenase have been available for a number of years. Therefore, the pathophysiological relevance of PMN lipoxygenases as sources of hyperalgesia-inducing factors in inflammatory pain remains doubtful.

In recent years a novel concept has emerged, suggesting that PMNs play an important role in the generation of lipoxins, lipid mediators that promote the resolution of the inflammatory process (Levy et al. 2001). According to this concept, 15-lipoxygenase products of arachidonic acid [15S-hydroxyperoxyeicosatetraenoic acid (15S-H(p)ETE), or the reduced alcohol form 15S-hydroxyeicosatetraenoic acid (15S-HETE)], derived from epithelial cells, eosinophils or monocytes serve as substrates for PMN 5-lipoxygenase, which results in the generation of lipoxin (LX)A_4 and LXB_4. During the synthesis of lipoxins, leukotriene synthesis is blocked at the 5-lipoxygenase level in PMN, resulting in an inverse relationship of lipoxin and LT biosynthesis. An alternative pathway involves an interaction between PMN and platelets, which convert PMN 5-lipoxygenase-derived LTA_2 via 12-lipoxygenase to LXA_4 and LXB_4 (Serhan 1997).

Lipoxins contribute to the resolution of inflammation by inhibiting neutrophil chemotaxis, and transmigration (Takano et al. 1997), stimulation of macrophage clearance of apoptotic PMN from an inflammatory focus (Godson et al. 2000), inhibition of cell proliferation, and modulation of metalloproteinase activity (Sodin-Semrl et al. 2000). Although there are no studies on the possible effects of lipoxins on the nociceptive threshold of primary afferent neurons, it can be expected that, by attenuating the inflammatory response, they can indirectly reduce inflammatory pain.

Since PMN 5-lipoxygenase is a key enzyme in the biosynthesis of lipoxins, pharmacological inhibition of 5-lipoxygenase in PMNs may in fact counteract processes that are involved in the resolution of inflammation. Theoretically, this may also be one possible explanation for the absence of obvious analgesic ef-

fects of 5-lipoxygenase inhibition in most types of inflammation.

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New Daily Persistent Headache

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Synonyms

Chronic Daily Headache; Daily Persistent Headache

Definition

New daily persistent headache (NDPH) is a form of chronic daily headache along with ► chronic migraine, ► chronic tension-type headache and ► hemicrania continua. The defining symptom of NDPH is a daily headache from onset, typically in an individual with minimal or no prior headache history. The headache will start one day and in most instances continue as daily unremitting pain.

Characteristics

New daily persistent headache (NDPH) was first described by Vanast in 1986 as a benign form of chronic daily headache that improved without therapy. Very little is known about this syndrome and only recently has it been recognized as a distinct entity by headache specialists. It is unique in that the headache begins daily from onset, typically in a patient with no prior headache history and can continue for years, without any sign of alleviation despite aggressive treatment. Proposed diagnostic criteria for NDPH are listed below. It appears that there maybe two subtypes of NDPH, a self-limited form, which typically goes away within several months without any therapy and never presents to a physician's office and a refractory form, which is basically resistant to aggressive outpatient and inpatient treatment schemes.

Proposed Criteria for New Daily Persistent Headache (Rozen)

A Daily head pain for >2 months

B Average headache duration of >4 h per day (if untreated). Frequently constant pain without medication

C No history of migraine or TTH that is increasing in frequency in association with a new daily persistent headache

D Prior history of any headache disorder is uncommon

1. Acute onset of constant unremitting headache (daily from onset)
2. At least 2 of the following pain characteristics
3. Pulsating or pressing/tightening quality
4. Moderate or severe pain intensity
5. Bilateral pain location
6. Aggravation by walking upstairs or similar routine physical activity

E At least one of the following

1. Nausea and / or vomiting
2. Photophobia or phonophobia

F Does not fit the criteria for hemicrania continua

There are only two case series in the literature dedicated to describing the clinical characteristics of NDPH, the largest completed by Li and Rozen in 2002. A retrospec-

tive chart review was carried out using a computerized database of patients from the Jefferson Headache Center (a large university based headache specialty unit). All patients who were seen at Jefferson between August 1997 and May 2000 and who met the criteria for NDPH were included. Unique to NDPH is that most patients are able to pinpoint the exact date when their headache started. Headache onset occurs in relation to an infection or flu-like illness in 30%, extracranial surgery (e.g. hysterectomy) in 12% and a stressful life event in 12%. Over one-third of patients cannot identify any precipitating event. NDPH had a female predominance (female to male ratio: 2.5:1). The peak age of onset of NDPH in women is in the second and third decades of life, while the largest incidence of NDPH in men comes in the third to fifth decade. A prior headache history is found in about 40% of patients, with episodic ► migraine being the most common type. In the majority of patients, the pain of NDPH is continuous throughout the day with no pain-free time noted. Baseline average pain intensity is moderate in most, while some patients experience severe pain all of the time. Headache location is typically bilateral and pain can occur anywhere on the head. Headache quality is usually throbbing or pressure-like. With regard to associated symptoms, nausea, photophobia, phonophobia or lightheadedness occur in more than 50% of patients. ► Aura-type symptoms can also occur but are uncommon. A family history of headache is documented in 30% of patients. In almost all instances, general and neurological examinations are normal. Neuroimaging and lumbar puncture are almost always negative studies.

Epidemiology

Even though NDPH has probably been around for centuries, it has only recently been diagnosed as an entity separate from chronic tension-type headache, hemicrania continua and chronic migraine. The prevalence of CDH from population-based studies in the United States, Asia and Europe is about 4% (Silberstein et al. 2001). In those epidemiologic investigations, primary CDH types are sometimes not mentioned in the analysis and NDPH is rarely stratified out from the data. Several studies have documented the prevalence of NDPH; Castillo et al. (1999) looked at the prevalence of CDH in 2,252 subjects in Spain and found that 4.7% of the population has CDH, of which 0.1% had NDPH. Bigal et al. (2002) noted that 10.8% of 638 patients with CDH in a headache specialty clinic had NDPH, while Koenig et al. (2002) found that 13% of a pediatric CDH population, surveyed from selected pediatric headache specialty clinics, had NDPH.

Etiology of NDPH

As at least a third of NDPH patients have a cold or flu-like illness when their headaches begin, an infectious etiology for NDPH has been hypothesized. Some authors have linked Epstein-Barr virus (EBV) infection with NDPH. Diaz-Mitoma et al. (1987) identified oropharyngeal secretions of EBV in 20 of 32 patients with NDPH compared with 4 of 32 age-and gender-matched controls. A history of mononucleosis was identified in 12 of the patients with NDPH. Almost 85% of the NDPH patients were found to have an active EBV infection as opposed to 8 in the control group. The authors hypothesized that activation of a latent EBV infection may have been the trigger for the development of a chronic daily headache from onset. Santoni and Santoni-Williams (1993) demonstrated evidence of systemic infection in 108 patients with NDPH including *Salmonella*, adenovirus, toxoplasmosis, herpes zoster, EBV and *E.coli* urinary tract infections. How an infection can induce NDPH is unknown. One may hypothesize an activated immune response to a new or reactivated viral or bacterial infection leading to an autoimmune-triggered headache, possibly by setting up a state of continuous neurogenic inflammation. The virus itself could in some way activate and damage the trigeminal system leading to daily pain.

Differential Diagnosis of NDPH

A diagnosis of primary NDPH is made only after secondary causes have been ruled out. Two disorders in particular can mimic the presentation of NDPH, ► spontaneous cerebrospinal fluid leak (CSF) and cerebral venous sinus thrombosis. Spontaneous CSF leaks typically present as a daily headache with a positional component (headache improved in a supine position, worsens in a sitting or standing position). However, the longer a patient suffers with a CSF leak-induced headache the less pronounced the positional component becomes. Thus if a patient is seen in a physician's office months to years after onset of a CSF leak, that patient may not even divulge a history of positional headaches, as that trigger may not have been evident to the patient for a very long time. In this setting, the CSF leak headache may mimic a primary NDPH picture.

In the patient who presents with new daily headache and is subsequently found to have cerebral venous thrombosis, in many instances none of the typical features recognized for cerebral venous thrombosis are present, including no history of new onset seizures, focal neurological deficits, change of consciousness, cranial nerve palsies or bilateral cortical signs and no evidence of papilledema on fundoscopic examination. The patient will just have a new headache that is daily from onset.

The evaluation of an NDPH patient should include neuroimaging, specifically brain MRI without and with gadolinium and MR venography (MRV). Gadolinium must be given to look for the ► pachymeningeal enhancement associated with spontaneous CSF leaks while MRV will help make the diagnosis of cerebral venous thrombosis. If a new daily headache begins after the age of 50 years, then giant cell arteritis must

be ruled-out. Headache is the most common reported symptom of the disorder occurring in up to 90% of individuals.

Treatment

NDPH can continue for years to decades after onset and be extremely disabling to the patient. Even with aggressive treatment many NDPH patients do not improve. In many circles, primary NDPH is felt to be the most treatment refractory of all headache disorders. Many patients with NDPH will fail every possible class of abortive and preventive medications without any sign of pain relief. Recently Rozen (2002) presented five patient cases in which successful treatment of NDPH was obtained with gabapentin or topiramate. This was the first ever published study recognizing a positive treatment response for the refractory form of NDPH (the self-limited form will alleviate without any therapy).

► Chronic Daily Headache in Children

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Newborn

Synonym

Neonate

Definition

Newborn infant who is less than 1 month postnatal age.

► Pain Assessment in Neonates

NGF

► Nerve Growth Factor

NGF -/- Mice

Definition

Mice that lack a functional gene encoding nerve growth factor, i.e. NGF knockout mice.

► Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain

NGF-OE mice

► Nerve Growth Factor Overexpressing Mice as Models of Inflammatory Pain

NGF, Regulation during Inflammation

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Definition

In inflamed tissue, there is increased expression of ► nerve growth factor (NGF), which affects afferent neuron function, and contributes to the development and resolution of the inflammatory process. Pharmacological tools that can modify inflammation-induced NGF biosynthesis are, therefore, of potential therapeutic value.

Characteristics

Nerve growth factor (NGF) belongs to a family of structurally related ► neurotrophins. During inflammation, there is a rapid increase in local NGF biosynthesis. The local increase in NGF in inflamed tissues leads to changes in the phenotype of a subset of ► primary afferent neurons, with consequences for the transmission of noxious afferent input (Mendell et al. 1999).

In addition to its neurotrophic properties, NGF has been shown to affect immune cell function (Aloe et al. 1999). Direct neuronal effects (Mendell et al. 1999), as well as effects on immune cells (Bennett et al. 1998; Schuligoi 1998), seem to contribute to NGF-induced sensitization of primary afferent neurons, which manifests itself as inflammatory ► hyperalgesia in the skin or bronchial hyperreactivity in the respiratory system (Renz 2001). Furthermore, it has been suggested that, by promoting keratinocyte proliferation and vascular neoangiogenesis, NGF contributes to cutaneous morphogenesis,

wound healing, and tissue response to inflammation (Aloe 2004).

Inhibition of NGF Biosynthesis

Attenuation of the inflammation-induced rise in tissue NGF may be effective in preventing longer-lasting hyperalgesic effects of NGF. Therefore, a number of studies were conducted in order to investigate the effects of anti-inflammatory drugs on NGF biosynthesis in inflamed tissue.

Glucocorticoids have been shown to suppress inflammatory edema as well as the inflammation-induced increase in NGF biosynthesis. This is in contrast to non-inflamed tissues, where acute glucocorticoid treatment seems to have no inhibitory effect on NGF biosynthesis (Safieh-Garabedian et al. 1995).

Non-steroidal anti-inflammatory drugs (► NSAIDs), belonging to the group of ► Cyclooxygenases (COX) inhibitors, suppress prostanoid biosynthesis, thereby reducing edema and pain. Therefore, it seems interesting that inhibition of prostanoid biosynthesis, although suppressing inflammatory edema, has no significant effect on NGF expression in inflamed paw tissue of the rat (Amann et al. 1996). This suggests that the potency of anti-inflammatory drugs to inhibit inflammatory edema, is not necessarily predictive of their ability to reduce the inflammation-induced increase in NGF. In contrast to the absence of significant influence on inflammation-induced NGF expression by COX inhibition, high dose NSAID treatment of rats has been shown to be effective in decreasing NGF in inflamed tissue (Safieh-Garabedian et al. 1995), an effect that may be ascribed to drug actions not related to COX inhibition (Tegeder et al. 2001).

Drugs Acting at Adrenergic Receptors

It has been shown that treatment of rats with adrenergic antagonists inhibits the increase in NGF observed in endotoxin-induced paw inflammation (Safieh-Garabedian et al. 2002), suggesting that endogenous adrenergic tone can be sufficient to stimulate local NGF biosynthesis (see "Stimulation of NGF biosynthesis"). However, a participation of endogenous adrenergic mechanisms in stimulating NGF biosynthesis in inflamed tissues does not seem to be a general feature of the inflammatory response. In contrast, there are even models of inflammation where adrenergic agonists can attenuate the inflammation-induced rise in NGF:

a) In allergic inflammation of the rat paw or respiratory system, the beta adrenoceptor agonist terbutaline attenuates edema as well as the increase in local NGF formation. In contrast to its inhibitory effect in allergic inflammation, terbutaline does not significantly affect NGF in carrageenan-induced inflammation of the rat paw, suggesting no general inhibitory effects of terbutaline on NGF biosynthesis in inflammation (Amann et al. 2001).

b) Neurogenic inflammation is caused by experimental stimulation of capsaicin-sensitive primary afferent neurons, leading to the peripheral release of neuropeptides (e.g. calcitonin gene-related peptide and ► tachykinins) that cause vasodilation, plasma protein extravasation, and also an increase in the NGF content of the innervated skin (see "Stimulation of NGF biosynthesis"). It can be shown that terbutaline inhibits the increase in NGF of rat skin in capsaicin-induced neurogenic inflammation, and the NGF increase following local injection of the tachykinin NK1 receptor agonist substance P (Amann et al. 2004).

At the transcriptional level, beta adrenergic agonists can stimulate rather than decrease the expression of NGF (see "Stimulation of NGF biosynthesis"). The observed inhibitory effects on NGF biosynthesis of beta adrenergic stimulation in the abovementioned studies are, therefore, not caused by specifically inhibiting NGF transcription, but by interference with the inflammatory stimulus itself.

Tachykinin NK1 receptor antagonists inhibit the increase in NGF caused by experimental neurogenic inflammation, but do not detectably affect the NGF response to carrageenan or allergic inflammation. In non-inflamed skin, treatment with tachykinin NK1 receptor antagonists has no detectable effect on NGF biosynthesis (Amann et al. 2000).

Stimulation of NGF Biosynthesis

Beta Adrenergic Agonists

A number of studies have shown that, depending on cell type, beta adrenergic agonists stimulate the expression of NGF mRNA and protein. These effects seem to be caused by an agonist-induced rise in intracellular cAMP and activation of ► protein kinase A, which causes an increase in early inducible genes such as ► c-fos and the associated AP-1 binding. The AP-1 element is present within intron 1 of the NGF gene. In addition, the transcription factor C/EBPδ is activated by elevated cAMP levels and is one of the transcription factors for induced NGF expression (Riaz and Tomlinson 2000).

Vitamin D Receptor Agonists

There are several studies showing that vitamin D analogs, acting at vitamin D receptors, stimulate the expression of NGF mRNA, probably due to increased AP-1 binding. Vitamin D analogues may indirectly modulate NGF mRNA levels through increasing intracellular Ca^{2+} and promoting c-fos and c-jun induction; these ► immediate early genes are postulated to enhance NGF expression (Riaz and Tomlinson 2000).

Tachykinin NK1 Receptor Agonists

It has been shown in rodents that an intraplantar injection of the endogenous NK1 receptor agonist substance P stimulates NGF biosynthesis in the paw skin. The effect of substance P is blocked by a selective tachykinin NK1

receptor antagonist, indicating that NK1 receptor activation stimulates the NGF increase. Further experiments have provided evidence of the involvement of NK1 receptors in the NGF increase caused by neurogenic inflammation, suggesting that endogenous substance P, released from primary afferent nerve terminals during stimulation, acts on NK1 receptors to increase tissue NGF (Amann et al. 2000).

It seems to be of particular interest that primary afferent neuron activation, through local release of neurotransmitters, augments NGF biosynthesis. This may not only constitute a regulatory feed back by which enhanced peripheral release of neuropeptides is signaled to dorsal root ganglia where NGF stimulates neuropeptide biosynthesis (Donnerer et al. 1993), but may be of importance in inflammation and/or the local response to tissue injury. However, the ► cellular targets of substance P have not been determined so far.

The vanilloid ► VR1 receptor agonist ► capsaicin is a potent stimulant to induce excitation and local neuropeptide release from small-diameter primary afferent neurons, and thus induces neurogenic inflammation. When applied by either intraplantar injection (Saade et al. 2003) or by topical application to the paw skin (Amann et al. 2004), capsaicin stimulates NGF biosynthesis in the skin. NK1 receptor antagonists prevent this effect of capsaicin, indicating that substance P, released from primary afferent neurons, is the mediator of the capsaicin-induced NGF response. Capsaicin would seem, therefore, to be a suitable pharmacological tool to increase skin NGF biosynthesis, especially since it is a constituent of various drug formulations made for topical administration to the skin. However, a single topical application of capsaicin to the skin results in reduced responsiveness to subsequent applications of capsaicin (Amann et al. 2004). Desensitization may, therefore, limit the use of vanilloid receptor agonists as tools for producing longer lasting stimulation of skin NGF biosynthesis.

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NGF, Sensitization of Nociceptors

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Definition

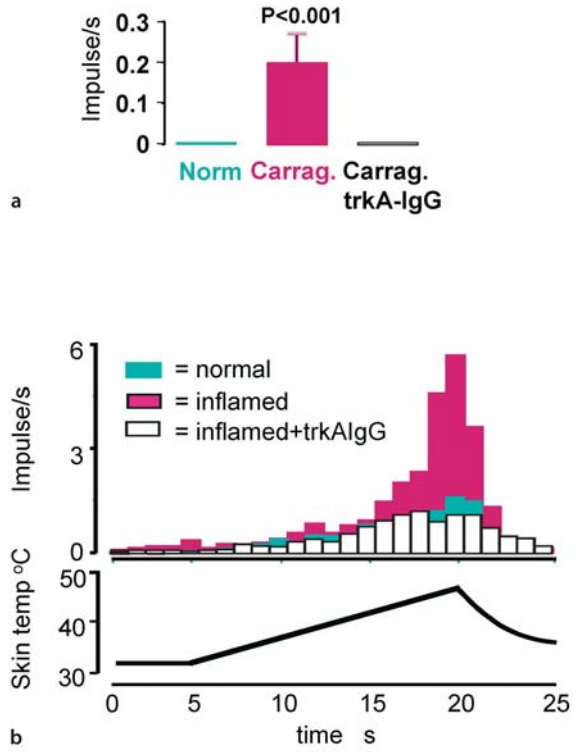
NGF is a secreted protein of molecular mass of 13 kD which exists as a homodimer. It is a member of the neurotrophin family, which also includes brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5). NGF binds to both a high affinity tyrosine kinase receptor trkA and a low affinity receptor p75. Nociceptors are primary afferent neurons that respond to potentially tissue damaging stimuli. NGF can sensitize these neurons so that they show an increased response to thermal and chemical stimuli.

Characteristics

It has now been established that NGF is a key mediator involved in the generation of inflammatory pain; for a full discussion of this please see the chapter 'Inflammatory pain and NGF'. Administration of NGF either locally or systemically results in both thermal and mechanical hyperalgesia. NGF administration produces both a peripheral sensitization (increased response of primary

afferent neurons to noxious stimuli) and central sensitization (a facilitation of the spinal processing of noxious stimuli). This chapter will deal with the mechanisms by which NGF sensitizes nociceptors.

An *in vitro* skin nerve preparation has been used to study the effects of NGF on cutaneous nociceptors. Direct application of NGF to the receptive fields of these afferents increased their sensitivity to noxious thermal stimuli but had no effect on their mechanical sensitivity (Rueff and Mendell 1996). In particular some fibres, which were thermally insensitive, developed a novel, heat sensitivity after NGF treatment. NGF administration did not induce ongoing activity. Application of NGF to dissociated DRG cells results in a sensitization of these neurons to noxious heat and capsaicin that occurs within minutes (Shu and Mendell 2001; Galoyan et al. 2003). NGF potentiates the response to a given heat stimulus and prevents the tachyphylaxis normally seen when paired stimuli are given. Visceral afferents innervating the bladder have been shown to display increased mechanical sensitivity following NGF administration (Dmitrieva et al. 1997) and high threshold group IV muscle afferents develop ongoing activity following intramuscular NGF injection (Hoheisel et al. 2005).



NGF, Sensitization of Nociceptors, Figure 1 The effects of carrageenan inflammation on the properties of primary afferent nociceptors. Recordings were made from an isolated skin-nerve preparation. Carrageenan inflammation led to increased spontaneous activity in primary afferents (a), which could be blocked by trkA-IgG treatment. Afferents were also tested for their response to a ramp increase in skin temperature (b). Afferents innervating inflamed skin showed an enhanced response to this heat ramp. This thermal sensitisation was prevented by administration of a trkA-IgG, which sequesters endogenous NGF. Figure adapted from Koltzenburg et al. 1999.

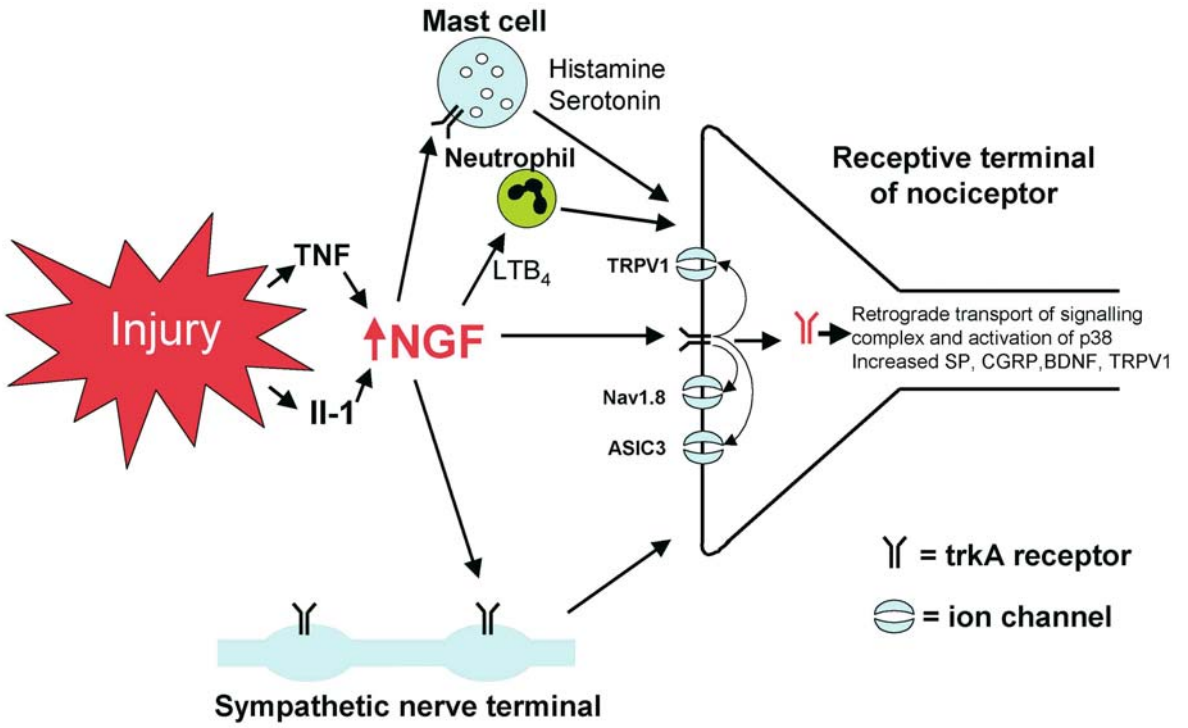
We have studied the role of NGF in producing primary afferent sensitisation following intraplantar carrageenan, which evokes an acute inflammatory reaction (Koltzenburg et al. 1999). A trkA-IgG fusion protein was used to sequester endogenous NGF. 3 h after carrageenan administration there was a marked increase in spontaneous activity (50% of nociceptors innervating inflamed skin *vs* 4% in control). This could be almost completely prevented by administration of trkA-IgG (Fig. 1). Interestingly, as described above, NGF administration alone does not induce spontaneous activity in nociceptors and therefore in the context of inflammation it must be acting co-operatively with other chemical mediators. Inflammation also produced an increased response of nociceptors to a standard heat ramp and the proportion of fibres responding to the inflammatory mediator bradykinin increased. Both of these changes could be prevented by administration of trkA-IgG (Fig. 1). We did not find any significant mechanical sensitisation of cutaneous nociceptors following inflammation. Other groups have found varying degrees of mechanical sensitisation following inflammation. It is likely that the mechanical hyperalgesia seen after NGF administration is due to central mechanisms.

N

Cell Types Involved in the Generation of NGF Mediated Hyperalgesia

As described in chapter 'Inflammatory pain and NGF' there are two receptors for NGF, a high affinity tyrosine kinase receptor trkA and a low affinity receptor p75. It is likely that the hyperalgesic effects of NGF are principally mediated by binding to trkA. Animals that lack p75 continue to develop thermal and mechanical hyperalgesia in response to NGF. A substantial proportion of peripheral nociceptor terminals express the trkA receptor and I will discuss the direct sensitizing action of NGF on these neurons in the following section. A number of other cellular elements within peripheral tissues also express trkA and some of the sensitizing actions of NGF may therefore be indirect (for a schematic representation of the sensitizing effects of NGF on nociceptors see Fig. 2).

Mast cells expressing trkA and NGF can induce mast cell degranulation (resulting in the release of histamine and serotonin) and the expression of a number of cytokines including Il-3, Il-4, Il-10 and TNFα. Mast cell degranulators and serotonin antagonists have been shown to reduce the thermal, but not the mechanical, hyperalgesia that occurs following NGF administration (Lewin et al. 1994; Woolf et al. 1996). There may also be an interaction between NGF and sympathetic efferents, which also express the trkA receptor. Surgical or chemical sympathectomy can reduce the short latency thermal and mechanical hyperalgesia evoked by NGF (Andreev et al. 1995; Woolf et al. 1996). The produc-



NGF, Sensitization of Nociceptors, Figure 2 Schematic representation of the role of NGF in nociceptor sensitisation. Following tissue injury there is a rapid increase in NGF expression, which is secondary to increased levels of Il-1 and TNFα. Some of the effects of NGF on nociceptor terminals are mediated indirectly *via* mast cells, sympathetic efferents and neutrophil chemotaxis. NGF can also act directly on nociceptors following binding to the trkA receptor. This leads to activation of multiple second messenger pathways resulting in the potentiation of a number of ion channels including TRPV1, Nav 1.8 and ASIC3. An activated trkA-NGF complex is also transported to the cell nucleus and modulates gene expression causing more long-term effects on nociceptor excitability and function. See text for details.

tion of eicosanoids by sympathetic efferents has been suggested to contribute to hyperalgesia under some inflammatory conditions. This does not appear to be the case for NGF induced hyperalgesia as this is unaffected by treatment with non-steroidal anti-inflammatory agents.

A further mechanism by which NGF produces peripheral sensitization is through activation of the 5-lipoxygenase pathway. The enzyme 5-lipoxygenase converts arachidonic acid into leukotrienes. Leukotriene B_4 (LTB_4) is a powerful chemotactic factor for neutrophils and has been shown to sensitize nociceptive afferents to thermal and mechanical stimuli. NGF increases the production of (LTB_4) in the skin and inhibitors of the 5-lipoxygenase enzyme prevent the development of thermal hyperalgesia following intraplantar NGF injection (Amann et al. 1996).

Direct Actions of NGF on Nociceptor Function

40% of adult DRG cells express trkA and it is selectively expressed in a population of small diameter DRG cells which express neuropeptides such as CGRP and which are predominantly nociceptors. The recent rapid advances in characterising both the second messenger pathways and the molecular identities of signal transducing elements within nociceptors has led to a much greater understanding of the sensitising actions of NGF on these neurons.

NGF binding to trkA results in the cross-linking of two adjacent receptors. This triggers each trkA molecule to phosphorylate tyrosine residues on the cytoplasmic domain of its cross-linked neighbour. Phosphorylation causes conformational changes, which expose binding sites for proteins with SH2 domains. Multiple second messenger pathways are subsequently activated including phospholipase C-γ (PLC-γ), protein kinase C and members of the mitogen-activated protein kinase (MAPK) pathway. NGF can have acute effects on ion channel function *via* posttranslational modification such as phosphorylation and long-term effects based on transcriptional regulation and transport of ion channels to the nerve terminal.

One of the most intensively studied areas which demonstrating these multiple levels of regulation by NGF is the potentiation of TRPV1 function. TRPV1 is expressed in nociceptors and is a capsaicin- and proton-sensitive cation-selective channel that transduces noxious heat stimuli. Although its role in thermal nociception in the normal state is controversial, it has been shown to

be important in the generation of thermal hyperalgesia following inflammation and following NGF administration (Chuang et al. 2001). NGF rapidly (within minutes) sensitises TRPV1 responses to low pH, capsaicin and noxious heat. This sensitising effect of NGF is clearly established, however there is some confusion in the literature as to the exact second messenger pathways mediating this effect. One proposed pathway is *via* activation of phospholipase C (PLC). PLC-γ activation results in reduced levels of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) in the plasma membrane. As PtdIns(4,5)P2 exerts a tonic inhibitory action on TRPV1 this can rapidly enhance the sensitivity of TRPV1 and this effect has been demonstrated both in heterologous cells expressing TRPV1 and DRG cells (Chuang et al. 2001; Galoyan et al. 2003). Another second messenger pathway activated by NGF is phosphatidyl 3-kinase (PI3K). This is a lipid kinase that phosphorylates the D-3 position of phosphatidylinositol lipids to produce PI(3,4,5)P3. One of the down stream effects of PI3K activation is activation of extracellular signal-regulated protein kinase (ERK) a member of the MAPK family. NGF activates PI3K and ERK in DRG cells. These pathways have been shown to have a role in mediating both the potentiation of TRPV1 current and the thermal hyperalgesia induced by NGF (Zhuang, Xu et al., 2004). Another group studying the sensitization of capsaicin-induced $[Ca^{2+}]_i$ by NGF in neonatal DRG cells also reported an important role for PI3K and also for PKC, but did not find any effect of blocking PLC (Bonnington and McNaughton 2003).

Following binding of NGF to trkA at the nerve terminal, the complex is internalised and retrogradely transported in a signalling endosome to the cell body, where it regulates further second messenger pathways. Signals may also be activated by NGF at the cell surface and then transported back to the cell body in the absence of NGF. Once this signal has reached the cell body, it can modulate gene transcription and the targeting of signal transducing molecules back to nociceptor terminals. 24 h after induction of inflammation, there is an activation of p38 MAP kinase within DRG cell bodies that has been shown to be NGF dependent. This activation of p38 by NGF results in increased levels of TRPV1 protein (but not mRNA), which is transported to the peripheral terminals of C-fibres. This increased TRPV1 expression makes a contribution to the late phase of thermal hyperalgesia during inflammation (Ji et al. 2002).

A second group of molecular targets within sensory neurons that are regulated by NGF are the tetrodotoxin resistant (TTXR) sodium channels (Nav 1.8 and 1.9). These are exclusively expressed within nociceptive neurons. Inflammatory mediators have been shown to increase the TTXR current in DRG cells. Post-translational modulation of TTXR is *via* phosphorylation of the channel mediated by both PKA and PKC (Gold et al. 1998). NGF may also regulate the expression of Nav 1.8 in DRG cells. The importance of this mechanism in mediating the sensitising effects of NGF on nociceptive neurons is demonstrated by the fact that animals that carry a null mutation of the Nav 1.8 gene show reduced thermal hyperalgesia following NGF administration (Kerr et al. 2001). NGF can also regulate the expression of other ion channels such as the acid sensing ion channel ASIC 3 within nociceptors. This and the potentiation in TRPV1 function (which is also activated by protons) are likely to increase the sensitivity of nociceptors to the low pH seen in inflammatory tissue.

In summary, NGF has been shown convincingly to sensitise nociceptive afferents to thermal stimuli and in the context of inflammation also contributes to the development of ongoing activity. There are likely to be a number of direct and indirect mechanisms that lead to such sensitisation. A greater understanding of this process, and in particular the means by which NGF potentiates the function of TRPV1 and Nav 1.8 in nociceptors, may ultimately lead to novel analgesic therapies for inflammatory pain.

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Nick Model of Cutaneous Pain and Hyperalgesia

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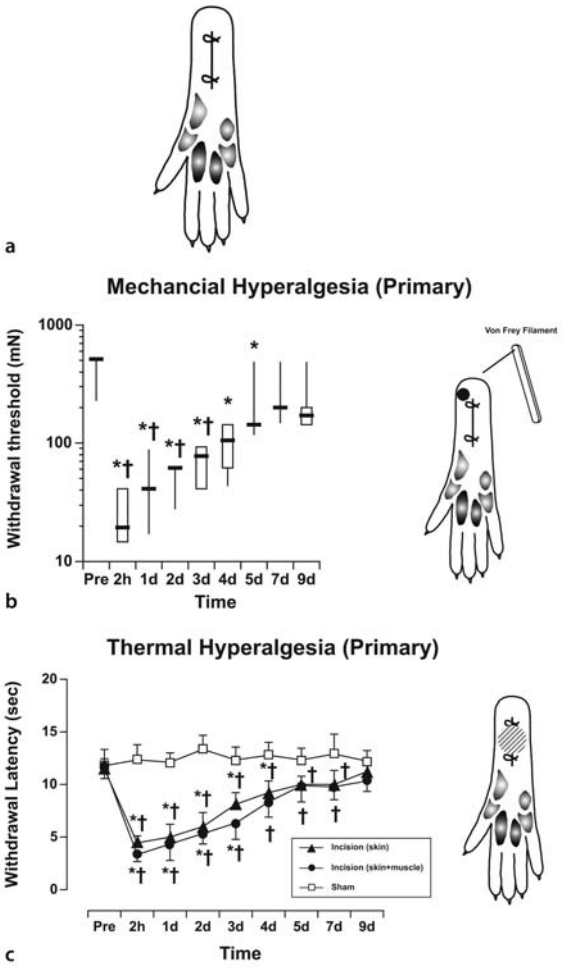
Synonyms

Plantar Incision Model; Incision Model for Postoperative Pain; Postoperative Pain Model; Brennan Pain Model

Definition

Postoperative pain is a unique and common form of acute pain. There is ample evidence that pains caused by inflammation, nerve injury or incision is based on different pathophysiological mechanisms. This explains why many treatment strategies are efficacious only against specific types of persistent pain (Hunt and Mantyh 2001). Recognizing this gap between preclinical models of persistent pain and postsurgical pain, new rodent skin incision models have been developed (Brennan et al. 1996; Martin et al. 2004; Pogatzki et al. 2002b). In the plantar incision model developed by Brennan and co-workers, a simple incision is made in the plantar aspect of the hind paw of a rat (Brennan et al. 1996). The plantar hind paw incision is performed under halogenated volatile anesthesia using sterile technique. After induction of anesthesia, a 1 cm longitudinal incision is made with a number 11 blade, through skin and fascia of the paw, starting 0.5 cm from the proximal edge of the heel and extending toward the digits (Fig. 1a). Subsequently, the underlying flexor muscle is elevated with the forceps and incised longitudinally with the scalpel blade. The muscle origin and insertion remain intact. After hemostasis with gentle pressure, the skin is apposed with two mattress sutures of 5-0 nylon and the wound site is covered with antibiotic ointment. The rats recover for 30 min to 1 hr from anesthesia in clean bedding. Nylon is used for closure to minimize the inflammatory response to the suture material. The sutures are removed on postoperative day 2 to prevent any persistent responses caused by the sutures.
Mouse models of postoperative pain have been performed (Pogatzki and Raja 2003). The behaviors caused by plantar incision in the mouse roughly parallel those in the rat.



Nick Model of Cutaneous Pain and Hyperalgesia, Figure 1 Mechanical and thermal hyperalgesia after incision. (a) Schematic of the incision in the plantar aspect of the rat foot. (b) Punctate mechanical hyperalgesia adjacent to the incision. The results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). Primary punctate hyperalgesia after an incision of skin, fascia and muscle. The site of incision and of testing is illustrated in a schematic accompanying the graph. *P<0.05 *vs.* pre; †P<0.05 *vs.* sham. (c) Heat hyperalgesia after incision. Primary heat hyperalgesia after an incision of skin, fascia and muscle. The site of testing and incision are illustrated to the right of the graph. * P<0.05 *vs.* pre; †P<0.05 *vs.* sham. The symbols represent the mean ± standard deviation (SD).

Characteristics

Primary and Secondary Hyperalgesia after Incision Injury

An incision made in the plantar aspect of the rat hind paw causes persistent, reduced withdrawal thresholds to mechanical stimuli suggesting hyperalgesia (decreased pain threshold to suprathreshold stimuli). Primary mechanical and heat hyperalgesia, enhanced pain to mechanical and thermal stimuli in the area of the incision, is present after a surgical incision in this model (Fig.

1b, c). Whereas primary mechanical hyperalgesia after plantar incision in rodents lasts for 2 to 3 days, primary heat hyperalgesia lasts up to 7 days after incision (Zahn and Brennan 1999b).

► Secondary hyperalgesia, enhanced pain to stimuli applied adjacent to the area of incision, was observed to punctate mechanical stimuli but not to a blunt mechanical probe or to a thermal stimulus (Pogatzki et al. 2002b; Zahn and Brennan 1999b).

Increased mechanical sensitivity after incision in rodents has striking similarities to an experimental incision in humans (Kawamata et al. 2002) and to patients' pain reports after surgery (Stubhaug et al. 1997). Hyperalgesia from the area of incision (► primary hyperalgesia) probably contributes to mechanically evoked pain in postoperative patients. A reduction of primary hyperalgesia with a specific treatment should therefore affect pain induced by mechanical stimuli (e.g. coughing, movement or ambulation after surgery).

Peripheral and Central Sensitization After Incision Injury

A number of neurophysiological studies using the rodent incision models have revealed some of the underlying mechanisms inherent in incision-induced pain. From these studies mechanical hyperalgesia after incision appears to involve activation and sensitization of primary afferent ► nociceptors (► peripheral sensitization) (Pogatzki et al. 2002a), and neurons in the spinal dorsal horn (central sensitization) (Vandermeulen and Brennan 2000; Zahn and Brennan 1999a).

Peripheral Sensitization after Incision (Fig. 2a–c):

In general, the characteristic features of experimentally induced peripheral sensitization of primary afferent fibers are a lowering of response threshold, an increase in response magnitude to suprathreshold stimuli, an increase in spontaneous activity or an increase in RF size. Recording mechanosensitive afferent fibers innervating the plantar aspect of the rat hind paw using standard teased-fiber techniques revealed an increase in the ► receptive field size and the occurrence of spontaneous activity of A-delta and C fibers after incision; activation thresholds of A-delta fibers, but not of C-fiber nociceptors, decreased after incision (Pogatzki et al. 2002a). Importantly, the conversion of mechanical insensitive ► silent nociceptors to mechanically activated fibers was indicated 24 h after incision (Pogatzki et al. 2002a). A-beta fibers did not sensitize after an incision. In conclusion, spatial summation of modestly increased response magnitude may contribute to the reduced withdrawal threshold after incision. Spontaneous activity in A-delta- and C-fibers may not only account for spontaneous pain behavior but may also contribute to mechanical hyperalgesia by amplifying responses centrally. Furthermore, the conversion of mechanical insensitive silent nociceptors to mechanically activated fibers probably has a role in the maintenance of hyperalgesia after an incision.

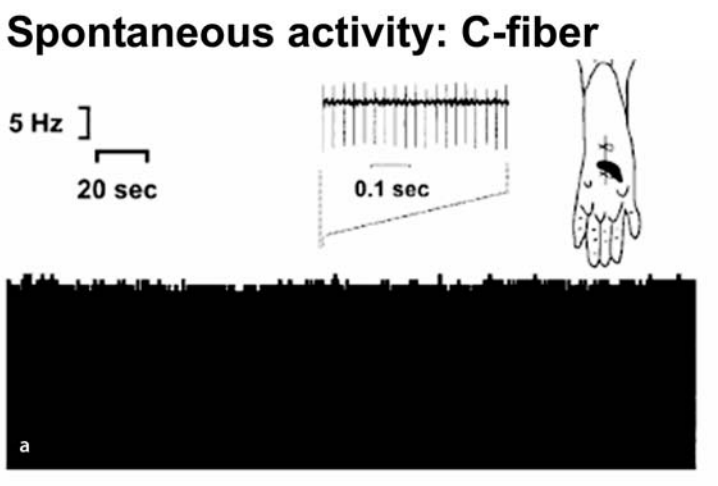
Spinal Sensitization after Incision (Fig. 2d):

Recording action potentials from dorsal horn neurons (DHN) after incision revealed unique characteristics of ► spinal sensitization defined by decreased withdrawal thresholds, increased neuronal activity, enlarged receptive field (RF) size and increased background activity. DHNs receiving input from the plantar aspect of the hind paw using natural stimuli in anesthetized rats were characterized as ► wide-dynamic-range (WDR), and ► high-threshold (HT) neurons based on their responses to brush and pinch. WDR neurons respond to both brush and pinch whereas HT neurons respond only to pinch. The results from DHN recording demonstrate that a plantar incision caused dorsal horn cell activation and central sensitization (Vandermeulen and Brennan 2000; Zahn and Brennan 1999a). Both WDR and HT neurons have increased background activity that is driven by activated primary afferent fibers from the incision area (Pogatzki et al. 2002c) and probably transmits evidence for nonevoked ongoing pain like guarding behaviors. Because the threshold of HT neurons did not decrease to the range of the withdrawal responses in behavioral experiments, particular WDR dorsal horn neurons probably contribute to the reduced withdrawal threshold observed in behavioral experiments. In contrast to other tissue injuries there is no evidence that after an incision HT neurons convert to WDR cells or low threshold neurons are sensitized.

Pharmacology of Incisional Pain

Excitatory amino acids (EAA), such as glutamate and aspartate, are important in the processing of nociceptive inputs in the dorsal horn of the spinal cord. These EAA, contained in primary afferent fibers and spinal interneurons, activate spinal N-methyl-D-aspartate (NMDA), non-NMDA and metabotropic EAA receptors to facilitate transmission of sensory inputs and contribute to the enhanced excitability of nociceptive pathways in the dorsal horn of the spinal cord in persistent pain states. These neurotransmitters are also candidates to facilitate dorsal horn neuron activity and contribute to the hyperalgesia following surgical injury.

Pharmacological studies (Zahn et al. 2002) demonstrated that intrathecally administered non-NMDA receptor antagonists blocked non-evoked pain behaviors and mechanical hyperalgesia after incision (Fig. 3b). In contrast to other tissue injuries, both the ongoing pain behavior and primary mechanical hyperalgesia that occurs after incision were found not to be dependent on activation of spinal NMDA receptors or spinal metabotropic EAA receptors (Fig. 3c). Furthermore, studying the role of different glutamate receptors for secondary hyperalgesia after incision indicates that non-NMDA receptors, but not NMDA receptors, are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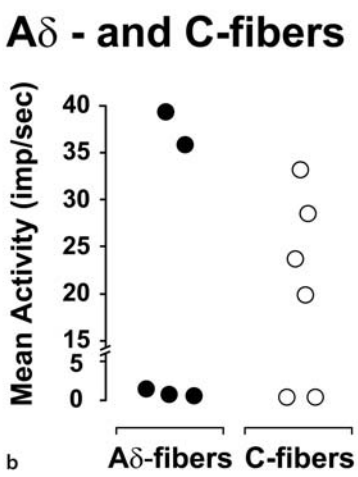
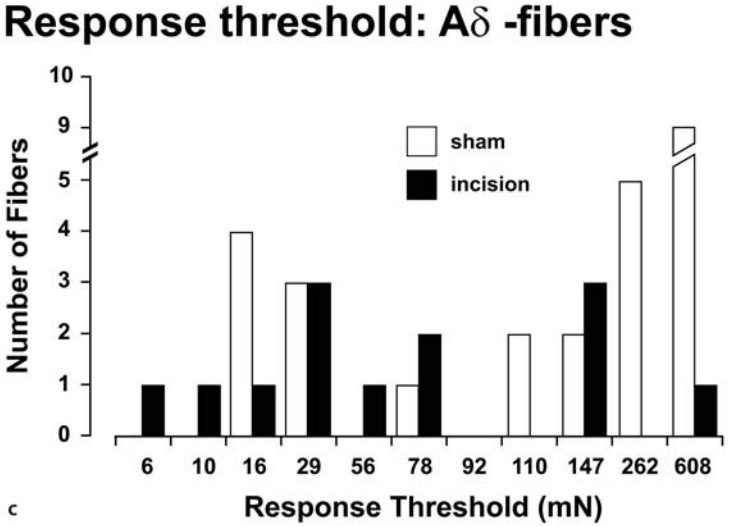
5 Hz
20 sec
0.1 sec
a


Aδ - and C-fibers
Mean Activity (imp/sec)
40
35
30
25
20
15
5
0
b
Aδ-fibers
C-fibers


Response threshold: Aδ -fibers
Number of Fibers
10
9
5
4
3
2
1
0
sham
incision
6
10
16
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56
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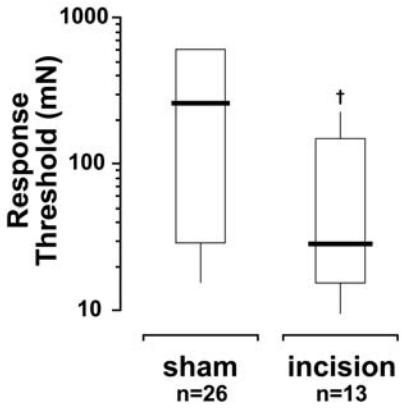
92
110
147
262
608
Response Threshold (mN)
c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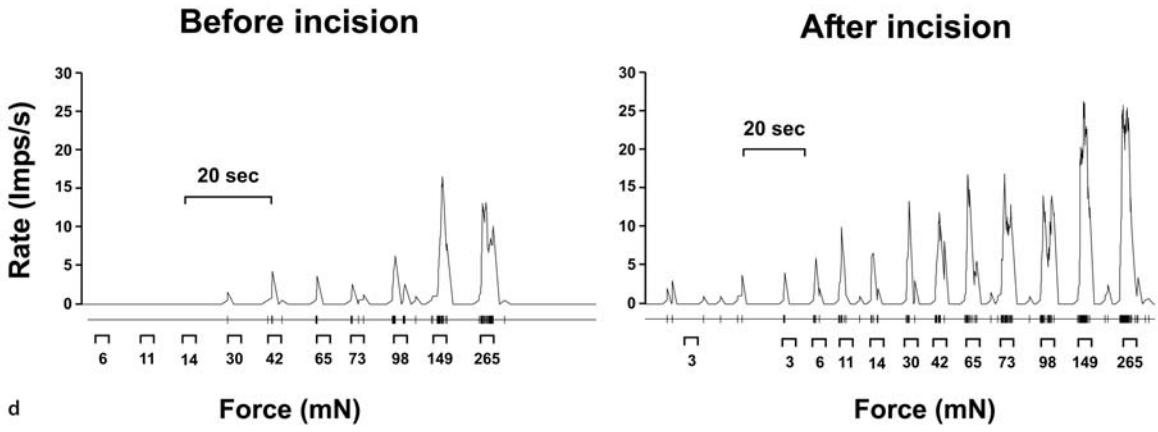
100
10
†
sham
n=26
incision
n=13


Dorsal horn neuron activity
Before incision
After incision
Rate (Imps/s)
30
25
20
15
10
5
0
20 sec
6
11
14
30
42
65
73
98
149
265
3
Force (mN)
d

◄ **Nick Model of Cutaneous Pain and Hyperalgesia, Figure 2,** (a-d) Sensitization of peripheral afferent fibers and dorsal horn neurons after incision. (a, b) Spontaneous activity of afferent fibers 1 day after plantar incision. A total of 67 fibers have been recorded. None of 39 fibers in the sham group had spontaneous activity, whereas 11 of 28 fibers (39%) in the incision group were spontaneously active. Both A-delta - and C-fibers in incised rats exhibited spontaneous activity. An example of a spontaneously active C-fiber with a high rate of activity is shown in (a); Mean firing frequency in this example was 33.2 imp/s. The mechanical receptive field (RF) of this fiber (black area) includes the incision. (b) The mean activity (imp/s averaged over 5 min) of 11 spontaneously active fibers in the incision group is shown. Two of 5 spontaneously active A-delta fibers, and 4 of 6 spontaneously active C-fibers had firing rates greater than 15 imp/s. (c)Mechanical response thresholds of 39 A-delta fibers. Median response thresholds of A-delta fibers after incision (n=13) were significantly decreased compared to A-delta fiber response thresholds after sham procedures (n=26). The results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). †P < 0.05 *vs.* sham. (d) Recording of dorsal horn neurons. Increased activity to mechanical stimuli of one WDR dorsal horn neurons and 1 hour after incision.

important for enhanced mechanical responses outside the area of an incision. Recently it has been demonstrated that spinally administered jorospider toxin (JSTX), an antagonist for calcium-permeable non-NMDA receptors, reduced secondary but not primary hyperalgesia after incision. This, again, differs from other types of tissue injuries. Furthermore, these data indicate that different spinal receptors are involved in maintaining primary and secondary hyperalgesia after an incision (Pogatzki et al. 2003).

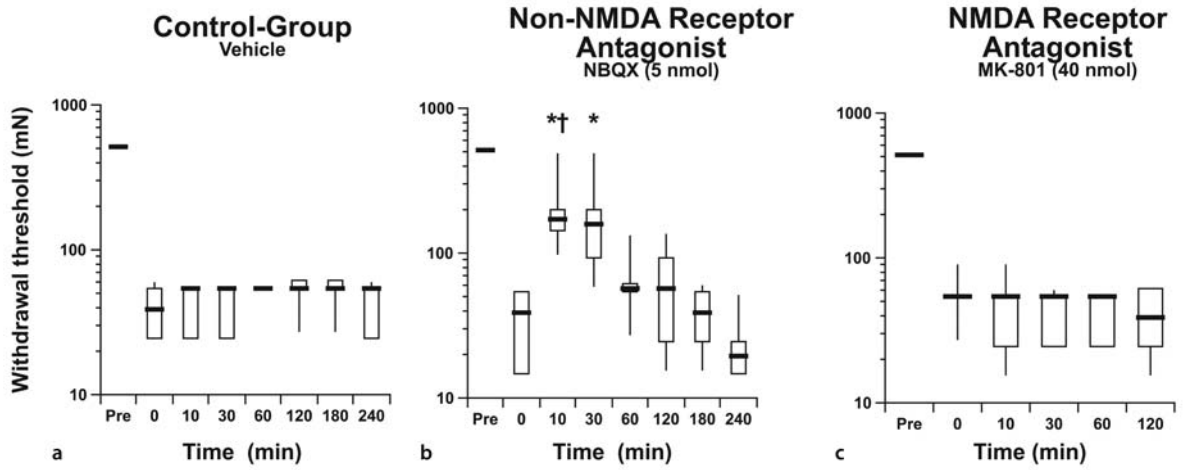
Thus, the mechanism(s) for maintaining pain behaviors following an incision are different from mechanisms described for inflammatory or neuropathic models of hyperalgesia; only the non-NMDA EAA receptors are required for the maintenance of pain behaviors after plantar incision. These data also suggest that models that rely on different receptor systems for the development and maintenance of pain behavior may not predict analgesia for patients with postoperative pain.

Other spinally administered drugs producing analgesia in this model include L-type calcium channel receptor antagonists (Wang et al. 2000), alpha-2 adrenoceptor antagonists (Onttonen and Pertovaara 2000), and prostaglandin receptor antagonists (Omote et al. 2002).

Pre-emptive Analgesia

Because central sensitization may be important for postoperative pain, many have proposed that a blockade of noxious input to the spinal cord before the tissue injury will reduce postoperative pain more than blockade after injury (► pre-emptive analgesia).

The usefulness of pre-emptive analgesia has been reported in several animal models of inflammation, chemical irritation or neuropathic pain but results from clinical studies on postoperative pain have been disappointing (Moiniche et al. 2002). In the incision model spinal administration of morphine, bupivacaine, and EAA receptor antagonists did not reduce pain behaviors beyond the expected duration of the analgesic effect (Zahn et al. 2002). These data indicate that when the early effect of a pharmacological treatment diminishes, the surgical wound appears capable of generating pain behaviors equivalent to the untreated group. In fact, ongoing input from the periphery after incision, not the afferent barrage during the injury, is critical for the expression of behaviors and sensitization of DHN after incision (Pogatzki et al. 2002c). This may explain why clinical studies using treatments attempting to prevent the development of postoperative pain have yielded mostly negative results.



Nick Model of Cutaneous Pain and Hyperalgesia, Figure 3 Effect of IT vehicle, NBQX (non-NMDA receptor antagonist) and MK-801 (NMDA receptor antagonist) on punctate mechanical hyperalgesia caused by incision. The results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). Withdrawal threshold after incision in rats treated with vehicle (a), 5 nmol of NBQX (b) and 40 nmol MK-801 (c) on the day of surgery. *P<0.05 vs. 0 min by Friedman and Dunnett's test. †P<0.05 vs. saline by Kruskal-Wallis and Dunnett's test. POD1=postoperative day 1.

Duration of treatment, rather than time initiated, may be important.

Conclusion

In conclusion, it is important to recognize that pain caused by different tissue injuries is probably a result of distinct neurochemical and electrophysiological mechanisms. Basic scientific studies on mechanisms of incisional pain have called attention to postoperative pain as an important clinical problem, distinguish postoperative pain from other experimental models by mechanism(s) and have facilitated pharmaceutical research aimed specifically towards treating incisional pain. Pain and hyperalgesia even from a simple cutaneous incision are only beginning to be understood; therefore, it is not surprising that despite the simple nature of incisional pain, postoperative pain remains a costly, poorly understood problem.

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Nicotinic receptors

► nAChr

NIRS

► Near-Infrared Spectroscopy

Nitric Oxide (NO)

Definition

Nitric oxide is a colorless, radical-free gas that reacts rapidly with O_2 to form other nitrogen oxides (e.g. NO_2, N_2O_2, and N_2O_4) and ultimately is converted to nitrite (NO_2-) and nitrate (NO_3-). Nitroc oxide itself is formed from L-arginine in bone, brain, endothelium, granulocytes, pancreatic beta cells, and peripheral nerves by a constitutive nitric oxide synthase, and in hepatocytes, Kupffer cells, macrophages, and smooth muscle by an inducible nitric oxide synthase (e.g. induced by endotoxin). NO- activates soluble guanylate cyclase. Nitric oxide is a gaseous mediator of cell-to-cell communication and acts as a second messenger in some neurons. It can diffuse from one neuron to another, and so participates not only in intracellular signaling but also in intercellular signaling. It may be the first known retrograde neurotransmitter. Nitric oxide is also a potent vasodilator, mediates penile erection, and it can participate as a reactive oxygen species in free radical actions.

► Headache Attributed to a Substance or its Withdrawal
► Opiates During Development
► Satellite Cells and Inflammatory Pain
► Spinothalamic Tract Neurons, Role of Nitric Oxide

Nitric Oxide Synthase

Synonyms

NOS

Definition

Nitric oxide synthase is an enzyme that synthesizes nitric oxide. The reaction involves arginine, which is transformed into citrulline accompanied by the release of nitric oxide.

► Spinothalamic Tract Neurons, Role of Nitric Oxide

Nitrous Oxide Antinociception and Opioid Receptors

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Synonyms

N_2O; Laughing Gas

Definition

An inorganic chemical gas with clinical anesthetic, analgesic and anxiolytic properties, as well as potential for inhalant abuse and chemical dependency.

Characteristics

Inhalation of N_2O results in activation of a number of neuronal systems and neurotransmitter receptors in the central nervous system. The actual molecular action of N_2O is not known, but many of these receptors appear to lie distal to the main sites of action of N_2O. One mechanism that has been extensively studied is that of the antinociceptive action of N_2O in animals. There is a substantial body of evidence that suggests that N_2O produces analgesia in humans through opioid mechanisms.

The analgesic properties of N_2O have been known for over two hundred years (Frost 1985). The English scientist Humphry Davy conducted N_2O experiments on himself, and suggested that N_2O might "probably be used with advantage during surgical operations in which no great effusion of blood takes place." However, it was some four decades later when the American dentist Horace Wells advocated N_2O for analgesia in dental procedures, and was the first to clinically demonstrate its use by having one of his own teeth extracted without pain.

The first significant insight into the mechanism of action of N_2O analgesia was provided by Berkowitz and his research group. N_2O ► antinociception in the mouse abdominal constriction test was partly antagonized by the ► opioid antagonist naloxone, thus implicating an opioid mechanism (Berkowitz et al. 1976). They also uncovered a unilateral cross-tolerance, in which mice and rats that were tolerant to morphine were cross-tolerant to N_2O, but animals tolerant to N_2O were not tolerant to morphine, leading to the hypothesis that N_2O caused the release of ► endogenous opioid peptides with subsequent activation of opioid receptors. Tolerance to N_2O resulted from exhaustion of the reservoir of opioid peptide, leaving the response to exogenously applied morphine intact (Berkowitz et al. 1979). Berkowitz' findings were soon reproduced in human subjects and also in various ► Antinociceptive Models using experimental animals.

Early studies of N_2O–induced antinociception utilized the non-selective opioid antagonist naloxone. The introduction of newer, subtype-selective opioid antagonists has made it possible to identify the ► endogenous opioid receptors that mediate N_2O antinociception. The most systematic investigation of opioid receptors and N_2O antinociception has been conducted employing the mouse abdominal constriction test (Quock and Vaughn 1995). N_2O antinociception was attenuated by (–)–naloxone but not (+)–naloxone, demonstrating that the antagonism is stereo-specific and attributable to blockade of opioid receptors. N_2O antinociception was also antagonized by naltrexone, a longer acting analog of naloxone, but not by systemically administered methylnaltrexone, the quaternary ammonium derivative of naltrexone that cannot cross the blood-brain barrier. Continuing studies in the abdominal constriction test have implicated ► κ opioid receptors in N_2O antinociception (Quock and Vaughn 1995). MR–2266, which blocks both κ and ► μ opioid receptors, reduced the antinociceptive response to N_2O. Norbinaltorphimine, which is highly selective for κ opioid receptors, also antagonized N_2O antinociception following either ► intracerebral (i.c.) or ► intrathecal (i.t.) pretreatment, thus implicating both supraspinal and spinal cord κ opioid receptors. However, β-funaltrexamine, a selective μ opioid antagonist, was without effect on N_2O antinociception after either i.c. or i.t. pretreatment. CXBK/ByJ mice, which are deficient in μ opioid receptors, were eight times less responsive to morphine antinociception than control mice, but when exposed to N_2O exhibited a strong antinociceptive response (Quock et al. 1993). These findings collectively supported the view that κ but not μ opioid receptors are involved in N_2O antinociception in the mouse abdominal constriction test.

Results of *in vivo* receptor protection experiments also supported the involvement of κ over μ opioid receptors in N_2O antinociception (Quock and Mueller 1991). ► Intracerebroventricular (i.c.v.) administration of β-chlornaltrexamine (β-CNA), a non-selective, non-equilibrium opioid receptor blocker, alkylates — μ, δ and κ opioid receptors and abolishes the antinociceptive response of mice to N_2O. When the κ opioid ligand U–50,488H was co-administered with β–CNA, the κ opioid receptors were spared from alkylation, and N_2O evoked its expected antinociceptive response. When the μ opioid ligand CTOP was co-administered

with β–CNA, the μ opioid receptors were protected from alkylation, yet the mice failed to exhibit an antinociceptive response to N_2O.

Results implicating other opioid receptor subtypes were apparent in other antinociceptive models. It required fairly high concentrations of N_2O to cause antinociception in rats in the 55°C hot plate test. The prolongation in response time was significantly reduced by i.c.v.–administered naltrexone, CTOP (a μ opioid antagonist) and β–endorphin$_{1\text{-}27}$ (a putative ϵ opioid partial agonist/antagonist), but not by the δ opioid antagonist naltrindole or the κ opioid antagonist norbinaltorphimine (Quock et al. 1993). Yet in another thermal nociceptive test — the warm water tail withdrawal test, the prolongation in latency time was blocked by the μ and κ opioid antagonist MR–2266, and the δ opioid antagonist ICI–174,864, but not by β–funaltrexamine (Quock et al. 1993). These studies indicate the specific opioid receptors involved in N_2O antinociception may be dependent on the antinociceptive model that is used, and/or the animal species being studied.

No such studies have been carried out to identify the type of opioid receptor that mediates cerebrospinal fluid analgesia in humans. However, the ability of N_2O to ameliorate abstinence symptoms from the mixed (κ) agonist/(μ) antagonist drug pentazocine (Kripke and Hechtman 1972), strongly suggests that κ opioid mechanisms may play a major role in the pharmacology of N_2O in humans.

Exposure to N_2O, increased levels of immunoreactive methionine-enkephalin (ME) in an artificial cerebrospinal fluid (CSF) perfusate collected from anesthetized, ventricular-cisternally-perfused rats (Quock et al. 1985). This was the first chemical evidence in support of Berkowitz' hypothesis that N_2O might induce release of opioid peptides. Selective increases in levels of ME and ME-Arg^6-Phe^7, but not other opioid peptides, were found in CSF collected from the third cerebral ventricle of dogs exposed to N_2O (Finck et al. 1990). However, increasing concentrations of N_2O increased the release of ► Beta(β)-Endorphin from superfused rat basal hypothalamic cells *in vitro* (Zuniga et al. 1987). The release of ► dynorphins by N_2O has not been shown directly; however, antisera against various fragments of rat dynorphins, but not other opioid peptides, significantly reduced the antinociceptive response of mice to N_2O (Branda et al. 2000; Cahill et al. 2000). Further, pretreatment with phosphoramidon, an inhibitor of endopeptidase 24.11, which is involved in the degradation of dynorphin, significantly enhanced N_2O antinociception (Branda et al. 2000). These findings suggest that opioid receptors are largely activated by N_2O indirectly, i.e. via stimulated neuronal release of multiple endogenous opioid peptides.

There is mounting evidence that the activation of central opioid receptors in the ► periaqueductal gray by N_2O, results in the activation of a ► descending noradrenergic pathway that modulates pain pathways in the spinal cord (Fujinaga and Maze 2002). Blockade of α_2 adrenergic receptors by yohimbine reversed N_2O antinociception in the rat tail-flick test (Orii et al. 2002). N_2O induced a naltrexone-sensitive increase in the norepinephrine concentration in the rat spinal cord (Fujinaga and Maze 2002). Depletion of norepinephrine in the spinal cord reduced the responsiveness of rats to N_2O antinociception. These descending noradrenergic pathways also appear to be modulated directly by GABAergic neurons at supraspinal and spinal levels (Hashimoto et al. 2001; Orii et al. 2003).

In conclusion, there is evidence that N_2O can stimulate the neuronal release of endogenous opioid peptides. Dynorphin and κ opioid receptors are involved in the antinociceptive effect of N_2O in the mouse abdominal constriction test. Other opioid receptors may be involved in other species and other antinociceptive models. Further, N_2O activation of central opioid receptors may produce some of their effect by activating descending noradrenergic pathways that modulate spinal cord processing of pain.

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NK–1 Blockers

Definition

Neurokinin–1 (NK–1) receptor inhibitors lessen emesis after cisplatin, ipecac, copper sulfate, apomorphine, and radiation therapy; e.g. aprepitant.

► Cancer Pain Management, Chemotherapy

NK-1 Receptor

Definition

One of the three types of G-protein coupled receptors where tachykinins act. Substance P is the preferred ligand, although neurokinin A (NKA) can also activate the NK1 receptor.

► Wind-Up of Spinal Cord Neurons

NMDA

Definition

N-methyl-D-aspartate (NMDA), a chemical analogue of glutamate that gives its name to the receptor. This receptor is formed of at least 4 subtypes, one of which is the NR2B.

► GABA and Glycine in Spinal Nociceptive Processing
► N-methyl-D-aspartate
► Opioids in the Spinal Cord and Central Sensitization

NMDA Glutamate Receptor(s)

Synonyms

N-methyl-D-aspartate receptor; NMDA Receptor

Definition

One of 4 general classes of glutamate receptors. Activated by N-methyl-D-aspartate with high affinity. It is involved in inducing long-term changes in the function of the neurons due to an influx of Ca^{++} through it. This receptor requires a release of glutamate (ligand-gated) and a change in the membrane voltage (voltage-gated) concomitantly to be activated. The NMDA receptor is found in high concentrations in the anterior horn of the spinal cord, where it is associated with the process of central sensitization, one of the precursors of neuropathic pain. Agonists include glutamate and aspartate, and antagonists include ketamine and dextromethorphan, and to a much lesser extent methadone. The receptors may be the site of action of dissociative anesthetics such as ketamine. The NMDA ion channel has a relatively high Ca^{2+} permeability, and this may be important in the initiation of plastic changes necessary for several types of learning and memory.

► Acute Pain Mechanisms
► Amygdala, Pain Processing and Behavior in Animals
► Descending Modulation and Persistent Pain
► GABA and Glycine in Spinal Nociceptive Processing
► Metabotropic Glutamate Receptors in the Thalamus
► Nociceptive Neurotransmission in the Thalamus
► NSAIDs, Adverse Effects
► Opiates During Development
► Opioids in the Spinal Cord and Central Sensitization
► Pain Control in Children with Burns
► Pain Modulatory Systems, History of Discovery
► Postoperative Pain, Transition from Parenteral to Oral
► Somatic Pain
► Wind-Up of Spinal Cord Neurons

NMDA Receptors in Spinal Nociceptive Processing

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Synonyms

Glutamate receptors; spinal dorsal horn; calcium channel; ionotropic receptor

Definition

The NMDA receptor is a tetrameric molecule provided with a channel that upon glutamate binding allows the outflow of potassium ions as well as the inflow of sodium and, characteristically, calcium ions across the neuronal

membrane. The NMDA receptor is responsible for excitatory synaptic transmission in nociceptive pathways and circuits.

Characteristics

Glutamate is the main excitatory transmitter in the central nervous system. When released by presynaptic terminals, glutamate reaches the postsynaptic membrane, where various types of receptor molecules may be found. Glutamate receptor molecules can be divided in two broad types, the ionotropic type, which possesses an ion channel that opens upon glutamate binding, and the metabotropic type, which has no channel but is coupled to G-proteins that transduce glutamate binding into modifications of intracellular messengers and enzymes.

Molecular Biology and Biophysics of NMDA Receptors

The ionotropic glutamate receptors (Kandel and Siegelbaum 2000) are named after their main pharmacological ligands, that is AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), kainate and NMDA (*N*-methyl-D-aspartate). In all three types, the ion channel permits the flow of sodium and potassium ions. Although a minority of AMPA receptors also permit the flow of calcium ions, a large calcium inflow is characteristic of all NMDA receptors and constitutes the key to their physiological role. NMDA receptors are also characterized by the fact that, at resting membrane potential (*ca.* -65 mV), the entrance to the ion channel is blocked by a magnesium ion. At resting membrane potential, glutamate binding does not lead to any ion current through the NMDA receptor; first the magnesium ion must be dislodged from this channel. The ionotropic glutamate receptors then function in the following manner.

The glutamate released from presynaptic terminals first activates AMPA and kainate receptors (usually called non-NMDA receptors), which leads to a fast flow of sodium and potassium ions and thus to depolarization of the postsynaptic neuron. If (and only if) this depolarization is large enough, the magnesium ion will no longer be attracted into the NMDA channel entrance and the channel will finally become free for sodium and potassium to flow through; this causes a prolonged depolarization. Most importantly, the unblocked NMDA channel now lets large amounts of calcium ions flow into the neuron.

Ionotropic glutamate receptor molecules are made of two pairs of subunits, each subunit in turn with four intramembranous segments. Subunits in NMDA receptors are called NR1, NR2 and NR3. The predominant NR2 subunit is the NR2B. These subunits can be sensitized through phosphorylation by protein kinases (see below). NR1 binds glycine and NR2 binds glutamate (Furukawa et al. 2005). Glycine is essential for the NMDA receptor to function. Normally there is enough glycine in the extracellular fluid and the function of the NMDA receptor then depends only on the glutamate released presynaptically and on the membrane potential of the postsynaptic neuron (Kandel and Siegelbaum 2000).

Involvement of NMDA Receptors in Normal Nociception

Some authors have found that NMDA receptor antagonists, whether given systemically or intrathecally onto the spinal cord, do not modify behavioral baseline responses to acutely painful stimuli (Yaksh 1999). Also, conditional deletion of the NR1 subunit in the spinal cord had no influence upon behavioral responses to tactile stimulation or to acute, high intensity non-damaging thermal stimuli applied to the skin (South et al. 2003). However, recordings from spinal cord nociceptive neurons have shown (Dougherty et al. 1992; Neugebauer et al. 1993a; Neugebauer et al. 1993b) that NMDA receptors participate in responses to various types of cutaneous and deep somatic noxious stimuli. It therefore seems that NMDA receptors will contribute to normal nociception whenever the postsynaptic neurons become sufficiently depolarized for the magnesium ion to be dislodged from the receptor. This will of course be more intense in cases of persistent nociception.

Involvement of NMDA Receptors in Persistent Nociception

In cases of persistent damage to a peripheral tissue, such as during inflammation, trauma or nerve lesions, the continuous and often large barrage of action potentials coming into the spinal cord causes a considerable release of glutamate and this in turn, *via* non-NMDA receptors, causes a sufficiently large neuronal depolarization for the magnesium ion to be evicted from the NMDA channel. Calcium then flows into the postsynaptic neurons and a whole series of events is triggered, which results in a considerable and long lasting increase in spinal neuronal excitability (central sensitization). The hyperalgesia (increased pain upon noxious stimulation) and allodynia (pain elicited by normally non-painful stimuli) of chronic pain conditions are thus thought to arise not only from an increased nociceptive input into the spinal cord, but also from an increased synaptic relay of nociceptive messages towards supraspinal structures.

It must be made clear that activation of NMDA receptors is not the only mechanism whereby central sensitization comes about. Concomitant mechanisms are, for example: (1) activation of receptors for pronociceptive neuropeptides such as ► substance P (NK1), neurokinin A (NK2), ► calcitonin gene related peptide (CGRP receptor), cholecystokinin (mainly CCK_B) and vasoactive intestinal peptide (VIP receptor); and (2) induction of cyclooxygenase-2 (Vanegas and Schaible 2001), with the resulting enhancement of the pronociceptive effects of prostaglandins and thromboxanes (► Prostaglandins, Spinal Effects). It must also be made clear that, in addition to the NMDA receptor, there are voltage activated

calcium channels (Vanegas and Schaible 2000) through which considerable amounts of calcium can flow into the depolarized neuron (► Calcium Channels in the Spinal Processing of Nociceptive Input). Compounds that antagonize NMDA receptors or the other mechanisms just mentioned have been shown to attenuate pain messages and are, of course, potential analgesic agents.

Electrically Induced Spinal Neuronal Hyperexcitability

Two types of electrophysiological manipulation have shown that the NMDA receptor plays a key role in spinal neuronal hyperexcitability. Both of these involve stimulating the primary afferents with electrical pulses whose intensities are generally large enough to fire all fiber types, from Aβ to C. Each stimulus pulse elicits in spinal cord neurons a depolarizing synaptic potential that lasts up to 20 s and is due to the activation of non-NMDA, NMDA and substance P receptors (Woolf 1996). If the pulse is applied about once per second, these potentials summate one on top of the other, thus giving rise to increasingly larger neuronal discharges. This is known as windup and shows that the excitability of neurons may increase as result of previous activity (thus a form of "learning"), that this involves NMDA receptors and that low frequency but persistent discharges in pain afferent fibers may cause increasing pain. Windup is not a product of neuronal plasticity; it happens by virtue of mechanisms that are normally present in the spinal cord. The enhanced excitability quickly returns to baseline if the electrical stimulation is terminated.

On the other hand, an increase in synaptic strength that may last for hours can be obtained if the stimulus pulse is applied about 100 times per second during a few seconds. This is known as long-term potentiation (LTP), and is the most widely and deeply studied form of activity dependent synaptic enhancement (the basis of learning) (Sandkühler 2000). The presence of LTP is shown after the inducing pulse train by applying a single test pulse to the presynaptic axons every few minutes - each test pulse elicits an enhanced excitatory postsynaptic response in the recorded neuron. Antagonists to NMDA, NK1 or glutamate metabotropic receptors prevent induction of LTP.

Induction of Hyperexcitability by Persistent Tissue Damage

In the spinal cord, LTP of responses to electrical pulses can be elicited by strong "natural" noxious stimuli (burning or crushing of paws, crushing of nerves) in anesthetized rats (Sandkühler 2000), provided that the nociceptive inhibition that normally descends from the brain stem is blocked (► Long-Term Potentiation and Long-Term Depression in the Spinal Cord). Another way of investigating the role of NMDA receptors in central sensitization and hyperalgesia is to use natural noxious stimuli instead of electrical pulses to test the responses of dorsal horn nociceptive neurons in anesthetized animals or the behavioral nociceptive responses in conscious animals. As mentioned above, inflammation or trauma of peripheral tissues or lesions to peripheral nerves induce an enhancement of spinal nociceptive responses in animals that is akin to the hyperalgesia and allodynia of clinical chronic pain conditions.

Plasticity of the NMDA Receptor

During persistent damage or LTP, the key to an increase in neuronal excitability is an increase in intracellular calcium concentration. This is brought about not only by the inflow of calcium through the NMDA receptor channel, but also by inflow through voltage dependent calcium channels or calcium permeable AMPA receptors and by activation of G-protein coupled receptors (to glutamate, prostaglandins, substance P), which results in calcium inflow and/or release from intracellular stores towards the cytosol (Ikeda et al. 2003; Woolf and Salter 2000). The paramount role of NMDA receptor activation, however, has been demonstrated in numerous studies where application of pharmacological antagonists or deletion of the NR1 subunit of the NMDA receptor in the spinal cord completely prevent induction of LTP or central sensitization (Sandkühler 2000; South et al. 2003; Woolf and Salter 2000). Nevertheless, blockade of other receptors, like the NK1 receptor, may also prevent LTP (Ikeda et al. 2003; Woolf 1996).

The increased intracellular calcium leads to activation of calcium-calmodulin dependent protein kinase II (CaMKII), protein kinase A (PKA) and protein kinase C (PKC), as well as nitric oxide synthase and the cyclooxygenases (Sandkühler 2000; Woolf 1996; Woolf and Salter 2000). This in turn leads to several events, including sensitization of both NMDA and non-NMDA receptors.

In spinal cord neurons, one important point of convergence for various intracellular pathways is PKC. CaMKII, another serine/threonine kinase that plays a key role in hippocampal and neocortical plasticity is less important in the spinal dorsal horn (Woolf and Salter 2000). Protein kinases phosphorylate the NMDA receptor and thereby sensitize it and partially dislodge the magnesium ion from the channel (Woolf 1996); as a result, binding of glutamate to the NMDA receptor will more easily cause calcium inflow. Tyrosine kinases such as Src also potentiate NMDA currents (Woolf and Salter 2000). Indeed, Src and PKC are involved in phosphorylation of the NR2B subunit induced by brain derived neurotrophic factor (BDNF) (Guo et al. 2004a), which binds to the tyrosine kinase B (trkB) receptor. Since peripheral inflammation induces expression of BDNF in primary afferents, this would contribute to central sensitization. It must be noted that increases in intracellular calcium as well as other forms of protein kinase activation do not necessarily result from an activation of the NMDA receptor and yet they feed back

and sensitize it. Indeed, activation of NK1, glutamate metabotropic and tyrosine kinase receptors may lead to nociceptive neuronal sensitization (Ikeda et al. 2003) and this has been shown to be associated with phosphorylation of the NMDA receptor (Guo et al. 2004a; Guo et al. 2004b).

Activation of spinal neuronal NMDA, glutamate metabotropic, NK1 and trkB receptors as a result of peripheral noxious stimulation and inflammation eventually leads to activation of ERK (extracellular signal regulated protein kinase, a mitogen activated protein or MAP kinase). ERK activation, by way of the cAMP responsive element binding (CREB) protein in the cell nucleus, results in gene expression mediated by the cAMP responsive element (CRE). This may in turn lead to medium- and long-term persistence of central sensitization to painful stimulation (Kawasaki et al. 2004) and to transition to chronicity in clinical pain.

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N-methyl-D-aspartate

Synonym

NMDA

Definition

N-methyl-D-aspartate (NMDA), a chemical analogue of glutamate that gives its name to the receptor. Agonist for the NMDA receptor for glutamate, which is the major excitatory neurotransmitter in the CNS.

► GABA and Glycine in Spinal Nociceptive Processing
► Opioids in the Spinal Cord and Central Sensitization
► Spinal Cord Nociception, Neurotrophins

N-methyl-D-aspartate (NMDA) Antagonist

Definition

There is accumulating evidence to implicate the importance of N-methyl-D-aspartate (NMDA) receptors to the induction and maintenance of central sensitization during pain states. However, NMDA receptors may also mediate peripheral sensitization and visceral pain. NMDA receptors are composed of NR1, NR2 (A, B, C, and D), and NR3 (A and B) subunits, which determine the functional properties of native NMDA receptors. Antagonists acting at the N-methyl-D-aspartate (NMDA) receptor can block the development of tolerance to the analgesic effects of [mu] opioid receptor (MOR) ligands, such as morphine, and can also enhance the analgesic efficacy of opioids. The last decade has seen significant progress in our understanding of the NMDA receptor complex and the site(s) of action of various uncompetitive antagonists. This has led to the development of a family of low-affinity, uncompetitive, cation channel antagonists that seem to offer many of the benefits of the older channel blockers but with a more acceptable adverse effect profile. Drugs such as memantine have shown beneficial effects in clinical trials for Alzheimer's disease and ischemia, with few adverse effects. Likewise, the NMDA receptor NR2B subunit antagonists derived from drugs such as ifenprodil, have proven beneficial in the treatment of neuropathic pain, and are also associated with few adverse effects.

► Multimodal Analgesia in Postoperative Pain

N-methyl-D-aspartate (NMDA) Receptor

Definition

Activation of the NMDA receptor sets in motion a series of events that increase the responsiveness of the nociceptive system (central sensitization). NMDA receptors are composed of NR1, NR2 (A, B, C, and D), and NR3 (A and B) subunits, which determine the functional properties of native NMDA receptors. Central sensitization lowers the activation thresholds of spinal neurones and is characterized by wind-up, whereby repeated C-fiber volleys result in a progressive increase in discharge of secondary dorsal horn nociceptive neurones. This contributes to the hyperalgesia. The N-methyl-D–aspartate (NMDA) receptor, at physiological Mg^{++} levels is initially unresponsive to glutamate, but following depolarization at the amino methyl propionic acid (AMPA) receptor by glutamate or the Neurokinin–1 receptor by Substance P, and the trkB receptor by brain-derived neurotropic factor (BDNF), it becomes responsive to glutamate, allowing Ca^{++} influx. The action of glutamate on the metabotropic receptor (modulated by glycine) stimulates G-protein-mediated activation of phospholipase C (PLC), which catalyses the hydrolysis of phophatidylinositol 4,5-biphosphonate (PIP2) to produce inositol triphosphate (IP3) and diacylglycerol (DAG). DAG stimulates production of protein kinase C (PKC), which is activated in the presence of high levels of intracellular Ca^{++}. IP3 stimulates the release of Ca^{++} from intracellular stores within the endoplasmic reticulum. Increased PKC induces a sustained increase in membrane permeability, with Ca^{++} leading to the expression of proto-oncogenes (c-fos, c-jun). The proteins produced encode a number of peptides (enkephalins, dynorphins, tachykinins). Increased intracellular Ca^{++} leads to the activation of calcium/calmodulin-dependent protein kinase (which briefly increases membrane permeability), to the activation of phospholipase A–2 (PLA–2), as well as to the activation of nitric oxide synthetase (NOS) (via a calcium/calmodulin mechanism). The conversion of phosphatidyl choline to prostaglandins and thromboxane is catalyzed by PLA2. The lipooxygenase pathway also produces leukotrienes. NOS catalyses the production of nitric oxide (NO) and L-citrulline from L-arginine. NO activates soluble guanalyte cyclase, increasing the intracellular content of cyclic GMP, leading to the production of protein kinases and alterations in gene expression. NO diffuses out of the cell to the primary afferent terminal, where, via a guanalyte cyclase/cyclic-GMP mechanism, it increases glutamate release. NO is responsible for the cell death demonstrated after prolonged activation of nociceptor afferents. Among NMDA receptor subtypes, the NR2B subunit-containing receptors appear particularly important for nociception, leading to the possibility that NR2B-selective antagonists may be useful in the treatment of chronic pain.

- ▶ Opioid Responsiveness in Cancer Pain Management
- ▶ Postoperative Pain, Persistent Acute Pain
- ▶ Postoperative Pain, Postamputation Pain, Treatment and Prevention
- ▶ Spinothalamic Tract Neurons, Glutamatergic Input

NMR

- ▶ Magnetic Resonance Imaging

NNT

Definition

The NNT of oxycodone 15 mg is 2.3.

- ▶ Number Needed to Treat
- ▶ Postoperative Pain, Oxycodone

Nocebo

Definition

Nocebo (from the Latin: "I shall harm") can be thought of as an agent or intervention that results in harm, either in the form of adverse outcomes or adverse side-effects. A nocebo effect is the effect that such an agent or intervention ostensibly exerts.

The term – nocebo effect, is most commonly used to describe side-effects that occur in response to interventions for which there is no physiological mechanism, such as a placebo. These side-effects are the same as, and may be more prevalent, than those encountered with the active medication. Interestingly, the side-effect profile of placebos mimic the active drug that it is being compared with and also appear to be disease-specific (e.g. dizziness in psychiatric disorders, headache in angina, gastro-intestinal disturbance in peptic disease (Barsky et al. 2002). This is thought to be due to patients being informed, and therefore cued, to expect potential adverse effects of the medication that they might be taking in the research project. Suggestion, expectation and conditioning are the likely reasons. It is commonly seen in clinical practice when some patients assiduously study their consumer product information in their medicine packets, and read about all the possible side-effects of the medicine they have been prescribed. Upon taking the medication, they believe that they experience the expected side-effect.

The nocebo effect can be reduced by some simple steps (Weihrauch and Gauler 1999). Firstly, if patients with negative expectations can be identified, especially

in conditions such as anxiety, depression and somatisation, it is worthwhile attempting to shift their cognition to more positive expectations. For example, with serotonin uptake inhibitors, telling patients that the nausea they are likely to experience is a good sign (since it shows serotonin is increasing and, therefore, the drug is working) makes it more likely they will continue taking the medication. Secondly, commencing with low doses and increasing them slowly reduces the risk of side-effects. Thirdly, using other health-care professionals who will reinforce the messages given to the patient lessens the risk of nocebo.

The term – nocebo response, is used in another fashion, to describe the response of patients who know that they have been given or allocated to an inferior treatment. Under those conditions, they report failure to improve in order to indicate indirectly their disaffection with the way that they have been treated.

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Nocebo Effect

Definition

Harmful effects occurring from placebos.

► Placebo

Nociceptin

► Orphanin FQ

Nociception

Definition

Nociception (from the Latin word *nocere*, to injure) is the transduction, encoding, and transmission of neural information about tissue damage, or impending tissue damage, which would occur if a stimulus was maintained over time. Cognitive central processing identifies the location of the stimulus, its general character, and the severity of the associated tissue injury. Some of the biological participants in this process include: unmyelinated dorsal horn neurons [like A-delta and C-fibers], excitatory amino acids, neuropeptides, zinc, biogenic amines, nitric oxide, wide-dynamic range spinal neurons, the limbic system of the brain, and several regions of the cerebral neocortex cortex. Normal nociception depends on a delicate balance between pronociceptive and antinociceptive forces. Chronic noxious stimulation causes ► central sensitization in which there is an increase in sensitivity of nociceptive neurons function nociceptively [recruitment] and new, semi-permanent neural connections develop [neuroplasticity].

► Allodynia and Alloknesis
► Amygdala, Pain Processing and Behavior in Animals
► Consciousness and Pain
► Ethics of Pain, Culture and Ethnicity
► Forebrain Modulation of the Periaqueductal Gray
► Muscle Pain, Fibromyalgia Syndrome (Primary, Secondary)
► Nociceptive Withdrawal Reflex
► Pain in Humans, Psychophysical Law
► Psychological Aspects of Pain in Women
► Secondary Somatosensory Cortex (S2) and Insula, Effect on Pain Related Behavior in Animals and Humans
► Spinothalamic Tract Neurons, in Deep Dorsal Horn
► Spinothalamocortical Projections from SM
► Transition from Acute to Chronic Pain

Nociception in Genital Mucosa

► Nociception in Mucosa of Sexual Organs

Nociception in Mucosa of Sexual Organs

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Synonyms

Nociception in Genital Mucosa; Genital Mucosa, Nociception; Mucosa of Sexual Organs, Nociception

Definition

Pain perception and nociceptors in mucosal lining, including epithelium and underlying connective tissue, of human external genital organs. The external genital organs covered by mucosa comprise vulvar vestibule, clitoris and glans penis. The vagina belongs to the internal female genital organs but is included since it has partly somatic innervation.

Characteristics

Pain Perception in Sexual Mucosa

The vestibule is by origin visceral tissue but is considered to have a somatic innervation (Cervero 1994). Sensations for touch, temperature and pain are therefore similar to sensations evoked in skin. Heat pain threshold is approximately 43° C, which is slightly below heat pain thresholds in skin (45–46°C). There are

limited data on cold pain threshold. Mechanical pain assessment has been performed using various methods and devices. Normal values with von Frey monofilaments are defined in two independent studies using slightly different experimental setups. The results show a wide range in punctuate mechanical pain thresholds (185–430 mN) in healthy fertile women (Bohm-Starke et al. 2001; Pukall et al. 2002; Lowenstein et al. 2004; Giesecke et al. 2004).

Experimental data on pain thresholds in the vagina are sparse. However, in clinical practice it is obvious that thermal pain perception exists, although spatial discrimination is poor. Furthermore while it is possible to perform unanaesthetized surgical interventions such as biopsies without eliciting pain in the proximal vagina, pain is easily evoked in the distal part close to the introitus. Mean mechanical pain threshold in the lateral vaginal wall using a 12 mm diameter probe is 10.7 N (range 4.3–25.3) in healthy women (Baguley et al. 2003). The vagina, although considered a visceral organ, is not sensitive to distension (Bohm-Starke et al. 2001).

On the glans penis mechanically as well as temperature induced pain can be elicited. Mechanical detection thresholds seem to coincide with pain thresholds (Halata and Munger 1986).

Nociceptors in Sexual Mucosa

The external genital area is supplied both with myelinated and unmyelinated nerves. Free nerve endings are most frequently found at the labia minora lateral to Hart's line (Krantz 1958). Biopsies from young healthy women taken from the posterior vestibule revealed that the intraepithelial free nerve endings rarely branch and penetrate two thirds of the epithelium. These intraepithelial nerve fibres are not evenly distributed. Some areas are almost devoid of nerves, whereas other parts of the epithelium are densely innervated. In addition, one population of free nerve endings terminate within the basal layer of the epithelium. The intraepithelial nerves are immunoreactive to CGRP. Nerve fibres are also encountered in the subepithelial connective tissue. In this area it is, however, more difficult to evaluate the existence of free nerve endings. Most nerve fibres are found in connection to vascular or glandular elements (Bohm-Starke et al. 1998; Bohm-Starke et al. 1999; Tympanidis et al. 2003). The clitoris and the female urethral meatus are richly innervated by free nerve endings. They are located in or just below the basal layer of the epithelium (Krantz 1958). Free nerve endings in the vagina are rare and found in the distal part, the anterior wall being more densely innervated than the posterior. Most of these nerve terminals end after penetrating two thirds of the epithelium; however some terminate just a few cell layers from the surface (Hilliges et al. 1995). The hymenal ring is richly supplied with free nerve endings (Krantz 1958).

The human glans penis is richly innervated by free nerve endings. These endings are found in almost every connective tissue papilla (Halata and Munger 1986).

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Nociception in Nose and Oral Mucosa

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Definition

Nociception in the oral and nasal cavities is predominantly mediated through fibers of the ► trigeminal nerve. Different from other areas of the body, ► nociceptive afferents are easily accessible as they are not covered, e.g. by a corneal layer of epidermis. Trigeminal input is intimately involved in the processing of both olfactory and gustatory information. In turn, trigeminal sensitivity appears to depend on olfactory/gustatory stimulation that may play a role in, for example, ► Burning Mouth Syndrome.

Characteristics

Nasal Cavity

Anatomy of Nasal Trigeminal Afferents

The nasal cavity is innervated by the ophthalmic (1^{st} branch of the 5^{th} cranial nerve: V_1) and maxillary (V_2) branches of the trigeminal nerve. V_1 (anterior ethmoidal and infraorbital nerves) innervates the anterior portion of the nasal cavity; the posterior part of the nasal cavity is innervated by V_2 (posterior superior medial nasal and nasopalatine nerves). Cell bodies of trigeminal afferents lie in the Gasserian ganglion. Axons project to the trigeminal sensory nucleus extending from the rostral spinal cord to the midbrain. ► Chemosensory fibers from the nasal cavity have been shown to project to the subnucleus caudalis and subnucleus interpolaris (Anton et al. 1991). Trigeminal information is relayed to the amygdala via the lateral parabrachial complex. Neurons of the spinal nucleus project to the ventral posterior medial, intralaminar, and mediodorsal nuclei of the thalamus. While most ascending fibers cross to the contralateral side, some fibers also ascend ipsilaterally (Barnett et al. 1995) – similar to the olfactory system. Apart from projections to the ► primary somatosensory cortex (SI), trigeminal stimulation also produces activation of SII. Further, trigeminal stimulation leads to activity in the insular cortex and the ventral orbital cortex, with stronger right-sided activity.

Intranasal Pain Fibers

The nasal mucosa is highly sensitive to painful stimuli. This seems to be partly due to the fact that, other than in the skin, nociceptors innervating the mucosa are not covered by squameous epithelium, giving nociceptive stimuli almost direct access to the nerve endings (Finger et al. 1990). Compared to noxious thermal or mechanical stimuli, this is of particular importance with regard to chemical irritants. Trigeminal chemoreceptors act as a sentinel of the airways, where they prevent inhalation of potentially life-threatening substances, with intranasal trigeminal activation producing an inspiratory stop. This correlates with the finding of an area of increased chemosensitivity in the anterior third of the nasal cavity, whereas the posterior nasal mucosa is more sensitive to mechanical stimuli.

The physico-chemical properties of most chemicals (e.g. molecular size, lipophilicity) determine the degree to which they activate the intranasal trigeminal system (Abraham et al. 1998). Nevertheless, chemical stimulation may also activate specific receptor types. Stinging sensations are likely to be mediated by A_{delta}-fibers, whereas burning sensations are largely mediated by ► C Fiber. In addition, a variety of receptors are involved in the coding of different qualities of trigeminal mediated sensations, e.g. tingling, stinging, or burning. These receptive structures include the ► TRPV1 receptor, the nicotinic receptor (which can be activated in a stereoselective manner), the ASIC receptors, the M2 receptor, and the ► P2X receptor. Most excitingly, solitary chemosensory receptor cells have been found to be attached to trigeminal afferents (Finger et al. 2003). Thus, it seems that the trigeminal system allows discrimination between numerous different chemical stimuli – although the number of discriminable stimulus qualities is an order of magnitude below that of the olfactory system.

Measures of Nasal Nociceptive Sensation

Techniques to study intranasal trigeminal function in humans include the psychophysical lateralization paradigm, electrophysiological recordings of the ► negative mucosal potential, or recordings of the ► EEG-based ► event-related potential (Hummel 2000). However, especially in a clinical context, to-date there is no rapid and reliable standardized test of trigeminal function in humans. A major limitation of many studies, however, is that only CO_2 has been used as the chemical pain stimulant, although this gas has the advantages of being virtually odorless, inexpensive, and non-toxic. Other stimuli of the intranasal trigeminal system include ► capsaicin, ► menthol, or nicotine. Recent research on the genetic variability of trigeminal receptors (e.g. TRPV1 polymorphisms), or opioid receptor polymorphisms helps to explain the heterogeneity of individual responses to trigeminal stimulation.

Intranasal Trigeminal Sensations and Smell

Intranasal trigeminal activation has been shown to influence the perception of odors, although suppression between the olfactory and the trigeminal systems can be mutual. Importantly, odors typically produce trigeminally mediated sensations when presented at higher stimulus concentrations. In a clinical context, it has been shown that olfactory activation increases sensitivity to trigeminally mediated stimuli. In turn, ► loss of olfactory function may result in a decrease of trigeminal responsiveness. Several possible mechanisms have been identified by which trigeminal activity may influence olfactory processing (Hummel and Livermore 2002). The systems may interact centrally (e.g. mediodorsal thalamus), at the level of the olfactory bulb, at the level of the olfactory epithelium through the release of substance P/other peptides from trigeminal fibers, or indirectly via reflexes designed to minimize potentially damaging exposure of the olfactory epithelium to noxious substances (e.g. alteration of nasal patency, change of the constitution of the olfactory mucus).

Oral Cavity

Anatomy of Oral Pain

Three cranial nerves serve oral pain. The two most important are the trigeminal (V) and the glossopha-

ryngeal (IX) nerves. The trigeminal nerve, which also innervates the nasal cavity, the face, and much of the scalp, provides sensitivity to temperature, touch and pain from the base of the tongue forward. The trigeminal nerve projects to the tongue via the lingual nerve, which it shares with the chorda tympani nerve (VII), a taste nerve that innervates the front of the tongue. The density of trigeminal endings is greatest in the fungiform taste ► papillae at the tip and sides of the tongue, where they greatly outnumber chorda tympani nerve endings. Two other branches of V, the maxillary (V2) and the mandibular (V3) nerves, innervate the upper and lower surfaces of the anterior oral cavity (e.g. the gingiva, buccal mucosa, and lips), and thus mediate all non-lingual oral pain, including dental pain. The glossopharyngeal nerve innervates the posterior 1/3 of the tongue as well as the soft palate, palatal arch, and anterior oral pharynx. Glossopharyngeal innervation is particularly dense in circumvallate taste papillae on the back of the tongue. Classified as a visceral nerve, the glossopharyngeal differs from the trigeminal in that it contains gustatory as well as somatosensory fibers. The receptive field of the vagus nerve (X) overlaps with the glossopharyngeal in the oral pharynx and posterior soft palate. The vagus is also sensitive to touch, temperature and pain, but appears not to contribute to taste perception in humans. The vagus, nevertheless, contributes to the perception of the somatosensory qualities of all foods and beverages as they are swallowed. Accordingly, IX and X function as the final gatekeepers of the upper alimentary canal, and trigger the protective reflexes of gagging and coughing.

Intra-Oral Pain Fibers

Although concentrated in the fungiform and circumvallate papillae, lingual pain fibers are present throughout the oral mucosa and gingiva. Evidence of TRPV1 and peptidergic C-fiber nociceptors has been found in both types of papillae (Ishida et al. 2002). In addition to signaling acute pain, neurons that express TRPV1 also contribute to hyperalgesia to temperature caused by inflammation. Evidence of other types of nociceptors, including some that respond to cold, menthol, carbonation, nicotine and high concentrations of salts (e.g. NaCl, KCl) and acids (e.g. citric acid), have also been found in various lingual nerve preparations (Wang et al. 1993).

Consistent with the variety of nociceptors that have been identified in the lingual nerve, salts and acids as well as menthol, carbonation, cinnamic acid and nicotine can produce burning, stinging or tingling when applied to the anterior tongue. Collectively, the sensitivity to all chemicals that produce sensations, other than (or in addition to) taste or smell, is referred to as ► chemesthesis (Green 2002).

Measures of Oral Nociception

By far the best studied oral pain stimulus is the vanilloid capsaicin, which has been used as a model for oral pain. As it occurs naturally in red (”hot”) peppers, capsaicin is also of interest as a flavor stimulus. Not surprisingly, capsaicin, which acts via TRPV1, is perceived most acutely in the areas of the mouth most heavily innervated by the trigeminal nerve, such as the tongue tip. In addition, as TRPV1 is expressed on C-polymodal nociceptors, capsaicin also serves as an indicator of sensitivity to nonchemical pain stimuli such as intense heat. Thus, heat sensitivity is also greatest in the front of the mouth, particularly on the tongue tip and lips. Pain sensitivity is lowest in the superficial buccal mucosa. The sensitivity to chemical irritants and heat is also less acute on the back of the tongue, where most glossopharyngeal pain fibers are located in the basement membranes of the circumvallate papillae. Interestingly, however, swallowing capsaicin or piperine (black pepper) produces burning sensations in the throat, which are at least as strong as those produced on the front of the tongue (Rentmeister-Bryant and Green 1997), indicating that the glossopharyngeal and vagus nerves contribute significantly to chemical pain during consumption.

As capsaicin has the capacity to desensitize the neurons it stimulates (Szolcsanyi 1993), desensitization has been used to determine the importance of capsaicin-sensitive neurons for perception of chemical irritants. The results have shown that much, but not all, of oral chemesthesis is mediated by such fibers.

Oral Chemesthesis and Taste

Like olfactory stimuli, most taste stimuli evoke chemesthetic sensations as well as taste, particularly at high concentrations. Notable in this regard are acids, salts and alcohols, which can produce burning, stinging or tingling even at moderate concentrations. On the other hand, some taste stimuli (particularly sucrose) have been shown to partially suppress the burn of capsaicin, suggesting that taste stimulation has the potential to inhibit some types of oral pain.

Oral Pain Syndromes

Injuries to cranial nerves V, IX or X can result in oral neuropathologies that range in severity from annoying to debilitating. Trigeminal and glossopharyngeal neuralgias can occur following injuries or insults to the deep tissue of the mouth or mandible (e.g. dental extractions), or inflammatory disorders such as herpes zoster. However, trigeminal neuralgias most often affect perioral and facial skin rather than oral structures. Glossopharyngeal neuralgias, which are relatively rare, are usually secondary to pathologies such as abscesses and tumors that affect IX, are characterized by pain at the base of the tongue, the pharynx, or soft palate.

Perhaps the most common oral pain disorder is Burning Mouth Syndrome (BMS), which manifests as consistent or episodic burning or tingling localized in the front of the mouth, particularly the tongue and/or lips (Kapur et al. 2003). BMS generally occurs in older individuals (>50 years old), and is much more common in women than in men. Although it is believed to have more than one etiology, recent research has pointed to an association between BMS and a loss of taste function, particularly in post-menopausal women (Grushka et al. 2003). Such a link is consistent with other evidence that the taste system may normally exert an inhibitory effect on the oral pain system.

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Nociception Induced by Injection of Dilute Formaldehyde

- ► Formalin Test

Nociceptive

Definition

Related to the neuronal mechanisms involved in the detection, encoding and transmission of noxious stimuli and hence to the sensation of pain or pain behavior. A nociceptive stimulus elicits pain or pain behavior such as withdrawal, vocalization, etc. and might be potentially or overtly injurious (from the Latin word nocere, to injure).

- ► Allodynia and Alloknesis
- ► Chronic Pelvic Pain, Musculoskeletal Syndromes
- ► Insular Cortex, Neurophysiology and Functional Imaging of Nociceptive Processing
- ► Nociception
- ► Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology

Nociceptive Afferents

- ► Nociception in Nose and Oral Mucosa
- ► Nociceptive Circuitry in the Spinal Cord
- ► Nociceptive in Mucosa of Sexual Organs
- ► Nociceptive Sensory Neurons

Nociceptive Circuitry in the Spinal Cord

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Definition

Pain is generally perceived as a result of stimuli that either damage or threaten to damage peripheral tissues. Sensory information resulting from tissue damage in the limbs and trunk is transmitted through nociceptive ► primary afferent axons to the dorsal horn of the spinal cord, and subsequently relayed to various sites in the brain, including the thalamus. The term nociceptive circuitry is used to describe the arrangement and functional interconnections of neurons that are responsible for conveying this information. Nociceptive circuits, including those in the dorsal horn, also involve inhibitory control mechanisms.

Characteristics

Background

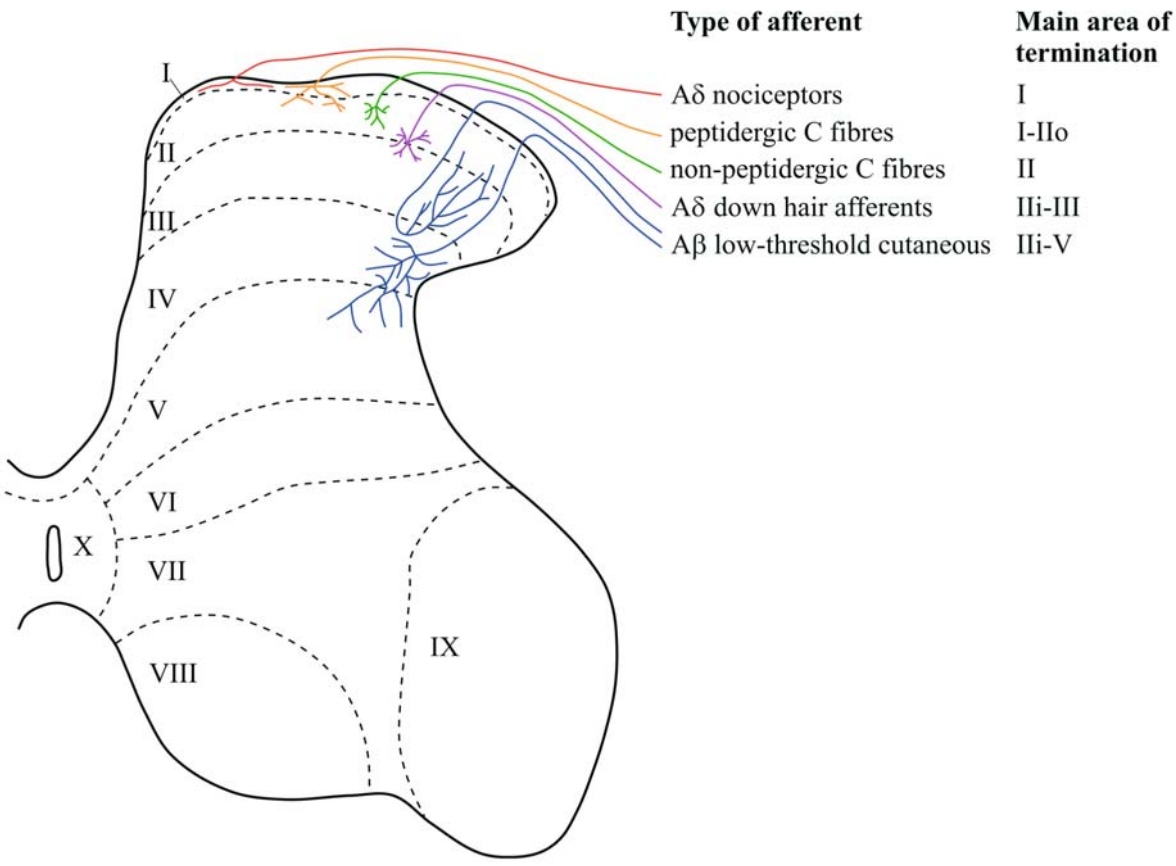
Nociceptive primary afferent axons terminate in the dorsal horn of the spinal cord, and this region therefore contains the first synapse in pathways that convey nociceptive information to the brain, as well as those

involved in reflexes. The dorsal horn is also an important site for modulation of sensory input. This modulation involves both local neurons and axons that descend from the brainstem and is thought to contribute to various types of analgesia. Changes affecting dorsal horn neurons occur in certain pathological states and are likely to contribute to the abnormal pain that follows peripheral inflammation and certain types of nerve injury. A knowledge of the nociceptive circuits in the dorsal horn would therefore be of fundamental importance for our understanding of the mechanisms underlying pain and analgesia and also for the development of new treatments for pain. However, despite extensive research on this subject we still know very little about the neuronal organisation and circuitry of the spinal dorsal horn.

Neuronal Components in the Dorsal Horn

Rexed (1952) divided the grey matter of the ► Rexed's laminae, (► ADLs laminae) and this scheme is generally used in anatomical studies (Fig. 1). The dorsal horn contains four different neuronal components: (1) central terminals of primary afferent axons, (2) ► projection neurons, (3) ► interneurons and (4) axons that descend from various brain regions.

All primary afferent axons that innervate tissues in the trunk and limbs form synapses in the spinal dorsal horn. Many different classes of primary afferent can be recognised based on the peripheral tissue that they innervate, the types of stimulus that activate them, their axonal diameter and the chemical messengers that they use (Todd and Koerber 2004). Each type of primary afferent has a characteristic distribution pattern within the dorsal horn (Fig. 1). The majority of nociceptive afferents have unmyelinated or fine myelinated axons, and these are referred to as C and Aδ fibres, respectively. Intra-axonal injection of Aδ nociceptive afferents has shown that these terminate mainly in lamina I, although they also give rise to terminals in lamina V (Light and Perl 1979). Because of their small size, intracellular labelling of C fibres has proved extremely difficult, and most anatomical studies of these afferents have used an indirect "neurochemical" approach. Many fine afferents, including approximately half of those that give rise to C fibres, express neuropeptides, such as ► substance P (SP) and



Nociceptive Circuitry in the Spinal Cord, Figure 1 A diagrammatic representation of the laminar distribution of certain types of primary afferent in the spinal cord. The dorsal horn is divided into 6 parallel laminae, and each type of afferent has a characteristic area of termination in one or more of these laminae. Note that the mediolateral and rostrocaudal distribution of afferents is related to the region of the body that they innervate. The body is thus "mapped" onto the spinal cord in these two dimensions.

► calcitonin gene-related peptide (CGRP). In the rat, all CGRP-containing axons in the dorsal horn are primary afferents, and these can be identified by immunocytochemistry. C fibres that do not express peptides can be labelled with a lectin (IB4) derived from *Bandeiraea simplicifolia*. Both types of C fibre terminate in the superficial laminae of the dorsal horn; those with peptides end mainly in lamina I and the outer part of lamina II (lamina IIo), while those that lack peptides arborise in the central part of lamina II. It is likely that the majority of both peptidergic and non-peptidergic C fibres function as nociceptors. However, little is known about the functional differences between the two classes. Tactile and hair afferents convey innocuous (low-threshold) information from the skin and most have myelinated axons. In some pathological conditions (e.g. ► neuropathic pain), activation of these afferents can be perceived as painful. The majority of low-threshold cutaneous afferents have large myelinated (Aβ) fibres and these terminate in a zone extending from the inner part of lamina II (lamina IIi) to lamina V. Fine myelinated afferents that innervate down hairs have a more restricted central distribution in laminae IIi and III.

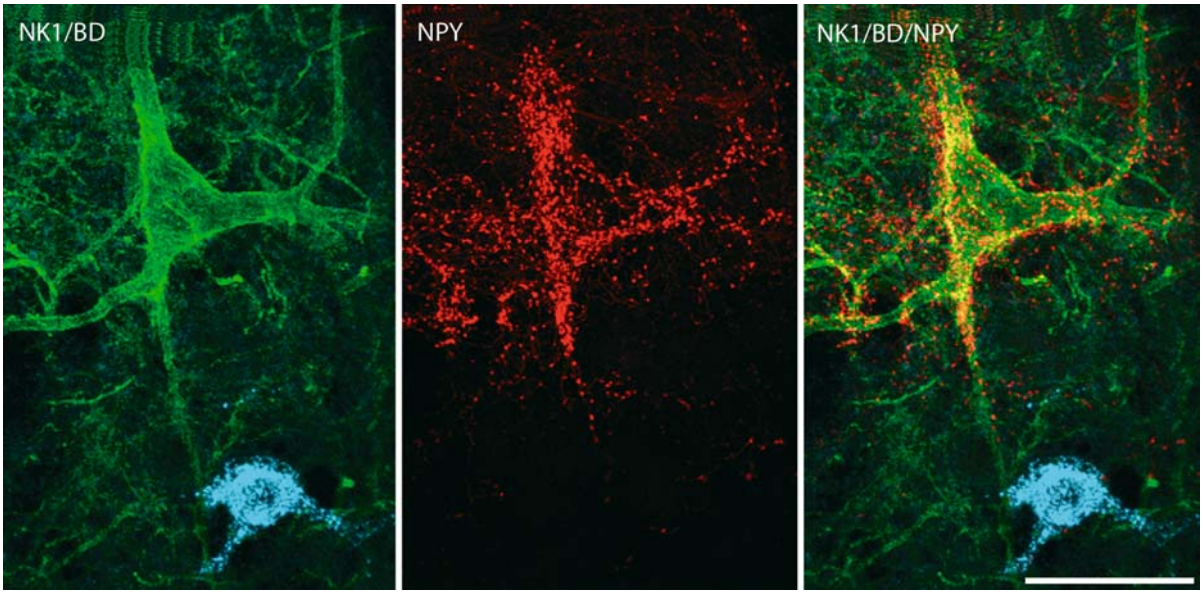
Projection neurons in the dorsal horn send their axons to several brain regions, including the thalamus, the periaqueductal grey matter of the midbrain, the lateral parabrachial area in the pons, and various parts of the brainstem reticular formation (Willis and Coggeshall 2004; Villanueva and Bernard 1999). It is likely that many projection neurons send axons to more than one of these targets (Spike et al. 2003). Projection neurons are concentrated in lamina I of the dorsal horn, and are also scattered throughout the deeper parts of the grey matter (laminae III-VII). However, in all parts of the spinal cord they are greatly outnumbered by interneurons. For example, it has been estimated that only around 5% of neurons in lamina I are projection cells (Spike et al. 2003). Projection neurons in lamina I have received considerable attention, as they are relatively numerous and most respond to noxious stimulation. The majority of projection neurons in lamina I, as well as some of those in laminae III and IV, express the neurokinin 1 (NK1) receptor, on which substance P acts. Substance P is present in many nociceptive primary afferents and is released into the dorsal horn following noxious stimulation. Intrathecal administration of substance P conjugated to the cytotoxin saporin leads to death of NK1 receptor-expressing neurons in the dorsal horn, as well as a dramatic reduction of ► hyperalgesia in both inflammatory and neuropathic conditions (Mantyh et al. 1997). This suggests that projection neurons with the NK1 receptor play an important role in the generation of hyperalgesia.

Interneurons make up the great majority of the neuronal population in each lamina of the dorsal horn and most are thought to have axons that arborise close to the cell body. These cells are therefore involved in local processing of information. Interneurons can be divided into two main classes, inhibitory cells, which use GABA and/or glycine as their neurotransmitter(s) and excitatory (glutamatergic) cells. Laminae I-III of the dorsal horn contain a particularly high density of small interneurons, and the majority of these (60-75%, depending on the lamina) are excitatory. The axons of inhibitory interneurons form two types of synaptic connection: (1) axoaxonic synapses, where they are presynaptic to a primary afferent terminal on which they exert presynaptic inhibition and (2) axodendritic or axosomatic synapses onto other dorsal horn neurons, which are responsible for post-synaptic inhibition. Blocking the actions of GABA or glycine with intrathecal antagonists leads to ► allodynia (Yaksh 1989), and this suggests that one of the roles of inhibitory interneurons is to prevent activity conducted in tactile and hair afferents from being perceived as painful. Much less is known about the function of excitatory interneurons, although it is thought that they convey information from primary afferents to other dorsal horn neurons through polysynaptic pathways. For example, they are likely to be responsible for transmitting nociceptive information from C fibres (which terminate mainly in laminae I-II) to neurons in deeper laminae. The organisation of dorsal horn interneurons is very complex (Todd and Koerber, 2004); for example Grudt and Perl (2002) have described 7 different populations in lamina II on the basis of physiological and morphological criteria. It is also possible to identify interneuron populations using neurochemical characteristics, for example patterns of neuropeptide or receptor expression.

Among the various populations of descending axons, those that contain the monoamine transmitters serotonin or norepinephrine have attracted particular attention, because of their role in stimulation-produced analgesia. Serotoninergic axons arise from cells in the raphe nuclei of the medulla, while those with norepinephrine are derived from cells in and around the locus coeruleus of the pons. Both types of axon are distributed throughout the dorsal horn, but are concentrated in the superficial laminae (I-II). The monoamine transmitters are likely to act through ► volume transmission, and therefore knowing the distribution of monoamine receptors on dorsal horn neurons will be important for understanding their roles in modulating sensory transmission.

What We Know About Neuronal Circuitry in the Spinal Cord

As stated above, our knowledge of specific neuronal circuits within the dorsal horn is still very limited. It has been shown that primary afferents that contain substance P form numerous synapses with projection neurons in laminae I and III that express the NK1 receptor (Todd et al. 2002). Since all substance P-containing



Nociceptive Circuitry in the Spinal Cord, Figure 2 This figure shows selective innervation of one of two different types of projection neuron in lamina III of the dorsal horn by axons belonging to a population of inhibitory interneurons. Two cells can be seen in the left image: the large cell in the upper part of the field is stained with an antibody against the NK1 receptor (green). All cells of this type are projection neurons. The lower cell was retrogradely labelled with biotin dextran (BD; blue) injected into the gracile nucleus and therefore belongs to the post-synaptic dorsal column (PSDC) pathway. The middle image shows the same field scanned to reveal axons that contain neuropeptide Y (NPY), and the image on the right is a merge of the three colours. NPY-containing axons in the dorsal horn are derived from a population of GABAergic interneurons in laminae I and II, and these axons can be seen to form numerous contacts with the NK1 receptor-immunoreactive cell, but not with the PSDC neuron. Scale bar = 50 μm. (Modified from Polgár et al. 1999, J Neurosci 19:2637-2646. Copyright 1999 by the Society for Neuroscience).

afferents respond to noxious stimulation (Lawson et al. 1997), this provides a direct route through which nociceptors can activate brain regions involved in pain mechanisms. The nociceptive afferents release both glutamate and substance P and these act through different mechanisms. Glutamate will be released across the synaptic cleft and act on receptors in the postsynaptic membrane, whereas substance P will diffuse to nearby NK1 receptors (► volume transmission). Nociceptive primary afferents presumably also form synapses with both excitatory and inhibitory interneurons, although much less is known about these connections.

There is some evidence to indicate that different populations of inhibitory interneurons have specific postsynaptic targets. One group of inhibitory cells in laminae I and II is characterised by the presence of GABA and neuropeptide Y. Axons of these cells form numerous synapses with projection neurons in laminae III and IV that express the NK1 receptor, but not with another population of projection cells that occupy the same laminae, those belonging to the ► post-synaptic dorsal column pathway (Polgár et al. 1999) (Fig. 2). As mentioned above, axoaxonic synapses are responsible for presynaptic inhibition of primary afferent terminals. Different classes of primary afferent appear to receive axoaxonic synapses from axons belonging to different types of inhibitory interneuron (Todd and Koerber 2004).

Much less is known about the synaptic connections between different types of interneuron, or between excitatory interneurons and projection neurons. It is likely that these are fairly specific (at least in some cases) and also very complex. Clearly, a great deal of research will be needed to unravel the details of nociceptive circuits in the spinal cord.

► Nociceptive Processing in the Spinal Cord

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Nociceptive Coding in Lateral Thalamus

► Thalamic Nuclei Involved in Pain, Human and Monkey

Nociceptive Masseter Muscle Afferents

► Nociceptors in the Orofacial Region (Temporomandibular Joint and Masseter Muscle)

Nociceptive Nerve Endings

Definition

The terminal branches of the peripheral axon of nociceptive neurons located in sensory ganglia.

► Nociceptor Generator Potential

Nociceptive Neuroplasticity

Definition

At its most general level, nociceptive neuroplasticity denotes the changes in nervous system processing resulting from nociceptive inputs. Used in this way, the term includes both functional and structural, reversible and irreversible changes. Other groups would use this term in a narrower sense, and only include alterations in nervous system function that are due to structural change.

► Central Sensitisation
► Quantitative Sensory Testing

Nociceptive Neurotransmission in the Thalamus

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Synonyms

Chemical Transmitter; neuromodulator; Thalamus, Nociceptive Neurotransmission

Definition

Neurotransmitters are chemical messengers that are released from one neural element (e.g. a nerve terminal) to then act upon a receptor located on or in another neural element (e.g. a dendrite). This transfer of information is neurotransmission, and this contribution describes neurotransmitter mechanisms which mediate the transmission and integration of nociceptive information in the thalamus.

Characteristics

The integrative role of the thalamus in the processing of nociceptive information is complex and diverse. A variety of different neurotransmitters and an array of receptors take part in this process, and it has become clear that the nature of these processes is pivotal to the function of the thalamo-cortico-thalamic circuitry (McCormick 1992; Broman 1994; Salt and Eaton 1996; Millan 1999). The majority of work carried out in the field of thalamic neurotransmitters has been in the so-called relay nuclei, of which the ventrobasal complex (ventroposterolateral and ventroposteromedial nuclei) is the somatosensory representative. Some of this function pertains to nociception, but it is important to remember that the ventrobasal complex (VB) has an important role in the processing of non-nociceptive somatosensory information and that many other thalamic nuclei (whose detailed transmitter functions are much less well studied) also participate in nociceptive functions.

Ascending Sensory Input

There is overwhelming neuroanatomical evidence, at both the light-microscopical and ultrastructural levels, to favour a neurotransmitter role for glutamate in the ascending afferent fibres in several mammalian species including rodents and primates (Broman 1994). These fibres impinge upon ionotropic ► glutamate receptors of the ► NMDA and ► AMPA varieties located upon proximal dendrites of VB thalamic relay neurones (Broman 1994; Liu 1997). Ascending afferents to other thalamic nuclei that may be important in nociception are probably also glutamatergic (Broman 1994). Electrophysiological studies confirm a functional role for

these receptor types in somatosensory transmission to the VB in rodents and primates (Salt and Eaton 1991; Dougherty et al. 1996) and it appears probable that, as in many other central synapses, the initial synaptic response is mediated *via* ► AMPA receptors with a following longer duration NMDA receptor mediated component which may become more prominent upon repetitive stimulation (Salt and Eaton 1991).

Cortico-Thalamic Input

The cortical inputs to thalamic relay neurones have been a focus of much study and speculation over many years (Sherman and Guillery 2000). Electrophysiological studies of these pathways have focussed on the role of NMDA receptors and, latterly, ► metabotropic glutamate (mGlu) receptors (Salt 2002). A particular focus has been the function of mGlu1 receptors, as these have been localised postsynaptically beneath terminals of cortico-thalamic fibres (Martin et al. 1992). However, it is also evident that there is a contribution from AMPA receptors to cortico-thalamic transmission (Golshani et al. 2001), a finding supported by ultrastructural evidence which indicates that there are AMPA receptor subunits that are predominantly GluR2/3 and GluR4 located postsynaptically at cortico-thalamic synapses in VB (Golshani et al. 2001). More recently a low level of ► kainate receptor subunits (GluR5/6/7) has been found postsynaptically beneath corticothalamic synapses in VB, although a synaptic role for these receptors has not been detected at this location.

Inhibitory Interneurones

► GABAergic inhibitory interneurones are a prominent feature of thalamic relay nuclei, and it is well known that ► GABAA and ► GABAB receptors play a prominent part in synaptic processing at both the pre- and post-synaptic level (Crunelli and Leresche 1991). There are two major groups of GABAergic neurones in the thalamic relay nuclei: the intrinsic Golgi II type interneurones, and the neurones of the ► thalamic reticular nucleus (TRN) which exert their influence *via* their projection into the relay nuclei (Ralston 1983). In rodents, only the latter population appears to be present (Ralston 1983) and performs a profound gating function upon thalamic transmission (Sherman and Guillery 2000). These GABAergic mechanisms may play an important part in the processing of sensory information in both acute and chronic nociception (Roberts et al. 1992). Intriguingly the GABAergic output from TRN is itself modulated by metabotropic ► glutamate receptors (Salt 2002) and ► kainate receptors (Binns et al. 2003). Such mechanisms indicate that sensory transmission through VB is not only dependent upon excitatory transmission, but that reduction of inhibitory transmission (i.e. functional disinhibition) could also have a significant potentiating influence on transmission.

Glia

The concept that glial cells or astrocytes may be active participants in brain function is supported by several findings, including that astrocytes possess ion channels and neurotransmitter receptors for a variety of neurotransmitters, and that astrocytes contain and can release excitatory amino acids such as glutamate and homocysteate (Haydon 2001). It is known that, in the ventrobasal thalamus, activity in astrocytes can evoke NMDA-receptor mediated responses in thalamic relay neurones *in vitro* (Parri et al. 2001), and that homocysteate can be released from thalamus *in vivo* and activate NMDA receptors (Do et al. 2004). This raises the possibility that astrocytes play a key role in the responses of thalamic neurones to sensory stimuli.

Neurotransmitters and Thalamic Integrative Function in Nociception

A role for NMDA receptors in the signalling of acute thermal and mechanical nociceptive responses in the VB thalamus at the single-neurone and behavioural level is now well established (Salt and Eaton 1996; Millan 1990). However, it is important to note that transmission of non-nociceptive sensory information to the thalamus can also show substantial NMDA receptor involvement (Salt and Eaton 1996). In addition, *both* Group I mGlu (mGlu1 and mGlu5) receptors also participate in the signalling of acute nociceptive information but not in the signalling of non-nociceptive mechanoreceptor input to the VB thalamus (Salt and Binns 2000). This functional distinction is intriguing, as there appears to be remarkable anatomical and neurochemical similarity between lemniscal (which carries non-nociceptive information) and spinothalamic (which carries nociceptive and convergent multimodal somatosensory information) inputs to the ventrobasal thalamus (Ralston 1983; Ma et al. 1987; Liu 1997). In view of this, it is conceivable that the recruitment of additional neural circuitry during noxious stimuli underlies the Group I mGlu receptor involvement in nociceptive responses. A possible source of this additional input could be the dense cortico-thalamic projection (Eaton and Salt 1995), which is known to be glutamatergic and which impinges upon mGlu receptors, particularly mGlu1 (see above). This is a particularly attractive hypothesis in the case of mGlu1 receptors, as these are restricted to corticothalamic synapses and because NMDA-receptor mediated responses have been shown to be modulated by activation of Group I (i.e. mGlu1 / mGlu5) receptors in several brain areas, as has modulation of AMPA-receptor mediated responses. In the VB, activation of mGlu1 receptors potentiates responses mediated *via* either AMPA or NMDA receptors *in vivo* (Salt and Binns 2000). It is probable that this is due to the direct effects of mGlu1 activation on neuronal membrane potential and resistance rather than a specific interaction at the receptor level, or that the potentiation that is seen is

a combination of these factors (Salt and Binns 2000). Thus the cortico-thalamic input would be able to exert a profound influence on ionotropic receptor mediated responses, if the sensory stimulus was appropriate to recruit activity in the cortico-thalamic output. This may well be the case for nociceptive stimuli (Eaton and Salt 1995; Millan 1999). A further enabling factor could be the removal of the inhibitory influence arising from the TRN (see above) (Roberts et al. 1992), and in this respect it is interesting to note that TRN neurones are inhibited by noxious peripheral stimulation (Peschanski et al. 1980).

Modulatory Systems

Transmission through the thalamic relay nuclei, including those serving somatosensation and nociception, can be modulated by amine neurotransmitters such as serotonin, noradrenaline or acetylcholine (McCormick 1992). These systems appear to be associated with activating systems that govern states of wakefulness and arousal, and it is unclear to what extent these specifically affect nociceptive processing at the thalamic level. In addition, the nitric oxide (NO) system is associated with some of these activating systems (Vincent 2000), and it has been shown that NO can modulate somatosensory transmission through the thalamus (Shaw and Salt 1997). Similarly, a number of ► neuropeptides have been located in thalamic nuclei and afferents, but their function remains unclear (Sherman and Guillery 2000). Of particular interest to nociceptive processing is the finding that ► cannabinoid receptors modulate acute nociceptive responses of VB neurones in the rat (Martin et al. 1996). However the precise mechanisms of this action remain to be elucidated.

Thalamic Transmitter Mechanisms and Adaptive Changes

It is known that thalamic neurones change their response and firing characteristics in conditions of chronic pain or chronic pain models, and a role for mGlu receptors and NMDA receptors in models of synaptic plasticity has been known for some time. Thus it is conceivable that changes in thalamic neurone responses may be due to changes in glutamate receptor function and may even be a consequence of activation of these receptors as has been suggested for the spinal cord (Willis 2002). Indeed there is already evidence to suggest that NMDA receptors in the thalamus are involved in inflammation-produced hyperalgesia in the rat. In arthritic rats, decreases in thalamic expression (including VB) of mRNA for mGlu1, mGlu4 and mGlu7 receptors have been observed (Neto et al. 2000). Interestingly, in these same animals, mGlu3 mRNA expression is elevated in the TRN and this expression appears to be both in presumed GABAergic neurones and in glial cells (Neto et al. 2000). Furthermore, injection into TRN of an antagonist for this receptor was found to be anti-hyperalgesic in such rats (Neto and Castro-Lopes 2000). Thus it may be that thalamic mGlu receptor mechanisms are important in both the induction of hyperalgesia and in the expression of hyperalgesic behaviours. It is noteworthy, however, that changes in other thalamic transmitter systems may also occur in chronic pain conditions, for example in the serotonergic system (Goettl et al. 2002). It is therefore important to note that transmitter systems should not be regarded in isolation.

Conclusions

It is evident that glutamate receptors are of fundamental importance in the transmission of nociceptive and other sensory information through the thalamus. Activation of NMDA receptors and certain mGlu receptors may be particularly important in the signalling and processing of nociceptive information, as well as in the induction of longer-term plastic changes in response to chronic noxious stimulation or injury. Molecular intervention at some of these sites may have considerable therapeutic potential.

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Nociceptive Pain

Definition

Pain caused by ongoing activation of Aδ and C nociceptors in response to a noxious stimulus of somatic or visceral structures such as inflammation, trauma, or disease.

- ► Analgesic Guidelines for Infants and Children
- ► Cancer Pain, Goals of a Comprehensive Assessment
- ► Cancer Pain Management, Treatment of Neuropathic Components
- ► Complex Chronic Pain in Children, Interdisciplinary Treatment
- ► Guillain-Barré Syndrome
- ► Opioids in Geriatric Application
- ► Opioid Responsiveness in Cancer Pain Management

Nociceptive Pathways

Definition

Neural circuits, including long sensory tracts, which convey information related to noxious stimuli are called nociceptive pathways. The consequences of nociceptive processing can include pain sensation, motivational-affective responses, reflex behavior, endocrine changes, and learning and memory of painful events.

- ► Nociceptive Circuitry in the Spinal Cord
- ► Spinothalamic Tract Neurons, Descending Control by Brainstem Neurons

Nociceptive Primary Afferents

Definition

Primary afferent neurons that respond to tissue damaging stimuli.

- ► Nociceptive Afferents
- ► Opioid receptors at postsynaptic sites

Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology

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Synonyms

Amygdala, Nociceptive Processing

Definition

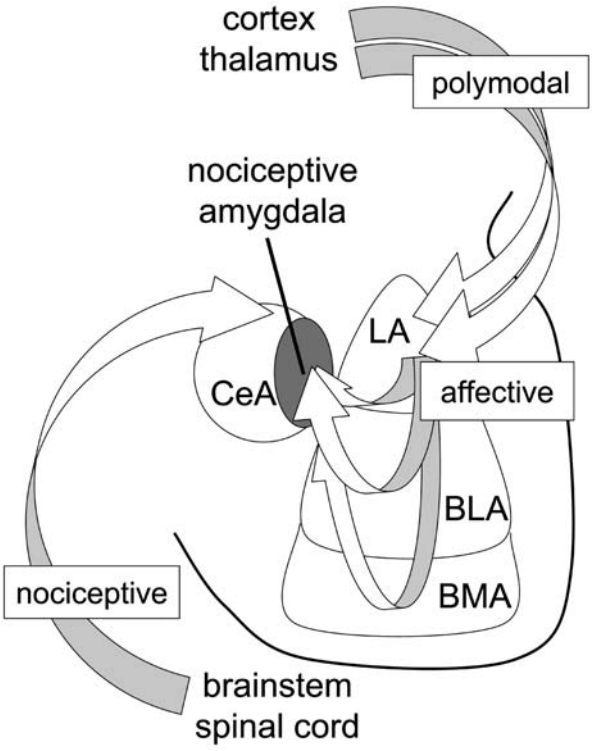
The amygdala is an almond-shaped structure in the medial temporal lobe and consists of several functionally and pharmacologically distinct nuclei. The central nucleus of the amygdala (CeA), which has been designated as the "► nociceptive amygdala", plays an important role in pain processing and pain modulation.

Characteristics

As part of the limbic system the amygdala plays a key role in attaching emotional significance to sensory stimuli, emotional learning and memory and affective states and disorders. The amygdala receives information from all sensory modalities; it also processes nociceptive information and projects to pain modulatory systems through forebrain and brainstem connections. Accumulating evidence suggests that the amygdala integrates nociceptive information with affective content, contributes to the emotional response to pain and serves as a neuronal interface for the reciprocal relationship between pain and affective states and disorders.

Anatomy and Circuitry

The amygdala includes at least 12 different nuclei. The lateral, basolateral, basomedial and central nuclei of the amygdala (LA, BLA, BMA and CeA, respectively) are of particular importance for the processing and evaluation of sensory information (Fig. 1). The LA is an input region; receiving sensory information from the thalamus; particularly the posterior areas and cortex, including insular cortex and association cortical areas (LeDoux 2000; Neugebauer et al. 2004; Pare et al. 2004; Price 2003). The LA represents the initial site of sensory convergence, processing and associative learning and plasticity in the amygdala (LeDoux 2000; Pare et al. 2004; Shi and Davis 1999). This highly processed information, which is a key element of the fear- and anxiety-related circuitry, is then transmitted to other amygdaloid nuclei, including the CeA (LeDoux 2000; Neugebauer et al. 2004; Pare et al. 2004).



Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology, Figure 1 Circuitry of information processing in the principal sensory nuclei of the amygdala. The lateral nucleus of the amygdala (LA) receives and integrates polymodal information from thalamic and cortical areas. This highly processed information with affective content is then distributed to other amygdaloid nuclei, including the central nucleus (CeA), either directly or through the basolateral (BLA) and basomedial (BMA) nuclei. The CeA is the major output nucleus of the amygdala and forms widespread connections with forebrain and brainstem areas. The laterocapsular division of the CeA represents the "nociceptive amygdala".

The CeA serves as the output nucleus of major amygdala functions. The CeA integrates inputs from other amygdala nuclei without forming reciprocal intra-amygdaloid connections. Sensory information reaches the CeA from the LA, either directly or indirectly, as well as from the brainstem (parabrachial area, PB) and spinal cord (Bernard et al. 1996; Burstein and Potrebic 1993; Gauriau and Bernard 2002; Neugebauer et al. 2004). Contextual representations are transmitted from the hippocampus to the CeA through the BLA and BMA (LeDoux 2000).

The CeA forms widespread connections with various forebrain and brainstem areas that are involved in emotional behavior and emotional experience and regulate autonomic and somatomotor functions. Targets of CeA projections include the cholinergic basal forebrain nuclei and the ► bed nucleus of the stria terminalis, midline and mediodorsal thalamic nuclei and paraventricular hypothalamus *via* the ► stria terminalis and lateral hypothalamus and brainstem areas such as periaqueductal gray (PAG) and parabrachial area (PB) *via* the ► ventral amygdaloid pathway (LeDoux 2000; Neugebauer et al. 2004; Price 2003).

Nociception and Nociceptive Plasticity

Within the CeA, the laterocapsular division is defined as the "nociceptive amygdala" because of the high content of neurons that respond exclusively or predominantly to ► noxious stimuli (Bernard et al. 1996; Neugebauer et al. 2004). The latero-capsular CeA receives nociceptive-specific information from the spinal cord and brainstem through the spino-parabrachio-amygdaloid pain pathway (Bernard et al. 1996; Gauriau and Bernard 2002) as well as through direct projections from the spinal cord (Burstein and Potrebic 1993).

Electrophysiological single-unit analysis in anesthetized rats has shown several characteristics of neurons in the nociceptive amygdala (Bernard et al. 1996; Gauriau and Bernard 2002; Neugebauer et al. 2004; Neugebauer and Li 2002). The majority of these neurons (80%) respond either exclusively ("nociceptive-specific" [NS] neurons) or predominantly ("multireceptive" [MR] neurons) to noxious stimuli. More neurons are excited than inhibited by noxious stimuli. A significant number

of "non-responsive" (NR) neurons (up to 20%) also exist in the latero-capsular CeA; they do not respond to somatic stimuli. NS and MR CeA neurons have large, mostly symmetrical bilateral receptive fields in the superficial and deep tissue; they respond to mechanical and thermal stimuli. Their stimulus-response functions are not monotonically increasing linearly but sigmoidaly. These properties argue against a sensory-discriminative function of CeA neurons. Among neurons with predominantly cutaneous input, there appear to be more NS than MR neurons whereas a larger percentage of MR neurons can be found among neurons with receptive fields mainly in the deep tissue. It is believed that NS neurons receive input from the spino-parabrachio-amygdaloid pathway whereas MR neurons integrate nociceptive information with affective content from the polymodal LA-BLA circuitry (Fig. 1).

Accumulating evidence now suggests that neurons in the nociceptive amygdala develop plasticity in models of persistent inflammatory pain such as arthritis and colitis (Bird et al. 2005; Han and Neugebauer 2004; Han et al. 2005; Neugebauer and Li 2003; Neugebauer et al. 2003). Extracellular single-unit recordings in anesthetized rats showed that MR neurons and NR neurons, but not NS neurons, become sensitized to afferent inputs in a model of arthritis pain induced in one knee joint by the intraarticular injection of kaolin and carrageenan. Characteristics of the pain-related sensitization of MR neurons are as follows: the processing of mechanical, but not thermal, pain-related information is increased (upward shift of the stimulus-response functions); responses to stimulation of the arthritic knee as well as of non-injured tissue in other parts of the body are enhanced; the total size of the receptive field expands; a constant input evoked by orthodromic electrical stimulation in the PB produces greater activation; background activity is increased. Unlike changes in the peripheral nervous system and spinal cord in this arthritis model, changes in MR amygdala neurons develop with a biphasic time course; the first phase (1–3 h) reflects changes at the spinal cord and brainstem levels whereas the persistent plateau phase (>5 h) involves intra-amygdala plasticity. MR neurons serve to integrate and evaluate sensory-affective information in the context of pain. NS neurons would continue to distinguish between noxious and ► Innocuous Input/Stimulus at the stage of plasticity.

Evidence that the sensitization of amygdala neurons involves plastic changes within the amygdala comes from electrophysiological studies in brain slices *in vitro*. Coronal slices containing the amygdala were obtained from normal rats, from rats with a ► Kaolin-Carrageenan Induced Arthritis and from rats with a zymosan-induced colitis (Han and Neugebauer 2004; Han et al. 2004; Neugebauer et al. 2003). Whole-cell patch-clamp recordings in slices from rats with arthritis or colitis (6 h post induction) showed enhanced synaptic transmission and increased neuronal excitability of CeA neurons with input from the PB and from the BLA (resembling the MR neurons *in vivo*). Synaptic plasticity in the reduced preparation is thus maintained at least in part independently of continuous input from the site of the somatic or visceral inflammation.

Pharmacology of Nociception and Plasticity

The roles of ionotropic and metabotropic glutamate receptors in brief nociceptive processing and persistent pain have been studied in CeA neurons.

Ionotropic Glutamate Receptors (Bird et al. 2005; Li and Neugebauer 2004b)

Extracellular single-unit recordings of CeA neurons in anesthetized animals showed that non-NMDA receptors are involved in the responses to innocuous and brief (15 s) noxious stimuli whereas NMDA receptors contribute only to the processing of nociceptive information. In the kaolin / carrageenan arthritis pain model (6 h post induction), activation of NMDA and non-NMDA receptors is required for the pain-related sensitization of CeA neurons. In these studies, antagonists at NMDA receptors (AP5) and non-NMDA (NBQX) receptors were administered into the CeA by microdialysis.

Pain-related synaptic plasticity recorded (patch-clamp) in the CeA in brain slices from arthritic rats involves enhanced function of postsynaptic NMDA receptors through PKA-dependent phosphorylation of the NR1 subunit.

Metabotropic Glutamate Receptors (Li and Neugebauer 2004a; Neugebauer et al. 2003)

Electrophysiological studies of amygdala neurons *in vivo* and *in vitro* have shown an important role of group I metabotropic glutamate receptors (mGluRs), which include the mGluR1 and mGluR5 subtypes and couple to G-proteins to activate phospholipase C, PKC and MAP kinases such as ERK. Extracellular single-unit recordings of CeA neurons in anesthetized rats suggest a change of mGluR1 function in the amygdala in pain-related sensitization, whereas mGluR5 is involved in brief as well as prolonged nociception. Activation of group I mGluR1 and mGluR5 by the agonist DHPG enhances the responses of CeA neurons to brief (15 s) innocuous and noxious stimuli under normal conditions. This effect can be mimicked by an mGluR5 agonist (CHPG). In the kaolin / carrageenan arthritis pain model (6 h post induction), the facilitatory effects of DHPG, but not CHPG, increased. Block of mGluR1 by CPCCOEt inhibits the responses of sensitized CeA neurons in the arthritis pain state but has no effect under normal conditions before arthritis. An mGluR5 antagonist (MPEP) inhibits both brief nociceptive responses under normal conditions and prolonged nociception in the arthritis pain model. Agonists and antagonists were

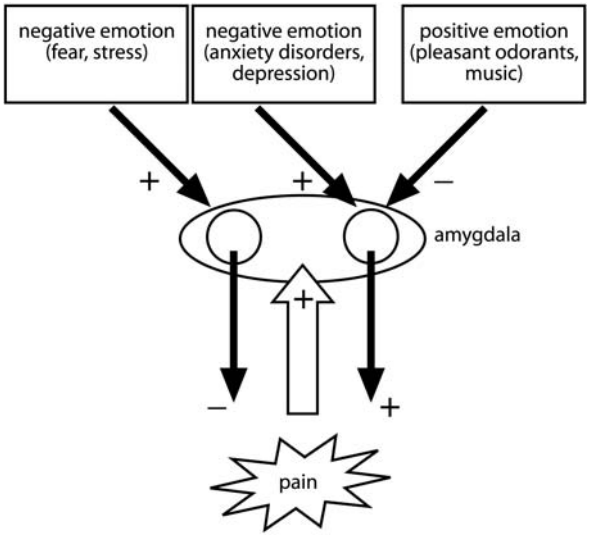
administered into the CeA by microdialysis. The roles of group II and III mGluRs in nociception and plasticity in the amygdala are not yet clear.

The contribution of group I mGluRs to normal synaptic transmission and pain-related synaptic plasticity in the amygdala was analyzed in brain slices *in vitro* using whole-cell voltage-clamp recordings of neurons in the nociceptive amygdala (Neugebauer et al. 2003). Synaptic transmission was studied at the nociceptive PB-CeA synapse and the polymodal-affective BLA-CeA synapse (Fig. 1). A group I mGluR1 and mGluR5 agonist (DHPG) and a mGluR5 agonist (CHPG) potentiate normal synaptic transmission similarly in CeA neurons in slices from normal rats. In slices from arthritic rats (6 h post induction), the effects of DHPG, but not CHPG, are increased, suggesting an enhanced function of mGluR1 rather than mGluR5. Block of mGluR1 with an antagonist (CPCCOEt) has no effect on synaptic transmission in CeA neurons in slices from normal rats but inhibits synaptic plasticity in slices from arthritic rats. An mGluR5 antagonist (MPEP) inhibits basal synaptic transmission in CeA neurons in slices from normal rats and synaptic plasticity in slices from arthritic rats. Thus, enhanced receptor activation of mGluR1 appears to be a key mechanism of pain-related synaptic plasticity in the CeA. Importantly, these agents had no effect on membrane properties and neuronal excitability but affected paired-pulse facilitation, suggesting a pre- rather than post-synaptic site of action. These data suggest that pain-related plastic changes in the amygdala involve a critical switch of presynaptic mGluR1 function.

Pain Modulation by the Amygdala

As part of the pain system and a key player in affective states and disorders, the amygdala contributes to the emotional response to pain and its modulation by affective state (Fig. 2). The CeA, including the latero-capsular division, forms direct and indirect connections with descending pain-modulating systems in the brainstem (Neugebauer et al. 2004). Descending pain control can be facilitatory (pro-nociceptive) and inhibitory (anti-nociceptive) (Gebhart 2004; Heinricher and McGaraughty 1999). Recent behavioral studies suggest that the consequence of pain-related plasticity in the CeA is increased pain. Pharmacologic inactivation of the CeA with mGluR1, mGluR5 or CGRP1 receptor antagonists inhibited pain behavior of arthritic rats (Han and Neugebauer 2005; Han et al. 2005).

Activity in the amygdala can be modified by negative and positive emotions, which in turn are known to reduce (stress, fear; music) or enhance (anxiety) pain (Fig. 2). The dependence of amygdala activity on affective state and the dual coupling of the amygdala to inhibitory and facilitatory pain control may explain some of the differential effects of amygdala stimulation and / or activation on pain behavior.



Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology, Figure 2 Pain, emotions, and the amygdala: a hypothetical model. Pain produces plastic changes in the amygdala. Affective states also modify activity in the amygdala; negative emotions generally increase amygdala activity, whereas positive emotions have been shown to deactivate the amygdala. The amygdala is linked to facilitatory and inhibitory pathways to modulate pain. Negative emotions associated with pain reduction (fear and stress) would activate amygdala-linked inhibitory control systems, whereas negative affective states that correlate with increased pain (depression and anxiety disorders) would activate pain-facilitating pathways. Positive emotions inhibit amygdala coupling to pain facilitation. Reprinted with permission of Sage Publications, Inc., from Neugebauer et al. (2004) The amygdala and persistent pain. Neuroscientist 10, p 232.

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Nociceptive Processing in the Cingulate Cortex, Behavioral Studies in Animals

► Cingulate Cortex, Nociceptive Processing, Behavioral Studies in Animals

Nociceptive Processing in the Cingulate Cortex, Behavioral Studies in Human

► Pain Processing in the Cingulate Cortex, Behavioral Studies in Humans

Nociceptive Processing in the Hippocampus and Entorhinal Cortex, Neurophysiology and Pharmacology

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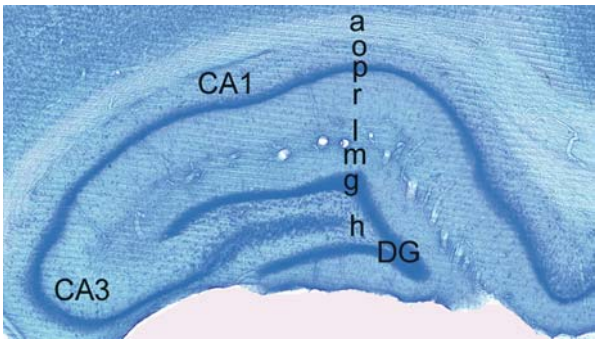
Definition

The hippocampus is the simplest part of the cortex, the ► allocortex, which, in humans, is arched around the mesencephalon, while in rodents it arches over the thalamus (Amaral and Witter 1995). The various fields of the hippocampus and its layers are illustrated in Figure 1. The principal neurons in the hippocampus are



Nociceptive Processing in the Hippocampus and Entorhinal Cortex, Neurophysiology and Pharmacology, Figure 1 Digitized image of Nissl stained transverse section taken through dorsal hippocampus and dentate gyrus (DG) of rat. The hippocampus is sub-divided into various fields, of which the prominent ones are CA1 and CA3. In a dorsal to ventral order in the transverse section, the layers of the hippocampus and DG are: a, alveus (which is as a fiber bundle marking the outer boundary of the hippocampus); o, stratum oriens; p, stratum pyramidale; r, stratum radiatum; l, stratum lacunosum-moleculare; m, stratum moleculare; g, stratum granulosum; h, hilus.

the ► pyramidal cells that are localized in the stratum pyramidale (Fig. 1).

Both anatomical and physiological studies suggest that the entorhinal cortex is a major source of afferent input to the hippocampus, either directly or indirectly via the dentate gyrus (Amaral and Witter 1995). Indeed, stimulation of perforant path fibers from the entorhinal cortex evokes both short- and long-latency excitatory responses in CA1, which are suggested to involve direct entorhinal to CA1 projection, and a ► sequential relay from the entorhinal cortex to CA1 via the dentate gyrus and CA3, respectively.

Characteristics

Melzack and Casey (1968) proposed that the limbic forebrain structures, including the hippocampus, are involved in 'aversive drive and affect that comprise the motivational dimension of pain'. Indeed, lesions of the hippocampus-dentate gyrus region reduce aversive foot shock-induced ► conditioned place avoidance (Selden et al. 1991), while intra-hippocampal administration of a NMDA receptor antagonist attenuated nociceptive behaviors to hind paw injection of the algogen formalin (► formalin test), a model of persistent clinical inflammatory pain (McKenna and Melzack 2001).

Consistent with a role in affect-motivation, a functional magnetic resonance imaging study reported that the anxiety-induced hyperalgesia in man was associated with bilateral activation in the entorhinal area, which correlated with activity in anterior cingulate cortex and insular cortex (Ploghaus et al. 2001). Furthermore, the hippocampus was also activated on peripheral application of high intensity noxious heat stimulation, relative to similar application of the stimulus at a lower intensity (Ploghaus et al. 2001). Indeed, and consistent with a role as a central intensity monitor, electrophys-

Nociceptive Processing in the Brainstem

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Introduction

The craniofacial region is principally innervated by the trigeminal (V) nerve, which terminates in the trigeminal brainstem nuclear complex (TBNC). The caudal portion of the TBNC is largely homologous to the spinal cord dorsal horn in terms of anatomy, neurochemistry and physiology and is termed the subnucleus caudalis (Vc) or medullary dorsal horn. It is the major region involved in the processing of pain and temperature sensations from the head. However, the more rostrally located subnuclei interpolaris (Vi) and oralis (Vo) also receive some nociceptive afferents and contain neurons responding to noxious stimuli. There is evidence that these subnuclei contribute to the perception of pain and especially pain of intraoral origin. There is no obvious homologous region in the spinal cord.

The craniofacial region contains several unique structures which include the tooth pulp and the cornea, as well as several other deep and intraoral tissues such as the temporomandibular joint (TMJ), the intracranial meninges and vessels and the intraoral mucosa. Pathological changes in these structures or their central representations can result in several pain conditions unique to the trigeminal region and these are described in the field ► orofacial pain. There is mounting evidence for sex differences in pain and analgesia, e.g. temporomandibular disorders (TMD) and migraine headaches are much more prevalent in females. Experimental studies in both animals and humans are revealing clear sex differences in peripheral and central neural processes underlying craniofacial nociception (e.g. Cairns et al. 2003; Okamoto et al. 2003). For further details see ► trigeminal brainstem nuclear complex, anatomy ► trigeminal brainstem nuclear complex, physiology ► trigeminal brainstem nuclear complex ► immunohistochemistry and neurochemistry ► nociceptors in the dental pulp ► ocular nociceptors ► nociceptors in the orofacial region (temporomandibular joint and masseter muscle) ► nociceptors in the orofacial region (meningeal/cerebrovascular) ► nociceptors in the orofacial Region (skin/mucosa).

Role of the Vc in Orofacial Nociceptive Processing

Several of the essays point out that most of the small diameter trigeminal (V) primary afferents terminate in the Vc, which is a laminated structure with many morphological and functional similarities with the spinal dorsal horn (although see below). There are 7 lines of evidence that the Vc is critical in V brainstem nociceptive processing. These include a direct projection to higher brain centers involved in pain perception and other aspects of pain behavior. In addition, by virtue of these ascending projections, the Vc has recently been documented to be essential for the expression of central sensitization in rostral elements of the V brainstem complex and ventrobasal thalamus (e.g. the Vo) (Chiang et al. 2002; Chiang et al. 2003). The extensive range of convergent afferent inputs to the Vc contributes to the development of the central sensitization that can be induced by inflammation or injury of peripheral tissues or nerves. Moreover, most Vc nociceptive neurons have in addition to a cutaneous receptive field (RF) also a deep RF (e.g. in the TMJ, muscle, tooth pulp, dura). The particular efficacy of deep nociceptive afferent inputs in inducing central sensitization, including an expansion of both their cutaneous and deep RFs, represent neuronal properties that may explain the poor localization of deep pain, as well as contribute to the spread and referral of pains that are typical of deep pain conditions such as TMD, toothaches and headaches (see ► orofacial pain).

The neurons in the Trigeminal Complex (TBNC) and particularly those in the Vc are subject to modulatory influences originating locally within the Vc or in more rostral parts of the complex as well as descending modulatory influences from brainstem, in particular the rostral ventromedial medulla (RVM) (Dubner and Ren 2004). These descending influences are very similar to those directed at the spinal dorsal horn and are described in ► descending modulation of nociceptive processing.

As mentioned above, a unique feature of the V system is the processing of nociceptive inputs from afferents supplying structures not found elsewhere in the body; these include the tooth pulp, periodontal tissues, cornea and nasal mucosa. Another unique feature is the dual organization of the Vc in the representation of some orofacial tissues. Some noxious stimuli (e.g. to cornea or TMJ) evoke a bimodal distribution of c-fos labeled neurons in the Vc that includes c-fos expression in the rostral Vc / caudal Vi transitional zone as well as in the transitional region between the caudal part of the Vc and the upper cervical spinal cord (see Bereiter et al. 2000; Dubner and Ren 2004). These findings are consistent with electrophysiological evidence of neurons responsive to corneal or TMJ inputs in one or both of these regions. The caudal Vc merges

without clear boundaries with the cervical dorsal horn, while the rostral Vc forms a distinctive transition region with the Vi. This transition region has a ventral Vi / Vc transition area, which is especially clear in rodents, and a dorsomedial transition area. It is becoming apparent that these 2 areas of the rostral Vc and the caudal Vc each have their own unique morphological and functional features that may be differentially involved in contributing to perceptual, autonomic, endocrine and muscle reflex responses to noxious stimulation of different orofacial tissues, in particular involving ophthalmic structures (Bereiter et al. 2000; Dubner and Ren 2004).

Further investigation of these different areas within the Vc, as well as caudal to the Vc, is needed to determine their specific functional roles in orofacial pain processes. It has been over 50 years since the trigeminal spinal nucleus was divided into Vo, Vi and Vc subdivisions and over 25 years since the structural homologies between the Vc and the spinal dorsal horn were emphasized. These studies have shaped discussions of the special relationship between the Vc and craniofacial pain (see Sessle 2000; Sessle 2005), although this useful homology may need some revision as recent evidence cited above indicates that select portions of the Vc are organized differently from the spinal system.

The region ventral to the Vc, which is part of the medullary reticular formation and is outside the main projection area of trigeminal primary afferents, also contains neurons that respond to noxious facial stimulation. Their receptive fields are usually larger than those in Vc, often bilateral and sometimes include the entire body (e.g. see Fujino et al. 1996; Nord and Kyler 1968; Yokota et al. 1991). In the rat, a population of neurons with large nociceptive receptive fields including the face is located in the subnucleus reticularis dorsalis. It has been suggested that this region is involved in circuits mediating ► diffuse noxious inhibitory controls (Villanueva et al. 1996).

Role of Rostral Components of V Brainstem Complex

Morphological, physiological, neurochemical and behavioral evidence support the involvement of more rostral components of the TBNC in orofacial nociceptive processing, especially in the case of the Vo. For example, lesions of rostral components may disrupt some pain behaviors and substantial numbers of NS and WDR neurons exist in the rostral components. These neurons have cutaneous RFs that are usually localized to intraoral or perioral areas and many can be activated by tooth pulp stimulation. These findings have raised the possibility that the rostral nociceptive neurons, particularly those in the Vo, are principally involved in intraoral and perioral nociceptive processing. Moreover, it is probable that their nociceptive afferent inputs are predominantly relayed through more caudal regions, such as the Vc, which exerts a considerable modulatory influence over Vo nociceptive processes.

Although some rostral neurons may directly receive primary afferent inputs from the tooth pulp, it is not clear that these inputs are nociceptive. How then can nociceptive phenomena occur in the rostral components when, especially in the case of the Vo, they lack an anatomical and in many instances a neurochemical substrate generally considered necessary for nociceptive processing? One possible explanation is that these substrates, although typical of the Vc and the spinal dorsal horn, are not essential for all types of nociceptive phenomena. In support of this possibility are the observations that nociceptive neurons contributing to nociceptive behavior occur in some spinal cord and brainstem areas devoid of some of these substrates (e.g. lateral cervical nucleus, reticular formation) (Dubner and Bennett 1983).

Another explanation lies in the anatomical and neurochemical framework for nociceptive processing that typifies the Vc and in its ascending projections to the rostral components of the TBNC. Parada et al. (1997) have argued that the cutaneous C fiber related nociceptive responses of Vo cutaneous nociceptive neurons that can be blocked by systemic administration of the NMDA antagonist MK-801 may depend on the well-documented ascending projection from the Vc that exerts a net facilitatory modulatory influence on Vo neurons (for review see Sessle 2000), since the Vc does have the features (NMDA receptors, C-fiber afferent terminals, substantia gelatinosa) considered necessary for these nociceptive phenomena. Furthermore, local application of MK-801 or morphine to the Vc can block the C-fiber related activity of some Vo nociceptive neurons. An analogous argument has also been used as an explanation for the neuroplastic changes that have been documented in the Vo and in the main sensory nucleus subsequent to C-fiber depletion induced by the neonatal application of capsaicin. Nonetheless, MK-801 applied directly to the Vo itself can antagonize Vo nociceptive neuronal changes induced by afferent inputs evoked from the tooth pulp (Park et al. 2001), which suggests that NMDA receptor mechanisms do operate within the Vo (see Sessle 2000 for references and further details).

Collectively, these various findings raise the possibility that, on the one hand, the cutaneous RF and response properties of rostral nociceptive neurons, particularly in the Vo, may be dependent on caudal regions such as the Vc for the relay of nociceptive signals from primary afferents supplying superficial craniofacial tissues. On the other hand, some of the extensive pulp afferent inputs to and effects upon neurons in the rostral TBNC may be dependent on relays in both rostral and

caudal components. Such a view, nonetheless, is still largely speculative and the relative roles of the rostral and caudal TBNC components, not only in cutaneous nociceptive mechanisms but also in nociceptive responses to noxious stimulation of deep craniofacial tissues and tooth pulp, represent an important issue requiring further research.

Ascending Projections and Higher Level Processing of Trigeminal Nociceptive Inputs

The nociceptive signals from the TBNC are relayed on to higher levels and in particular to the thalamus and from there to cerebral cortex. The thalamus receives direct contralateral input from each of the TBNC subnuclei (Kemplay and Webster 1989; Mantle-St John and Tracey 1987). The majority of the TBNC neurons projecting to the contralateral thalamus are found in the trigeminal principal nucleus, terminate in the ventroposterior medial nucleus (VPM) and are primarily involved in tactile sensation. The remaining neurons are located in the Vi, the Vo and the Vc. However, the major thalamic projection related to pain and temperature perception arises from neurons in the contralateral Vc and is equivalent to the spinothalamic tract (for references see Discussion in Craig 2004).

The trigeminothalamic neurons in the Vc are located primarily in laminae I and V. Those in lamina V have a major projection to the VPM (or its borders in the cat), but those in lamina I terminate in several other regions, which are species dependent. For example, in the monkey, lamina I neurons project primarily to the posterior ventromedial nucleus (VMpo) as well as to the ventrocaudal medialis dorsalis (MDvc), but have only a sparse projection to the VPM (Craig 2004). In the cat, the main projections of lamina I neurons are to the ventral border of the VPM and adjacent nuclei, the dorsomedial VPM and the nucleus submedius (Craig 2003). In the rat, lamina I neurons project largely to the VPM, the nucleus submedius, the posterior nucleus and the posterior triangular nucleus (Iwata et al. 1992; Jasmin et al. 2004; Yoshida et al. 1991).

The main cortical targets of thalamic nuclei receiving nociceptive inputs are the insula, the primary and secondary somatosensory cortices and the cingulate. There are extensive connections between cortex and thalamus, which play an important although poorly understood role in processing nociceptive inputs. Most of the electrophysiological studies on responses of thalamic and cortical neurons to noxious stimuli have focused on inputs from the limbs and have revealed neurons with both NS and WDR type responses. These responses are generally similar to those of neurons in the spinal dorsal horn and the trigeminal Vc, but tend to have increased and fluctuating spontaneous activity (see reviews by Kenshalo and Willis 1991; Willis 1997). Sections ► Nociceptive Processing in the Thalamus and ► Cortical and Limbic Mechanisms Mediating Pain and Pain-Related Behavior provide further details of the roles of thalamus and cortex in pain.

In addition to the thalamus, TBNC neurons also project to several diencephalic and brain stem areas that are involved in regulation of autonomic, endocrine, affective and motor functions. In the rat, all TBNC subnuclei contain neurons that project directly to the hypothalamus (Malick and Burstein 1998). Most of these hypothalamic tract neurons in the Vc and C1-2 respond preferentially or exclusively to noxious mechanical and thermal stimulation to the facial skin and to electrical, mechanical and chemical stimulation of the dura mater (Burstein et al. 1998). There also are projections from the TBNC to the parabrachial and Kölliker-Fuse nuclei (Bernard et al. 1989; Feil and Herbert 1995; Hayashi and Tabata 1990). In particular, neurons in the Vc, including those in the superficial laminae, project to the external portion of the lateral parabrachial area, where many respond exclusively to noxious stimuli (Hayashi and Tabata 1990). It has been proposed that this projection is part of a trigeminopontoamygdaloid pathway involved in the affective, behavioral and autonomic reactions to noxious stimuli (Bernard et al. 1989). Anatomical studies also have shown that there are projections from the TBNC to the adjacent reticular formation, the RVM, the periaqueductal gray, various brain stem autonomic nuclei, the superior colliculus, the ipsilateral cerebellum, the contralateral inferior olive and the nucleus of the solitary tract (Bruce et al. 1987; Craig 1995; Jacquin et al. 1989; Mantle-St John and Tracey 1987; Marfurt and Rajchert 1991; Keay and Bandler 1998; Renehan et al. 1986). Several of these projections are likely to be important in mediating nonperceptual (e.g. autonomic) effects elicited by nociceptive stimuli.

In summary, a great deal of information regarding the representation and processing of craniofacial nociceptive inputs in the TBNC and higher levels has been gained in recent years. Although there are many similarities between the spinal and trigeminal systems, there are also some important differences and some of these relate to unique structures and likely contribute to these pain conditions that are unique to this region. Nevertheless, there are still important gaps in our knowledge and further experimental studies are necessary to fully understand the mechanisms underlying the normal and pathological processing in the TBNC of nociceptive inputs from the craniofacial region.

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iological studies conducted in anaesthetized rat provided evidence that noxious stimulus evoked intensity-dependent changes in ► excitability of CA1 pyramidal cells (Khanna and Sinclair 1989, Wei et al. 2000) that were ► non-topographic (Khanna and Sinclair 1992). Interestingly, Khanna (1997) reported that following hind paw injection of formalin, a population of dorsal CA1 putative pyramidal cells was selectively excited against the background of widespread pyramidal cell suppression, reflecting 'signal-to-noise' processing by the hippocampal network that enhanced the 'signal' to noxious stimulus relative to 'background' noise. Such processing was observed in correlation with ► theta ► rhythm (Khanna 1997), which is sinusoidal rhythmic extracellular oscillations that reflect rhythmic oscillations of CA1 neurons in processing of information. Hippocampal theta activation has been linked to ► sensorimotor integration and animal motivated behavior (Bland and Oddie 2001). In addition, CA1 'signal-to-noise' processing, in parallel with theta acti-

vation, has been linked to ► mnemonic function of the hippocampus (Buzsaki 1989). In this context, a noxious stimuli-induced increase in levels of the transcription protein Egr1 has been observed in the hippocampus, especially in field CA1 (Wei et al. 2000). The enhanced level of Egr1 in CA1 was linked to facilitation of ► long-term potentiation (LTP) of excitatory synaptic transmission in the region (Wei et al. 2000), LTP being a cellular model of learning and memory.

Consistent with findings from electrophysiological studies, ► c-Fos mapping techniques in anaesthetized and behaving rats also indicated that neural changes in hippocampus, especially field CA1 and medial entorhinal cortex, are noxious intensity-dependent (Funahashi et al. 1999, Khanna et al. 2004). The changes in CA1 were bilateral and were observed along the length of the region. However, the noxious stimuli-induced effect in entorhinal cortex was significant only ipsilateral to the stimuli, though a trend was also observed in the contralateral entorhinal cortex (Funahashi et al. 1999).

A role for acetylcholine in hippocampal nociceptive processing has been proposed. In this context, acetylcholine is released in the hippocampus in the formalin test (Ceccareli et al. 1999), and intra-hippocampal administration of the muscarinic antagonist, atropine, attenuates peripheral noxious heat-induced suppression of CA1 pyramidal cell synaptic excitability (Zheng and Khanna 2001). Furthermore, destruction of ► cholinergic input to the hippocampus attenuated the pyramidal cell suppression, without an apparent effect on cell excitation to hind paw injection of formalin (Zheng and Khanna 2001). This points to the possibility that the hippocampal cholinergic input influences the background 'noise' of 'signal-to-noise' processing to formalin.

The evidence, that intra-hippocampal administration of NMDA antagonist attenuated animal behavior in the formalin test (McKenna and Melzack 2001), favors a role for glutamate in nociceptive processing in the hippocampus-dentate gyrus. Further, the noxious stimuli-induced increase in Egr1 in hippocampus was blocked by systemic administration of an NMDA receptor antagonist (Wei et al. 2000). Another molecule that has drawn some attention and may be in a position to influence hippocampal processing of noxious information is the cytokine, ► tumor necrosis factor-alpha (TNFα). The levels of this molecule are elevated in the hippocampus-dentate gyrus region after the development of thermal ► hyperalgesia in the rat chronic constriction nerve injury animal model of neuropathic pain (see ► Neuropathic Pain Model, Chronic Constriction Injury) (Ignatowski et al. 1999). Interestingly, TNFα induces thermal hyperalgesia when administered intracerebroventricularly in otherwise normal animals.

In summary, the evidence so far suggests that nociceptive processing in hippocampus and the entorhinal cortex is, at least in part, distributed, non-topographic, and noxious stimulus intensity-dependent, which is in line with the postulated role of these regions in affect-motivation to pain. The processing of noxious information in these regions is likely to be influenced by multiple transmitters/modulators.

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Nociceptive Processing in the Nucleus Accumbens, Neurophysiology and Behavioral Studies

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Synonyms

Nucleus Accumbens; NAcc

Definition

The nucleus accumbens (NAcc) is a brain structure that forms part of the ventral striatum. It has been established that the NAcc is a critical structure for the rewarding and reinforcing properties of addictive drugs. It also appears to play an important role in modulating pain sensations.

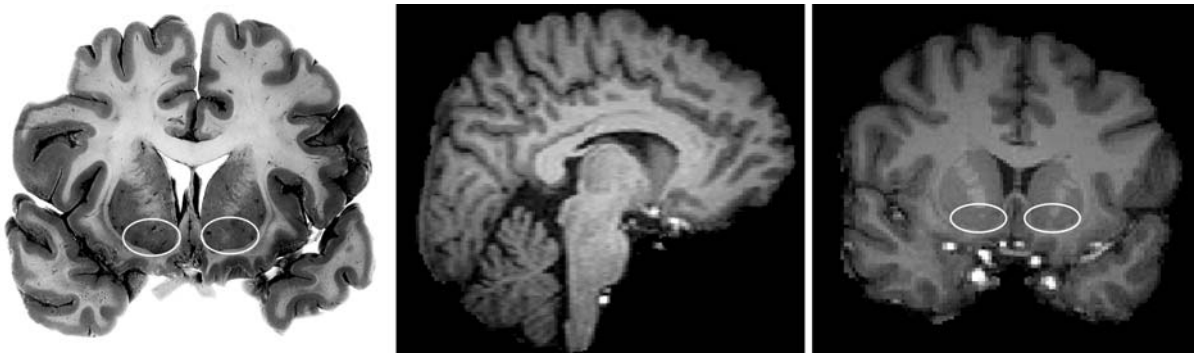
Characteristics

The human ventral striatum encompasses a series of structures of which the nucleus accumbens (NAcc) is the primary one (Nolte 2002). This definition is functionally based on its connectivity. In rodents, the NAcc has been clearly defined by cytoarchitecture, neuroactive transmitters and receptor distributions (Otake and Nakamura 2000). Two main substructures have been identified, a core and a shell. The core is located in the dorsolateral portion of the ventral striatum and the shell is in the medioventral portion (Otake and Nakamura 2000). The core projects directly to the lateral ► ventral tegmental area (VTA), while the shell projects to the ventromedial ventral pallidum, which in turn projects to the VTA. In primates, however, the core region appears contiguous with the rest of the striatum and cannot be easily distinguished. The shell has several histochemical features that make it different from the rest of the striatum (Prensa et al. 2003). The NAcc in humans is located at the base of the ► caudate nucleus and the ► putamen (Fig. 1) (see Buchsbaum et al. 1998 for a systematic approach to identify the NAcc in MRI images). In humans, several histological and chemical studies have given mixed results and concluded that separation of core and shell substructures is difficult, probably due to the complexity and heterogeneity of the structure and its innervation (Prensa et al. 2003).

The NAcc is involved in evaluating probability assessments and the emotional valence of information. Disturbances at this level have been implicated in a number of affective disorders including drug abuse (Altier and Stewart 1999). Recent functional imaging investigations in humans have shown that the NAcc is activated in situations related to drug-associated reward and to monetary, and other rewarding stimuli (Breiter et al. 1997; Zink et al. 2004).

Opioids such as morphine and heroin and psychostimulants such as amphetamine are known drugs of potential abuse. However, it is also clinically known that these drugs are effective in the treatment of pain (Altier and Stewart 1999). These results suggest that reward and aversion share a common neural substrate. Opioids are known to inhibit neural systems that transmit nociceptive information (Fields 2004); the descending pain pathway relays information from the ► periaqueductal gray (PAG) to the ► rostroventral medulla (RVM) and through the spinal cord to the dorsal horn, where peripheral nociceptive information is inhibited from reaching supraspinal structures (Fields 2004). Microinjections of morphine into the PAG produce deep analgesia allowing the performance of major surgery on subjects. Interestingly, opioids as well as psychostimulant drugs increase transmission in mesocorticolimbic dopamine (DA) neurons that are known to be activated by rewards such as food or sex (Altier and Stewart 1999). These neurons arise from cell bodies in the ventral tegmental area (VTA) and project to various sites, among them the NAcc, amygdala and frontal cortex.

A number of studies have suggested a significant role for the NAcc in the processing of pain and analgesia. Experiments in rodents subjected to the ► formalin test (subcutaneous injection of diluted formalin into an animal's hind paw to produce persistent nociceptive pain) have been performed to elucidate the role of NAcc in analgesia. Injection of morphine, amphetamine or substance P agonist into the VTA produces analgesia, i.e., the animal displays fewer effects of pain due to the formalin injection. However, lesions of the NAcc or injection of DA receptor antagonists in the NAcc diminishes the analgesia produced following intra-VTA injections (Altier and Stewart 1999).



Nociceptive Processing in the Nucleus Accumbens, Neurophysiology and Behavioral Studies, Figure 1 Histological and MRI slices of the NAcc. Left panel depicts a brain slice through the anterior part of the caudate nucleus and putamen with the NAcc bridging both structures (Adapted from DE Haines, Neuroanatomy). Right panel shows a corresponding MRI slice of the NAcc.

The role of the NAcc in aversive stimuli such as pain may be as a result of both dopaminergic projections from the VTA region and inputs from spinal levels. Dopamine release in the NAcc modulates information about tonic noxious stimulation coming from the periphery. The broader neuroanatomical circuitry underlying this effect may be explained by looking at the NAcc afferent and efferent connections. One possibility arises from direct projections of spinal cord neurons into the NAcc (Cliffer et al. 1991). Several studies have implicated an ascending pain control pathway that produces analgesia if stimulated by intense noxious stimuli or by peripheral injection of local anesthetic or opioids (Gear and Levine 1995). Another possibility arises from NAcc projections into the medial thalamus known to receive input from dorsal horn neurons sensitive to noxious stimuli. The NAcc has projections to the amygdala, which has connection to the brainstem and from there to the spinal cord (Altier and Stewart 1999).

Other mechanisms, besides dopaminergic ones, have been found to be involved in processing pain by the NAcc. In a series of experiments, animals were subjected to intense noxious stimuli (injection of high concentrations of capsaicin subcutaneously or immersion of hind paws in hot water) and tested for pain in a different body part (Gear and Levine 1995). Under intense stimuli, animals displayed less pain in the stimulated body part than without the stimuli. The mechanism is based on an ascending pain pathway projecting to the NAcc that disinhibits opioid neurons in the NAcc and produces antinociception. There is no direct connection between the NAcc and the RVM; however, the NAcc projects to other structures such as the hypothalamus, which in turn sends projections to the brainstem (Gear and Levine 1995).

In animal studies, it has been established that the NAcc plays an important role in processing pain and several mechanisms have been elucidated and are continuously studied. Human studies, however, present a much larger challenge, given that none of the manipulations done in animals are possible. Yet new neuroimaging techniques have made possible the study of responses in the CNS, including the NAcc, non-invasively in conscious human subjects (Becerra et al. 2001; Breiter et al. 1997). Most of the studies reporting NAcc activation have been devoted to the role of NAcc in processing rewarding stimuli such as drugs of abuse (Breiter et al. 1997) or monetary reward (Zink et al. 2004). For positive valence hedonic stimuli (e.g. food, money), the NAcc was found to produce a positive signal change in ► functional MRI (fMRI) studies (Zink et al. 2004).

Recently, fMRI studies of noxious stimuli in normal subjects have indicated that the NAcc displays negative signal changes (Becerra et al. 2001), opposite to those observed for rewarding stimuli. Further studies will allow dissection of the role of the NAcc in human physiological and pathological pain processing. In a broader scope, the NAcc is part of a group of structures involved in the processing of motivational and emotional information and the structure may be implicated in maladaptive behaviors observed in chronic pain such as depression and anxiety.

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Nociceptive Processing in the Secondary Somatosensory Cortex

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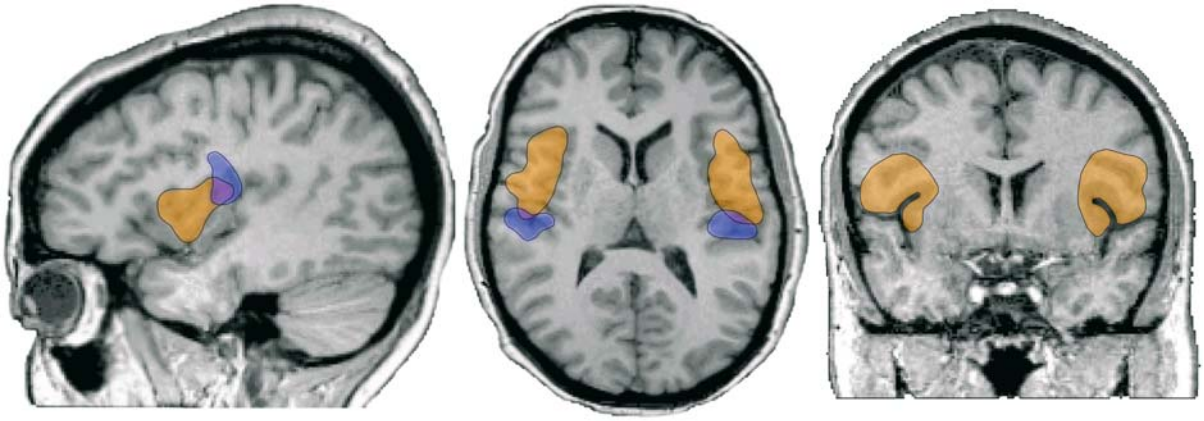
Synonyms

Secondary Somatosensory Cortex; second somatosensory cortex; second somatic area; S2; SII; SII/PV

Definition

The term "secondary somatosensory cortex" refers to a cortical representation of the body outside the primary somatosensory cortex (SI). Such a secondary representation was initially found in two separate locations (for detail see Burton 1986; Caselli 1993), near the midline in the human parietal lobe (supplementary sensory



Nociceptive Processing in the Secondary Somatosensory Cortex, Figure 1 Location of the secondary somatosensory cortex in the human brain. This figure shows schematic drawings of the classical secondary somatosensory area SII as identified using tactile stimulation (blue) in comparison with those areas in the operculo-insular cortex that respond to nociceptive stimulation (orange). Note that the nociceptive cortex extends further anterior than the tactile areas and includes both opercular and insular parts. Left: Sagittal section passing slightly medial of SII in order to show the insula also. Centre: horizontal section through SII. Right: Coronal section through nociceptive regions in the anterior insula and in the inner vertical face of the frontal operculum.

area) and within the parietal ► operculum in the superior bank of the Sylvian or lateral fissure (secondary somatosensory area SII); additional somatosensory areas are situated in the superior parietal lobule and in the ► insula. The somatosensory area in the parietal operculum (Fig. 1) has retained the designation as SII, because historically it was the first example of a second representation of any of the senses in the brain (Adrian 1941).

Characteristics

SII is located on the lateral curvature of parietal cortex in rodents and rabbits, in the anterior ectosylvian gyrus in cats and dogs and along the superior bank of the ► Sylvian fissure in primates (Burton 1986). Human SII does not usually reach the convexity of the brain, but was explored by Wilder Penfield during procedures in which it did reach the convexity or in which the fissure was split (Penfield and Jasper 1954). Functional localization of SII in humans with imaging techniques such as PET and fMRI confirmed its location predominantly in the upper bank of the Sylvian fissure (Burton et al. 1993), opposite the auditory cortex in Heschl's convolutions (Özcan et al. 2005).

The SII region probably contains more than one somatosensory area as suggested by the presence of two separate face areas in the lateral parietal operculum and a common foot area in medial parietal operculum. SII proper is in the caudal (posterior) part of this region, the parietal ventral area PV may be in its rostral (anterior) portion (Disbrow et al. 2000; Fitzgerald et al. 2004). Cytoarchitectonic classification of the parietal operculum has been undertaken only recently (Young et al. 2004), yielding four subregions OP1–OP4, of which SII is probably situated in OP1 / 2 and PV in OP3 / 4. Brodmann areas are not defined in this region. In the stereotactic coordinates of the atlas of Talairach and Tournoux, SII extends 40–60 mm lateral of the midline, 15–30 mm behind the anterior commissure (AC) and 5–25 mm above the AC-PC line. The Sylvian fissure runs obliquely through this coordinate system and its location varies considerably across individuals (Özcan et al. 2005). Therefore, either the auditory cortex or the Sylvian fissure and the central sulcus should be used as anatomical landmarks when referring to the location of SII.

The secondary somatosensory cortex receives direct thalamocortical input from the ventral posterior nuclear group, in particular the ventro-postero-inferior nucleus VPI (Apkarian and Shi 1994; Gauriau and Bernard 2004; Jones and Burton 1976). In addition, there is a prominent projection from SI to SII, particularly in primates (Friedman et al. 1986; Jones et al. 1978).

Single neuron recordings in SII have largely focussed on the tactile representation (Fitzgerald et al. 2004; Robinson and Burton 1980). Although most neurons in SII have contralateral ► receptive fields, a sizable proportion of bilateral receptive fields has been observed. Most imaging and electrophysiological studies in humans have found a bilateral response to unilateral stimulation, with a contralateral preponderance. Functionally, SII is considered to play a role in tactile object recognition and memory (Caselli 1993), as well as the perception of vibrotactile stimuli (Burton et al. 1993; Ferrington and Rowe 1980). The cortico-cortical output connections of SII into the insula and parahippocampal gyrus are similar to those of the inferior temporal cortex, which is implicated in visual object recognition (Friedman et al. 1986).

In human imaging studies, enhanced perfusion as a sign of activation by phasic nociceptive stimuli was found more regularly in SII and adjacent parts of the insula than

in SI (Peyron et al. 2002; Treede et al. 2000). Evoked potential recordings showed that SII was activated simultaneously with or even earlier than SI (Schlereth et al. 2003). Hence, SII appears as a major site for the early cortical encoding of pain in the human brain. SII is considered to be important for the recognition of the noxious nature of a painful stimulus, for intensity coding and other sensory-discriminative aspects of pain and as part of a sensory-limbic pathway for pain memory and affective motivational aspects of pain (Berthier et al. 1988; Lenz et al. 1997; Treede et al. 2000; Ohara et al. 2004). In contrast to the abundance of evidence for nociceptive activation of the SII region from human evoked potential and imaging studies, there are few single neuron recordings in this region showing specific nociceptive responses (Dong et al. 1994). In monkey, some cells in the SII region respond to the approach of a sharp object to the face, suggesting that this region may represent the position of a painful or threatening stimulus in extrapersonal space. These neurons, however, were located in area 7b, which is adjacent to SII in monkey but not in humans, in whom Brodmann areas 39 and 40 separate SII and area 7b. Since search stimuli in all these animal studies were tactile, an intriguing alternative possibility is that tactile and nociceptive inputs are represented in different areas within the SII region (Treede et al. 2000). The insula has been suggested to contain such a separate representation of nociception at its dorsal junction with the parietal and frontal operculum (Craig 2002). The insula subserves sensory integrative functions for pain, taste and other visceral sensations, as well as visceral motor and other autonomic functions (Treede et al. 2000). Direct comparisons of vibrotactile and painful heat stimulation in humans showed activation of SII by both stimuli and a more pronounced activation in anterior insula by painful stimuli (Coghill et al. 1994). It has been suggested that the dorsal and anterior insula may be part of a multisensory interoceptive pathway signalling the internal state of the body. In this view, pain is an emotion or interoceptive sensation resulting from disequilibrium of this internal state (Craig 2002). This hypothesis is called into question by recent studies suggesting that interoceptive sensations and emotions are infrequently evoked by stimulation of this system (Lenz et al. 2004). Subdural and depth electrophysiological recordings in patients undergoing epilepsy surgery have identified a region in the inner vertical face of the frontal operculum that receives nociceptive input with a latency of about 150 ms, corresponding to the earliest portion of the whole cerebral response (Frot and Maguiere 2003; Vogel 2003). The centre of this region is located about 15–20 mm anterior of the tactile SII area, and lateral of the anterior insula which lies on the other side of the circular sulcus of the insula (Fig. 1). Because of this proximity, many neuroimaging studies may mislabel the inner vertical face of the frontal operculum as either SII or anterior insula. In current meta-analyses of neuroimaging studies (PET, fMRI, MEG and EEG source analysis) it was found that the resolution of the techniques was not sufficient to separate insular from opercular activity (Peyron et al. 2002). More studies are needed to clarify how many functionally and cytoarchitectonically separate areas are contained within the parasylvian cortex. It is evident, however, that the parasylvian cortex plays an important role in the cortical representation of pain. This region includes SII, but extends further anterior into the frontal operculum and medially into the insula.

Acknowledgements

Drs Treede, Baumgärtner and Lenz and some of the studies reviewed here were supported by DFG Tr 236 / 13-3 and by NIH-NINDS 38493.

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Nociceptive Projecting Neurons

- ► Spinoannular
- ► Spinohypothalamic Tract, Anatomical Organization and Response Properties
- ► Spinomesencephalic Tract
- ► Spinoparabrachial Tract
- ► Spinoreticular Neurons
- ► Spinothalamic Tract Neurons, Visceral Input

Nociceptive Reflex

Definition

A reflex that is elicited by noxious stimuli, is mediated by nociceptive (Aδ, C) afferents, and exerts a defense reaction.

- ► Jaw-Muscle Silent Periods (Exteroceptive Suppression)
- ► Nociceptive Withdrawal Reflex

Nociceptive Selective Stimulation

Definition

Stimulation selectively activates nociceptive (Aδ, C) fibers without simultaneously activating tactile-mediated large myelinated Aβ fibers, e.g. laser stimulation.

- ► Insular Cortex, Neurophysiology and Functional Imaging of Nociceptive Processing

Nociceptive Sensory Neurons

Definition

Nociceptive sensory neurones are specialized sensory nerve fibers innervating peripheral tissues that are normally only activated by noxious stimuli (i.e. the stimuli capable of causing tissue damage) and not innocuous stimuli.

- ► Inflammation, Modulation by Peripheral Cannabinoid Receptors
- ► Nociceptive Afferents
- ► Nociceptors
- ► Nociceptors, Action Pontentials and Post-Firing Excitability Changes

Nociceptive Specific Neurons

Definition

Nociceptive specific sensory neurons are a type of nociceptive neuron, which are excited by stimulus intensities that are sufficiently intense to cause injury to the body if sustained for a sufficiently long period of time. In the absence of injury, this type of neuron will not normally respond to innocuous thermal or innocuous mechanical stimulation of its receptive field. This type of neuron is defined physiologically by its response properties and can be found at spinal, thalamic, and cortical sites important in the processing of noxious information.

- ► Arthritis Model, Kaolin-Carrageenan Induced Arthritis (Knee)
- ► Encoding of Noxious Information in the Spinal Cord
- ► Freezing Model of Cutaneous Hyperalgesia
- ► Human Thalamic Nociceptive Neurons
- ► Referred Muscle Pain, Assessment
- ► Thalamic Nuclei Involved in Pain, Human and Monkey
- ► Thalamus, Nociceptive Cells in VPI, Cat and Rat
- ► Trigeminal Brainstem Nuclear Complex, Physiology

Nociceptive Processing in the Spinal Cord

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Nociceptive Functions of the Spinal Cord

Nociceptive primary afferent fibres project to the spinal cord, where they form synapses with second order neurons. The spinal cord has several functions in nociceptive processing. Firstly, ascending axons of nociceptive neurons in the gray matter project to supraspinal sites. These projections activate neuronal circuits in the brainstem that are involved in descending inhibition and facilitation and they activate the thalamocortical system that produces the complex conscious pain response. Secondly, the spinal cord generates motor responses to noxious stimuli. Motor responses are nociceptive reflexes and also more complex motor behaviour such as avoidance of movements. Thirdly, the spinal cord is involved in the generation of autonomic reflexes that are elicited by noxious stimuli.
Nociceptive sensory spinal cord neurons are located in the superficial and deep dorsal horn. Neurons with nociceptive properties are also found in the ventral horn. The majority of nociceptive neurons are interneurons that do not project to supraspinal sites (Willis and Coggeshall 2004).

Structure of the Dorsal Horn and Spinal Projection of Nociceptive Afferents

The spinal cord gray matter contains neuronal cell bodies and fibres, whereas the white matter consists of fibres including axons of ascending and descending tracts. The gray matter has been divided into laminae I–X by ► Rexed's Laminae (1952, 1954). The dorsal horn consists of laminae I–VI. For a detailed description see ► nociceptive circuitry in the spinal cord.

Laminae I–VI

Lamina I (► Laminae I and V Neurones), also called the marginal zone of the dorsal horn, is characterized by large horizontal neurons (the Waldeyer cells) and a plexus of numerous horizontally arranged fine axons and dendrites. However, many cells appear much smaller. Lamina II (► Laminae II_{outer} and Lamina II_{inner}), also called ► substantia gelatinosa, consists of numerous small neurons and their processes; myelinated axons are almost absent. Stalked cells and islet cells have been identified, but many interneurons have other morphologies. ► Lamina III forms a broad band across the dorsal horn, and it has slightly larger and more widely spaced cells. Lamina III contains many myelinated fibres. ► Lamina IV is relatively thick and contains many small and numerous large neurons. Lamina V (► lamina I and V neurones) extends as a thick band across the narrowest part of the dorsal horn. Its lateral boundary is indistinct because of the many bundles of myelinated fibres coursing longitudinally through this area, giving the lateral part of this lamina a reticulated appearance. Cells are more varied than in lamina IV. Lamina VI cells are arranged much like those in lamina V (Rexed 1952, 1954; Willis and Coggeshall 2004).

Spinal Termination of Non-nociceptive and Nociceptive Primary Afferent Fibres

In general, non-nociceptive and nociceptive primary afferent fibres have different termination sites in the dorsal horn. Nociceptive Aδ fibres (see ► A Afferent Fibers (Neurons)) project mainly to lamina I (and II). Some Aδ fibres have further projections into lamina V. C fibres (► C afferent axons/fibers) project mainly to lamina II. By contrast, non-nociceptive primary afferents with Aβ fibres (► A afferent fibers) project to lamina III and deeper (Willis and Coggeshall 2004).
It should be noted that the patterns of inputs from different tissues are not identical. The main inputs into lamina II are primary afferents from skin, but visceral and muscular unmyelinated afferents project to lamina II as well (Sugiura et al. 1989). However, visceral and muscular afferents also terminate in laminae I and deeper laminae. It is thought that visceral afferents distribute to a wider area of the cord, but that numbers of terminals for each fibre are much sparser for visceral than for cutaneous fibres (Sugiura et al. 1989).

Inputs of Neurons in Laminae I–VI

Lamina I neurons get inputs from primary afferent fibres, interneurons and descending fibres. Approximately 50% of the synapses are lost following dorsal rhizotomy and thus arise presumably from primary afferents (Chung et al. 1989). All of the primary afferents entering lamina I arise from Aδ and C fibres. The input to lamina II consists of unmyelinated fine primary afferent fibres arising from the tract of Lissauer and the marginal plexus, from propriospinal neurons and from descending fibres. About 50% of the synapses in lamina II survive dorsal rhizotomy (Chung et al. 1989). The primary afferent input into lamina III is largely from collaterals of large sensory axons (Scheibel and Scheibel 1968). These are from different types of non-nociceptive primary afferents such as hair follicle afferents, Pacinian corpuscle afferents, rapidly and slowly adapting afferents etc. However, some projection of fine primary fibre input into lamina III has also been noted (Sugiura et al. 1986). Many of the large neurons

in lamina IV and lamina V have long spiny dendrites that pass dorsally, laterally and ventrally. The dorsal dendrites penetrate the substantia gelatinosa to be contacted by axons from interneurons and from fine primary afferents. In addition they receive inputs from terminal ramifications of large myelinated primary afferent fibres that make synaptic contacts to lamina IV cells. There is not much fine primary afferent input directly into lamina IV. Neurons in lamina VI often send dendrites across the width of the dorsal horn but the dendrites do not penetrate laminae I and II. The primary afferent input is from collaterals from primary afferent axons destined to reach ventral horn cells (for references see Willis and Coggeshall 2004).

Output of Neurons in Laminae I–VI

Most neurons are interneurons with axons ending in the same or adjacent laminae. For example, some of the laminae III–V cells are antenna-type neurons that send dendrites to lamina II and are thus output neurons from lamina II. However, lamina I, includes the dentrites to lamina III–VI neurons that form long axons projecting to supraspinal sites (Willis and Coggeshall 2004). Lamina I neurons project to various sites in the brain stem. Within laminae III to VI are spinocervical tract neurons and ► postsynaptic dorsal column cells. Neurons in laminae IV to VI send their axons into the lateral white column or across the midline, presumably to the opposite ► spinothalamic tract through the anterior white commissure. They may bifurcate before they go to the white matter and collaterals of these axons ramify in laminae III–V and deeper laminae, including the contralateral side and lamina X.

Response Properties of Nociceptive Spinal Cord Neurons

Recordings have shown responses of spinal cord neurons to electrical stimulation of nerve fibres and to natural innocuous and noxious stimulation (for references see Willis and Coggeshall 2004). Upon electrical nerve stimulation lamina I neurons were excited by cutaneous Aδ fibres and sometimes by volleys in C fibres, in line with the morphological studies. However, some neurons were excited (polysynaptically?) by Aβ fibre stimulation. Neurons in the substantia gelatinosa were activated primarily by C fibres; however, Aβ and Aδ fibre stimulation also activated some of them. Neurons in laminae III to VI respond either to A and C fibre or only to A fibre stimulation. Many neurons show convergence of Aβ, Aδ and C fibre inputs.

It is noteworthy that neurons in laminae I and V in the thoracic and sacral spinal cord also show responses to stimulation of visceral nerves and neurons in the superficial and deep lumbosacral enlargement are activated by cutaneous fibres, muscular group III fibres and joint afferents.

Neuronal responses were also recorded during natural stimulation (for references see Willis and Coggeshall 2004). It appeared that neurons in the different laminae are heterogeneous in their response properties. This is not surprising on the one hand, because each of the Aβ, Aδ and C fibre classes has different modalities (mechanoreception, nociception, thermoreception). On the other hand, the response properties do not seem simply to reflect the input into the laminae. This has to be expected because only about 50% of the synapses are from primary afferent fibres.

Lamina I contains neurons that are only activated by intense mechanical stimulation of the skin, neurons that are activated by intense mechanical stimulation of skin and noxious heat applied to the skin and non-nociceptive thermoreceptive neurons that respond to innocuous warming or cooling. Other neurons are wide dynamic range neurons responding weakly to innocuous and strongly to noxious stimulation. Furthermore, neurons in lamina I show convergent inputs from cutaneous and deep tissue or convergence from cutaneous and visceral inputs.

Information on identified lamina II neurons is sparse. Cells were excited by innocuous or noxious mechanical stimuli. However, several authors reported that spontaneously active neurons in this lamina were inhibited by natural stimulation or they were inhibited and then excited by weak mechanical stimuli. Furthermore, neurons showed phenomena such habituation, long afterdischarges following brief stimuli and variable receptive fields.

Neurons in laminae III–VI are of different types. Concerning thresholds and encoding ranges some neurons are low threshold, responding only to innocuous stimulation. Many of these neurons are located in laminae III and IV. Most neurons are wide dynamic range, responding weakly to innocuous stimuli and strongly to noxious stimuli and a further group of neurons are high threshold responding only to noxious intensities. Thus, at least the wide dynamic range neurons seem to receive inputs from non-nociceptive as well as from nociceptive afferents, in line with morphological studies showing projection of non-nociceptive afferents into deep laminae, extension of dendrites of deep cells up to the superficial laminae and interneurons transmitting information from superficial into deeper laminae. Many neurons in laminae III–VI receive inputs from deep tissue. These cells are solely excited by deep tissue stimulation or show convergent inputs from deep tissue and skin, or they are excited from skin and viscera. All neurons with visceral input seem to receive input from the skin as well. Lamina X neurons are also either low threshold,

wide dynamic range or high threshold. Many of these neurons respond to visceral stimulation such as colorectal distension, in addition to cutaneous stimuli. Some cells in lamina X have bilateral receptive fields.

Encoding of Noxious Stimuli in the Spinal Cord

The understanding of the encoding of nociceptive information in the spinal cord is not very advanced. On the basis of single neuron recordings both ► wide dynamic range neurons and ► nociceptive specific neurons seem to be suitable to encode the intensity of a noxious stimulus to a specific site. However, wide dynamic range neurons in particular often have large receptive fields and a stimulus of a defined intensity may elicit differently strong responses when applied to different sites in the receptive field. It is questionable whether the activity of a single neuron reflects more the magnitude of a stimulus or the site in the receptive area where a stimulus of a defined intensity is applied. Furthermore, the situation becomes more complicated when inputs from different tissues to a neuron are studied. It seems impossible that e.g. a single wide dynamic range neuron with convergent cutaneous and visceral inputs can unequivocally signal that the skin or a visceral organ has been challenged with a noxious stimulus. This uncertainty in the message of a neuron could in fact be the reason why pain in viscera and to some extent in the deep tissue is often referred to a cutaneous area, namely into a so-called Heat zone. However, it is quite clear that the precise location of a noxious stimulus, its intensity and character cannot be encoded by a single nociceptive neuron. It is assumed therefore, that encoding of a noxious stimulus is only achieved by a population of nociceptive neurons (for further discussion see Price et al. 2003). This topic is addressed in detail in ► encoding of noxious information in the spinal cord. Samples of neurons were studied by the recording of field potentials in the dorsal horn, but these data have not contributed to the understanding of encoding. Furthermore, changes in metabolic activity and the expression of immediate early genes such as ► C-Fos have been used to map regions in the spinal cord that are activated by a noxious stimulus. The expression of FOS protein (► c-fos) has been used extensively because individual neurons can be visualised. The expression of C-FOS in a neuron is thought to show its activation (Willis and Coggeshall 2004). For example, noxious heat stimulation evokes expression of C-FOS in the superficial dorsal horn within a few minutes and staining shifts to deeper laminae of the dorsal horn thereafter (Menetréy et al. 1989; Williams et al. 1990). Noxious visceral stimulation evokes C-FOS expression in laminae I, V and X, thus resembling the projection area of visceral afferent fibres and injection of mustard oil into muscle elicits C-FOS expression in laminae I and IV to VI (Hunt et al. 1987; Menetréy et al. 1989). These data show therefore, in which spinal laminae and segments neurons were activated by noxious stimulation. It should be noted however, that excitatory as well as inhibitory neurons may express C-FOS.

Plasticity in the Nociceptive Processing in the Spinal Cord

Importantly, spinal cord neurons show changes in their response properties, including the size of their receptive fields, when the peripheral tissue is sufficiently activated by noxious stimuli or when thin fibres in a nerve are electrically stimulated. In general, it is thought that plasticity in the spinal cord contributes significantly to clinically relevant pain states.

Wind-up

► Wind-up is the increase in the response of a spinal cord neuron when electrical stimulation of C-fibres is repeated at intervals of about 1 s (Mendell 1966; Mendell and Wall 1965). The basis of wind-up is a prolonged EPSP that builds up as a result of a repetitive C-fibre volley and thus it rests on temporal summation of synaptic potentials within the cord (Sivilotti et al. 1993). Other neurons show wind-down (Alarcon and Cervero 1990; Fitzgerald and Wall 1980; Woolf and King 1987). Wind-up disappears quickly when repetitive stimulation is stopped. Wind-up is likely to contribute to short lasting increases in response to painful stimulation (see ► wind-up of spinal cord neurons).

Long-term Potentiation (LTP) and Long-term Depression (LTD)

These are long lasting changes in synaptic activity after peripheral nerve stimulation (Randic et al. 1993; Rygh et al. 1999; Sandkühler and Liu 1998; Svendsen et al. 1997). They can be observed as increases in field potentials in the superficial dorsal horn. The most pronounced ► LTP with a short latency can be elicited after application of a high frequency train of electrical stimuli that are suprathreshold for C-fibres when the spinal cord has been transected in order to interrupt descending inhibitory influences from the brain stem. However, LTP can also be elicited with natural noxious stimulation, although the time course is much slower (Rygh et al. 1999). By contrast, LTD in the superficial dorsal horn is elicited by electrical stimulation of Aδ fibres. This latter form of plasticity may be a basis of inhibitory mechanisms that counteract responses to noxious stimulation (Sandkühler et al. 1997). LTP and LTD will be addressed in detail in the essay ► long-term potentiation and long-term depression in the spinal cord.

Central Sensitization in the Course of Inflammation and Nerve Damage

Changes in responses of spinal cord neurons have been studied in models of inflammation and neuropathy. Pronounced changes in response properties of neurons in the superficial dorsal horn, the deep dorsal horn and the ventral cord have been described. ► Central sensitization, originally described by Woolf (1983), has been observed in neurons with cutaneous input during cutaneous inflammation and other forms of cutaneous irritation such as capsaicin application. Pronounced central sensitisation of spinal cord neurons with deep input has been shown during inflammation in joints, muscle and viscera. Typical changes in responses of individual neurons are (a) increased responses to noxious stimulation of inflamed tissue, (b) lowering of the threshold of spinal cord neurons with an initially high threshold (initially nociceptive spinal neurons will change into wide dynamic range neurons), (c) increased responses to stimuli applied to non-inflamed tissue surrounding the inflamed site and (d) expansion of the receptive field. In particular the enhanced responses to stimuli applied to non-inflamed tissue in the vicinity of the inflamed zone indicate that the sensitivity of the spinal cord neurons is enhanced, so that previously subthreshold input is sufficient to activate the neuron under inflammatory conditions. The sensitisation of individual spinal cord neurons will lead to an increased percentage of neurons in a segment that respond to stimulation of an inflamed tissue. Thus the population of responsive neurons increases. Central sensitisation can persist for weeks judging from the recording of neurons at different stages of acute and chronic inflammation (for review see Coderre et al. 1993; Dubner and Ruda 1992; Mense 1993; Schaible and Grubb 1993).

In ► neuropathic pain states, findings in the spinal cord are dependent on the neuropathic model used. Evidence for central sensitisation has been observed in neuropathic pain states in which conduction in the nerve remains present and thus a receptive field of neurons can be identified. In these models, more neurons show ongoing discharges and on average higher responses can be elicited by innocuous stimulation of receptive fields (Laird and Bennett 1993; Palacek 1992 a, b). In some models of neuropathy, neurons with abnormal discharge properties can be observed. For more information see chapters on neuropathic pain.

During inflammation and neuropathy, more neurons that express C-FOS are observed in the spinal cord, supporting the finding that a large number of neurons are activated. At least at some time points, enhanced ► metabolism can be seen in the spinal cord during inflammation and neuropathy. Both of these findings underscore the plasticity that occurs in the spinal cord under these conditions (Price et al. 1991; Schadrack et al. 1999).

Transmitters and Receptors Involved in the Spinal Nociceptive Processing

Numerous transmitters and receptors are involved in spinal nociceptive processing. They mediate processing of noxious information arising from noxious stimulation of normal tissue and they are involved in plastic changes in spinal cord neuronal responses when the peripheral tissue is inflamed or when a nerve is damaged in a neuropathic fashion. Other transmitters are inhibitory and control spinal processing. In general, transmitter actions have either fast kinetics (action of glutamate on ionotropic AMPA and kainate receptors, action of ATP at ionotropic purinergic receptors, action of GABA at ionotropic GABA receptors) or slower kinetics (in particular neuropeptides that act through G-protein coupled metabotropic receptors). Actions with fast kinetics evoke immediate and short-term effects on neurons, thus encoding the input to the neuron, whereas actions with slow kinetics rather modulate synaptic processing (Millan 1999; Willis and Coggeshall 2004). The following paragraphs summarize the main findings on synaptic transmission of nociceptive information (for references see Willis and Coggeshall 2004).

Glutamate

This excitatory amino acid is a principal transmitter in the spinal cord that produces fast synaptic transmission. ► Glutamate is a transmitter of primary afferent neurons and of dorsal horn interneurons. Many spinal cord interneurons are excited by glutamate and by agonists at glutamate receptors. Glutamate activates ionotropic AMPA / kainate (non-NMDA) and ► NMDA receptors as well as ► metabotropic glutamate receptors. They are expressed all over the spinal cord grey matter, although differences in regional densities are found. Glutamate receptors are found in both excitatory and inhibitory interneurons.

Glutamate receptors are involved in the excitation of substantia gelatinosa neurons by Aδ and C fibres in slice preparations. Usually these actions are mainly blocked by CNQX, an antagonist at non-NMDA receptors, whereas ► NMDA receptor antagonists usually cause a small reduction in EPSPs and reduce later components of the EPSP. *In vivo* recordings showed that both non-NMDA and NMDA receptors are involved in the synaptic activation of neurons by noxious stimuli. In addition, both of these receptors are involved in forms of functional plasticity. For example, both wind-up and central sensitisation by inflammation are blocked by spinal application of NMDA antagonists (and non-NMDA antagonists). Metabotropic glu-

tamate receptors can potentiate the action of ionotropic glutamate receptors (for review see Fundytus 2001; Millan 1999; Willis and Coggeshall 2004). A detailed discussion of glutamate receptors is provided by the essays ► NMDA receptors in spinal nociceptive processing and ► metabotropic glutamate receptors in spinal nociceptive processing.

Adenosine Triphosphate

► ATP depolarises some dorsal horn neurons in the superficial dorsal horn. ATP has been implicated in the fast synaptic transmission of innocuous mechanoreceptive input but evidence has also been provided for an involvement in nociceptive synaptic transmission. Some spinal cord neurons seem to express purinergic receptors for actions of ATP, but other reports rather described presynaptic actions of ATP that caused an enhanced release of glutamate. The latter finding is consistent with the localisation of purinergic receptors in dorsal root ganglion cells. ► P2X3 Receptor immunoreactivity in the inner part of lamina II is reduced following dorsal rhizotomy (for review see Willis and Coggeshall 2004).

GABA and Glycine

► GABA is probably the most important fast inhibitory transmitter in the spinal cord. Application of GABA to neurons causes IPSPs and inhibition of the activity of spinal cord neurons. GABA occurs in inhibitory interneurons throughout the spinal cord. ► GABAergic inhibitory interneurons can be synaptically activated by primary afferent fibres and this explains why strong nociceptive inputs can induce, in addition to excitation, inhibition of neurons (usually following initial excitation) that are under the control of these inhibitory interneurons. Noxious stimuli can cause FOS expression in GABAergic interneurons. Some of the GABAergic interneurons also contain other mediators such as glycine, acetylcholine, enkephalin, galanin, neuropeptide Y or nitric oxide synthase (NOS).

Both the ionotropic ► GABAA receptor and the metabotropic ► GABAB receptor are located presynaptically on primary afferent neurons or postsynaptically on dorsal horn neurons. Responses to both innocuous mechanical and noxious stimuli can be reduced by GABA receptor agonists (for review see Willis and Coggeshall 2004). It is under discussion whether reduced inhibition may be a mechanism of neuropathic pain (Polgár et al. 2004).

Some of the inhibitory effects are due to glycine and indeed, the ventral and the dorsal horn contain numerous glycinergic neurons. Glycine may be colocalized with GABA in synaptic terminals. The roles of GABA and glycine are addressed in more detail in ► GABA and glycine in spinal nociceptive processing.

Acetylcholine

Many small DRG neurons, some large DRG ones and some neurons in the dorsal horn are cholinergic. *Vice versa*, many DRG neurons and neurons in the dorsal horn express nicotinergic and muscarinergic receptors. Application of ► acetylcholine to the skin is pronociceptive (*via* nicotinic and muscarinergic receptors) whereas spinal acetylcholine produces pro- or anti-nociception (for review see Willis and Coggeshall 2004).

Excitatory Neuropeptides

A number of peptides are colocalised with excitatory transmitters, in particular with glutamate. Excitatory neuropeptides evoke EPSPs, but these differ from EPSPs evoked by glutamate in several respects. Usually they occur after a latency of seconds, but they last longer. They may not be sufficient to evoke action potential generation. Because glutamate and excitatory peptides are coreleased from synaptic endings, they are thought to act in a synergistic way (Urban et al. 1994).

Substance P

This excitatory peptide is colocalized with glutamate in a proportion of thin diameter primary afferents and in a proportion of spinal cord interneurons. SP-containing endings are concentrated in laminae I and II and in lamina X. They terminate on cell bodies and dendrites of dorsal horn neurons. SP is released mainly in the superficial dorsal horn following electrical stimulation of unmyelinated fibres and during noxious mechanical, thermal or chemical stimulation of the skin and deep tissues such as the joints. In part, release of SP is dependent on NMDA receptors on primary afferent endings (for review see Willis and Coggeshall 2004).

SP acts on ► neurokinin-1 (NK-1) receptors that are located on dendrites and cell bodies of dorsal horn neurons in laminae I, IV–VI and X. Fewer neurons in laminae II and III have NK-1 receptors. The vast majority of neurons with NK-1 receptors are excitatory, while a few contain GABA and glycine and are thus inhibitory. Some of the neurons with NK-1 receptors are projection neurons including spinothalamic, spinoreticular and spinobrachial neurons. Upon strong activation by SP, NK-1 receptors can be internalized. Such an internalization is blocked by NK-1 receptor antagonists and by NMDA receptor antagonists.

NK-1 receptors are G-protein coupled receptors, i.e. the action of SP on ion channels is indirect. Application of SP evokes a prolonged excitation of nociceptive dorsal horn neurons. These depolarizations are presumably caused by Ca^{2+} inward currents and inhibition of K^+ currents and possibly by other currents. Several second messengers such as PKA and PKC are involved.

There is general agreement that SP and NK-1 receptors are involved in the plasticity of nociceptive processing, whereas the involvement of this system in normal nociception is controversial. Responses of dorsal horn neurons to C-fibre volleys and to noxious stimulation of skin and deep tissue are enhanced by SP and receptive fields can show an expansion. Antagonists at NK-1 receptors reduce responses to C-fibre volleys and to noxious stimulation of skin and deep tissue and they attenuate central sensitisation. Mice with a deletion of preprotachykinin A have intact responses to mildly noxious stimuli but reduced responses to moderate and intensely noxious stimuli. Mice with a deletion of the gene responsible for the production of NK-1 receptors respond to acutely painful stimuli, but lack intensity coding for pain and wind-up (for review see Willis and Coggeshall 2004).

Neurokinin A

In addition to substance P, neurokinin A (NKA) is found in small DRG cells and in the spinal dorsal horn. NKA is released in the spinal cord upon noxious stimulation. Due to its resistance to enzymatic degradation, NKA spreads throughout the grey matter. Interestingly, it has not so far been possible to identify NK-2 receptors immunohistochemically in the dorsal horn, leading to the unresolved question as to where NKA acts. It was proposed that NKA activates NK-1 receptors, because these are internalized after application of both SP and NKA. However, specific NK-2 receptor antagonists suggest that specific binding sites for NKA should be present.

The literature on NKA effects is controversial, because some authors found an involvement of NKA in nociceptive processing whereas others did not. Intrathecal application of NKA produces nocifensive behaviour and iontophoretic application of NKA activates nociceptive and non-nociceptive dorsal horn neurons. NKA facilitates behavioural nociceptive responses to heat stimulation, which are blocked by NK-2 receptor antagonists. While some authors could not antagonize responses of dorsal horn neurons to noxious mechanical stimulation, others were able to show such an effect. Antagonists at NK-2 receptors were able to attenuate central sensitisation during knee inflammation and colon inflammation (for review see Willis and Coggeshall 2004).

Neurokinin B

This peptide is found in synaptic terminals and dendrites in laminae I–III, independently of SP-containing elements and is not contained in dorsal root ganglion cells. NK-3 receptors are found in the most superficial part of the dorsal horn. Their function is unclear.

Calcitonin Gene-Related Peptide (CGRP)

This peptide is found in many small DRG neurons and is often colocalized with substance P. Probably the primary afferent neurons are the only source of CGRP in the dorsal horn. CGRP-containing afferents project mainly to laminae I, II and V. However, CGRP is also contained in motoneurons. CGRP is released in the spinal cord by electrical stimulation of thin fibres and by noxious mechanical and thermal stimulation. During joint inflammation, the pattern of CGRP release changes in that innocuous stimuli to the joint are sufficient to elicit CGRP release.

CGRP binding sites are concentrated in lamina I and in the deep dorsal horn. CGRP enhances actions of substance P. It inhibits enzymatic degradation of SP and it seems to potentiate release of SP. Enhanced Ca^{2+} influx may be important in this respect. CGRP activates nociceptive dorsal horn neurons with a slow time course. Blockade of CGRP effects reduces nociceptive responses and attenuates inflammation evoked central sensitisation. The effects of CGRP are discussed in more detail in the essay ► CGRP and spinal cord nociception.

Vasoactive Intestinal Polypeptide (VIP)

► Vasoactive intestinal polypeptide is found in small diameter afferent fibres especially in the sacral spinal cord. Terminals with VIP are concentrated in laminae I, II, V, VII and X. In addition, neurons with VIP are located in laminae II–IV and X. VIP bindings sites are concentrated in laminae I and II. VIP excites nociceptive dorsal horn neurons.

Neurotensin

Neurotensin is located in interneurons in lamina I and the substantia gelatinosa and neurotensin binding sites are present in the dorsal horn. The peptide excites neurons in the superficial dorsal horn.

Cholecystokinin (CCK)

► Cholecystokinin (CCK) is located in DRG neurons and in neurons in several laminae of the dorsal horn. Binding sites reach their highest concentration in laminae I and II. CCK can excite neurons in laminae I–VII and an antagonist at CCK-B receptors is antinociceptive. Antinociceptive effects of CCK have also been described.

Thyrotropin Releasing Hormone (TRH)

Thyrotropin releasing hormone (TRH) is located in the ventral and dorsal horn. Many TRH-containing neurons also contain GABA. TRH facilitates responses of nociceptive neurons to glutamate at NMDA receptors, wind-up and responses to noxious stimuli.

Corticotropin-releasing hormone (CRH)

(CRH)-immunoreactive fibres are present in the sacral spinal cord (laminae I, V–VII, X, intermediolateral column) and CRH immunostaining is abolished after dorsal rhizotomy. CRH binding sites are found in the superficial dorsal horn.

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)

► Pituitary adenylate cyclase activating polypeptide (PACAP) is localized in small DRG cells and many axons in the superficial dorsal horn. PACAP is released after intrathecal capsaicin, and intrathecal PACAP causes nocifensive behaviour. Nociceptive dorsal horn neurons are excited by PACAP (for review see Willis and Coggeshall 2004).

Inhibitory Neuropeptides

Numerous neuropeptides are inhibitory. They may reduce release of transmitters by presynaptic actions or inhibit postsynaptic neurons.

Opioid Peptides

The dorsal horn contains leuenkephalin and metenkephalin, ► dynorphin and ► endomorphins 1 and 2. Enkephalin containing neurons are particularly located in laminae I and II and dynorphin containing neurons in laminae I, II and V. Endomorphin II has been visualised in terminals of primary afferent neurons in the superficial dorsal horn and in dorsal root ganglia but also in postsynaptic neurons.

► Opiate Receptorsμ, δ, κ) are concentrated in the superficial dorsal horn and in particular μ and δ receptors are not only located in interneurons but also on primary afferent fibres. The activation of these opiate receptors reduces release of mediators from primary afferents (presynaptic effect). This effect is mediated by inhibition of Ca^{2+} channels. Other opiate receptors are located on intrinsic spinal cord neurons and mediate postsynaptic effects. The activation of a K^+ conductance could be the relevant mechanism. In general, enkephalins are ligands at δ-receptors, endomorphins are ligands at μ-receptors and dynorphin is a ligand at κ-receptors. However, dynorphin may also activate NMDA receptors. Actions of all opiates are antagonized by naloxone. Specific antagonists at different receptors are available (Waldhoer et al. 2004).

Application of opioids into the dorsal horn reduces responses to (innocuous) and noxious stimulation and responses of neurons to iontophoretic application of excitatory amino acids, showing postsynaptic effects of opioids. Depending on the site of application (superficial or deep laminae), μ-, κ- or δ-receptor ligands are more or less effective in producing neuronal effects. In addition many dorsal horn neurons are hyperpolarised by opiates. While agonists at μ- and δ-receptors usually evoke inhibitory effects, dynorphin may produce either inhibitory or excitatory effects.

In addition to these "classical" opiate receptors, nociceptin / orphanin FQ receptors (see ► Orphanin FQ) have recently been discovered. These proteins share greater than 90% sequence identity and about 60% homology with the classical opiate receptors (Waldhoer et al. 2004). An endogenous ligand at these receptors is ► Nociceptin. This peptide has similar cellular actions to classical opioid peptides. It causes presynaptic inhibition of glutamate release in the spinal cord and reduces FOS expression in the superficial dorsal horn. However, pronociceptive effects have also been described. A related peptide is nocistatin. At present it is unknown at which receptor nocistatin acts (for review see Willis and Coggeshall 2004).

Somatostatin

This peptide is expressed in primary afferent neurons, in dorsal horn interneurons and in axons that descend from the medulla. ► Somatostatin is released mainly in the substantia gelatinosa, by heat stimulation. Actions of somatostatin on nociceptive neurons in the dorsal horn are inhibitory. It is an intriguing question as to whether inhibitory somatostatin is released in the spinal cord from primary afferent fibres or from interneurons.

Galanin

This peptide is expressed in a subpopulation of small DRG neurons and galanin binding sites are also expressed on DRG neurons. Both facilitatory and inhibitory effects of galanin have been described in inflammatory and neuropathic pain states.

Neuropeptide Y

This is normally only expressed at very low levels in DRG neurons, but DRG neurons express Y1 and Y2 receptors. It was proposed that Y1 and Y2 receptors contribute to presynaptic inhibition.

Other Mediators

A number of other mediators influence synaptic transmission in the spinal dorsal horn. Most attention has given to ► NO, ► prostaglandins and ► neurotrophins. These mediators have actions in non-neuronal tissues as well as in neuronal tissues in the peripheral and central nervous systems including the spinal cord. They play significant roles in pathophysiological pain states. The role of prostaglandins is addressed in another section, and a recent review (Vanegas and Schaible 2001) provides a comprehensive summary. The role of

neurotrophins is addressed in the essay ► spinal cord nociception, neurotrophins.

Involvement of Calcium Channels in Release of Transmitter and Postsynaptic Excitability

The release of transmitters is dependent on the influx of Ca^{2+} into the presynaptic ending through ► Voltage-Dependent Calcium Channels. In addition Ca^{2+} also regulates excitability of postsynaptic neurons. High voltage activated N-type channels, which are mainly located presynaptically but also on the postsynaptic side and P / Q-type channels that are located on the presynaptic side are important for nociceptive processing. In particular, blockers of N-type channels reduce responses of spinal cord neurons and behavioural responses to noxious stimulation of normal and inflamed tissue and blockade of N-type channels can also reduce neuropathic pain. There is some evidence that P / Q-type channels are mainly involved in the generation of pathophysiological pain states. A role for high voltage activated L-type channels and low voltage activated T-type channels has also been discussed. The role of calcium channels is addressed in detail in the essay ► calcium channels in the spinal processing of nociceptive input.

Final conclusions

This review concentrated on spinal cord neurons that are excited by noxious stimuli applied to the peripheral tissue. The significance of particular types of neurons in the transmission of sensory information to supraspinal sites through ascending tracts will be addressed in another section. The modulation of responses of spinal cord cells by descending inhibition and facilitation is described in another section. In addition, the particular aspects concerning deep somatic and visceral pain are also covered elsewhere. As briefly outlined here, the spinal cord is subject to considerable changes during pathophysiological pain states. These changes will also be addressed in other sections. Moreover, the spinal cord is the major site at which antinociceptive compounds work. Again this will be addressed in other sections.

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Nociceptive System

Definition

Peripheral, spinal and cerebral structures involved in the processing of noxious stimuli. Sensory-discriminative aspect of pain: Perceptual component of pain perception including the perception of location, quality, intensity and duration of the painful stimulus.

- ► Noxious Stimulus
- ► Primary Somatosensory Cortex (S1), Effect on Pain-Related Behavior in Humans

Nociceptive Temporomandibular Joint Afferents

- ► Nociceptors in the Orofacial Region (Temporomandibular Joint and Masseter Muscle)

Nociceptive Threshold

Definition

Nociceptive thresholds in experimental animals are usually defined as the threshold (temperature, mechanical force) at which a withdrawal response is evoked, measured either by active withdrawal of a limb or the tail, for example, or measurement of the electrical activity of a muscle in an anaesthetized animal.

- ► Arthritis Model, Adjuvant-Induced Arthritis

Nociceptive Transduction

Definition

Generation of the nociceptive information in nociceptors in response to noxious stimuli by generation of depolarizing currents.

- ► Nociceptor Generator Pontential
- ► NSAIDs, Mode of Action

Nociceptive Withdrawal Reflex

Definition

Nociceptive withdrawal reflexes denote an integrated reflex to avoid potential tissue injury. The reflex response is dependent on stimulus site, stimulus intensity, and functional context. During standardized experimental conditions, the reflex is correlated to the pain intensity.

- ► Pain in Humans, Electrical Stimulation (Skin, Muscle and Viscera)

Nociceptor Accommodation

- ► Nociceptor, Adaptation

Nociceptor Desensitization

Definition

Decreased sensitivity to noxious stimuli elicited by application of capsaicin. Short-term desensitization is due to the inactivation of TRPV1, preventing the generation of action potentials. On the other hand, long-term desensitization evoked by large doses of capsaicin onto polymodal nociceptor endings, is due to the destruction of a subset of small diameter primary afferent fibers and their cell bodies.

- ► Polymodal Nociceptors, Heat Transduction
- ► TRPV1 Receptor

Nociceptive Processing in the Thalamus

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The thalamus (Jones 1985) is the primary gateway for nociceptive information transmission to the cortex, similarly to most other sensory systems other than olfaction. However, in contrast to other sensory systems, nociceptive information is also transmitted to the cortex through pathways outside of the ► spinothalamic-thalamocortical projections. Recent new advances have pointed to nociceptive inputs to the brainstem, which in turn project to the thalamus and then to the cortex in a very different pattern from that of the spinothalamic inputs. Nociceptive information transmission to the cortex through thalamocortical projections remains the most thoroughly examined, even though substantial gaps remain in current understanding of this system. The thalamus is the only CNS structure that contains ► mast cells. Their exact role remains unclear but they exist in thalamic nuclei with cortical projections and seem to increase or decrease in number under different conditions. They may be involved in regulating the ► blood-brain barrier. They are probably important in neural-endocrine interactions. (see ► spinothalamic projections in rat). The state of current knowledge regarding the properties of nociceptive processing in the thalamus is outlined here, emphasizing the best-established facts, gaps in current knowledge and points of contention.

Cells of Origin and Tracts

► Spinothalamic and ► trigeminothalamic pathways are the main direct source of nociceptive information input to the thalamus. Multi-synaptic projections through the ► brainstem provide additional nociceptive inputs to the thalamus. Specific subpopulations of spinal cord cells within laminae I, V–VI and VII–VIII project contralaterally through the spinothalamic pathway and transmit mainly nociceptive inputs to medial and lateral thalamic nuclei. The axons of the spinothalamic tract show a topographic organization and a dorsoventral segregation, with axons of superficial lamina spinothalamic cells being located more dorsally than axons of deeper lamina spinothalamic cells. The vast majority of spinothalamic cells respond to nociceptive stimuli. These responses may be uniquely nociceptive and thus called nociceptive specific or convergently responding to both noxious and innocuous stimuli, called ► wide dynamic range type. The responses may be to heat, cold or tactile stimuli specifically or convergently. There is little evidence as to the spinothalamic cell responses to chemical irritants (see ► spinothalamic input, cells of origin (monkey)).

Spinothalamic Targets

The differential projections for innocuous and nociceptive inputs to the thalamus were first noted when the functional distinctions between the dorsal columns and the anterolateral tract were observed in humans following cordotomies, over a 100 years ago. The terminations of the spinothalamic pathway still remain controversial. There is disagreement as to the terminations of the pathway in the lateral and medial thalamus. In 1980s, the ► nucleus submedius (SM) in the medial thalamus was claimed to be the nociceptive specific nucleus of the thalamus (Craig and Burton 1981), since it was thought to receive inputs only from spinal cord lamina I nociceptive neurons. This idea has been mostly discounted, at least in the rat and monkey. However, the primary medial thalamic termination site, which has traditionally been reported to be in ► MD has been put into doubt in recent reports as well; instead it is claimed that the main spinothalamic input is to ► CL. Lateral thalamic terminations have classically been reported to target ► VP, VPI and ► PO and there is ample evidence for this idea. However, this was recently challenged by the claimed existence of another nociception, thermoception and itch specific nucleus ► VMpo within the lateral thalamus, which seems to receive spinal cord and trigeminal lamina I inputs somatotopically and is most evident in the monkey and man (Craig et al. 1994). This notion has recently been questioned as well, by demonstrating that VMpo may simply be part of the periphery of ► VPM. At one level, these disputes seem simply a consequence of disagreements over how one delineates various borders of thalamic nuclei, which are invariably ambiguous and poorly defined, especially the transition zones between nuclei. On the other hand, they reflect philosophical differences as to the rules of the organization of the central nervous system regarding pain perception. The claim of the existence of nociceptive specific nuclei in the thalamus has the consequence of implying that these regions together with their inputs and output targets constitute the pain specific network of the brain. This claim in turn denies the contribution of other thalamic nuclei and their inputs and outputs to pain perception (see ► Spinothalamocortical Projections from SM).

Periphery of VP, Rod and Matrix of VP and VMpo

Staining thalamic tissue for ► cytochrome oxidase (CO) showed that lateral thalamic somatosensory regions, VP and its surrounding nuclei, can be subdivided into two compartments, a region densely la-

beled with CO and a surrounding periphery (VPp) labeled weakly with CO. This observation was first made in Kniffki's lab for the cat thalamus, in the late 1980s. Independently, E.G. Jones noted the same CO staining based distinction in the monkey lateral thalamus and extended the parcellation by combining CO staining with two calcium binding proteins (► calbindin and ► parvalbumin), which preferentially label the CO dense and CO sparse domains of ► VP and its periphery. Jones advanced the hypothesis that the CO dense region, dubbed the rod region, was involved in sensory information transmission to the cortex by terminating in layer IV, while the CO weakly labeled region, dubbed the matrix region, projected mainly to layer I of the cortex and was involved in modulating cortical responses. A subsequent study did not substantiate this idea (Shi et al. 1993). Kniffki and colleagues hypothesized, mainly by relating terminations of the spinothalamic tract to the CO sparse region of the cat VP, that neurons in the VP periphery (VPp) are mainly nociceptive, while the CO dense region receives inputs from the medial lemniscus and signals innocuous somatosensory information to the cortex. A series of studies indicated that the cat VP does not have nociceptive cells. However, the opposite claim has been harder to prove since the number of nociceptive cells characterized in the VPp of the cat has remained small (Horn et al. 1999; Martin et al. 1990). To further explore the segregation of VP and its periphery in the monkey, electrophysiological studies contrasted nociceptive neurons in the squirrel monkey VP and VPI (equivalent to VPp in the cat) and observed that most VP nociceptive cells were WDR type while VPI nociceptive cells were both NS and WDR types. Given the distinct white matter localization of spinal cord lamina I *vs.* deeper spinothalamic cells and the evidence that most NS type cells in the spinal cord are localized to lamina I, it was proposed that the monkey periphery of VP preferentially or exclusively receives inputs from spinal cord lamina I cells, while the VP proper receives inputs from deeper laminae spinothalamic cells. This claim remains unproven and does not match with earlier reports (in the macaque) that VP nociceptive cells are of both NS and WDR types; it also does not match with the prevalences of NS and WDR type spinothalamic cells in the monkey, which do not seem to differ between spinal cord laminae. Craig and colleagues extended these ideas to the extreme and proposed the existence of VMpo, a unique lateral thalamic nucleus that provides pain specific inputs to the insular cortex. The corollary to this idea is that nociceptive inputs to VP proper are modulatory in nature and do not signal nociceptive sensory information. This idea has been criticized by various groups and remains controversial. Human imaging shows robust activation of SII and posterior insula and more variable responses in SI for experimental pain in healthy humans. A recent meta-analysis, however, indicates no significant difference in activation incidence between these structures (Apkarian et al. 2005). Even if one assumes that posterior insular activity is due to inputs from VMpo, nociceptive inputs to SI and SII are undoubtedly from VP and VPI and hence nociceptive inputs to the latter nuclei participate in the cortical activity associated with pain perception (see essays ► corticothalamic and thalamocortical interactions; ► spinothalamic terminations, core and matrix; ► thalamic nuclei involved in pain, human and monkey; ► spinothalamic projections in rat; ► spinothalamic input, cells of origin (monkey); ► thalamic nuclei involved in pain, cat and rat; ► spinothalamocortical projections to ventromedial and parafascicular nuclei; ► thalamus, nociceptive cells in VPI, cat and rat).

Generally, this debate is the most modern version of specificity *vs.* pattern theories of coding for pain in the nervous system, a debate that goes back to Helmholz, Von Frey and Goldsheider, who were battling pain representation models based on the discovery of punctate receptive fields on the human skin. In the 1960s, the debate moved from psychophysics to the properties of spinal cord neurons and the contribution of nociceptive specific *vs.* wide dynamic range type cells to pain perception. One group of scientists staked the claim that the wide dynamic range neurons were necessary and sufficient for pain perception, while another group claimed that nociceptive specific cells were all that was needed for pain perception. There was probably always a silent majority of scientists who simply accepted that both types of neurons are involved in pain perception and that these cell types complement each other in the range of stimuli that could evoke pain perception. Thus, VMpo and its connectivity represent the latest effort in pinpointing a specific group of cells from the skin to the cortex that uniquely signal pain from neurons that are specifically involved in coding noxious, thermal and itch stimuli. The opponents of this idea question whether VMpo exists as a unique thalamic nucleus and argue that there is ample evidence that other spinal cord neurons, thalamic nuclei and cortical regions have repeatedly been demonstrated to have all the necessary characteristics to encode nociceptive information.

Brainstem Inputs

Spinoreticulothalamic projections, which are pathways conveying spinal cord inputs to different brainstem targets and then in turn projecting to the thalamus have been studied best in the rat. Some of these pathways seem specifically involved in conveying whole body nociceptive information to the thalamus. In the medullary brainstem, the subnucleus reticularis dor-

salis (SRD) seems to be one of the main nociceptive relays to the thalamus. Neurons from the SRD project to VMl and ► PF in the ► medial thalamus and neurons in VMl in turn project to the layer I of the ventrolateral cortex, while projections from PF project to the ► basal ganglia, the subthalamic nucleus and parts of the motor and parietal cortex (see ► brainstem subnucleus reticularis dorsalis neuron).

Another brainstem-thalamic projection, described in the rat, is through the internal lateral parabrachial nucleus, which receives nociceptive inputs from deep laminae of the spinal cord and projects to medial thalamic nuclei PC, CM and PF. Traditionally, CM and PF have been grouped together and implicated in affective modulation of pain. A large proportion of PF cells respond to nociceptive stimuli and stimulation in the region evokes pain-like reports in humans and pain-like behavior in animals. In humans, medial thalamic stimulation or lesioning has been used for pain relief and has targeted the CM-PF region. Such procedures report a fair incidence of success. PF and amygdala receive serotonergic inputs from ventral PAG and the three structures seem to interact in modulating the affective component of pain. Suppression of rats' affective reaction to noxious stimuli by injection of morphine into the ventral PAG is reversed by serotonin antagonists applied either to PF or amygdala (see ► parafascicular nucleus, pain modulation ► thalamus, nociceptive inputs in the rat (spinal) ► thalamo-amygdala interactions and pain).

Spinothalamo-Cortical Connectivity

Although the suggested role of the nucleus submedius (SM) in nociception has diminished over the years, its projection targets show that SM neurons (in the rat) terminate in the ventrolateral orbital cortex (VLO), a region where nociceptive neurons have been described in the rat. The VLO nucleus also receives inputs from the ventral ► periaqueductal gray (PAG) and dorsal raphe. A similar but smaller brainstem projection seems to exist for the SM as well. The rat SM does not receive spinal cord lamina I inputs as originally claimed; instead its inputs are from deeper laminae. Although a nucleus equivalent to SM was originally described in the monkey, this claim has been repeatedly refuted (see ► thalamus, nociceptive inputs in the rat (spinal); ► spino-thalamocortical projections from SM).

Potential connectivity between thalamocortical projecting neurons and spinothalamic terminations has been studied probabilistically at the light microscopy level (Gingold et al. 1991). Hand primary somatosensory cortex projecting cells are labeled retrogradely with a given marker and spinothalamic terminations from the upper cervical enlargement labeled anterogradely with a different marker. Given the known dendritic branching pattern of thalamocortical cells, one can then calculate how many of these cells can potentially receive spinothalamic inputs on their dendritic perimeter. Although such an analysis cannot establish the presence or absence of synapses, it provides quantitative bounds as to the influence of nociceptive inputs through the spinothalamic projections to the cortical target. The analysis shows that 87% of cervical enlargement spinothalamic terminations are localized to VPL, VPI and CL and 24% of the hand region of the primary somatosensory cortex is putatively contacted by these spinothalamic terminations. A more recent study examined connectivity between thalamic cells and spinothalamic afferents by intracellularly labeling individual thalamic neurons and examining the proximity of the labeled dendrites to spinothalamic terminals (Shi and Apkarian 1995). A similar study has also been done electrophysiologically and indicates that the probability of encountering spinothalamic terminations in the vicinity of nociceptive cells in VP is 33% while in VPI it is 73% (Apkarian and Shi 1994).

Synaptic Connectivity

Synaptic morphology, using electron microscopy, has been studied for spinothalamic inputs and contrasted to dorsal column inputs in VP (Ralston 2003). The study examined synapses for spinothalamic projections onto VP proper cells and contrasted them to medial lemniscal synapses. Spinothalamic synapses were found to be mainly on dendrites of projecting cells, in contrast to medial lemniscal synapses that formed triads between terminals and projecting and local GABAergic cells. This distinction between afferent input types and synapses is congruent with physiological connectivity differences observed for VP cell groups with and without nociceptive inputs (see ► thalamus, dynamics of nociception). More recently, similar electron microscopic studies were also done for spinal cord lamina I inputs to VMpo (Beggs et al. 2003). The synaptic profiles in VMpo were almost always triadic. Thus, the lamina I spinothalamic inputs to VMpo are different from spinothalamic inputs to VP. The difference is partly attributed to the targets and partly to inputs. Most likely most of the inputs examined in VP reflected terminations from deeper laminae than lamina I. These differences are consistent with the light microscopic observation regarding proximity of spinothalamic terminations to nociceptive cells in VP *vs*. VPI.

Species Differences

There are important species differences regarding the spinothalamic pathway, its terminations in the thalamus and response properties of thalamic nociceptive

cells. The rat spinothalamic tract (see ► Spinothalamic Projections in Rat) is composed of a third fewer cells than that of the monkey. Rat lateral thalamic nuclei are devoid of local interneurons. Thus, one can assert that terminations in these nuclei are synapses on cortex projecting cells. Also, the rat VP was not traditionally subdivided into a core and a periphery; as a result there is no doubt that some spinothalamic terminals are on VP cells that project to the primary or secondary somatosensory cortex. Rat spinothalamic terminations in the medial thalamus generally target the same nuclei as in the cat or monkey, perhaps with the exception of SM, which does not seem to receive spinal cord lamina I inputs. In the spinal cord, the lateral cervical nucleus (LCN) seems more prominent in the rat and these cells do project to the thalamus, although their functional role has remained unclear.

More recent studies have unraveled differences in terminations of superficial laminar spinal cord cells and deeper ones as to their targets in the rat thalamus. Lamina I inputs seem to be limited to lateral thalamic targets, where the region is subdivided into VP, VPpc, Po and PoT, where VPpc and PoT probably correspond to different portions of VP periphery. Direct deeper laminae projections in the rat seem to be limited to PoT and CL. (see ► thalamus, nociceptive inputs in the rat (spinal) ► spinothalamic projections in rat).

Neurotransmitters and Neuromodulators

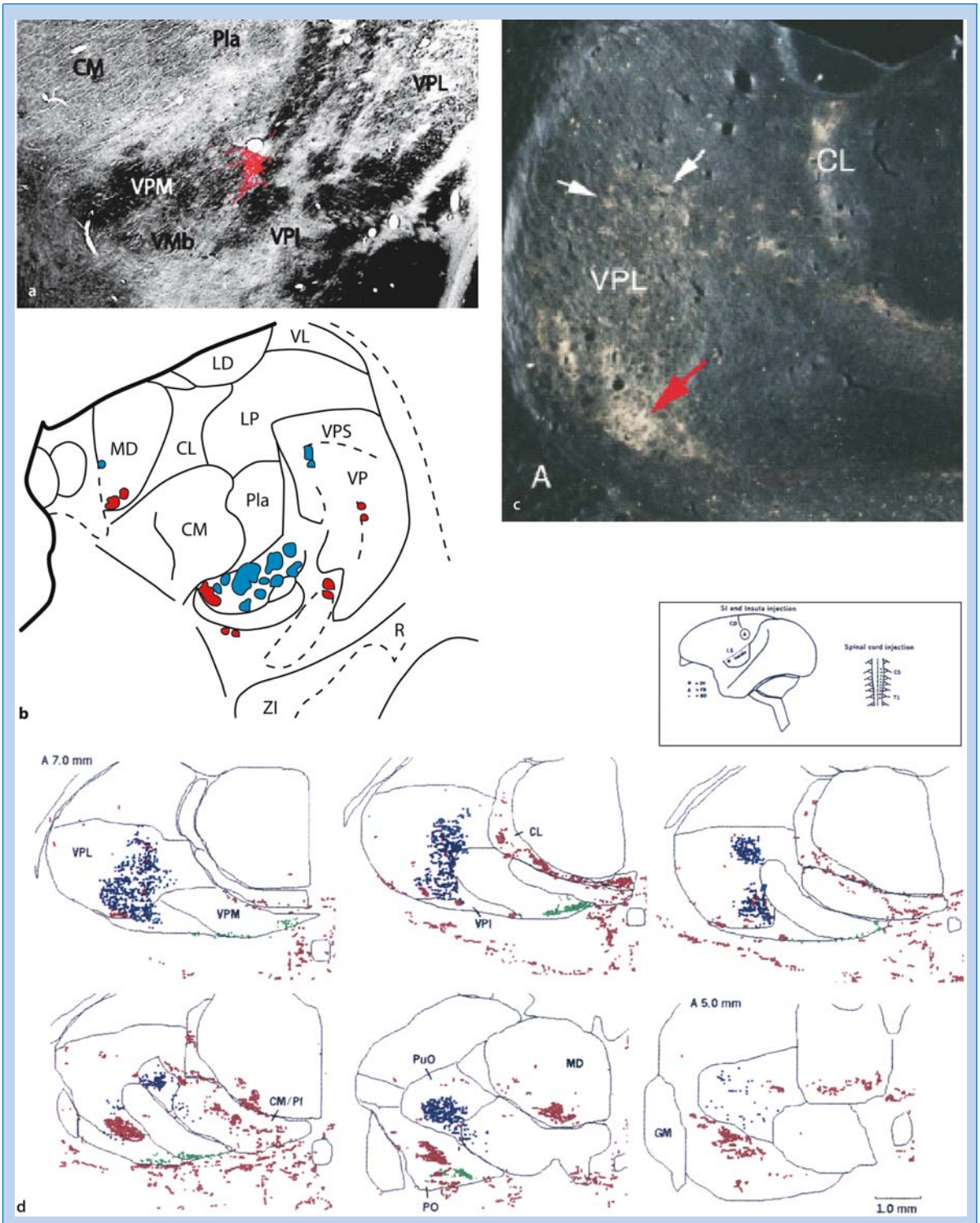
Like other sensory inputs to the thalamus, ► glutamatergic neurotransmission is assumed to transmit nociceptive information. Although such transmission has been demonstrated for other sensory modalities, as in somatosensory transmission, it is not proven in the case of nociception. The thalamocortical efferent pathway is made of neurons that are glutamatergic. Cortical inputs to the thalamus seem to be mediated through ► AMPA and ► mGLU receptors. ► GABAergic inhibitory interneurons and GABAergic thalamic reticular nucleus innervation provide inhibition on projecting neurons through ► $GABA_A$ and ► $GABA_A$ receptors. Most current knowledge regarding nociceptive neurotransmission is based on studies of VB neuronal properties and indicates that ► NMDA receptors signal acute thermal and mechanical responses. There is also evidence that thalamic mGLU receptor mechanisms are important in inflammation-induced hyperalgesia and in the expression of such behavior (see ► metabotropic glutamate receptors in the thalamus).

Eight metabotropic glutamate receptor subtypes (mGLU1–mGLU8) have been characterized, are divided into three groups (I, II, III) and are all found in the thalamus. Group I receptors mediate mainly postsynaptic actions, while Groups II and III regulate presynaptic transmitter release. Different subtypes are important in either cortico-thalamic inputs or thalamic reticular neuronal modulating of GABAergic transmission. Thus, mGLU receptors have a minimal role in ascending sensory transmission but are more important in modulating this transmission (see ► nociceptive neurotransmission in the thalamus).

Spinothalamic neurons contain glutamate and various ► neuropeptides. Of the large list of neuropeptides seen in spinothalamic cells, ► substance P (SP) is found abundantly in the medial thalamus, some of which may be due to spinothalamic inputs. Cholinergic, ► serotonergic and ► noradrenergic inputs from the brainstem are also found in the thalamus, all of which are probably part of the arousal modulatory system. Intrinsic SP neurons are described in thalamic regions receiving spinothalamic inputs. ► CGRP neurons are found in the periphery of VP and described as projecting to the amygdala or insula (see ► thalamus, visceral representation ► parafascicular nucleus, pain modulation ► thalamic neurotransmitters and neurochemical effector molecules).

Somatic Representation

Nociceptive representation in the thalamus has been described in multiple species, primarily in rat, cat and monkey and mainly in anesthetized preparations. The overall number of nociceptive cells described remains relatively small and the properties of cells located in medial in contrast to lateral thalamic nuclei seem distinct. Nociceptive cells found in the medial thalamic nuclei tend to have more nociceptive specific responses, with response patterns that are modulated with the sleep-wakefulness cycle, level of anesthesia and attentional manipulations. In contrast, nociceptive cells in the lateral thalamus have more divergent inputs, they can be nociceptive specific or wide dynamic range types, with response properties that seem more reproducible and less dependent on attentional manipulations. The receptive field size, location and properties seem labile in medial thalamic cells, while in lateral thalamic cells they seem more constant and correspond to the properties of similar cells described in the spinal cord or trigeminal nuclei. These response differences are generally consistent with the notion that medial thalamic nociceptive information may be providing cortical signals regarding the affective properties of pain and also providing a more general modulatory signal that may be important in biasing the cortex and in modulating the attentional circuitry of the cortex. In contrast, lateral thalamic nociceptive signals are consistent with the general idea that these are the ► sensory-discriminative information being transmitted to cortical regions specifically involved in pain perception. Even though both notions may generally be



true,there are important deviations regarding input-output properties and response properties of nociceptive cells in specific thalamic nuclei, implying that the medial *vs*. lateral thalamic functional differentiation is most probably too simplistic (see ► parafascicular nucleus, pain modulation ► thalamus, nociceptive inputs in the rat (spinal) ► spinothalamic input, cells of origin (monkey)).

◄ **Nociceptive Processing in the Thalamus, Figure 1,** Spinothalamic inputs to the lateral thalamus. Data are presented from four different labs. (a) A recent study (Graziano and Jones 2004) shows that trigeminothalamic projections in the monkey terminate in the periphery of VP, asserts that these terminations in turn project to primary and secondary somatosensory cortex and thus questions whether VMpo is a unique thalamic nucleus (from Graziano and Jones 2004). (b) An extensive study of spinal and trigeminal lamina I projections to the thalamus shows the location of VMpo and the somatotopic terminations within it (Craig 2004). Terminations from the cervical enlargement are in red, while those from trigeminal nucleus caudalis are in blue (from Craig 2004). (c) Site of dense terminations from cervical enlargement in monkey (Ralston 2003). The spinal cord injection does not distinguish between laminae. The author illustrates that the dense terminations in this case are more lateral and anterior than VMpo (from Ralston 2003). (d) Terminations from cervical enlargement in the monkey, in relation to neurons projecting to the hand region of the primary somatosensory cortex and in relation to neurons projecting to the insula (Apkarian and Shi 1998). Spinothalamic terminations are in red, cells projecting to hand primary somatosensory cortex in blue, cells projecting to insula green. Spinothalamic terminations in slice 4 from the most anterior section show a dense terminal patch at the border of VPL and VPI, closely corresponding to the labeling illustrated in (c). On the other hand, spinothalamic terminals in slice 6 are very similar to the labeling shown in (b), thus matching the VMpo label. At least at this slice location and from the specific insular injection, there is no overlap between these terminations and insular projecting cells. More anteriorly, there is some overlap between spinothalamic terminations and insula projecting cells. However, the overlap between primary somatosensory cortex projecting cells and spinothalamic terminations is more extensive. Comparing the four panels it should be evident that spinothalamic terminations in the lateral thalamus extend from VP proper to VPI and other VP periphery regions and most posteriorly are located at the interface between VP and PO, a region that has been called VMpo. At least in (d) this labeling seems continuous antero-posteriorly, casting doubt on the notion that the VMpo region is a unique nucleus with distinct projections to the insula. Figure from Apkarian and Shi, 1998.

Visceral Representation

Visceral stimulation induced thalamic activity is demonstrated in the thalamus in humans with brain imaging studies and in animals using electrophysiology. Visceral responsive cells are found in and around VP with no evidence for viscerotopy, although visceral topography for baro- and chemo-receptors has been suggested. Visceral responsive cells are also reported in the medial thalamus. However, thalamic regions with inputs from SRD seem to lack visceral inputs (see ► thalamus, visceral representation ► thalamus and visceral pain processing (human imaging) ► thalamus, clinical visceral pain, human imaging ► thalamus, nociceptive cells in VPI, cat and rat ► spinothalamocortical projections to ventromedial and parafascicular nuclei).

Thalamic Lesions in Animals

Thalamic lesions (thalamotomy) are used to relieve chronic pain in humans. On the other hand, stimulation within the human thalamus gives rise to pain sensations. Recent animal studies examined the behavioral effects of thalamic lesions, targeting either the lateral thalamus or both the lateral and medial thalamus. Generally, it seems, at least in the rat, that lesions involving any part of the thalamus give rise to increased sensitivity to mechanical and thermal noxious stimuli, reminiscent of 'thalamic syndrome' outcomes in humans. Moreover, when thalamic lesions are performed in animals with neuropathic pain-like behavior (partial peripheral nerve injury), this behavior is diminished only transiently and when the neuropathic injury is performed after a thalamic lesion, no significant change in neuropathic behavior is observed. These results challenge the idea that the thalamus is the main sensory transmission pathway for nociception, at least in the rat (see ► lateral thalamic lesions, pain behavior in animals ► thalamotomy, pain behavior in animals).

Thalamic Plasticity

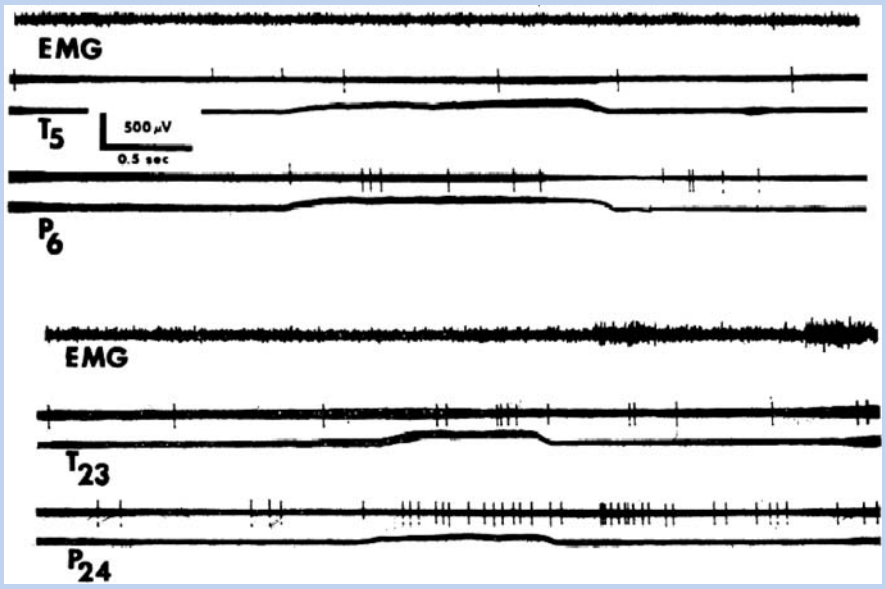
Plasticity of thalamic representation of innocuous and noxious inputs has been demonstrated following a variety of deafferentation procedures. The main effect is an expansion of intact adjacently represented regions into the areas of deafferentation. Spinothalamic tract lesions seem to increase spontaneous firing rates and increase sensitivity and bursting of thalamic cells with innocuous inputs. Similar observations have been made in humans with chronic pain (see ► thalamic plasticity and chronic pain).

Dynamics of Thalamic Coding for Nociception

A major function of the thalamus is state dependent modulation of incoming sensory information. Changes in intrinsic response properties of thalamic cells with the sleep and wake cycle have been documented extensively in many regions of the thalamus (Steriade et al. 1990). However, there is minimal such information regarding nociceptive inputs, the difficulty being that most thalamic nociceptive neurons are studied under anesthesia.

Thalamic neurons fire in two distinct modes, tonic and bursting. The bursting mode is due to a T-type calcium channel and such bursting activity is seen in conscious chronic pain patients. During sleep, thalamic neurons are mostly in burst mode and shift to tonic mode with wakefulness. Bursting activity in the conscious state is termed ► thalamocortical dysrhythmia and suggested to be a basis for pain and other neurological disorders (see ► burst activity in thalamus and pain).

A very early report documented that most nociceptive neurons in the thalamus switch response modes with the sleep cycle. Of 8 neurons that were characterized at different states of wakefulness, 5 began to respond to innocuous stimuli as well when the animal was more awake and 3 responded more specifically to noxious stimuli with increased wakefulness (Casey 1966).



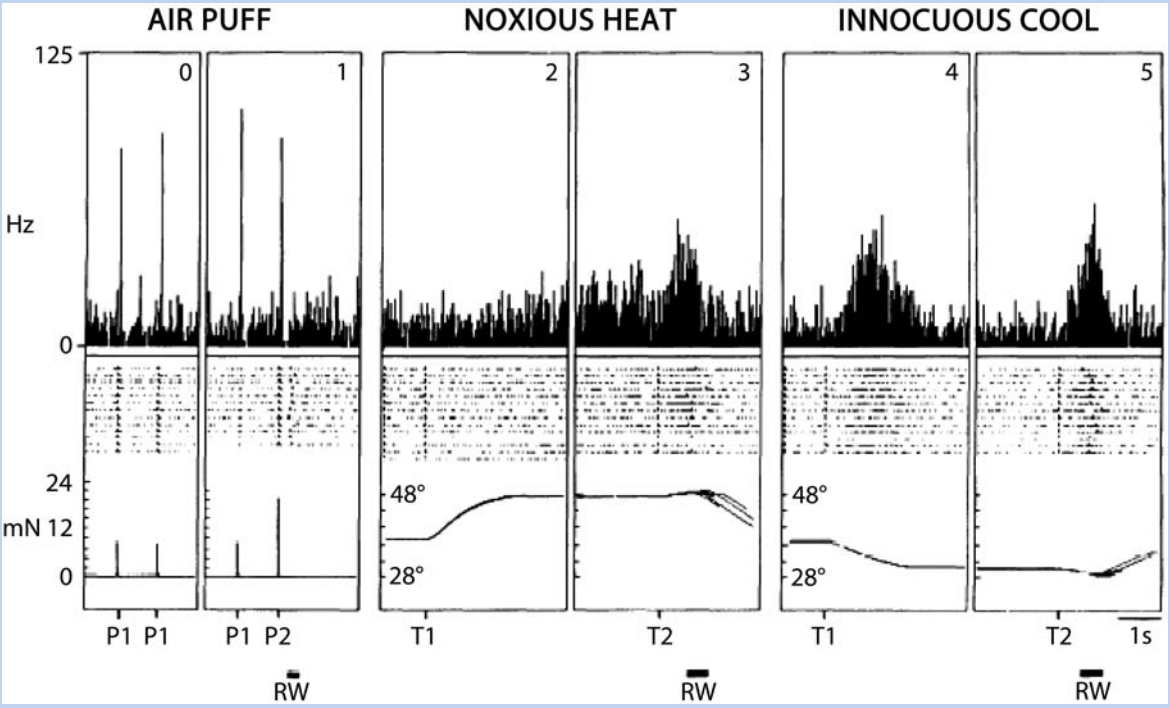
Nociceptive Processing in the Thalamus, Figure 2 Response properties of a neuron recorded in MD, in a conscious monkey (Casey 1966). Responses to innocuous and noxious stimuli are modulated with the wakefulness of the animal. In the top panel the animal is drowsy or lightly asleep, as a result the painful stimulus (delivered to the arm) does not evoke an EMG response. Tactile stimulation (T5) does not activate the neuron, but a painful stimulus does (P6). When the animal becomes more awake, the neuron responds at a higher frequency to both tactile (T23) and painful (P24) stimuli. Figure from Casey 1966.

Moreover, the author noticed and described changes in firing patterns for these nociceptive cells before and after the animal was given either an auditory or visual stimulus, which also changed the responses of the nociceptive cells to the noxious or innocuous somatic stimuli. Casey presented these results as evidence for refuting the notion of specificity of pain processing in the CNS. It is noteworthy that most neurons that changed responses with the state of wakefulness were located in the medial thalamus. Unfortunately, there are no new systematic studies on the topic. The effects of anesthesia on transmission of somatosensory inputs have recently been examined in VP, mainly for somatic innocuous inputs (Vahle-Hinz and Detsch 2002). Simultaneous recordings from groups of neighboring neurons in and around the VP in the anesthetized monkey indicate that the firing patterns of such cells change dynamically with every stimulus (see ► thalamus, dynamics of nociception).

Bushnell and colleagues (Bushnell et al. 1993) performed electrophysiological recordings in conscious monkeys trained in a thermal discrimination task on the lip. The recordings were mostly from the medial border region of VPM (which proponents of VMpo now claim was mislabeled and that in fact these were recordings from VMpo). They found 22 cells responding to heat, the majority of which also responded to noxious heat; some responded to mechanical stimuli and / or cooling. Thus, neurons in this region had both NS-type responses and WDR-type responses. The group average of some of the nociceptive responsive cells showed a well-definedthreshold and a linear increase in firing rate above threshold. Importantly, cells in this region were tested for modulation by attentional shifts and, in contrast to the observations by Casey (Casey 1966), these VPM cells were not affected by attention towards or away from the stimulus.

The most comprehensive physiological study of nociceptive thalamic neurons was done in anesthetized monkeys, where 73 nociceptive cells were characterized in 26 animals (Kenshalo et al. 1980). The study explored neurons in VPL and showed that nociceptive cells' receptive field properties generally matched the somatotopic organization of this nucleus, with nociceptive cells in medial VPL having somatic fields localized to the forelimbs, while nociceptive cells in lateral VPL usually had receptive fields limited to the lower body. They also showed that repeated thermal stimulation of these cells increased their responses, implying that such neurons may participate in perceptions associated with thermal sensitization. Moreover, the authors showed that only spinal cord lesions that severed the ventrolateral white matter ipsilateral to the recording in the thalamus and contralateral to the location of the receptive field on the skin would abolish the responses of these cells to noxious stimuli applied within the receptive field.

The stimulus-response curves for noxious thermal stimuli from Kenshalo et al. (Kenshalo et al. 1980) and from Bushnell et al. (1993) are presented together to emphasize the similarity of the results obtained in anesthetized and conscious monkeys and to show the sim-



Nociceptive Processing in the Thalamus, Figure 3 Response properties of a neuron recorded from VPM in conscious monkey (Bushnell et al. 1993). The neuron responds to air puffs (a), to noxious heat (b) and to innocuous cooling (c) of the skin on the maxilla. The initial heat response at 4˚C is small but increases with continued heating. The second heat change (T2 of 0.4, 0.8 or 1.0˚C) results in a robust response. From Fig. 2 of Bushnell et al. 1993.

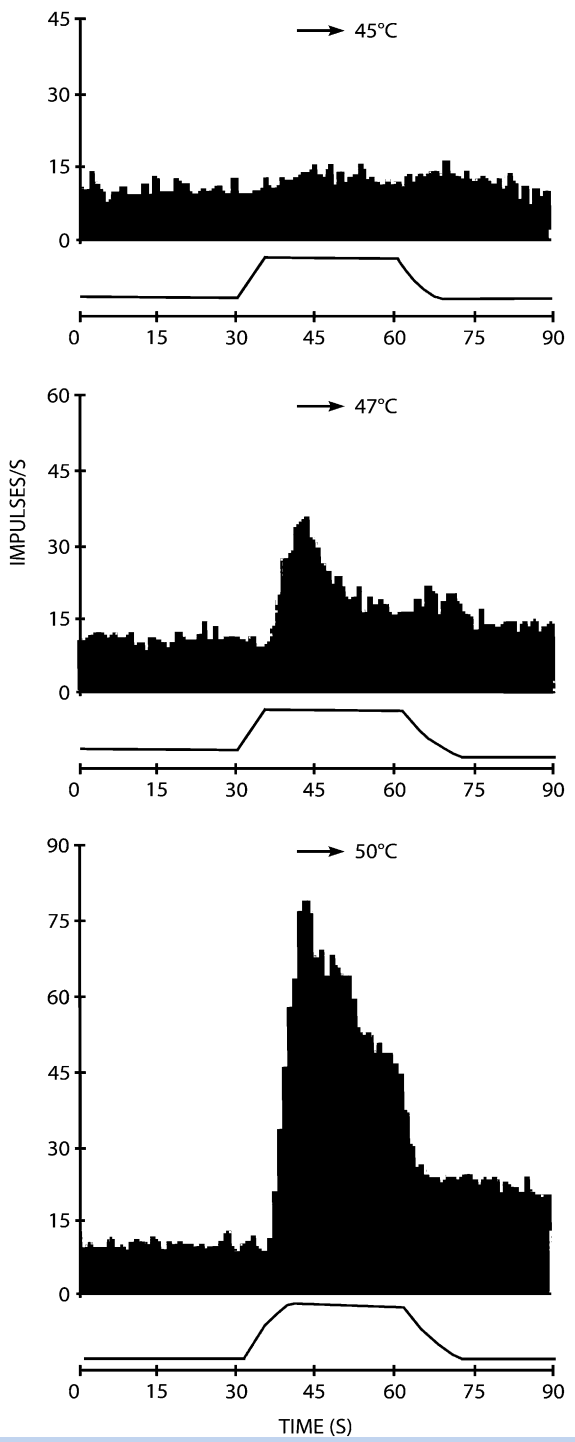
ilarity of responses for nociceptive cells in VPL and VPM thalamic nuclei. Both group-averaged curves increased positively for stimuli above 47˚C at approximately the same rate, although in the conscious preparation, threshold to painful thermal stimulus-responses seemed closer to 47˚C than in the anesthetized monkey, where the threshold was around 43–45˚C. Thus, in the lateral thalamus, nociceptive cells respond to thermal stimuli within a range that generally corresponds to human psychophysical studies for heat pain perception and at least the threshold of these neurons also corresponds to the heat response thresholds for peripheral nociceptive afferents.

Thalamic physiology, especially for nociception, has traditionally emphasized the response properties of individual neurons. Establishing such properties provides the basis for the kinds of information that different neurons in different parts of the thalamus have access to, but it by no means demonstrates the dynamic properties of such neurons when neurons are considered as part of a population and where interactions between members and modulatory influences from remote sites would change stimulus-response properties in time and space within and across neuronal assemblies. Populational recording studies show that even the notion of a receptive field is a function of the group of neurons and the time point at which the group interactions are considered and neighboring groups of VPcells with and without nociceptive inputs have distinct spatio-temporal response properties. Such populational coding properties must also be modulated with intrinsic thalamic conditions (burst or tonic mode), as well as modulatory functions of cortical and brainstem inputs (see ► thalamus, dynamics of nociception).

At a higher level of integration, one needs to consider the mode of interaction between cortical areas, especially given that a diverse set of cortical regions have been shown to participate in pain perception. The cortico-cortical interactions are usually assumed to be direct. Recent models, however, propose that all such interactions may be mediated through thalamocortical – corticothalamic chained loops. Such models have been advanced mainly for the visual thalamus and cortex and remain to be tested for pain (see ► thalamocortical loops and information processing).

Human Imaging Studies — Visceral

The participation of the human thalamus in various innocuous and noxious visceral sensations has now been demonstrated in human brain imaging studies. Thalamic activity in humans has now been observed in angina, silent ischemia and syndrome X, as well as in noxious esophageal stimulation, gastric distension and noxious gastrointestinal distension in healthy



Nociceptive Processing in the Thalamus, Figure 4 Average responses of 10 neurons in anesthetized monkey (Kenshalo et al. 1980). Recordings are from VPL. Responses to 45, 47 and 50°C thermal stimuli are shown. The stimulus time course is shown below each response. Figure from Fig. 5 of Kenshalo et al. 1980.

subjects and in irritable bowel syndrome patients (see ► thalamus and visceral pain processing (human imaging) ► thalamus, clinical visceral pain, human imaging).

Human Imaging Studies — Acute Pain and Clinical Conditions

Thalamic activity has been observed in some of the earliest brain imaging studies of pain (Jones al. 1991). There is now ample evidence that thalamic activity can be reproducibly observed in human studies of acute or experimental conditions. More recent studies have attempted to parcel this activity into lateral and medial activations. Spatial resolution of this technology limits our ability to state the specific thalamic nuclei activated in the human brain (see ► human thalamic response to experimental pain (neuroimaging)).

Inflammatory pain conditions *vs.* neuropathic pain conditions in animals show distinct reorganization of the peripheral and spinal cord circuitry. This has been only minimally studied in the thalamus (Vos et al. 2000) and the results indicate increased rates and more nociceptive responses for VP cells in neuropathic rats. Human brain imaging studies generally show a decreased baseline activity, noxious stimulus evoked activity and atrophy in chronic clinical pain states, but there is no evidence as to whether this pattern is generally applicable for inflammatory pain conditions as well (see ► thalamus, clinical pain, human imaging).

Human Thalamus: Recording, Stimulation and Lesion

Neurosurgical attempts to control chronic pain and tremor by thalamic lesions or stimulation, such as ► thalamotomy, ► deep brain stimulation, ► gamma knife procedures or stereotactic surgeries targeting the thalamus provide the opportunity to study thalamic neuronal properties in conscious humans or to examine the effects of localized electrical stimulation evoked perceptions. In the lateral thalamic ► Vc region, the human equivalent of VP, WDR and NS type nociceptive cells are described. A few nociceptive cells are also found in human medial thalamus. Unfortunately, the human studies cannot pinpoint the exact location of such neurons. In subjects with a history of existing painful conditions, there is now good evidence that incidence of stimulation-evoked pain is enhanced in Vc and this increase may be more prominent in the periphery of Vc (posterior-inferior area) (see ► lateral thalamic pain-related cells in humans; ► human thalamic nociceptive neurons)

Based on different traditions and theoretical ideas, different neurosurgeons have had their preferred targets for thalamic stimulation or lesion for pain relief. Jeanmonod and colleagues have targeted the CL region and within that region especially neurons that exhibit low threshold calcium spike bursts since they believe that

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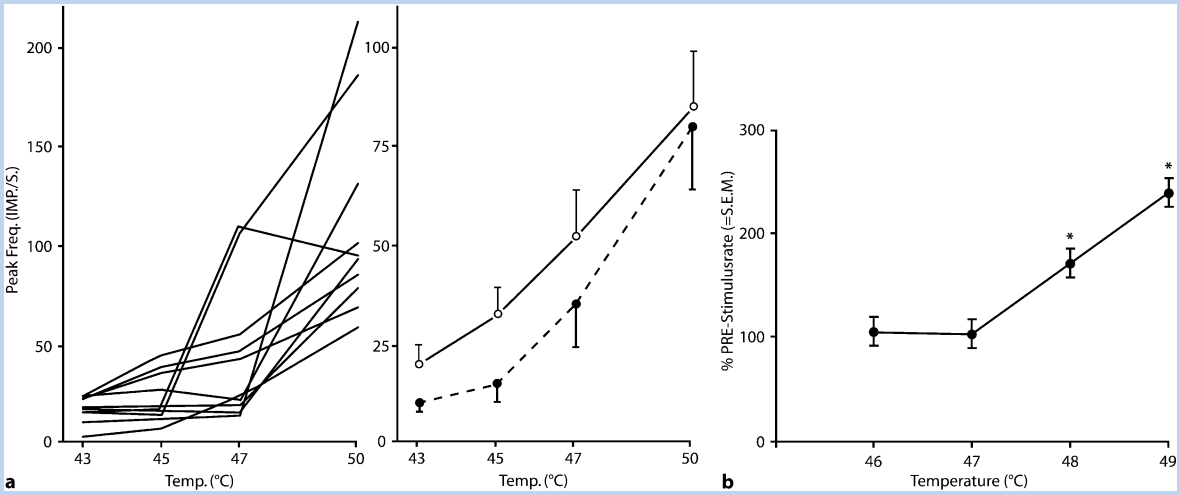


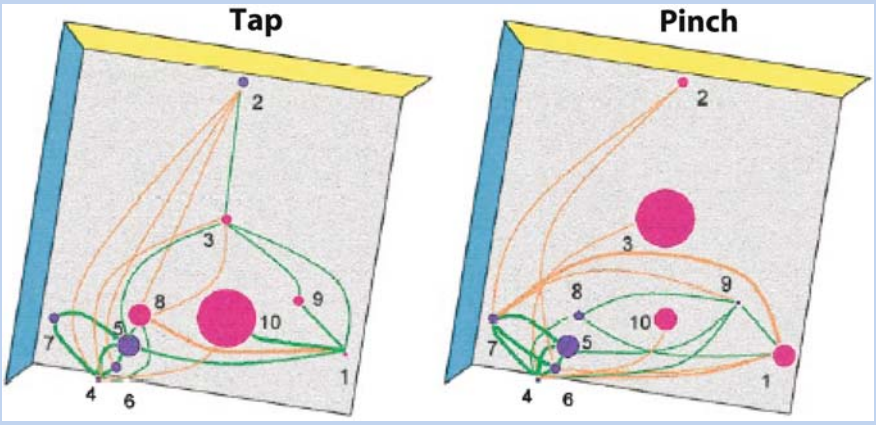
Nociceptive Processing in the Thalamus, Figure 5 (a) Stimulus-response for 10 neurons found in VPL in the anesthetized monkey (Kenshalo et al. 1980). The peak impulses / second are plotted for each corresponding temperature. Left panel are individual neurons, right panel is group average (from Fig. 4 of Kenshalo et al. 1980). (b) Average stimulus-response curve of 6 heat activated and heat / cold activated neurons to temperatures of 46–49°C, as compared to baseline activity (Bushnell et al. 1993). The neurons were characterized in the conscious monkey VPM. Stimulus-responses increase only for 48 and 49°C. Figure from Fig. 6 of Bushnell et al. 1993.

neurogenic pain of central or peripheral origin can be adequately controlled by silencing these neurons with a central lateral thalamotomy (CLT). Other neurosurgeons have targeted the CM-PF region. Lenz and colleagues, on the other hand, have targeted the Vc region and its periphery, based on observing increased bursting activity in the region in chronic pain conditions in humans and in monkey studies. Increased bursting activity seems most prominent in patients with spinal transection or thalamic cells that do not have receptive fields and that are located in the representation of the anesthetic part of the body. Given the location of these bursting cells, their specific role in pain perception remains to be established (see ► thalamotomy for human pain relief; ► thalamic bursting activity, chronic pain). Electrophysiological mapping of the thalamus during neurosurgical procedures provides the opportunity to characterize the neurons' receptive fields (RF) and compare them to the perceived location and quality of sensations evoked by stimulating these neurons (projected field or PF maps). This method can reveal plasticity of thalamic organization in humans with chronic pain. Such studies have been done mainly for Vc and its periphery. In chronic pain patients, in and around Vc, there is often a mismatch between RF and PF especially at the zone between the anesthetic site, the site of sensory loss and the transition to sites with normal sensations. Moreover, electrical stimulation in and around Vc has a higher probability of evoking pain in patients with chronic pain. These plastic changes probably contribute to the chronic pain condition (see ► thalamus, receptive fields, projected fields, human).

Nociceptive responsive cells have been described in and around Vc in humans. Most nociceptive cells in this region are characterized asWDR-type. Some respond to mechanical and heat stimuli, others to mechanical and cold. Microstimulation in the same region of the human thalamus results in sensations of pain and / or heat. Nociceptive neurons in the human CM-PF have also been described, most responding to noxious pinprick but not innocuous touch (see ► parafascicular nucleus, pain modulation ► human thalamic nociceptive neurons ► Lateral thalamic pain-related cells in humans). In a subject suffering from angina, thalamic micro-stimulation evoked a pain 'almost identical' to her angina, which started and ended in exact relationship to the electrical stimulation. The stimulation site was in the periphery of Vc. Thus, this region and its cortical connectivity can access the memory of angina pain (see ► angina pectoris, neurophysiology and psychophysics).

Overview

It should be clear from this overview that there remain large gaps in our knowledge regarding the role of the thalamus in nociception. Unfortunately, animal studies of the thalamic physiology of pain have dramatically decreased in the last few years. Perhaps this is due to the success of human brain imaging studies that provide us with information regarding thalamus and cortex in conscious human pain perception. There is no doubt that human brain imaging is providing exciting new insights into the role of the CNS in pain. On the other hand, the spatial and temporal resolution of these techniques severely limit the detailed information on neuronal and glial processes that remain to be uncovered



Nociceptive Processing in the Thalamus, Figure 6 Dynamics of nociceptive responses in a group of neurons in the lateral thalamus in the monkey (Apkarian et al. 2000). Responses of 10 neurons studied simultaneously in a 100 micron^3 space are shown. The relative locations are shown by the circles, the size and color indicate stimulus responses (red increased activity, blue decreased activity). Connections between cells indicate strength of correlations (orange positive, green negative). The figure illustrates that response magnitude and connectivity change dynamically with different types of stimuli for a cluster of cells that are involved in coding nociceptive inputs (from Apkarian et al. 2000).

in order to properly understand the functional roles of various thalamic structures and their interconnections with the cortex in pain perception.

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Nociceptor Generator Potential

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Synonyms

Receptor potential; generator current

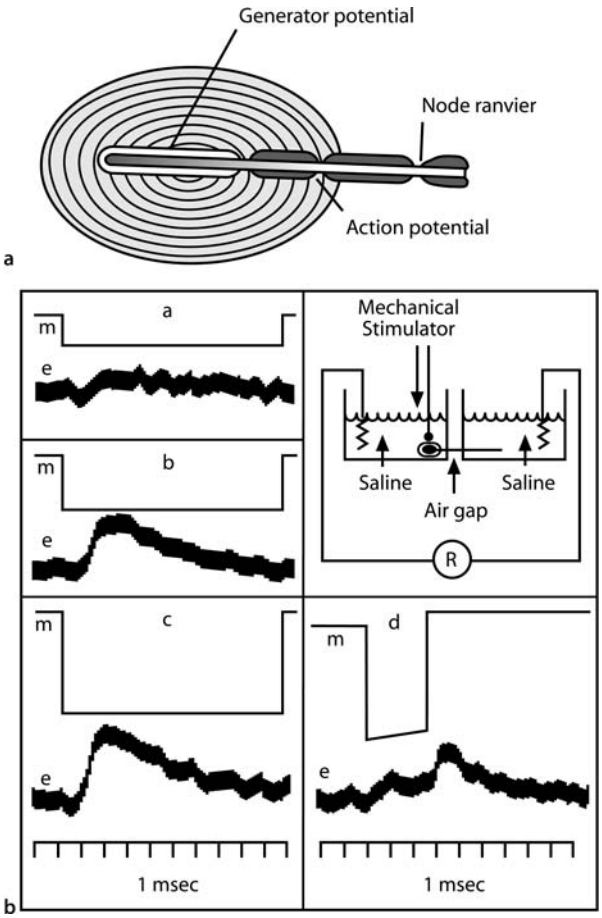
Definition

The local change in ► membrane potential caused by the opening of ion channels in the peripheral terminals of nociceptor neurons, when natural stimuli (mechanical, thermal, chemical) activate their transduction mechanisms.

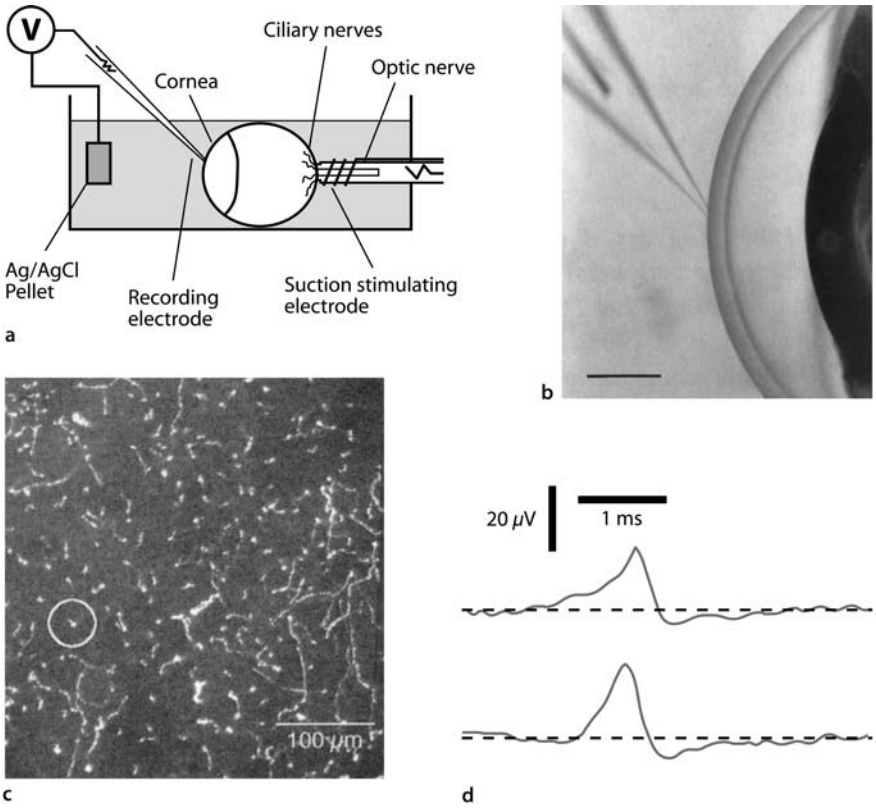
Characteristics

Transduction of natural stimuli by the specialized membrane of sensory receptor cells leads ultimately to the opening and closing (gating) of ion channels and to the generation of local electrical signals. These were already described in early studies using invertebrate stretch receptor cells (Eyzaguirre and Kufler 1955) as well as in the receptor cells of specialized sensory organs in mammals, such as the cochlear hair cells, the olfactory neurons or the retinal photoreceptors, taking advantage of their accessibility to direct electrophysiological recording (for review see Gardner and Martin 2000). In all cases, the gating of ► transduction channels triggered by the stimulus caused charge transfer across the membrane and a gradual depolarization (or hyperpolarization) of an amplitude proportional to the intensity of the stimulus called the 'receptor or generator potential'.

The receptor endings of primary sensory neurons in mammals are not easily accessible to the conventional biophysical methods that were successfully applied to the cell soma. An indirect approach aimed at recording the small current flows associated with the opening of transduction channels in sensory endings was made in the middle of the 20th century using the pacinian corpuscle, a specialized mechanoreceptor that responds to very low mechanical forces and can be easily visualized and isolated from the mesentery of the cat. The pacinian corpuscle is formed by the nerve terminal of a large myelinated sensory axon surrounded by a number of concentric lamellae, which loses its myelin sheath and Schwann cells upon entering the corpuscle, running subsequently as a straight, bare nerve ending. In a series of classical studies (Gray and Sato 1953; Loewenstein 1961), the pacinian corpuscle isolated 'in vitro' was employed to record extracellularly the ► membrane potential changes associated with mechanical stimulation of the corpuscle surface, establishing that in the most distal part of the nerve terminal the stimulus evoked a flow of generator current of an amplitude proportional to the magnitude of indentation (Fig. 1). ► Generator currents could summate temporally and propagate electrotonically (i.e. declining exponentially with distance) along the length of the axon. When the amplitude of the depolarization reaching the first node of Ranvier (located inside the corpuscle near to the point of entrance of the nerve) attained a critical level, an ► action potential was generated (Fig. 1). The conclusion of these and subsequent studies, was that the generator process in sensory receptor fibers takes place in a terminal portion of the nerve membrane that is not electrically excitable, i.e. cannot support a regenerative change in sodium conductance and that the process of transduction that leads to the generator potential is spatially separated from the point where propagated action potentials are produced. The firing frequency and the duration of the impulse discharge are proportional



Nociceptor Generator Potential, Figure 1 (a) The pacinian corpuscle. (b) Generator potentials recorded extracellularly in a pacinian corpuscle, '*in vitro*'. a, b, and c, generator potentials (e) elicited by mechanical compressions of increasing magnitude shown in m. d. Short mechanical pulses elicit both "on" and "off" responses which sum. ((a) from Schmidt RF & Thews G, 1990, Physiologie des Menschen, 24. Auflage, Springer Verlag; (b) after Gray JAB & Sato M, 1953, J Physiol 122:610–636)



Nociceptor Generator Potential, Figure 2 Recording of nerve terminal impulses in the guinea pig cornea. Schematic diagram of recording set-up (a) and photomicrograph (b) showing the location of the recording electrode. (c) confocal micrograph of nerve terminals in the cornea. (d) averages of spontaneously occurring nerve terminal impulses recorded from a mechano-nociceptor (upper trace) and a polymodal nociceptor (lower trace).

to the amplitude and duration of the generator potential (for details see Patton 1966; Gardner and Martin 2000). It has been speculated that the sequence of phenomena observed in the transduction process of mechanoreceptor fibers is general to all mammalian sensory receptor endings, including nociceptive terminals, where the transduction channels opened by mechanical, thermal or chemical stimuli are thought to generate a local receptor or generator potential that will ultimately lead to impulse firing in the parent axon (Belmonte 1996). However, direct evidence for this extrapolation is still lacking, due to the difficulty of applying the intracellular or extracellular recording techniques used in other receptor classes to nociceptive nerve fibers intimately embedded in their surrounding tissues and with a diameter below 1 µ.

In recent years new technical approaches have offered indirect evidence of the presence of generator currents in nociceptive terminals. Extracellular activity of single ► nociceptive nerve endings was successfully recorded in the cornea of the eye (Fig. 2) using a large tip microelectrode tightly applied against the corneal surface (Brock et al. 1998). Terminal sensory branches run between corneal epithelium cells ending close to the most superficial epithelium layers; the high resistance seal formed by the electrode allowed the recording of the spontaneous and stimulus-evoked propagated impulse activity of the ending located immediately below the electrode tip. In these experiments, a depolarization preceding the propagated nerve terminal impulse, suggestive of a local generator current was occasionally observed in polymodal nociceptor endings (Brock et al. 1998). Likewise, in single nociceptive fibers of the rat skin, Sauer et al. (2004) using the 'threshold tracking technique' reported that heat and ► bradykinin stimulation of polymodal nociceptor endings was preceded by a reduction of threshold suggestive of a local depolarization presumably corresponding to the generator potential.

Nevertheless, the location in nociceptors of the transformation site where generator currents give rise to propagated impulses when the depolarization exceeds threshold remains unknown. It has been suggested, based on morphological evidence that the patches of axonal membrane devoid of Schwann cell coating observed in the terminal portion of ► knee joint nociceptors correspond to the ► transduction sites where receptor potentials would be generated (Heppelmann et al. 1990) while action potentials occur at a more central point. However, in corneal polymodal nociceptor endings it has been shown that the most distant portion of the terminal possess sufficient density of ► tetrodotoxin-resistant sodium channels to sustain propagated action potentials (Brock et al. 1998), a characteristic that is not shared by cold-sensitive nerve fibers, whose peripheral terminals seem to lack regenerative properties

(Brock et al. 2001). Thus, the possibility exists that in nociceptor terminals, the ion channels responsible for generator currents and those sustaining the production of propagated action potentials are not spatially segregated. This may have a functional significance in the profusely ramified nociceptor fibers. Action potentials originated at the peripheral endings by a direct action of the stimulus also propagate antidromically and invade terminals of the same parent axon that were not directly excited by the stimulus. A large proportion of nociceptor terminals contain vesicles filled with ► neuropeptides (CGRP, SP), that are released by the entrance of calcium ions driven by the invading antidromic action potential, thereby contributing to neurogenic inflammation.

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Nociceptor Inactivation

► Nociceptor, Adaptation

Nociceptor Sensitization

Definition

Process by which there is a decrease in nociceptor threshold and enhanced responses to suprathreshold stimuli. This phenomenon is an increment of the excitability of the nociceptor, due to a metabolic change induced by sensitizing agents such as pro-inflammatory mediators.

► Capsaicin Receptor
► Polymodal Nociceptors, Heat Transduction
► Thalamus, Clinical Pain, Human Imaging

Nociceptor(s)

Definition

Harmful stimuli activate the peripheral endings of primary afferent neurons, also called nociceptors. Their cell bodies lie in the dorsal root ganglia (DRG) or the trigeminal ganglia. Distinct classes of nociceptors encode discrete intensities and modalities of noxious stimuli. Receptor molecules that lend these specific properties to diverse classes of nociceptors and mediate transduction have been cloned. One important molecule is the vanilloid receptor TRPV1, which serves as a transducer of noxious thermal and chemical (e.g. protons) stimuli, and can be activated by capsaicin, the active ingredient of hot chili peppers. Conduction of nociceptive signals in nociceptors is mediated via activation of voltage-gated sodium channels. A family of nociceptor-specific tetrodotoxin (TTX)-resistant sodium channels modulates the excitability of primary afferents and likely mediates pathophysiological alterations thereof.

► Acute Pain Mechanisms
► Cancer Pain, Animal Models
► Cancer Pain Management, Nonopioid Analgesics
► COX-1 and COX-2 in Pain
► Cytokines, Effects on Nociceptors
► Descending Modulation and Persistent Pain
► Dorsal Root Ganglionectomy and Dorsal Rhizotomy
► Drugs Targeting Voltage-Gated Sodium and Calcium Channels
► Fibromyalgia, Mechanisms and Treatment
► Freezing Model of Cutaneous Hyperalgesia
► Functional Imaging of Cutaneous Pain
► Hypoalgesia, Assessment
► Mechanonociceptors
► Muscle Pain Model, Inflammatory Agents-Induced
► Nociceptor, Axonal Branching
► Nociceptor, Categorization
► Nociceptors, Cold Thermotransduction
► Nociceptors in the Dental Pulp
► NSAIDs, Mode of Action
► Opioids, Effects of Systemic Morphine on Evoked Pain
► Opioids in the Periphery and Analgesia
► Postherpetic Neuralgia, Pharmacological and Non-Pharmacological Treatment Options
► Postoperative Pain, Acute Pain Management, Principles
► Postoperative Pain, Acute Pain Team
► Somatic Pain
► Spinohypothalamic Tract, Anatomical Organization and Response Properties

- ▶ Spinothalamic Tract, Anatomical Organization and Response Properties
- ▶ Thalamic Nuclei Involved in Pain, Cat and Rat
- ▶ Tourniquet Test
- ▶ TRPV1 Modulation by p2Y Receptors
- ▶ Visceral Nociception and Pain
- ▶ Visceral Pain and Nociception

Nociceptor, Adaptation

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Synonyms

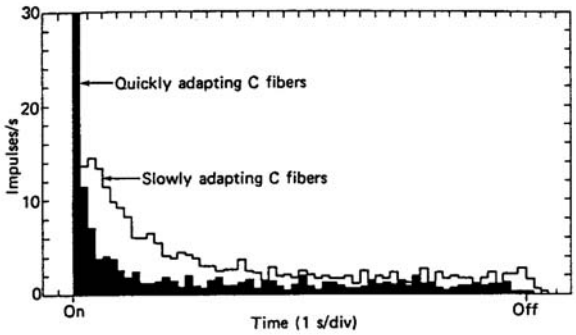
Nociceptor Accommodation; Nociceptor Inactivation

Definition

The gradual decrease over time in the response of a nociceptor to a maintained noxious stimulus of fixed intensity.
Nociceptors Action Potentials and Post-Firing Excitability Changes

Characteristics

The response of nociceptors to a constant-temperature heat stimulus adapts with time. Unmyelinated nociceptors innervating the hairy skin of monkey can be separated into two classes based on the rate of adaptation to heat stimuli (Fig. 1). In response to a 53˚C stimulus, the discharge rate of quickly adapting C fibers is 20% of the peak response within 4s, whereas slowly adapting C fibers take more than 15 s to reach this level (Meyer and Campbell 1981). Myelinated fibers can also be separated into two classes based on their heat response: Type II fibers adapt quickly in a manner similar to quickly adapting C-fibers, whereas Type I fibers actually exhibit an increase in their response with time (Treede et al. 1998).



Nociceptor, Adaptation, Figure 1 Adaptation of response to heat (53˚C) in C-fiber nociceptor afferents in the monkey (From Meyer and Campbell 1981).

Nociceptors also exhibit a slowly adapting response to mechanical stimuli applied to their receptive field (Slugg et al. 2000). An exception to this rule exists for mechanically-insensitive nociceptors, which can develop a response to tonic pressure (Schmidt et al. 2000). The mechanisms underlying adaptation in nociceptors are not well understood, but calcium-dependent and -independent mechanisms appear to be involved.

- ▶ Nociceptors Action Potentials and Postfiring Excitability Changes

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Nociceptor, Axonal Branching

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Synonyms

Axon reflex; neurogenic inflammation; Flare; Neurogenic Vasodilation; protein extravasation

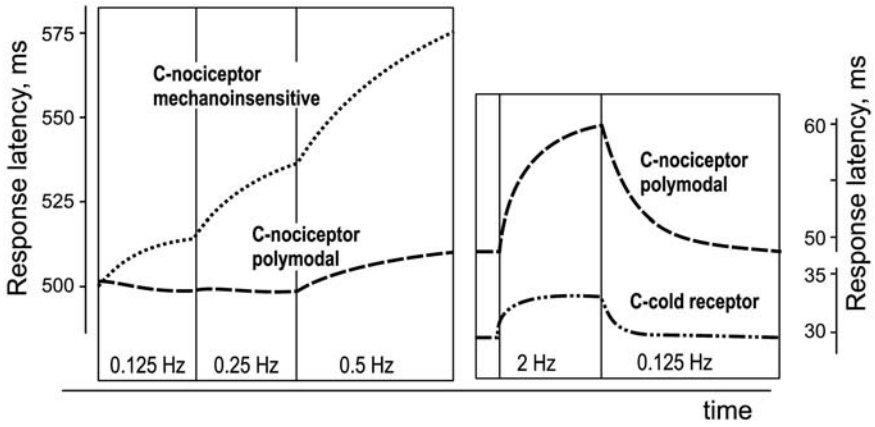
Definition

Single nociceptive nerve fibers branch extensively in the periphery to form their receptive fields: branches can measure up to 9 cm in human skin. In addition, their terminal endings can also inhibit extensive branching in the micrometer range. Axonal branching is the structural basis for antidromic action potential propagation (axon reflex), leading to neurogenic inflammation.

Characteristics

Innervation Territories of Nociceptors

The receptive field of primary afferent nociceptors has been found to be very small in rodents. However, in humans, skin innervation territories measuring up to 9 cm in diameter have been found (Schmelz et al. 1997). Most of the available data on the structure of innervation territories and branching derive from skin, as they can more easily be analyzed as compared to deep somatic or visceral nociceptors. Extensive branching of skin nociceptors has also been found in monkey skin, with branching



Nociceptor, Axonal Branching, Figure 1 Response latency of action potentials between electrical stimulation of the receptive endings in the skin and recording in the peripheral nerve (n. peroneaus) is shown. Left panel: Electrical stimulation at increasing frequency provokes increased response latency for different classes of afferent C-fibers (activity dependent slowing). The activity dependent slowing is most pronounced in mechano-insensitive C-nociceptors which slow down in conduction velocity even at low stimulatory frequencies of 0.125 or 0.25 Hz. The slowing of traditional mechano-responsive C-nociceptors ("polymodal") is far less pronounced and clearly separates between the two nociceptor classes (modified from Weidner et al. 1999). Right panel: At higher stimulation frequencies of 2 Hz polymodal nociceptors show a pronounced activity dependent slowing which clearly separates them from cold-sensitive C-fibers (C-cold receptor), which only slightly increase their response latency when stimulated at 2 Hz (modified from Serra et al. 1999).

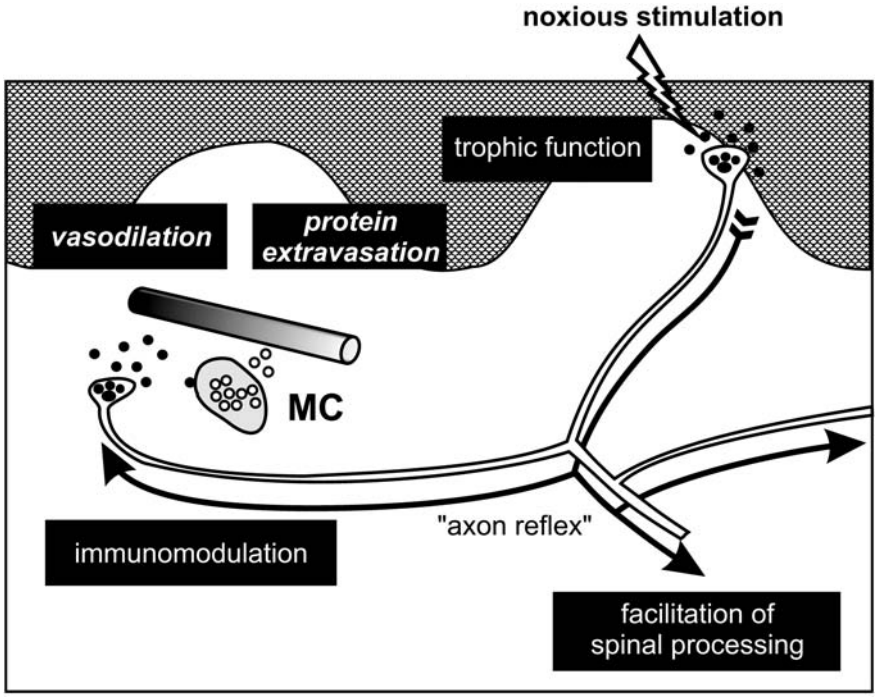
points being rather proximal from the actual receptive field; interestingly, frequently unmyelinated branches of A delta fibers were found, which had a length of about 5 cm (Peng et al. 1999).

Axonal Properties of Different Nociceptor Classes

In human skin unmyelinated nociceptors fall into two basic classes: the majority of the fibers are mechano-heat sensitive polymodal nociceptors; however, about 20% are unresponsive to mechanical stimulation (Schmidt et al. 1995). These "silent" or "sleeping" nociceptors differ from conventional polymodal nociceptors in their receptive properties, their biophysical characteristics and their function. They have higher activation thresholds for heat and are not activated even by intense mechanical stimuli (Schmidt et al. 1995). Their innervation territories in the leg are larger (6 vs. 2 cm^2), and conduction velocity is lower (0.8 vs. 1 m/s,) than in polymodal fibers (Schmidt et al. 1995). Most interestingly, their high transcutaneous electrical thresholds and their activity dependent hyperpolarization by far exceed the values observed in polymodal nociceptors. Although based on the axonal properties, analysis of activity dependent hyperpolarization allows classification of these two nociceptor classes (Weidner et al. 1999), and thus predicts sensory properties of their endings. Moreover, activity dependent hyperpolarization has also been shown to separate C-cold thermoreceptors from C-polymodal nociceptors (Fig. 1). It should be pointed out that this unexpected correlation between specific axonal properties and characteristics of sensory endings has a variety of implications. As mechanisms of activity-dependent hyperpolarization are currently being clarified on a molecular level, immunohistochemistry might in the future enable differential staining and functional identification of axons. First clinical results confirm that this approach can be used to improve characterization of neuropathic axonal changes (Boettger et al. 2002).

Neurogenic Inflammation

Decades ago Thomas Lewis described the erythema arising in human skin in the surroundings of trauma as part of the "triple response" to noxious stimuli (Lewis et al. 1927). This "flare response" is dependent on the integrity of primary afferent nerves, but not on their central nervous connections. From his own findings and earlier work Lewis developed the concept of "axon reflex flare", i.e. the notion that "nocifensive" nerve fibers excited by a trauma send impulses not only into the central nervous system, but also via axon branches into the surrounding skin, where they trigger the release of a vasodilating substance from the nerve endings. Neuropeptides are now held responsible for the vasodilatation, which are produced by small dorsal root ganglion cells and transported in their thin axons to the central and peripheral nerve terminals. The main vasodilatory agent is probably CGRP which induces vasodilatation, but no plasmaextravasation (Brain 1996). The edema, caused by increased permeability of the endothelia for plasma proteins (neurogenic protein extravasation) can be attributed to the release of substance P. However, a variety of other neuropeptides like neurokinin A, neurokinin B, somatostatin, galanin, and recently, endomorphins have been also be found in primary afferent neurons. It should also be noted that in addition to the acute vascular effects, neuropeptides have important trophic functions and modulate the activity of local immune cells (Fig. 2).



Nociceptor, Axonal Branching, Figure 2 Schematical drawing of mechanisms involved in dermal neurogenic inflammation. Noxious stimulation of the skin (hatched area) results in generation of action potentials in nociceptors. The action potentials reach the arborisations of the axonal tree via the axon reflex (black arrows). By depolarization of the terminals, neuropeptides (black circles) are released. Key effects of neuropeptides are given in black squares. The involvement of mast cell mediators (MC, open circles) in vasodilation and protein extravasation in neurogenic inflammation is controversial.

Analysis of Neurogenic Inflammation

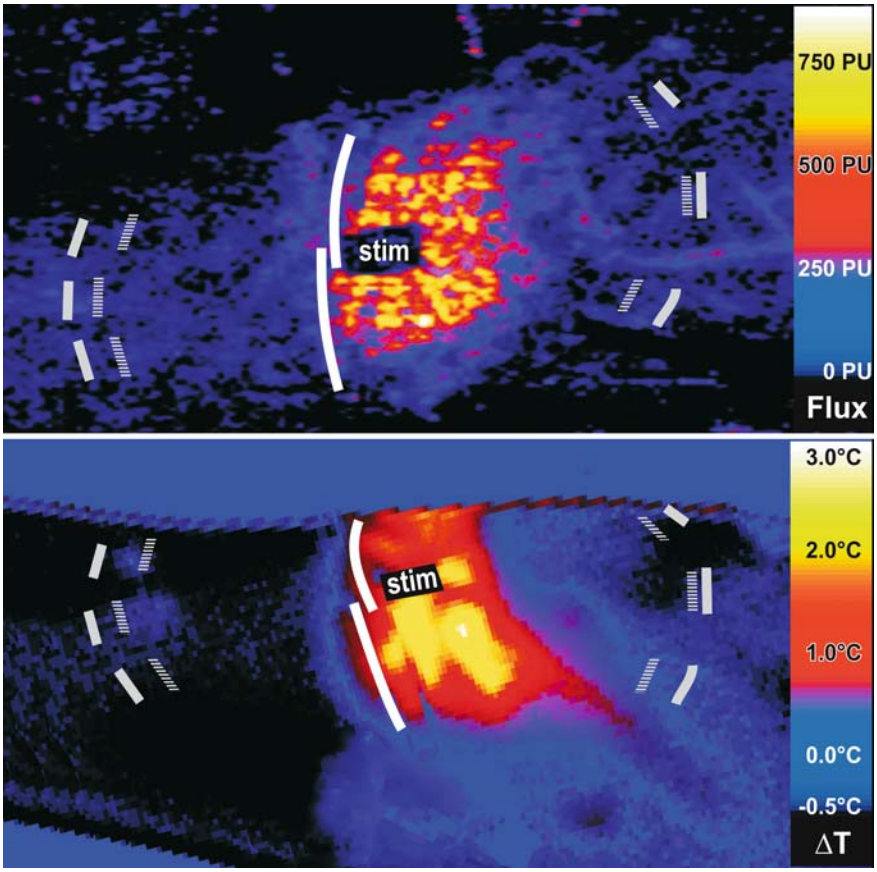
Chemical, thermal, and electrical stimulation has been widely used to elicit neurogenic inflammation, and direct evidence of neuropeptide release has been obtained using capsaicin as well as antidromic electrical nerve stimulation. In rodents, activation of polymodal nociceptors was sufficient to cause neurogenic inflammation (Gee et al. 1997). In contrast, mechano-insensitive, but heat- and chemosensitive C-nociceptors have been found responsible for the neurogenic vasodilation in pig skin (Gee et al. 1997; Lynn et al. 1996) and human (Sauerstein et al. 2000). The extent of the neurogenic erythema nicely matches the large cutaneous receptive fields of mechanoinsensitive nociceptors, and their high electrical thresholds also match the strong currents required to provoke neurogenic flare electrically. Thus, neurogenic inflammation in human differs from rodents, in which the neurogenic inflammation can be elicited by polymodal C nociceptors, and consists of a combination of vasodilation and protein extravasation (Sauerstein et al. 2000). In healthy volunteers no neurogenic protein extravasation could be induced (Sauerstein et al. 2000), but may develop under pathophysiological conditions (Weber et al. 2001).

Neurogenic Inflammation and Secondary Hyperalgesia

The areas of vasodilation and warming around a noxious stimulation site have been found to be similar to the areas of secondary mechanical hyperalgesia (punctate hyperalgesia) (Serra et al. 1998). However, recent results would suggest, that by blocking axonal action potential propagation by a local anesthetic, only the spread of axon reflex vasodilation and warming can be blocked. In contrast, areas of punctate hyperalgesia developed symmetrically, even beyond a peripherally located "anesthetic strip" (Fig. 3).

Epidermal Axonal Branching

Under physiological conditions unmyelinated human nerve fibers entering the epidermis are found to be oriented straight up, and reach the outermost layers of viable skin without pronounced branching (Hilliges et al. 1995). Interestingly, in some human skin diseases extensive branching of epidermal nerve fibers has been described. Increased intradermal nerve fiber density has been found in patients with chronic pruritus. In addition, increased epidermal levels of neurotrophin 4 (NT4) have been found in patients with atopic dermatitis, and massively increased serum levels of NGF and SP have been found to correlate with the severity of the disease in such patients (Toyoda et al. 2002). Increased fiber density and higher local NGF concentrations were also found in patients with contact dermatitis. Most interestingly, intraepidermal sprouting has also been found as a physiological response to circular skin incision (Rajan et al. 2003): „collateral sprouting" from axons at the incision margins lead to centripedal reconstituting of skin innervation, probably due to higher local NGF concentrations in the denervated skin (Rajan et al. 2003). A similar mechanism might also explain findings in patients with diabetic neuropathy characterized by reduced epidermal innervation density, but higher degree of epidermal branching (Polydefkis et al. 2003). It will be of major interest in the future to assess the



Nociceptor, Axonal Branching, Figure 3 Specimen of transcutaneous electrical stimulation (1 Hz, 50 mA, 0,5 ms; stimulation site ["stim"] is marked by a rectangle) provoking an area of increased superficial blood flow as assessed with a laser Doppler scanner (upper panel) and with an infrared thermocamera (lower panel). An anesthetic strip was induced by perfusing two intradermal microdialysis membranes (vertical white lines) with 2% lidocaine. The borders of hyperalgesia to punctate stimuli (grey lines) and to light stroking (dotted lines) are shown in the laser Doppler scan and the thermogram. (Modified from Klede et al. 2003).

effects of the local sprouting on the sensory function of the nociceptors. There is already evidence of increased epidermal nerve fiber sprouting in vulvodynia, and moreover, signs of nociceptor sensitization (Bohm-Starke et al. 2001). Taken together, these data would suggest that local inflammatory processes could initiate nociceptor sprouting and sensitization by increased NGF production.

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Nociceptor, Categorization

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Synonyms

Categorization of Nociceptors

Definition

Why categorize a mixed set of primary afferent neurons? As Lynn (1996) pointed out, classifying the components of a neuronal population into categories on the basis of shared features does more than one service. It facilitates communication by providing a shorthand to designate a subset with certain features. It also provides a way to deal with the large number of neurons in the mammalian nervous system that serves common functions. Moreover, appropriate classification of neurons can help facilitate concepts on development and the functional organization of nervous mechanisms.

Characteristics

Noxious and Nociceptor

Definition of certain classes of stimuli as noxious and creation of the term nociceptor (noci-receptor) were outgrowths of the dispute in the late 19th Century about the sensory nature of pain. Physicians and physiologists of those days generally accepted pain to be a sensation. On the other hand, philosophical critics of this idea argued that in contrast to accepted sensations, pain does not have a "well defined" physical or chemical stimulus (Perl 1996). Mechanical events, heat, cold, and chemical agents can all produce it. Charles Sherrington (1906), an eminent physiologist of the time, proposed an answer to this criticism with the logic that pain ordinarily results from tissue injury. Consequently, tissue damage represents a common denominator of natural stimuli for pain. He suggested that events producing disruption of tissue or representing a physical threat to its integrity could be labeled noxious regardless of their nature, thereby providing an encompassing definition for the stimuli evoking pain. In this concept, sense organs signaling the presence of noxious events were labeled noci-receptors (now shortened to nociceptors). Designation of stimuli as noxious creates its own problems. Tissues of the mammalian body are diverse with widely differing mechanical and thermal characteristics. This means that quantitatively the intensity of an environmental or circumstantial event necessary to cause tissue damage varies over a substantial range. Compare mechanical durability of the cornea of the human eye to the skin on the sole of a human foot. Furthermore, subcutaneous tissues and organs are protected from many environmental changes and potential insults; their exposure to some conditions that are innocuous for the skin would lead to tissue injury and thereby should be considered noxious. Thus, the nature of noxious events and their signaling by sense organs must be considered in the context of tissue type and location.

Classification of Sense Organs as Nociceptors

A primary afferent neuron is appropriately considered to be a nociceptor if the intensity of the most effective "natural" stimulus that evokes conducted action potentials approaches or exceeds the noxious (damaging) level for the innervated tissue. This criterion implies that such sensory units respond weakly or not all to innocuous stimuli of any type. Since the nature and intensity of noxious stimuli will vary for different tissues, the responsive characteristics of nociceptors will differ from one tissue to another.

Observations demonstrating nociceptors to be distinctive categories of somatic sense organs provide evidence that more than one type innervate many tissues. How are these different types distinguished? Actually, nociceptor classification has evolved as information about them expands and the changing criteria sometimes have led to ambiguity. Given the view that the function of nociceptors is to transmit to the central nervous system information about events dangerous to the physical integrity of the tissue they innervate, a first order in their classification, and one commonly used, is the nature of effective stimuli or the events signaled.

Classification of Nociceptors by Effective Stimuli

Much information about nociceptors has come from study of the innervation of epithelial tissue, particularly the skin. Early in the documentation of cutaneous nociceptors as distinctive types of sense organs, it became evident that the skin is innervated by more than one type (Perl 1996). As already suggested, nociceptors can be distinguished and classified according to the nature of stimuli activating them. On this basis, several categories are demonstrable in mammalian skin (Perl 1996; Campbell and Meyer 1996). In terms of effective stimuli, the cornea, another epithelial tissue, is innervated by closely similar varieties (Belmonte and Gallar 1996). One kind of cutaneous nociceptor responds vigorously to strong mechanical stimulation, positively grading the frequency and number of impulses in proportion to stimulus intensity. Extreme temperatures (e.g., noxious heat, freezing) excite such mechanical nociceptors (high threshold mechanoreceptors) only after a delay. A second category of cutaneous nociceptor responds more

globally, being promptly activated by heat, mechanical distortion and irritant chemicals including protons. The latter response pattern led to the designation of this class as polymodal nociceptors. A third kind, prominent in the innervation of glabrous skin, is excited by mechanical distortion and elevated skin temperature (mechanical-heat), but does not promptly respond to surface application of irritant chemicals or acid. A fourth type responds both to low skin temperatures and to noxious mechanical stimuli (mechanical-cold). In addition to these four categories, evidence exists for a class of primary afferents (labeled 'silent' nociceptors) that are only excited by mechanical stimuli when sensitized by local inflammation and for another category selectively excited by ► histamine (pruritus receptors).

Characterization by Conduction Velocity

Whereas the nature of effective stimuli represents an approach to classifying nociceptors that relates to function, it is not the only important criterion. Indications that peripheral stimuli evoke pain after distinctly different delays existed prior to documentation of nociceptors as a special set of peripheral sense organs. Transient application of a noxious mechanical or heat stimulus to distally located skin anecdotally and experimentally was noted to provoke a double pain response, one of short latency and a second delayed; these differences in latency can be attributed to differences in ► conduction velocity of peripheral nerve fibers responsible for the afferent messages. In addition to these distinctions of delay, "first" and "second" pain is reported to differ in quality of the sensation. This circumstantial evidence for conduction differences is consistent with findings that some categories of nociceptors have myelinated (A) and others unmyelinated (C) afferent fibers. Those with ► A fibers conduct much more rapidly (10–50×) than the ► C Fiber groups, and even though most A nociceptors have thinly thinly myelinated fibers (Aδ), a number from distal limb regions are in the medium myelinated range with Aβ (35–50 m/s) conduction velocities. A fiber and C fiber nociceptors also differ in other ways. For instance, several sets of C-fiber nociceptors express peptide mediators (e.g., substance P, CGRP) that are apparently absent in myelinated nociceptors. Furthermore, the central projections of A and C fiber nociceptors differ. These distinctions are consistent with certain differences in functions initiated by the A and C fiber categories.

Characterization by Tissue of Origin

Primary afferent neurons with responsive features of nociceptors innervate many mammalian tissues or organs, both somatic and visceral. In addition to skin and cornea these include teeth, skeletal muscle, tendon, joints, bone, urethra, ureter, urinary bladder, blood vessels, bronchi, heart, pleura and peritoneum, segments of the alimentary tract, meninges, and testis (Cervero 1996). These tissues differ substantially in physical and chemical attributes, differences that are reflected in part in the responsive and signaling features of innervating nociceptive fibers. The testis is innervated by nociceptors mimicking the broad activation of cutaneous polymodal nociceptors by being responsive to noxious mechanical, heat and chemical stimuli (Kumazawa 1996). In contractile tissues, unusually high tension is an effective excitant for part of the nociceptive innervation. Similarly, lowered pH (protons), by itself or in combination with anoxemia, activate or enhance the responsiveness of certain nociceptive afferents of skeletal muscle (Mense 1996). Circumstantial evidence suggests that subcutaneous tissues such as joints and muscle contain a number of primary afferent fibers that are unresponsive to mechanical or thermal stimuli until injury has induced inflammation and its chemical environment (Schaible and Schmidt 1996). The latter can be considered types of chemoreceptor. Thus, the classification of nociceptors must take into account the tissue innervated in addition to effective stimuli, and the diameter (conduction velocity) of the afferent fiber.

Characterization by Molecular Features

A presumption underlying hypotheses about differentiation and specialization of biological cells, in our case neurons, is that these processes are guided and controlled by the presence and expression of particular molecular entities. Relating factors associated with molecular expression to functionally important features of nociceptors is an ongoing effort and at present represents at most an emerging story with promise for future insights.

In one example, the heat responsiveness of polymodal nociceptors is attributed to a membrane receptor, ► TRPV1 (Caterina and Julius 2002). TRPV1 donates such reactivity when expressed in heterologous cells. TRPV1 is the endogenous receptor that is selectively activated by capsaicin, the substance that gives the sensation of heat upon ingestion of "hot" pepper. A structurally related membrane receptor, ► TRPV2, is predominantly expressed in different primary afferent neurons than TRPV1, and is proposed to provide a higher threshold heat response for a set of nociceptors different from the polymodal type. TRPV2 is neither excited by capsaicin nor acid (Caterina and Julius 2002). Other relationships between molecular features and categories of nociceptors include the immunocytochemical labeling of a subset of small diameter dorsal root ganglia (DRG) neurons and their processes for the peptides, ► substance P and ► CGRP. Both circumstantial and direct correlations indicate that at least some peptide-labeled elements are polymodal or mechanical-heat nociceptors (Lawson 1996; Lawson et al. 1997). The small substance P-containing neurons are mostly distinct from an isolectin IB4-binding population of presumed nociceptors (Lawson 1996). Thus, evidence for the common presence of TRPV1, TRPV2, or any other unique cellular constituent links the nociceptors

in question by a shared feature, and represents a basis for categorization.

Characterization by Central Projection

Nociceptors, as primary afferent neurons, project to the spinal cord or the brainstem with a heavy concentration of synaptic terminations in the superficial dorsal horn or the trigeminal equivalent. The bulk of nociceptors enter the spinal cord through the ► lateral division (of the spinal dorsal root). There is a distinct layering of terminations from different nociceptor subsets as defined by other criteria. Substance P (and CGRP)-labeled endings concentrate in the marginal zone (► lamina I) and outer ► substantia gelatinosa (► lamina II), as do the non-peptide terminations of cutaneous myelinated-fiber, mechanical nociceptors (Light 1992; Perl 1984). IB4-labeled fibers generally terminate more deeply in lamina II than the concentration of substance P terminals (Woodbury et al. 2000). In addition to these superficial dorsal horn terminations, nociceptive primary afferents contribute to other dorsal horn regions. Whereas these terminal synaptic regions are not definitive markers of receptive category, they have been used as guides to relate participation by particular types of nociceptors in experimental analyses.

Summary

Categorization of nociceptors is a multifactorial task. To be of value in facilitating understanding of their biology and place in function, categorization of nociceptive afferents should consider the nature of events signaled, the structural and functional details of the neuron (including its afferent fiber), the presence of unique molecules and features of the peripheral and central connections.

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Nociceptor, Fatigue

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Synonyms

Tachyphylaxis; deactivation; desensitization; suppression

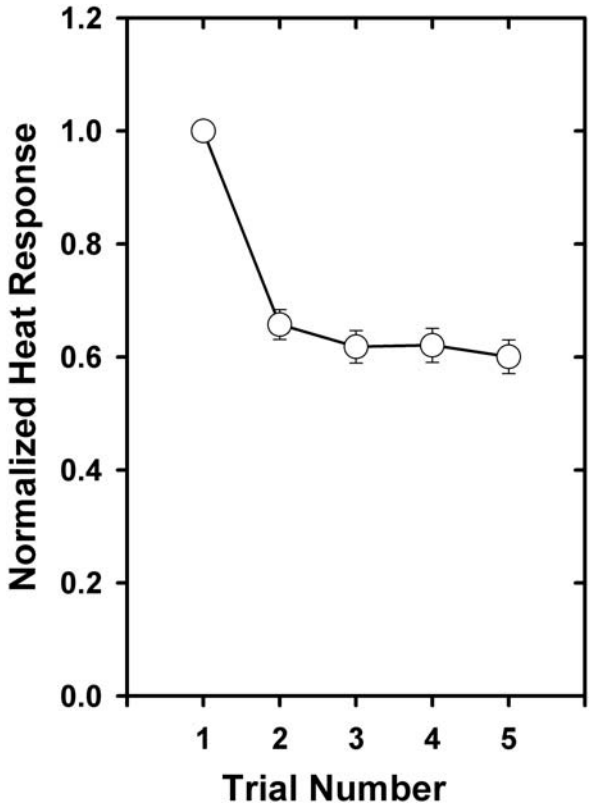
Definition

The decrement in response of nociceptors to natural stimuli that occurs upon repeated stimulation.

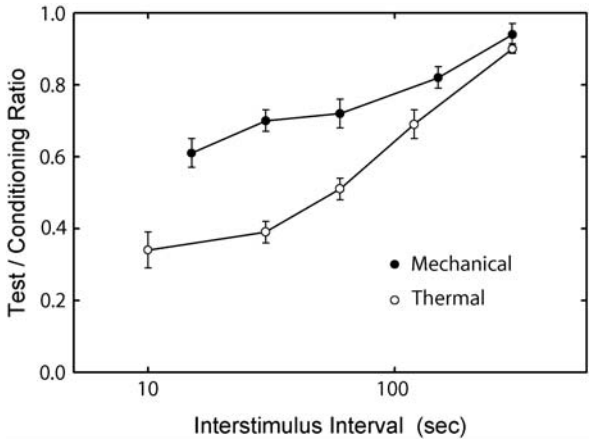
Characteristics

The response of a primary afferent nociceptor to a given stimulus is greatly reduced when the stimulus is applied a second time. For example, the response of a nociceptor to a heat stimulus applied to its receptive field is reduced by about 40% when presented a second time within 60 s of the first presentation (Fig. 1). Nociceptors exhibit fatigue to all stimuli that activate them, including heat, mechanical and chemical stimuli (Liang et al. 2001; Slugg et al. 2000; Tillman 1992). The magnitude of fatigue is dependent on the number of action potentials evoked by the conditioning stimulus, but is independent of the frequency of the evoked action potentials. Consequently, the magnitude of fatigue increases with the intensity of the applied stimulus (LaMotte and Campbell 1978; Peng et al. 2003). The time course for recovery from fatigue is slow, with full recovery taking more than 5 min (Fig. 2). Fatigue is also seen for repeated stimuli that have been applied to isolated dorsal root ganglion cells (Schwarz et al. 2000).

The psychophysical correlate of fatigue is suppression. Suppression corresponds to the decrement in pain rating to a noxious stimulus that occurs upon repeated stimulation at the same location. For example, the pain rating to a



Nociceptor, Fatigue, Figure 1 Normalized response of C-fiber mechano-heat sensitive nociceptors to five presentations of a heat stimulus with a repetition period of 60s. The response decreased between trials 1 and 2 and stabilized to about 60% of the response to the first heat stimulus. (Adapted from Peng et al. 2003).



Nociceptor, Fatigue, Figure 2 Slow recovery from fatigue. The magnitude of fatigue is dependent on the time between the conditioning and the test stimulus. The response to the test stimulus approaches the response to the initial identical conditioning stimulus as the interstimulus interval increases. The fatigue to heat stimuli is greater than the fatigue to mechanical stimuli (Adapted from Slugg et al. 2000; Tillman 1992).

heat stimulus applied to the hand is significantly lower than the pain rating to the same stimulus applied 30 s earlier (LaMotte and Campbell 1978). The time for full recovery is greater than 5 min.

Most nociceptors are polymodal and respond to more than one stimulus modality. Cross-modal fatigue occurs such that stimulation with one stimulus modality (e.g. mechanical stimuli) will lead to a decrement in response to another stimulus modality (e.g. heat). In addition, stimulation to one part of the receptive field can lead to fatigue in another part of the receptive field, presumably due to ► antidromic invasion of the adjacent terminals. The magnitude of fatigue is less, and recovery time is quicker, for cross modal fatigue compared to unimodal fatigue (Peng et al. 2003).

Fatigue may occur at the stimulus transduction and/or at the spike initiation site and may involve calcium-dependent and independent mechanisms.

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Nociceptors, Action Potentials and Post-Firing Excitability Changes

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Synonyms

Action Potential in Different Nociceptor Populations; Post-Firing Excitability Changes in Different Nociceptor Populations

Definition

The electrophysiological properties of nociceptive neurones.
primary afferent

Characteristics

This essay will consider the electrical properties of mammalian nociceptive neurones, covering both the action potential itself and the changes in excitability that follow it. Much of the work in this area has been concerned with major differences between nociceptors

on the one hand, and non-nociceptive afferent neurones on the other. There has also been a relatively small amount of work showing clear differences within nociceptor subclasses. As well as describing differences in such properties as time course or amplitude, an attempt will be made to look at likely differences in underlying membrane properties, especially in the sub-classes of ► ion channel present. Differences in electrical properties directly affect the ability of nociceptive neurones to encode information about stimuli, for example by limiting firing rates. In addition, however, the finding of differences in the key proteins controlling excitability between nociceptors and non-nociceptors, and between sub-classes of nociceptor, opens up important possibilities for the development of selective analgesic agents.

Action Potentials in Nociceptive Neurones

Axonal Action Potentials

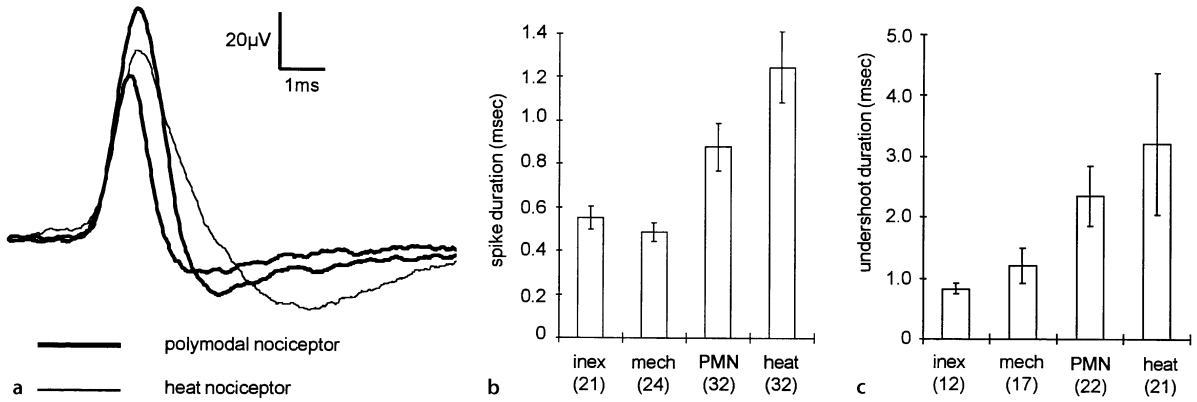
Extracellular recordings from unmyelinated fibres have revealed that there are differences in the action potential time course between sub-classes of nociceptor (Fig. 1). The only extensive study has been in the pig (Gee et al. 1999), where 2 major classes of C-fibre nociceptor have been found in the skin. Polymodal nociceptors respond to all types of nociceptive stimuli, whereas heat nociceptors respond to heat and chemicals, but not to pressure. The heat nociceptors have ► spikes of longer duration and with longer after potentials than do the polymodal nociceptors (Fig. 1). In the rat, there are mainly polymodal nociceptors, and these have wide spikes compared with non-nociceptive afferents (Gee et al. 1999). However, no comparisons have been made with other classes of C-fibre nociceptor in this species. In both pig and rat there are also A-fibre nociceptors, and these have narrow spikes compared with C-fibre nociceptors, as one would expect from the generally faster excitability changes seen in myelinated compared with unmyelinated ► axons. In man, microneurographic recordings have again revealed longer duration action potentials in C-nociceptor axons compared with C-cold afferent axons (Serra et al. 1999). Interestingly, a group of mechanically insensitive axons with small, short duration spikes was also observed (Serra et al. 1999). The functional identity of these fibres has not been established, but they might be a second group of very mechanically insensitive nociceptors with different axonal properties.

Soma Action Potentials

Intracellular recordings from dorsal root ganglion cells reveal that, as described above for the axons, nociceptive neurones have spikes with a slower time course than mechanoreceptor neurones with a similar conduction velocity (Lawson 2002). This is true for the ► soma spikes of neurones with myelinated and unmyelinated axons. Often the broad spikes of nociceptive neurones have a distinct inflexion (or "shoulder") on the falling phase. After-potentials are also of longer duration. No studies have been made of soma spike shape for sub-classes of nociceptor. One interesting observation is that following peripheral inflammation in the innervation territory of the neurone, the soma spike becomes shorter in duration (Djouhri and Lawson 1999).

Mechanism of Differences in Action Potential Duration

The longer action potential durations in nociceptive neurones may be due to a combination of slower



Nociceptors, Action Potentials and Post-Firing Excitability Changes, Figure 1 Axonal action potentials recorded from the saphenous nerve of the anaesthetised pig. (a) Examples of three C-fibre spikes recorded from the same filament. Two (thick lines) were polymodal nociceptor units, and one (wider spike with thinner line) was a heat nociceptor. (b) Duration of the main peak of the action potential at half maximum amplitude for different types of C-fibre (mean ± s.e.). Analysis of variance showed that heat nociceptors had significantly longer duration spikes than polymodal nociceptors, and that both classes of nociceptor had longer duration spikes than the 2 non-nociceptor classes. (c) Duration of action potential undershoots at half maximum amplitude. Analysis of variance showed that the two classes of nociceptor had longer duration after potentials than the non-nociceptors, but no significant difference between heat and polymodal nociceptors. Class labels: inex – unit with no afferent field to pressure or heat; mech – sensitive mechanoreceptor (non-nociceptive); PMN – polymodal nociceptor; heat – heat nociceptor. Number of units in brackets below the class labels. From Gee et al (1999).

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sodium channel kinetics and an additional calcium current (Blair and Bean 2002). The somas of nociceptive neurones contain much higher levels of the sodium channels NaV1.7, NaV 1.8 and NaV 1.9 (Djouhri et al. 2003a; Djouhri et al. 2003b; Fang et al. 2002). Of these, the ► TTX resistant channel NaV 1.8 (SNS) is probably the most important contributor to the action potential (Wood et al. 2004). This channel has slower kinetics than the TTX sensitive channels that dominate in non-nociceptive neurones, so may be part of the reason that spikes have a slower rise time and longer durations. There is also evidence that C-fibre, presumed nociceptor, axons have both TTX-resistant sodium current and calcium transients, so similar factors may operate at an axonal level as in the soma (Grosskreutz et al. 1996; Mayer et al. 1999).

Post-Firing Excitability Changes in Nociceptive Neurones

Following an action potential, the excitability of neurones undergoes a series of fluctuations. Typically, a period of subnormal excitability is followed by a supernormal period of slightly increased excitability, and then sometimes a further period of slightly reduced excitability. The effects of one action potential can be detected in some neurones for several minutes. A readily observed effect of these excitability changes is that subsequent action potentials propagate with a slowed conduction velocity during periods of subnormal excitability, and with a faster conduction velocity during the supernormal period.

Analysis of Conduction Velocity Slowing in C-Fibres

Conduction velocity changes during repetitive firing of both A-fibre and C-fibre afferents are clearly greater in nociceptors than in non-nociceptive axons (Gee et al. 1996). In human nerves, there are also differences between subclasses of nociceptor. Conduction velocity slowing is more marked in mechanically inexcitable C-fibre afferents (CMi) than in those with noxious pressure receptive fields (C-responsive or CMR). Note that such neurones are also usually heat sensitive, so correspond to C-polymodal nociceptors or may be designated CMH (Weidner et al. 1999). Conduction velocity slowing is also more marked in terminal branches of C-fibres than in the axons within the main nerve trunk (Weidner et al. 2003). A supernormal period is also seen at all stimulus frequencies in CMi afferents, whilst it was only present at faster stimulation frequencies, with greater pre-existing slowing, in CMH fibres. The supernormal period in C-nociceptor axons has the intriguing effect of causing action potentials to catch each other up at high frequencies of stimulation, from approximately 20 Hz upwards. This means that two stimuli given at, say, 50 ms intervals evoke action potentials that are only 10 ms apart by the time they reach the central nervous system (they cannot catch up completely as the short term subnormal period corresponding to the relative refractory period sets an upper limit to firing rate). This substantial change in frequency of firing could well increase the effectiveness of such inputs at synapses. In contrast, at lower frequencies, typically less than 10 Hz, spikes are in the late subnormal period and some slight decrease in final firing rate will occur during propagation of spikes to the central nervous system. Weidner et al. (2002) suggest that this phenomenon may lead to a degree of contrast enhancement, with the apparent firing rate for strong stimuli being enhanced relative to the firing evoked by weaker stimulation.

Mechanisms Underlying Post-Firing Changes in Nociceptor Excitability

The slow changes in excitability after an action potential will reflect: (a) which particular ion channels are operating, (b) any accumulation of ions intracellularly or in the immediate extracellular space, and (c) hyperpolarisation due to increased activity of the ► sodium pump. Immediate post-firing subnormal excitability is caused by residual sodium channel inactivation plus delay in closing of depolarisation-activated potassium channels. The supernormal period appears to be a passive consequence of the long time constant (around 100 ms) of the C-fibre membrane (Bostock et al. 2003; Weidner et al. 2002). In this respect, mammalian C-fibres resemble the internodal membrane of myelinated fibres. The very long term subnormality appears to reflect the hyperpolarising action of the sodium pump (Bostock et al. 2003). The studies to date have not examined why CMi neurones have greater subnormality at long interstimulus intervals than CMR neurones, but this may reflect either some difference in sodium pumping or other very slowly changing ion channels. In sympathetic C-fibres, hyperpolarisation-activated inward currents (Ih) appear to play a role. However, double pulse experiments with nociceptive C-fibres did not show any changes attributable to Ih (Bostock et al. 2003).

Concluding Comments

The action potentials, both in the axons and the soma, are of longer duration in nociceptors than in other classes of somatosensory afferent neurone. Intriguing differences also exist between sub-classes of nociceptor in their action potential shape and in their post-firing excitability changes. In general, the mechanically insensitive nociceptors show longer duration axonal spikes and more profound depression of excitability by slow (2 Hz or less) activation. The differences between nociceptors and non-nociceptors may reflect the presence of slower TTX-resistant currents, plus significant calcium influx, during nociceptor action potentials. The reasons why CMi differ from CMR nociceptors are not known. The importance of CMi fibres for ► secondary hyperalgesia (Serra et al. 2004), and their involvement in chronic pain conditions (Orstavik et al. 2003) makes

these differences worthy of further study. A tantalising possibility is that differences in electrophysiological properties between nociceptors and non-nociceptors, or even possibly between sub-classes of nociceptor, might provide a basis for novel analgesic drugs. Some work on ► sodium channel blockers is already published and this is likely to remain an active research field (Wood et al. 2004)

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Nociceptors, Cold Thermotransduction

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Synonyms

Cold Nociception; Noxious Cold Receptor; Cold Thermotransduction

Definition

A large fraction of nociceptors can be excited by application of cold temperatures to their peripheral endings. Most have the functional properties of C-type polymodal nociceptors. The molecular sensors involved in transducing strong cooling stimuli into an electrical signal are still unresolved; ► TRPA1 channels are contested candidates. Noxious and thermal signals are further processed in the brain to establish the intensity and quality of the sensation. Peripheral nerve injury can modify the process to give rise to ► cold allodynia or ► hyperalgesia.

Characteristics

Humans can feel a wide range of ambient temperatures. This capacity is fundamental for tactile recognition of objects and thermoregulation. Within the cold temperature range, the qualities of sensations evoked vary from pleasantly cool to extremely painful. The neutral zone, evoking no sensation upon temperature change of the skin ranges between 35˚C and 31˚C. Cutaneous temperatures of 30–15˚C are generally perceived as cool to cold. Upon further temperature reduction, the perceptual qualities of the sensation change, becoming painful. The sensation of cold pain can have a burning, aching, prickling or stinging quality, depending on temperature and stimulus duration, possibly reflecting the activation of different classes of afferents. In contrast to the sharp threshold temperature for heat pain, the threshold for cold pain is less clearly defined and influenced by several factors such as rate of temperature change and stimulus area, indicating that mechanisms of temporal and spatial summation participate in encoding these sensations. Generally, the sensation turns painful only after a considerable delay, many seconds after cold application, which further complicates the definition of a threshold. Focal skin temperatures above approximately 43–45˚C also evoke a ► paradoxical cold sensation in many individuals.

While significant advances in the cellular and molecular mechanisms responsible for temperature transduction by nerve terminals have taken place in recent years

(reviewed by Jordt et al. 2003; Patapoutian et al. 2003; Reid 2005), many important aspects of the function of ► cold thermoreceptors and nociceptors and how cold pain is encoded remain obscure. Thus, it is unknown which molecular and cellular factors determine the different temperature thresholds of individual sensory terminals. Also, the mechanisms involved in the development of pain by moderate cooling after nerve injury (► cold hyperalgesia) remain mysterious. These are important questions with implications in the treatment of disorders in which cold temperatures evoke pain.

The existence of separate, small, cold and warm cutaneous sensory spots has been known since the late 19th century. These findings lend support to von Frey's specificity theory of somesthesia, according to which sensory nerve fibers of the skin were sensitive to only one form of stimulation and acted as "labeled lines" for the transmission of information encoding a single perceptual quality. In contrast to the strict labeled line hypothesis, many studies also indicated that the perceptual quality of cold-evoked painful sensations is determined by the integrated activity of both nociceptive and non-nociceptive systems.

Psychophysical studies suggest that pure cooling and cold pain sensations result from the activation of different populations of receptors. In support of this view, humans show better detection ability in the innocuous cold compared to the noxious cold temperature range. Compression block of myelinated fiber conduction in cold-sensitive afferents shifts the pain threshold of cold stimulation towards higher temperatures, pointing to a convergent processing of thermal and nociceptive inputs (Yarnitsky and Ochoa 1990). The same occurs in certain diseases, including peripheral nerve lesions and neuropathic pain syndromes, which are often associated with cold hyperalgesia.

The anatomical substrates for these cold-sensitive spots are free nerve endings branching inside the superficial skin layers. Functional studies suggest that receptors sensing noxious cold may be located more deeply within the skin, some located along vein walls (Klement and Arndt 1992). The difference in location has important implications for interpreting experimental findings; the deeper location of nociceptive endings will result in a large discrepancy between the actual temperature of the receptor and the readings of the surface probe used to apply the cold stimulus. This lag in thermal readings will overestimate the apparent low temperature of activation of nociceptors to cold. As a matter of fact, temperature recordings inside the skin indicate that cold pain may be evoked with intracutaneous temperatures as high as 28°C (Klement and Arndt 1992).

Cold-Sensitive Fibers

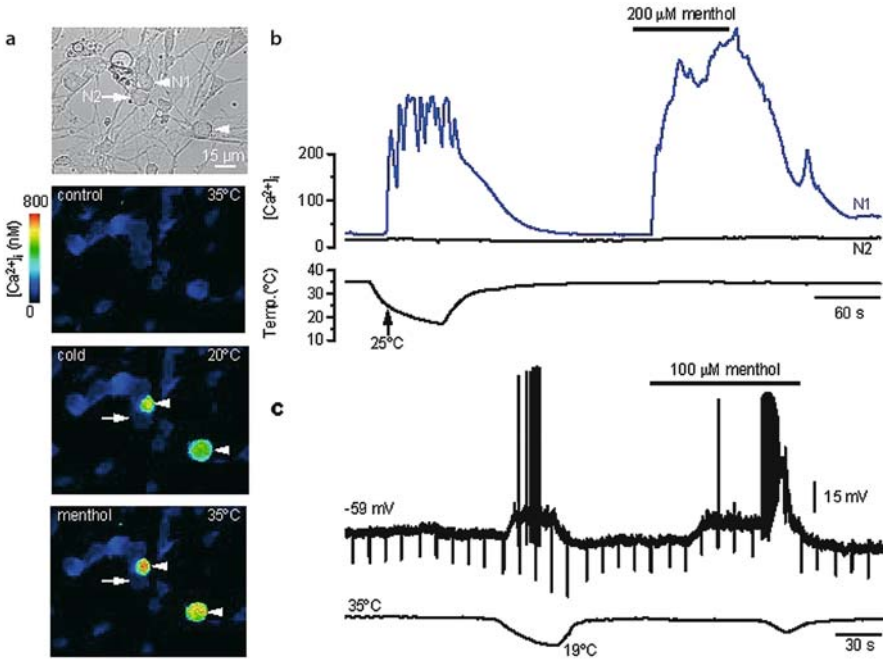
Extending the psychophysical studies, single fiber recordings in various species, including primates, have identified a population of myelinated Aδ fibers excited by moderate decreases in the temperature of their receptive field. These fibers are insensitive to mechanical stimuli. They are the prototypical "low-threshold" cold thermoreceptors (Hensel 1981). At normal skin temperatures (33–34°C), cold thermoreceptors show a static ongoing discharge. On sudden cooling, they display a transient peak in firing that adapts to a new static level, often characterized by short bursts of impulses separated by silent intervals. Rewarming of the skin leads to silencing of the receptors. A mirror response is observed in warm receptors. Activation of these receptors is the probable mechanism responsible for the sensation of innocuous cold. In humans, microneurographic recordings confirmed the existence of cold thermoreceptors; the principal difference in primates was the lower conduction velocity and the regular pattern of discharge (Campero et al. 2001). ► Menthol, a natural substance found in leaves of certain plants, evokes cold sensations when applied at low doses to skin and mucosae. This effect is due to sensitization of cold thermoreceptors; they shift their threshold of activation to higher temperatures. However, other studies also show that topical application of high concentrations of menthol can sensitize nociceptors and evoke pain (Green 1992; Wasner et al. 2004).

Greater decreases in temperature recruit an additional population of receptors. Many of these high-threshold cold-sensitive fibers are also heat- and mechanosensitive and conduct slowly, which would classify them as C-type polymodal nociceptors. In humans, they are characterized by low and irregular firing rates (< 1 impulse/s) during cooling (Campero et al. 1996). Activation of these nociceptors is thought to underlie the sensation of cold pain. However, other fiber types also augment their discharge during strong cold stimuli, including high threshold cold receptors and a fraction of slowly adapting low threshold mechanoreceptors. Thus, it is still an open question as to the contribution of these various fibers to noxious cold sensations. Terminal stumps of damaged sensory fibers can be excited by moderate cooling stimuli. The majority are C-type and many are also menthol sensitive (Roza et al. 2006)

Parallel input from primary sensory neurons carrying innocuous and noxious thermal information to the brain is suggested by the existence of anatomically and functionally distinct second-order neurons in lamina I of the spinal cord responsive to innocuous cooling, pure nociceptive stimuli or multimodal thermal and mechanical stimuli.

Cold Sensitive Neurons

To investigate the cellular mechanisms underlying cold sensing, many laboratories have turned to models that use primary sensory neurons maintained in culture (reviewed by Reid 2005). Activity in these cells can be monitored with calcium-sensitive fluorescent dyes (Fig. 1). Only a small fraction (10–15%) of sensory



Nociceptors, Cold Thermotransduction, Figure 1 Identification and response characteristics of cold sensitive trigeminal neurons in culture. (a) A neuronal culture (1 day *in vitro*) obtained from the trigeminal ganglion showing the bright-field image (top) and (in descending order) the pseudocolor images of the fura-2 ratio intensity at 35˚C, 20˚C and 35˚C in the presence of 200 µM menthol. Two of the neurons (marked with arrowheads) increased their resting calcium level during the cold stimulus, while the remaining cells did not change their calcium levels. (b) Intracellular calcium response to a cold ramp and 200 µM menthol application in the two neurons marked in (a). Only one of the cells responded to the stimuli. (c) Whole cell current clamp recordings of a cold sensitive neuron showing the potentiation of the cold response and the shift in temperature threshold by menthol.

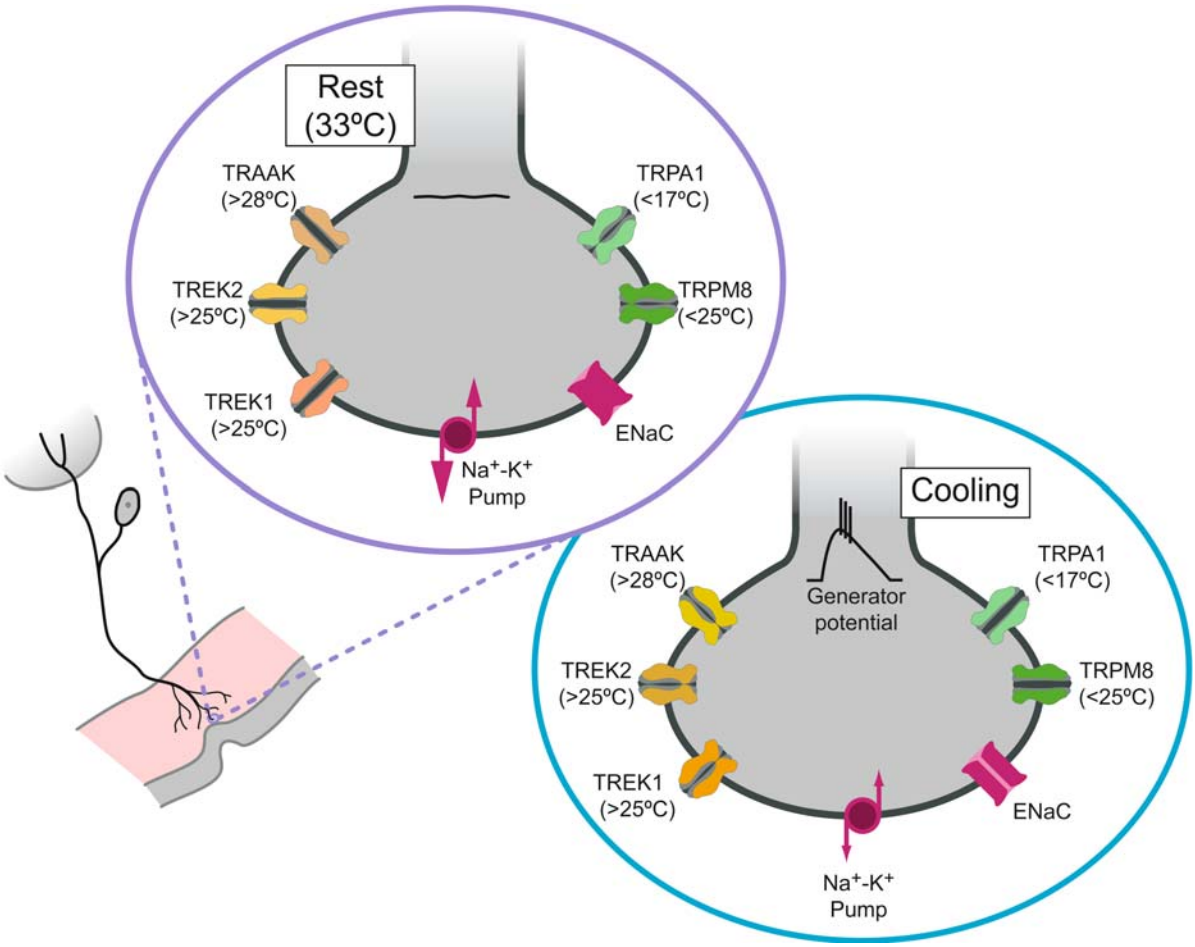
neurons respond to application of cold stimuli with an elevation in their intracellular calcium concentration. Many of these cold sensitive neurons are also activated by cooling compounds like menthol (Fig. 1), suggesting that they are indeed cold thermoreceptors (Viana et al. 2002; McKemy et al. 2002; Reid et al. 2002). These studies further showed that cold sensitive neurons have distinct electrophysiological properties. A hallmark is their high excitability; they require minute excitatory currents to reach firing threshold. The increased excitability reflects the relative low expression of subthreshold voltage gated potassium currents in comparison with other sensory neurons. A high percentage of cold and menthol sensitive neurons are also excited by the algesic compound capsaicin, which can be interpreted as further evidence for the expression and role of ► TRPM8 channel s in polymodal nociceptive neurons (Viana et al. 2002; McKemy et al. 2002).

Molecular Sensors for Cold

Work in the 1970's attributed the activation of nerve terminals by cooling to the depolarization produced by inhibition of the Na^+/K^+ pump. However, the realization that individual terminals can be activated by variable low temperatures hinted at the existence of more specific mechanisms, such as membrane ion channels with distinct temperature thresholds of activation, as likely molecular sensors of innocuous and painful cold signals. In principle, excitation of nerve terminals by a cold temperature could involve opening of cation channels or closure of resting potassium channels (Fig. 2). In either case, the net result is a depolarizing generator potential and firing of action potentials by the terminal.

Following on the landmark identification of ► TRPV1, a cation channel activated by heat and capsaicin (Caterina et al. 1997), two research groups cloned two additional transient receptor potential (TRP) channels activated by decreases in temperature. These two ion channels, known as ► TRPM8 and ► TRPA1, are expressed in discrete, non-overlapping, subpopulations of primary sensory neurons (Story et al. 2003). So far, the best characterized channel in the molecular machinery for neuronal cold sensing is TRPM8. The data argue strongly for a primary role of TRPM8 in non-noxious cold detection. TRPM8 is a calcium permeable, voltage gated, non-selective cation channel that is activated by temperature and natural cooling compounds like menthol and eucalyptol (McKemy et al. 2002; Peier et al. 2002). TRPM8 is expressed selectively in a small population (10–15%) of primary sensory neurons of small diameter and most cold sensitive neurons are also excited by menthol. Many of the same neurons also express TRPM8 mRNA transcripts. Moreover, many cold sensitive neurons manifest a non-selective cation current with many biophysical and pharmacological properties consistent with the properties of TRPM8-dependent currents (reviewed by Reid 2005).

One important difference between native cold activated currents and cloned ► TRPM8 channels is the temperature threshold (de la Pena et al. 2005). Activation threshold of TRPM8 channels is around 25˚C, a surprisingly low value. This is not a trivial issue for two reasons. First, it leaves unexplained the ability of many cold thermoreceptors to sense temperature decreases in the 33 to 26˚C range. Second, the high threshold observed suggests that TRPM8 may also be a candidate for thermal nociception



Nociceptors, Cold Thermotransduction, Figure 2 Cold sensing molecules in peripheral nerve endings. A schematic representation of a thin sensory fiber innervating the skin. The transduction of cold temperatures into an electrical signal takes place at free nerve endings. A free nerve ending with all the putative cold-sensing channels and their temperature thresholds. In the case of K^+ channels, closed by low temperature, the "threshold" value represents the lowest temperature at which the channels display significant activity. At normal skin temperatures, the terminal is kept hyperpolarized by the background activity of K^+ channels. Activity in the electrogenic Na^+-K^+ pump contributes to the hyperpolarization. Upon cooling, K^+ channels close and thermosensitive TRPs open, with a decrease in Na^+-K^+ pump activity, leading to a depolarizing receptor potential and spike firing.

as well. As already mentioned, this hypothesis is supported by some psychophysical studies in humans (Wasner et al. 2004; Green 1992). In turn, the discrepancy in thresholds may be explained by intrinsic modulation of TRPM8 channels by endogenous factors.

The identification of cold sensitive neurons that are insensitive to menthol points to additional cellular mechanisms in neuronal cold sensing (reviewed by Reid 2005). As a matter of fact, many high threshold cold sensitive neurons lack TRPM8 expression. The second cold sensitive TRP channel identified, ▶ TRPA1, is activated by much lower temperatures (< 17˚C) than TRPM8 and has therefore been suggested to be important in the transduction of high threshold, painful, cold stimuli (Story et al. 2003). This channel is not activated by menthol but is potently activated by pungent compounds like cinnamaldehyde and isothiocyanates present in cinnamon oil, wasabi and mustard oil. However, other investigators have not been able to replicate the cold sensitivity of TRPA1 channels (Jordt et al. 2004). Furthermore, the population of high threshold, menthol insensitive, cold activated neurons is not activated by mustard oil, which would suggest that their activation is not dependent upon TRPA1 activity (Babes et al. 2004). These findings make TRPA1 an uncertain candidate as a molecular sensor for noxious cold. In an interesting twist of events, recent studies suggest that TRPA1 channels are part of the mechanotransduction complex of vertebrate hair cells.

Cooling also activates ENaC channels, a member of the amiloride sensitive epithelial sodium channel family. However, a cold sensitive current matching the pharmacological profile of ENaC channels has not been documented in sensory neurons.

Alternatively, neuronal cold sensing may involve the closure of background potassium channels by cold (Fig. 2). TREK-1, TREK-2 and TRAAK, ▶ Two Pore Domain K+ Channels activated by fatty acids and me-

chanical stimuli, expressed in sensory neurons and with high sensitivity to temperature are good molecular candidates for this role (Kang et al. 2005; Maingret et al. 2000). The steep, rapid and gradual decrease in current flowing through these channels in a broad physiological temperature range (44–24°C) make them excellent candidates for thermal sensing in those terminals harboring them. In contrast to the direct temperature sensitivity of ► TRP channels, cell integrity is required for temperature sensitivity of these K^+ channels. Other background K^+ channels (i.e. TASK) are minimally affected by temperature. Experimental data implicating the closure of background potassium channels in cold transduction have been obtained in trigeminal and DRG neurons (Viana et al. 2002; Reid and Flonta 2001). However, the pharmacology or molecular nature of the conductance closed by cold temperature has not been addressed directly.

It is interesting that blockade of certain types of voltage-gated K^+ channels can render a population of sensory neurons cold sensitive. Experiments in trigeminal neurons showed that a slowly inactivating potassium current can act as a brake on excitability, reducing cold sensitivity (Viana et al. 2002). Neurons insensitive to cold and ► menthol could be transformed into cold sensitive neurons in the presence of low concentrations of 4-AP, a blocker for these channels.

It is important to emphasize that the various ionic mechanisms postulated in cold transduction are not mutually exclusive; if present in the same nerve terminal they could act synergistically to expand the dynamic range of temperature detection. Alternatively, activity of thermosensitive K^+ channels in those terminals with TRP channels opened by heat (i.e. TRPV2, TRPV3, TRPV4, TRPV1) would act as a brake on the excitatory actions of the latter.

Unfortunately, most recent data on thermosensitive ion channels have been obtained during *in vitro* animal studies, precluding a direct translation of these results to human physiology and pathophysiology. Much remains to be learnt about the differential expression pattern of the different thermosensitive channels and the functional properties of the sensory fibers expressing them. It is likely that other thermosensitive channels will be uncovered in the next few years, expanding the palette of putative molecular candidates for cold sensing.

In summary, psychophysical studies indicate that input from non-noxious thermal systems is essential for the thermal quality and the intensity of the pain sensation evoked by cold. Thermosensory afferent input normally inhibits cold evoked pain. At the molecular level, the diverse functional characteristics and broad range of temperature thresholds in the different afferent fibers suggests that each class of nerve terminal may operate with a combinatorial code of sensory receptors. The available evidence supports an important role for TRPM8 in sensing of innocuous cold temperatures in peripheral receptors (evidence reviewed by Reid, 2005). In addition, the participation of TRPM8 channels in certain forms of cold pain is a distinct possibility. Lacking genetic evidence (i.e. TRPM8-deficient mice) or specific pharmacological tools, this conclusion is not firm. The role of TRPA1 channels in the transduction of noxious cold pain is uncertain. Activity of background K^+ channels can certainly influence cold sensitivity, but their role as primary transducers has not been addressed directly. Not surprisingly, interest in the pharmacological profile of thermosensitive channels is very high. It is anticipated that modulators of these channels will provide new therapeutic options with which to treat certain forms of pain, including cold hyperalgesia and allodynia.

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Nociceptors, Immunocytochemistry

► Immunocytochemistry of Nociceptors

Nociceptors in the Dental Pulp

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Synonyms

Intradental Nociceptors; Pulpal Nociceptors

Definition

► Nociceptors that are located inside the tooth in the ► dental pulp and in the ► dentinal tubules in the most inner layers of ► dentin. The intradental afferent innervation consists of both myelinated and unmyelinated nerve fibers, which are mostly, if not exclusively, nociceptive. The nerve fibers originate from the mandibular (lower jaw) and maxillary (upper jaw) branches of the trigeminal nerve, and have their cell bodies in the ► trigeminal ganglion.

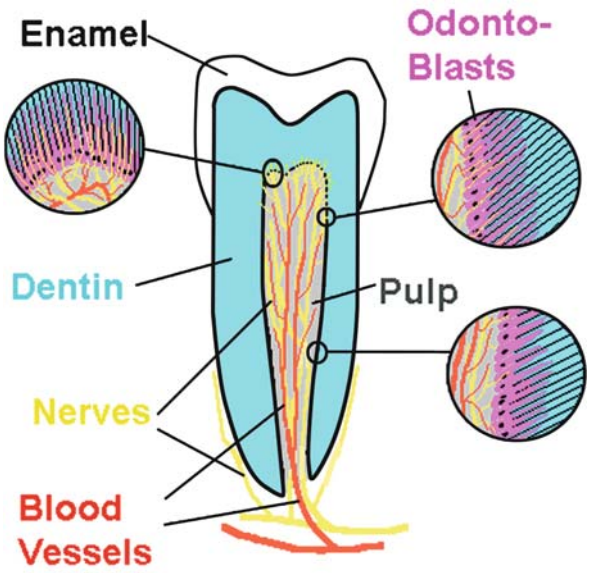
Characteristics

Pulpal inflammation can be extremely painful. Also, the intensity of the pain responses induced from teeth, e.g. from exposed dentin, by external stimulation can reach the maximum level of any pain score. The structure of the intradental innervation gives a basis for such high sensitivity. It should also be noted that pain is the predominant, if not the only, sensation that can be evoked by activation of intradental nerves.

Structure of Intradental Innervation

The innervation of the dental pulp is exceptionally rich (Fig. 1). Several hundred nerve fibers enter each tooth (Byers 1984). Approximately 20–30% of them are myelinated, mostly of smaller diameter, A–δ type, although there are also some larger A–β type axons. A great majority of the pulp nerve fibers are unmyelinated (C-fibers). A small proportion, approximately 10%, of the unmyelinated fibers are sympathetic efferents (see Byers and Närhi 1999). Their activation causes vasoconstriction and consequently reduction in the pulpal blood flow (Olgart 1996).

All intradental axons terminate as free nerve endings, the C-fibers in the pulp proper and A-fibers both in the pulp and a great number of them also in the ► predentin and inner layers of dentin (Fig. 1.). The myelinated nerve fibers branch abundantly and one axon may have endings in more than a hundred dentinal tubules (Byers 1984). The maximum distance that the fibers penetrate into the tubules is approximately 100 – 150 μm in the horns or tip of the coronal pulp. The pulp horns are also the most densely innervated areas of the pulp. At the pulp tip, approximately 50% of the tubules contain nerve terminals, some of them even multiple endings (Byers 1984). As there are 30000–40000 tubules/mm^2 of dentin (Brännström 1981), the density of the innervation of the pulp-dentin border at the tip of the coronal pulp is exceptionally dense. However, there are fewer nerve endings at the pulp-dentin border of the cervical region, and yet exposed dentin in these areas can be extremely sensitive, with innervation in the root being especially sparse (Fig. 1) (Brännström 1981).



Nociceptors in the Dental Pulp, Figure 1 Schematic presentation of the intradental innervation. A few branches from the alveolar nerve enter the pulp through the apical foramen. These bundles extend further through the root pulp and branch extensively, especially in the coronal pulp. The terminal branches of the axons are free nerve endings, and are located either in the pulp (especially the C-fibers) or in the tubules of the predentin and inner layers of dentin (many of the A-fibers). See text for more details.

Function of Intradental Nociceptors

The similarity of the structure of the intradental innervation in human teeth to that in cats, dogs and monkeys (Byers 1984) gives a morphological basis for studies, where the function of intradental nerves in experimental animals have been compared to the sensations evoked by dental stimulation in man. Electrophysiological recordings have revealed that A- and C-fibers of the pulp are functionally different (Närhi 1985; Byers and Närhi 1999). Comparison of those results to the sensory responses evoked by stimulation of human teeth also indicated that activation of pulpal A- and C-fibers may induce different types of pain sensations, namely sharp and dull pain, respectively (Ahlquist et al. 1985; Jyväsjärvi and Kniffki 1987). The intradental nerve activity recordings in human teeth indicate that the nerve function resembles that of experimental animals

(Edwall and Olgart 1976). The results of the single fiber recordings in cat and dog teeth also indicate that A-fibers are responsible for the sensitivity of dentin (Närhi 1985; Närhi et al. 1992), and that the intradental Aβ– and Aδ–fibers respond in a similar way to noxious dental stimulation (Närhi et al. 1992). Accordingly, they belong to the same functional group (Närhi et al. 1992 1996).

Pulpal A-fibers can be activated by a number of different stimuli applied at the tooth surface. Their responses are greatly enhanced if the dentin is exposed and the dentinal tubules are open (Närhi et al. 1992). Heat, cold, hypertonic solutions of various chemicals and desiccating air blasts, for example, applied to exposed dentin, evoke nerve responses with quite a similar pattern. The nerve firing starts immediately, or within a couple of seconds after the stimulus is applied, far before the stimuli, e.g. heat or cold, have reached the most inner layers of dentin and the pulp, where the nociceptors are located. So, the responses cannot be caused by a direct effect of the applied stimuli on the intradental nerve endings.

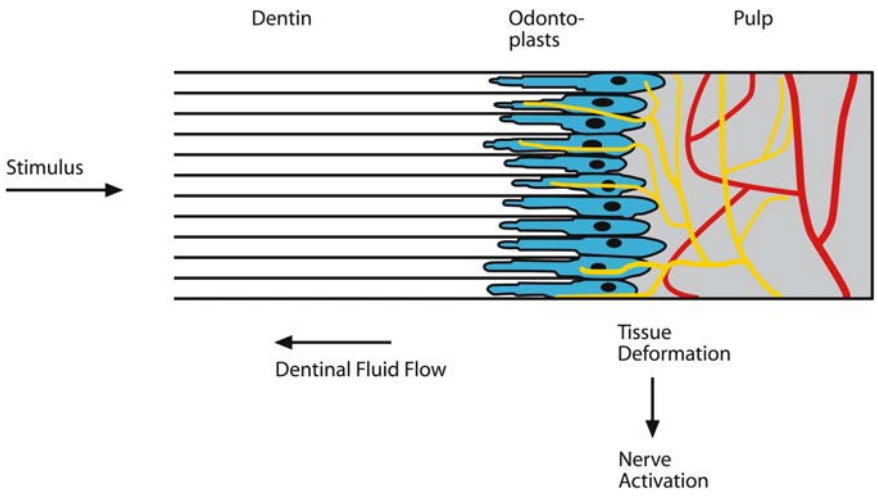
The stimuli, which induce pain from human teeth and activate the intradental nerves in experimental animals, are able to induce fluid flow in the dentinal tubules (Brännström 1981). Moreover, the fluid flow and the nerve responses induced by hydrostatic pressure are directly related (Vongsavan and Matthews 1994). Also, both the induction of the fluid flow, and the sensitivity of dentin are, to a great extent, dependent on the openness of the dentinal tubules (Brännström 1981; Närhi et al. 1992). Accordingly, the final stimulus for the nociceptors, which are responsible for the sensitivity of dentin (intradental A-fibers), seems to be the fluid flow in the tubules and, probably, the consequent mechanical deformation of the tissue and nerve endings in the pulp-dentin border (the so-called hydrodynamic mechanism of pulp nerve activation, Brännström 1981) (Fig. 2).

The hydrodynamic fluid flow is based on the strong capillary forces in the thin dentinal tubules (Brännström 1981). If dentinal fluid is extracted from the outer end of an open tubule by any stimulus, it is immediately replaced by a rapid outward fluid shift e.g. air-drying of dentine induces outward fluid flow and intense firing of the pulpal A-fibers (Närhi 1985). It also causes disruption of the tissues in the peripheral pulp (Brännström 1981). Thus, the capillary and hydrodynamic forces can considerably intensify the effect of the applied stimuli, and even a light stimulus such as an air blast can turn out to be noxious to the pulp.

The pulpal C-nociceptors are polymodal, and only activated if the external stimuli reach the pulp proper (Närhi 1985; Närhi et al. 1996). They do not respond to hydrodynamic stimulation. The C-fibers are activated by intense cold and heat applied to the tooth crown (Närhi et al. 1982; Jyväsjärvi and Kniffki 1987; Närhi et al. 1996). The response latencies are quite long, because the thermal stimuli have to reach the pulp where the nerve endings are located (Närhi 1985; Jyväsjärvi and Kniffki 1987; Närhi et al. 1996). The responses seem to be induced by a direct effect of heat and cold on the nerve endings. The C-nociceptors also respond to intense mechanical stimulation as well as to bradykinin, histamine and capsaicin applied to the exposed pulp (Närhi 1984; Närhi et al. 1992; Närhi et al. 1996).

Inflammation-Induced Changes in Pulpal Nociceptor Function

In healthy teeth the intradental nociceptors are well protected by the dental hard tissues and difficult to activate. Thus, pain is seldom induced from teeth during everyday activities. However, this is not the case when dentin with open tubules is exposed, because the effects of external stimuli are intensified by the hydrodynamic forces (Brännström 1981; Närhi et al. 1992). As in other tissue injuries, inflammation of the dental pulp can considerably sensitize the nociceptors (Brännström 1981;



Nociceptors in the Dental Pulp, Figure 2 Activation of the intradental A-fibers by the hydrodynamic mechanism. Various stimuli applied to the exposed dentin surface are able to remove fluid from the outer ends of the dentinal tubules. Due to the high capillary force within the thin tubules the removed fluid is immediately replaced by a rapid outward fluid shift. This, in turn, distorts the tissue in the pulp-dentin border and, consequently, activates the nociceptors in the area.

Närhi et al. 1996). This may lead to extremely intense spontaneous pain (toothache), and exaggerated painful responses to external stimuli e.g. to hot or cold food or drinks. The change in pulpal nociceptor activity and sensitivity is caused by a number of different inflammatory mediators, which are released and/or synthesized in response to the tissue injury (Närhi et al. 1996; Byers and Närhi 1999).

Intradental A– and C-nociceptors are activated and sensitized by inflammatory mediators in the initial stages of inflammation, which include neurogenic reactions, e.g. ► axon reflexes (Närhi 1984; Närhi et al. 1992; Närhi et al. 1996; Olgart 1996). However, there are other longer-term neurogenic mechanisms which may contribute to the increased sensitivity (Byers and Närhi 1999). These include activation of silent pulpal nociceptors (Närhi et al. 1996; Byers and Närhi 1999), i.e. the proportion of pulpal A-fibers responding to dentinal stimulation is significantly higher in inflamed compared to normal teeth, and is especially profound among the slow-conducting Aδ fibers (Närhi et al. 1996). Moreover, the receptive fields of the single nerve fibers in inflamed teeth become expanded (Närhi et al. 1996). Both of these changes may be related to sprouting of the nociceptive nerve fibers (Kimberly and Byers 1988) and/or activation of normally unresponsive nerve terminals. These changes may contribute to the increased sensitivity of inflamed teeth.

In view of the dense innervation of the dental pulp, and the structural neural changes that can occur in inflamed teeth, it is clinically puzzling to find that pulpitis may frequently be almost or even completely asymptomatic. This can be partially due to central regulatory mechanisms, which may inhibit the impulse transmission in the trigeminal pain pathways (Sessle 2000). However, local mechanisms also seem to exist in the dental pulp itself, which may be important not only for the regulation of the inflammatory reactions, but also the sensitivity of the nociceptors (Närhi et al. 1996; Olgart 1996; Byers and Närhi 1999; Dionne et al. 2001). Endogenous opioids may inhibit the nerve activation and neuropeptide release from the nociceptive nerve endings. Also, somatostatin may have an inhibitory effect on the nociceptor activation. Furthermore, sympathetic nerve fibers seem to prevent the release of the neuropeptides, by virtue of their preterminal connections to the nociceptive nerve endings (Olgart 1996). Accordingly, a number of different central and peripheral regulatory mechanisms may be operating, and contribute to the great variability of the pain symptoms connected with pulpal inflammation.

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Nociceptors in the Mucosa

► Nociceptors in the Orofacial Region (Skin/Mucosa)

Nociceptors in the Orofacial Region (Meningeal/Cerebrovascular)

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Synonyms

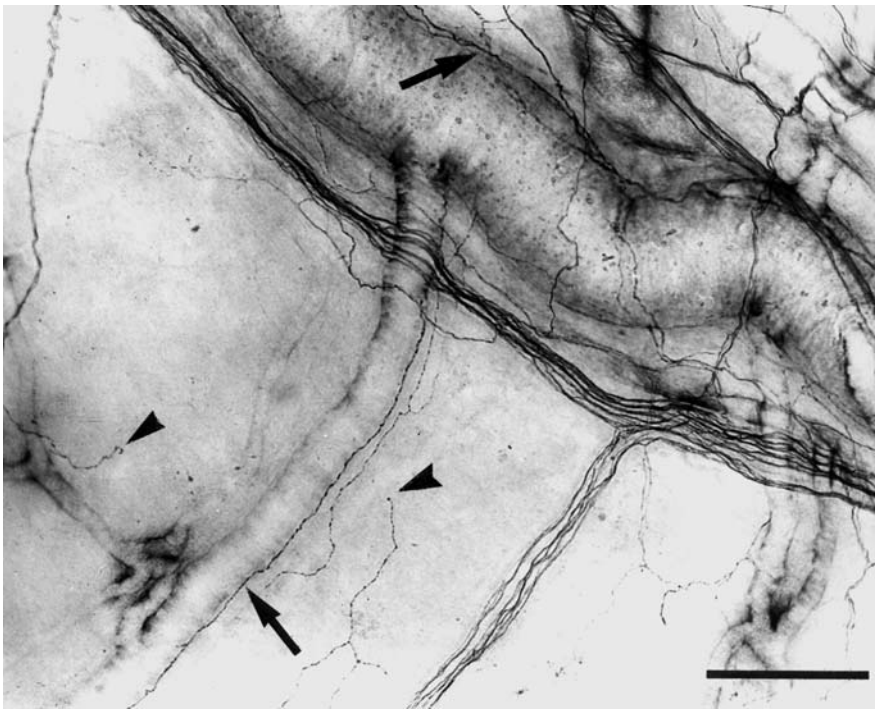
Meningeal Afferents; Meningeal Nociceptors; Intracranial Nociceptors; Dural Receptors

Definition

Trigeminal afferents that respond to noxious (painful) stimulation of intracranial structures (cranial meninges and intracranial blood vessels). These afferents originate in the trigeminal ganglion and project centrally to the spinal trigeminal nucleus, and to some extent to the spinal dorsal horn of the first cervical segments. Activation of meningeal afferents produces the sensation of headache.

Characteristics

The afferent innervation of the meninges and intracranial blood vessels has long been associated with the generation of ► headaches. Intraoperative exploration of patients undergoing open head surgery has revealed that intracranial structures are differentially sensitive to various stimuli (Ray and Wolff 1940). Noxious mechanical, thermal or electrical stimulation of dural blood vessels and the main intracerebral arteries, but not other intracranial tissues, has been reported to be painful in these experiments. Since headache-like pain was the only sensation evoked from stimulation of these intracranial structures, meningeal afferents are generally attributed a nociceptive function. This concept has been confirmed by morphological findings. Afferent innervation of intracranial structures is restricted to Aδ and C fibres, which are known to terminate as ► non-corpuscular sensory endings. These nerve endings do not form distinct corpuscular end structures but disperse as small bundles of sensory fibres, partly encased by peripheral glia (Schwann cells), along dural blood vessels and into the dural connective tissue (MeSSlinger et al. 1993; Fricke et al. 2001). Immunohistochemical preparations have shown that a considerable proportion of intracranial afferent fibres contain vasoactive neuropeptides, particularly ► calcitonin gene-related peptide (CGRP) (MeSSlinger et al. 1993). This vasodilatory neuropeptide is released from activated intracranial afferents, and has been suggested to be involved in the generation of ► migraine and other primary headaches (Edvinsson and Goadsby 1998). Experimentally, CGRP release from cranial dura mater can be quantitatively assessed *in vitro* and used as a measure for the activation of meningeal afferents by noxious stimuli. Using this method, it has been shown that not only classical noxious stimuli such as inflammatory mediators (Ebersberger et al. 1999); capsaicin, protons or heat, but also nitric oxide (NO) (Strecker et al. 2002) is able to release CGRP from rodent cranial dura. Another neuropeptide that has been identified immunohistochemically, in a smaller proportion of meningeal afferents, is substance P (MeSSlinger et al. 1993). Peripheral release of substance P is known to cause ► neurogenic inflammation, characterised by protein plasma extravasation, as well as other endothelial and perivascular changes. Neurogenic inflammation has been proposed to be a key factor in migraine pathophysiology (Moskowitz and Macfarlane 1993), but substance P was not found to be elevated during migraine attacks (Edvinsson and Goadsby 1998). In animal experiments, noxious stimulation of the meninges has failed to release detectable amounts of substance P in the periphery (Ebersberger et al. 1999), however, in



Nociceptors in the Orofacial Region (Meningeal/Cerebrovascular), Figure 1 Meningeal Afferents. CGRP immunoreactive nerve fibres in the rat dura mater encephali. Bundles and single immunopositive nerve fibres (arrows) run along the middle meningeal artery (MMA) and its branches, and terminate close to blood vessels (arrowheads). Scale bar 100 μm. Modified from (MeSSlinger et al. 1993).

the spinal trigeminal nucleus, increased substance P release during stimulation of rat dura mater with acidic solutions has been shown using a sensitive microprobe technique (Schaible et al. 1997). Therefore, substance P (and possibly CGRP), released from the central terminals of activated meningeal afferents, can be assumed to contribute to nociceptive transmission in the central trigeminal system.

Only a few direct electrophysiological recordings from intracranial afferents have been made in animal experiments. The teased fibre technique has been used to record from intracranial afferents running in the nasociliary nerve, which innervate fronto-medial parts of the supratentorial dura mater of the guinea pig (Bove and Moskowitz 1997). Another approach has been made in the rat to record with microelectrodes from the trigeminal ganglion, selecting neurons with dural receptive fields located around the middle meningeal artery or the large sinuses (Dostrovsky et al. 1991; Strassman et al. 1996). These studies are in accordance with the release studies mentioned above, and have shown afferent activation caused by noxious chemical stimuli (inflammatory mediators, capsaicin, and acidic buffer), heat and cold stimuli applied to the exposed dura mater, as well as mechanical stimulation of dural receptive fields. These studies also suggest that most, if not all, meningeal afferents can be regarded as ► polymodal nociceptors, which can be sensitized to mechanical stimuli through a cAMP mediated intracellular mechanism (Levy and Strassman 2002). There is evidence that most meningeal sensory endings express tetrodotoxin-resistant sodium channels, as has been reported frequently for visceral nociceptors (Strassman and Raymond 1999). In a new *in vitro* preparation of rat cranial dura, electrophysiological recordings from meningeal nerves are now made routinely in our laboratory. These experiments will provide additional insight into the response properties of meningeal afferents to stimuli such as nitric oxide and histamine, which are suggested to be key mediators in the generation of primary headaches.

Extracellular recordings from second-order neurones in the cat and the rat spinal trigeminal nucleus have shown that there is high convergence of afferent input from the meninges and orofacial region, most typically from the periorbital area (Davis and Dostrovsky 1988; Schepelmann et al. 1999). This observation has led investigators to assume that ► referred pain and hyperalgesia associated with headache may result from a ► central sensitization (Yamamura et al. 1999; Ellrich et al. 1999). The morphological and electrophysiological data taken from meningeal afferents suggest that intracranial pain (headache) may share more characteristics with visceral, rather than somatic, nociception and pain.

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Nociceptors in the Orofacial Region (Skin/Mucosa)

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Synonyms

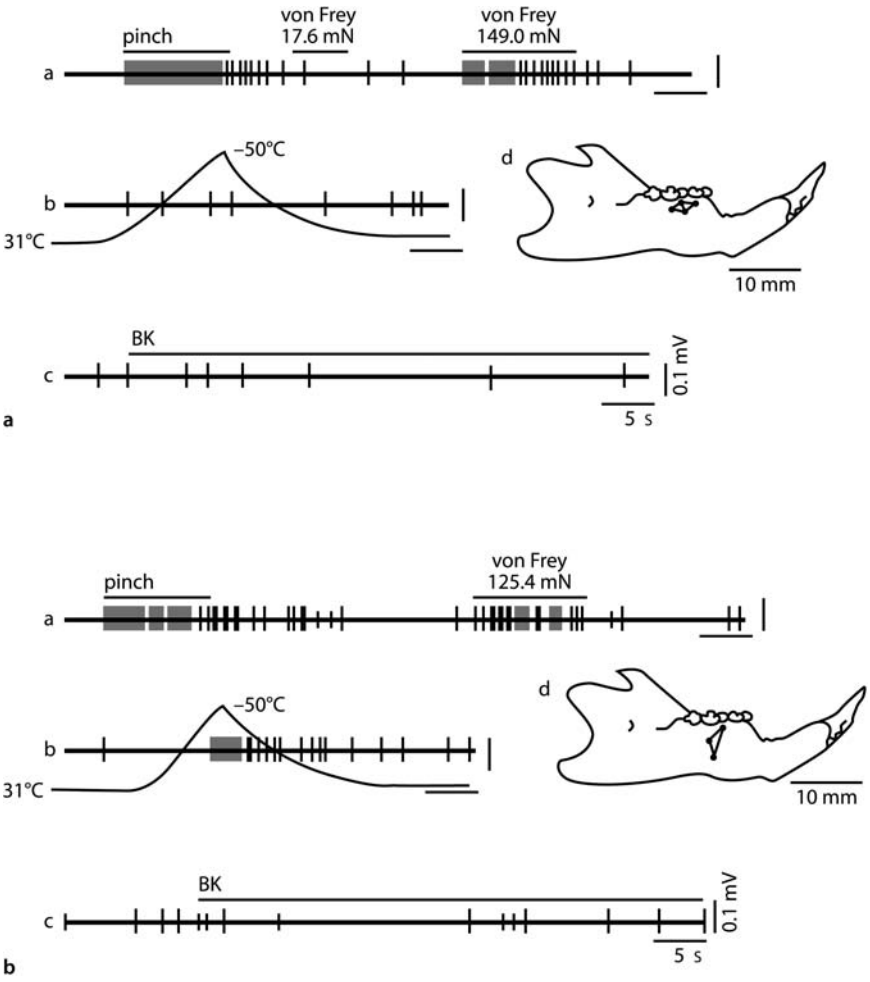
Polymodal nociceptor; High Threshold Mechanoreceptor; mechanonociceptor; Mechanoheat Nociceptor; nociceptors in the skin; Nociceptors in the Mucosa

Definition

Nociceptors detect and encode stimuli with actual or potential tissue damaging properties. Many are also responsive to chemicals released from traumatized tissue, inflammatory and immune system cells. Nociceptors are notable among sensory afferents for their plasticity. Detection and encoding capacities undergo rapid quantitative and slower qualitative up regulatory adaptations (sensitization). These contribute to both peripheral and central nervous system changes, which mediate increased pain sensitivity following tissue or nervous system injury. These adaptations serve to protect tissue from further damage and promote healing. Orofacial nociceptors supply a diverse set of tissues that include skin, muscle, bone and joints, but also highly specialized structures such as cornea, mucosa (oral and nasal) and teeth. Accordingly, some of the anatomic and physiologic features are unique. The reader should examine the essays on corneal and tooth ► pulp nociceptors for further details.

Characteristics

Trigeminal nociceptors of skin and mucosa are a diverse population of mechanical, thermal and chemically responsive afferents that detect and encode intense physical, thermal and chemical events associated with actual or near tissue damage. The trigeminal root ganglion (TRG, gasserian ganglion, semilunar ganglion) contains cell bodies of orofacial skin and mucosal nociceptors. Trigeminal nociceptors are anatomically distinct from spinal nociceptors (derived from dorsal root ganglia), in that their central projections terminate in the brainstem rather than in the spinal cord. In other respects, they are very similar (Hu 2000; Sessle 2000). In addition to detection and encoding of noxious stimuli, nociceptors of the skin and mucosa are able to release neuropeptides at both the peripheral and central processes. Peripherally, these peptides are involved in a variety of pro-inflammatory events (► plasma extravasation, mast cell degranulation, PLA_2 activation, healing),



Nociceptors in the Orofacial Region (Skin/Mucosa), Figure 1 Mucosal nociceptors respond differentially to mechanical, chemical and thermal stimulation. (a) Brisk response of an Aδ HTM of the rat mucosa to mechanical stimulation. The fiber is less responsive to other modalities. (b) An Aδ MH responds to both mechanical and thermal stimuli. Reprinted from Neuroscience Letters 228, K. Toda, N. Ishii and Y. Nakamura, 'Characteristics of mucosal nociceptors in the rat oral cavity: an in vitro study', pp. 95-98, 1997 with permission from Elsevier. BK, bradykinin 0.1 uM.

while centrally they are released with primary neurotransmitters (e.g. glutamate) to modify the synaptic pain message at the first synapse (Cooper and Sessle 1993). The neuropeptides substance P and/or CGRP (calcitonin gene related peptide) are expressed in nociceptors; including, myelinated and unmyelinated nociceptors (Aδ and C class) that have been described in both facial and oral mucosa tissues (Aδ ► HTM, Aδ ► MH, Aδ PMN, C PMN) (see ► MH, ► HTM, ► PMN) (Cooper et al. 1991; Bongenhielm and Robinson 1996; Toda et al. 1997; Flores et al. 2001). Many nociceptors contain neither of these peptides. In the trigeminal ganglion, the small and medium sized cells represent the main populations of nociceptive neurons. The large neurons of the TRG may also contain nociceptive populations. Nociceptors are highly specialized to detect chemical agents associated with tissue trauma, and may sensitize following exposure to chemical algesics. Both peptidergic and non-peptidergic nociceptive afferents express cholinergic (► nAChr) (Liu et al. 1993; Carstens et al. 2000), ► purinergic (► P2X3 ; Eriksson et al. 1998, Xiang et al. 1998) or acid sensitive receptors and ► ion channels (► ASIC) (Ichikawa and Sugimoto 2002). These channels enable nociceptors to detect the presence of ATP, ACh or protons that are associated with tissue damage and inflammation. ATP and ACh are released from damaged cells, while high levels of protons may be associated with infection or ischemia due to compromise of the vascular supply. Many of these same populations express the ► capsaicin sensitive protein ► VR1 or the capsaicin insensitive ► VRL1 (TRPV1, TRPV2) (see ► VR1, ► VRL1, ► TRPV1, ► TRPV2), which transduce noxious heat stimuli and are critical to the development of heat sensitization (Ichikawa and Sugimoto 2000; Stenholm et al. 2002). Responses to cooling, ► bradykinin, ► PGE2 and histamine have also been described (Toda et al. 1997; Viana et al. 2002) (Fig. 1).

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Nociceptors in the Orofacial Region (Temporomandibular Joint and Masseter Muscle)

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Synonyms

Nociceptive Temporomandibular Joint Afferents; Nociceptive Masseter Muscle Afferents

Definition

Primary afferent fibers that innervate the ► temporomandibular joint (TMJ) and masticatory muscles, and are activated by noxious mechanical, chemical or thermal stimuli applied to these tissues. These afferent fibers transduce and convey information about potential or actual tissue injury from the orofacial region to the central nervous system.

Characteristics

TMJ Nociceptors

The TMJ is innervated by thinly myelinated and unmyelinated afferent fibers, with non-specialized endings, which contain clear and dense core vesicles. This

suggests some of these afferent fibers release neurotransmitters and neuropeptides, such as calcitonin gene related peptide (CGRP) and substance P, from their terminal endings (Kido et al. 1995). These small-diameter afferent fibers project, via the gasserian or trigeminal ganglion, to the trigeminal subnuclei interpolaris and caudalis (Casatti et al. 1999; Capra 1987; Widenfalk and Wiberg 1990), areas of the caudal brainstem which appear to be most important for the integration of nociceptive input from deep orofacial tissues. Electrophysiological studies have confirmed the projection of a subpopulation of TMJ afferent fibres with conduction velocities of less than 25 m/sec (Aδ and C fibres) to the caudal brainstem (Cairns et al. 2001 a,b). These fibres are activated by noxious mechanical and/or chemical stimuli and appear to function as nociceptors (Cairns et al. 2001 a,b).

TMJ afferent fibers, identified as mechanical nociceptors by their response to noxious protrusion or lateral movement of the TMJ, have been described (Cairns et al. 2001a; Loughner B et al. 1997). These fibers are not activated by innocuous jaw opening, but begin to discharge as lateral rotation of the jaw exceeds the normal range, and exhibit a progressively increased discharge with supra-normal rotation of the jaw. Some of these nociceptors also appear to encode rate of jaw rotation (Loughner B et al. 1997). The threshold of TMJ nociceptors to noxious mechanical rotation of the jaw is lower in females than males; however, this is apparently due to sex-related differences in the biomechanical properties of the TMJ tissues (Loughner B et al. 1997).

TMJ nociceptors respond to injection of algogenic substances such as mustard oil, potassium chloride and ► glutamate into the TMJ, which also evokes a ► nociceptive reflex response in the jaw muscles (Cairns et al. 2001a; Cairns et al. 1998) The activity of TMJ nociceptors precedes, but has a markedly shorter duration, than reflex jaw muscle activity evoked by injection of glutamate into the TMJ. This finding has led to the speculation that a brief activation of TMJ nociceptors, by algogenic compounds such as glutamate, is sufficient to induce ► central sensitization, a period of prolonged increase in the excitability of trigeminal subnucleus caudalis neurons (Cairns et al. 2001a). Such a phenomenon may explain the diffuse referral pattern of TMJ pain, which may spread to include the masticatory and neck muscles, and why acute joint pain can sometimes significantly outlast the duration of nociceptive stimulation.

Sex-related differences in the chemical response characteristics of TMJ nociceptors have also been noted. The greatest response to algogenic compounds has been observed in small, mechanically sensitive afferent fibers with conduction velocities of less than 10 m/s, which suggests that these particular fibers function as polymodal nociceptors, i.e. nociceptors that respond to more than one type of noxious stimulation. Sex-related differences in response to injection of glutamate into the TMJ have been best characterized. Injection of glutamate into the TMJ has been found to evoke significantly greater nociceptive reflex responses and discharge in polymodal nociceptors in females than in males (Cairns et al. 2001a) Such sex-related differences in TMJ nociceptor excitability may explain, in part, the increased prevalence of certain orofacial pain syndromes in women (Dao and LeResche 2000).

Algogenic compounds, such as mustard oil and glutamate, excite TMJ nociceptors in part through activation of peripheral NMDA and non-NMDA receptors (Cairns et al. 1998). This suggests that peripheral glutamate receptor antagonists may be of use in modifying the excitability of TMJ nociceptors under certain pathological conditions. In contrast, the peripheral endings of TMJ nociceptors are not excited by γ- ► aminobutyric acid (GABA), which is thought to depolarize the central endings of nociceptors (Cairns et al. 2001a). Indeed, the current evidence suggests that GABA may in fact decrease the excitability of TMJ nociceptors through activation of peripheral $GABA_A$ receptors (Cairns et al. 1999). This unexpected effect of GABA suggests that the activation of peripheral $GABA_A$ receptors may result in a local analgesia of the TMJ.

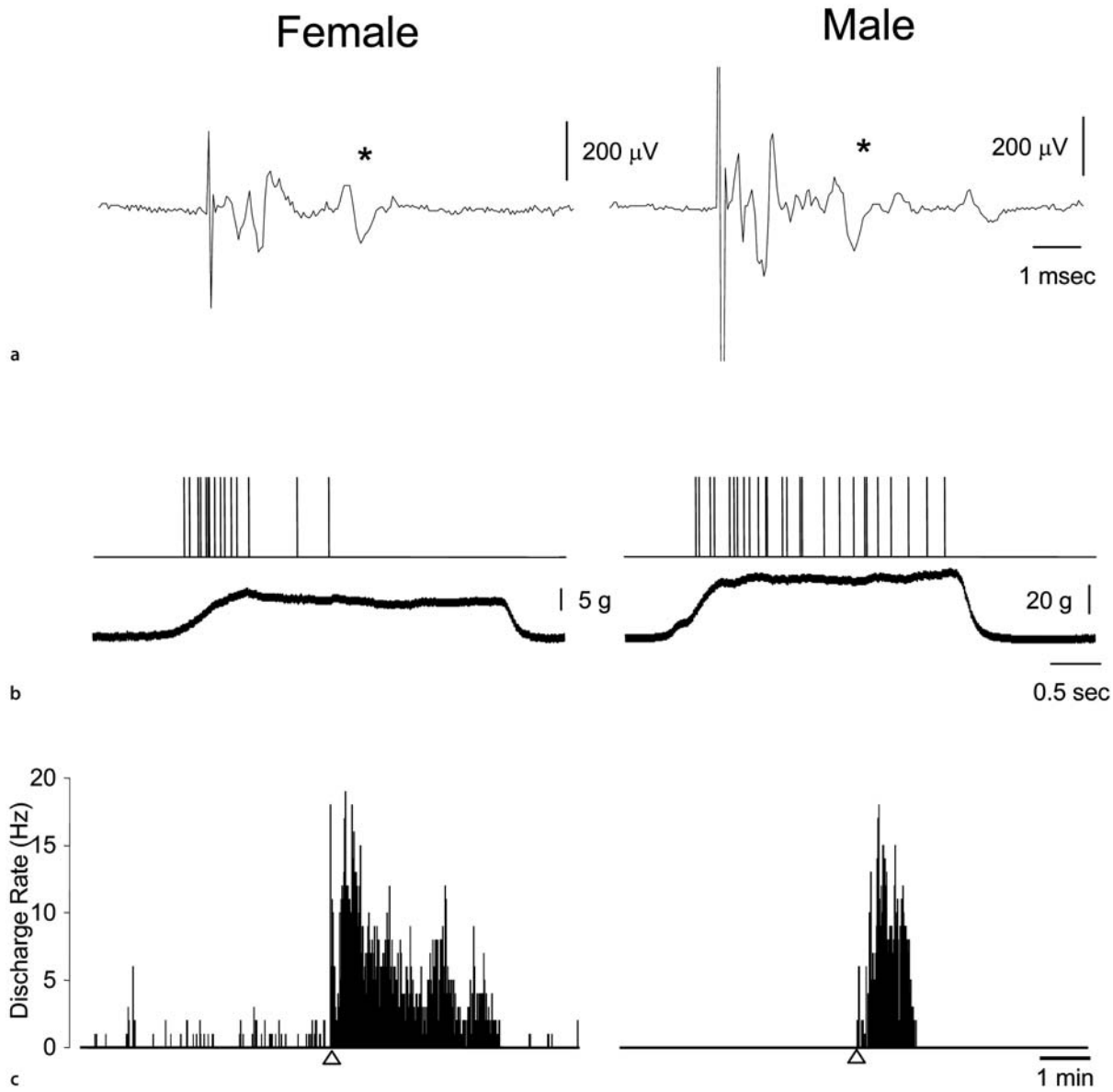
N

Masseter Muscle Nociceptors

Anatomical and electrophysiological studies have indicated that the ► masseter muscle is also innervated by thinly myelinated and unmyelinated trigeminal afferent fibers with non-specialized endings, which project to the trigeminal subnucleus interpolaris and caudalis (Cairns et al. 2002; Cairns et al. 2001b; Cairns et al. 2003; Capra and Wax 1989; Nishimori et al. 1986) These fibers are activated by noxious mechanical and/or chemical stimuli and appear to function as nociceptors (Cairns et al. 2002; Cairns et al. 2001b; Cairns et al. 2003).

About one-third of masseter muscle afferent fibers that project to subnucleus caudalis have mechanical thresholds that exceed the human pressure pain threshold (Cairns et al. 2003; Svensson et al. 2003). In uninjured masseter muscle, these nociceptors are predominantly Aδ fibers with conduction velocities of less than 10 m/sec (Cairns et al. 2002; Cairns et al. 2003). Most of these nociceptors exhibit slowly adapting responses to sustained noxious mechanical stimulation (Fig. 1). A significant sex-related difference in the mechanical threshold of these nociceptors has not been found.

Like TMJ nociceptors (see above), masseter muscle mechanical nociceptors also respond to the injection of algogenic substances such as ► hypertonic saline and glutamate, but not GABA, into their mechanoreceptive field (Cairns et al. 2002; Cairns et al. 2001; Cairns et al. 2003). The afferent discharge evoked by these algongenic substances is greatest in C fibers and in Aδ fibers with con-



Nociceptors in the Orofacial Region (Temporomandibular Joint and Masseter Muscle), Figure 1 Examples of deep orofacial tissue nociceptor response characteristics. (a) The line drawing illustrates antidromic action potentials (*), evoked by electrical stimulation of the caudal brainstem, to confirm the central projection target of these masseter muscle Aδ nociceptors. (b) Sustained noxious mechanical stimulation of the masseter muscle with an electronic Von Frey hair (lower trace), resulted in a slowly adapting discharge (upper trace). (c) The peri-stimulus histograms illustrate the effect of injection of the algogenic substance glutamate into the masseter muscle. Note that glutamate-evoked nociceptor discharge was markedly greater in the female than in the male.

duction velocities of less than 10 m/s. Thus, these particular fibers appear to function as polymodal nociceptors. Glutamate consistently evokes significantly greater nociceptor discharges and pain responses in females than in males (Cairns et al. 2002; Cairns et al. 2001; Cairns et al. 2003) (see examples, Fig. 1). Thus, sex-related differences in masseter muscle nociceptor excitability appear to underlie, at least in part, the increased prevalence of masticatory muscle pain conditions suffered by women (Dao and LeResche 2000). Prolonged mechanical sensitization of the masseter muscle and its nociceptors has also been demonstrated to occur after injection of glutamate into the masseter muscle, although there do not appear to be sex-related differences in this phenomenon (Cairns et al. 2002; Svensson et al. 2003).

Unlike TMJ nociceptors, current evidence suggests that glutamate-evoked afferent discharge in masseter nociceptors is predominantly mediated through activation of peripheral NMDA receptors (Cairns et al. 2003). Glutamate-induced mechanical sensitization is also mediated through activation of peripheral glutamate receptors (Cairns et al. 2002). Thus, peripheral NMDA

receptor antagonists may prove to be particularly effective analgesics for the treatment of masticatory muscle pain.

Conclusion

The role of orofacial nociceptors is to transduce and convey information about the intensity and quality of orofacial pain. The characteristics of TMJ and masseter muscle nociceptors suggest that they may play a role not only in the development, but also in the maintenance of certain types of orofacial pain, and contribution to sex differences in TMJ and masticatory muscle pain.

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Nociceptors, Perireceptor Elements

► Perireceptor Elements

Nocifensive

Definition

Denoting a process or mechanism that acts to protect the body from injury.

► Nocifensive Behaviors of the Urinary Bladder
► Secondary Somatosensory Cortex (S2) and Insula, Effect on Pain Related Behavior in Animals and Humans

N

Nocifensive Behavior

Definition

Nocifensive behaviors are those that are evoked by stimuli that activate the nociceptive sensory apparatus. They are associated with protection against insult and injury typically in response to a noxious stimulus. Responses to noxious stimuli in animals may include behaviors resembling responses to pain in humans, such as limping, flinching, vocalization and reflexive withdrawal. Other specific pain-related responses in animals include tail and paw flicks, licking, and scratching. Responses to increased deep muscle and joint pain may include reduced exploration activity. In the viscera, nocifensive behaviors can be produced by hollow organ distension, ischemia, traction on the mesentery or stimulation of inflamed organs.

► Muscle Pain Model, Inflammatory Agents-Induced
► Nocifensive Behaviors (Muscle and Joint)
► Sensitization of Visceral Nociceptors

Nocifensive Behaviors Evoked by Myositis

► Muscle Pain Model, Inflammatory Agents-Induced

Nocifensive Behaviors, Gastrointestinal Tract

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Synonyms

Pseudaffective; pseudoaffective; Gastrointestinal Tract, Nocifensive Behaviors

Definition

Nocifensor, a term introduced by Lewis (1936; see Lewis 1942; LaMotte 1992 for discussion), describes a system of "nerves" associated with local defense against injury. Nocifensive has since expanded as a term to describe behaviors associated with protection against insult and injury. Nocifensive behaviors are more complex than simple nociceptive flexor withdrawal reflexes, such as the tailflick reflex, and the term is particularly appropriate in the visceral realm, where stimuli considered to be adequate (e.g. hollow organ distension, ischemia, traction on the mesentery) are different from those Sherrington (1906) defined as adequate for activation of cutaneous nociceptors. The nocifensive behaviors produced by visceral stimulation are also considered pseudaffective (Sherrington 1906) (pseudoaffective), because responses to visceral stimulation are organized supraspinally.

Characteristics

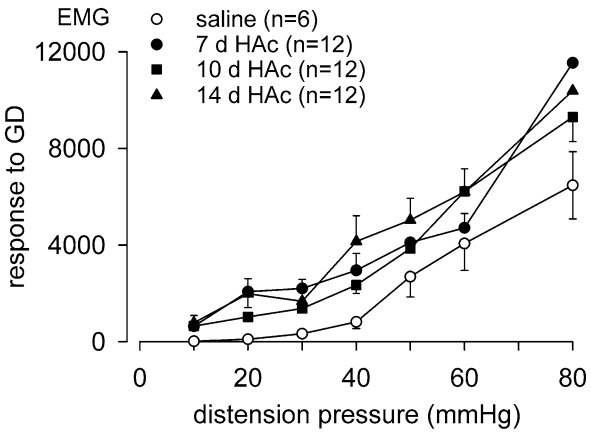
Balloon distension of gastrointestinal tract organs has been widely employed in both human and non-human animal studies. Significantly, balloon distension of the esophagus, stomach or large bowel reproduces in humans the distribution of referred sensations, as well as the quality and intensity of discomfort and pain, arising from pathological visceral disorders (see Ness and Gebhart 1990 for review). In addition, balloon distension of hollow organs is a stimulus that is easy to control in terms of onset, duration and intensity, as opposed, for example, to a chemical or ischemic visceral stimulus. The nocifensive behaviors produced by balloon distension of hollow organs include changes in blood pressure, heart rate and respiration, and visceromotor reflexes (► visceromotor reflex/response). All of these responses are absent in spinally transected animals, but present following mid-collicular decerebration, thus revealing that they are responses integrated in the brainstem. All of these response measures are intensity-dependent and can be quantified as indices of visceral nociception.

Although balloon distension of hollow organs has been widely used, it wasn't until Ness parametrically characterized in the rat responses to colorectal distension that this model of visceral pain became widely accepted and widely used (Ness and Gebhart 1988). Similar parametric evaluation of responses to colorectal distension in the mouse (Kamp et al. 2003) and gastric distension in the rat (Ozaki et al. 2002) have since been described.

Colorectal Distension (CRD)

Balloons of different lengths and varying durations of distension have been reported in the literature. As Lewis (1942) noted, distension of hollow organs is most painful in humans when long, continuous segments of the gut are distended, which emphasizes the importance of ► spatial summation as an important consideration in CRD as a stimulus. Results using different length balloons in the rat are qualitatively similar, although greater intensities of stimulation are generally required with smaller balloons to produce quantitatively equivalent responses. The human literature (see Ness and Gebhart 1990 for citations and discussion) also established that constant pressure distension, rather than constant volume, was the appropriate stimulus. It should be appreciated that hollow organs throughout the viscera, and particularly within the gastrointestinal tract, accommodate as they fill. Thus, constant volume distension produces an inconstant intensity of stimulation as the organ musculature relaxes.

Balloon CRD produces contraction of the peritoneal musculature, representing what has been termed a visceromotor reflex. Among the responses to CRD, the visceromotor reflex recorded from the external oblique peritoneal musculature is perhaps the most reliable and experimentally least complicated measure to quantify. Indwelling arterial catheters to measure changes in blood pressure or heart rate produce equally robust and quantifiable measures of response to distension, but are more difficult to maintain than electromyographic electrodes sewn into the peritoneal musculature. Figure 1 illustrates graded visceromotor responses to increasing intensities of CRD in the mouse. The response threshold for CRD is typically between 20 mmHg and 25 mmHg in normal rat colon. In consideration of the response threshold for a subset of pelvic nerve afferent fibers that innervate the colon and spinal dorsal horn neuron responses to CRD, the intensity of CRD in the rat considered to be noxious is $\geq$ 30 mmHg. This interpretation is consistent with results from psychophysical studies in humans (e.g. Ness et al. 1990), in which increases in heart rate and blood pressure were produced by colon distension at intensities of distension less than reported as painful. Accordingly, quantification of pseudaffective responses to visceral stimulation requires interpretation with respect to the quality of the nocifensive behavior produced (e.g. visceromotor response). That is, changes in response measures may be apparent before a behavior is nocifensive, although thresholds for response are similar.



Nocifensive Behaviors, Gastrointestinal Tract, Figure 1 Visceromotor responses recorded as electromyographic activity in the peritoneal musculature of the mouse to graded intensities of colon distension. Distending pressures (15 – 60 mmHg) are illustrated; the period of colorectal distension is 20 sec.

Gastric Distension (GD)

Unlike CRD, in which a lubricated balloon can be inserted via the anus into the descending colon and rectum of a rodent, insertion of balloons for GD requires a surgical procedure in advance of the experiment. Like CRD, responses to balloon GD in the rat are graded with stimulus intensity (Ozaki et al. 2002). The visceromotor response in studies of GD is recorded from the acromiotrapezius muscle; responses to GD recorded in other muscles (e.g. external oblique peritoneal muscles, spinotrapezius muscles or sternomastoideus muscles) were unreliable and not graded with stimulus intensity. In addition to being able to surgically implant a balloon into the stomach, one can surgically place a small diameter polyethylene tube into the stomach to deliver chemical substances. Thus, one can examine the effect of chemical stimulation of the stomach in the absence or presence of an ulcer, and/or before and after GD. For example, intragastric administration of hydrochloric acid produces concentration-dependent (0.05–0.3 mol/l) activity in the acromiotrapezius muscle, which can be quantified and used to assess a chemically-evoked nocifensive behavior. Interestingly, we (Lamb et al. 2003) were able to show that responses to balloon gastric distension were conveyed to the central nervous system by the splanchnic innervation of the stomach, whereas chemonociceptive stimulation of the stomach by hydrochloric acid was conveyed to the central nervous system via the vagus nerve. The importance of these observations relates to support for a role of the vagus nerve in chemonociception, an area of growing investigation.

Small Bowel Distension

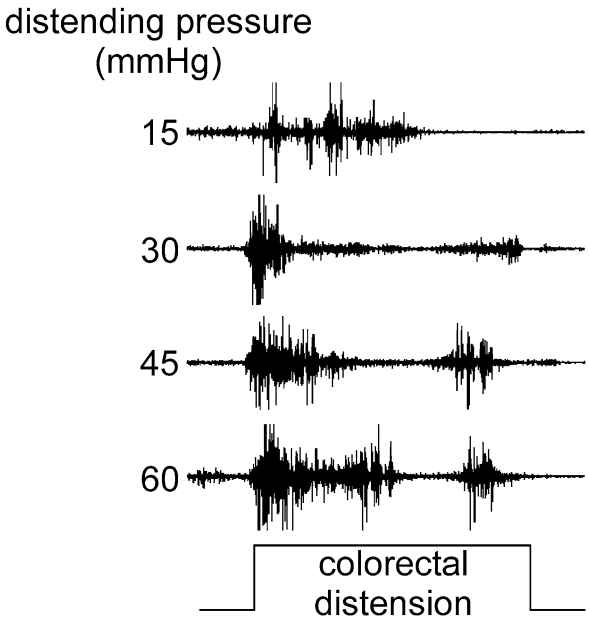
Balloon distension of the small bowel has also been studied in rodents. Colburn and colleagues (1989) described a model of duodenal distension in which responses to distension were graded from 0–4, based on interpretation of the nocifensive behavior produced. A difficulty with models of small bowel distension is that permanent implantation of balloons tends to lead to obstruction. This is avoided in studies of GD by placing the balloon in the antrum, where it does not significantly interfere with ingestion of food or weight gain.

Pseudaffective Reflexes and Anesthesia

The concept of a nocifensive behavior implies evaluation of response to stimulation in the awake animal. However, changes in blood pressure, heart rate or the visceromotor response can also be interpreted as a behavior, and responses to gastrointestinal distension have been carried out in anesthetized animals. As initially reported by Ness and Gebhart (1988), the presence of anesthesia typically converts pressor and tachycardic responses to CRD to depressor and bradycardic responses, and attenuates the visceromotor response to distension. Depressor and bradycardic responses to CRD in anesthetized rats have been quantified and reported, but their relevance to nociception and pain is less clear than vigorous nocifensive behaviors produced in the awake, behaving animal.

Gastrointestinal Hypersensitivity

Responses to balloon distension of the gastrointestinal tract have been well characterized in behavioral and electrophysiological studies (and results from both behavioral and electrophysiological studies have been important to understanding mechanisms of visceral nociception). Gastrointestinal hypersensitivity, such as associated with dyspepsia and gastrointestinal inflammatory disorders, in addition to so-called functional disorders, which are not associated with either biochemical or pathological markers of organ pathology, comprise a significant proportion of patients reporting gastrointestinal discomfort and pain. Accordingly, a variety of models of gastrointestinal organ irritation and insult have been reported, and thus to study the presumed processes underlying visceral hypersensitivity. Experimental gastric ulceration or gastritis produced by ingestion of a dilute solution of iodoacetamide both produced long-lasting gastric hypersensitivity, as assessed by balloon distension and quantification of the visceromotor response (Fig. 2). Importantly, the gastric hypersensitivity to balloon distension in these models long outlasts recovery from ulceration or gastritis (Ozaki et al. 2002). In the colon, a variety of chemicals have been instilled into the lumen to produce insult ranging from mild irritation to ulceration. In such models, in which substances such as mustard oil, turpentine, zymosan, trinitrobenzene sulfonic acid, etc. have been placed intracolonically, hypersensitivity to balloon CRD can be shown within hours, and can last for days to weeks. For example, in the mouse, intracolonic instillation of zymosan can produce hypersensitivity to



Nocifensive Behaviors, Gastrointestinal Tract, Figure 2 Responses to gastric distension (GD) 7-14 days after injection of acetic acid (HAc) into the stomach wall. Relative to saline-injected animals, the visceromotor response, quantified as the EMG recorded in the acromiotrapezius muscle, responses to gastric distension are significantly increased after ulceration of the stomach.

balloon CRD that lasts for 5–7 weeks, and does so in the absence of histological evidence of damage to the colon (R.C.W. Jones III and G.F. Gebhart, unpublished observations). In addition to these models of relatively acute to chronic colon hypersensitivity, models have also been developed which are intended to, or closely replicate, contributions to gastrointestinal hypersensitivity in humans. For example, about 30% of individuals with irritable bowel syndrome report a previous gastrointestinal infectious event, and models of gastrointestinal infection (e.g. *Trichinella spiralis*) have been developed in which both changes in gastrointestinal motility and sensation are altered (Barbara et al. 1997). Patients that suffer from irritable bowel syndrome also often report that symptoms are exacerbated by stressful life events, and a model of maternal separation of rat pups produced visceral hypersensitivity in adult rats as assessed by visceromotor responses to CRD (Coutinho et al. 2002). Al Chaer and colleagues (2000) exposed rat pups to either mechanical balloon distension of the colon, or treatment with mustard oil during a critical neonatal period, and were able to document hypersensitive responses to CRD in adult rats.

Conclusions

Balloon distension, at constant pressure, produces robust and readily quantifiable pseudaffective responses and nocifensive behaviors. Development and use of these models has improved understanding of visceral nociceptive mechanisms, visceral hypersensitivity, and modulation by pharmacological and other manipulations.

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Nocifensive Behaviors, Muscle and Joint

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Synonyms

Hyperalgesia; Guarding; spontaneous pain

Definition

Nocifensive behaviors are the response of the animal to noxious or painful stimuli.

Characteristics

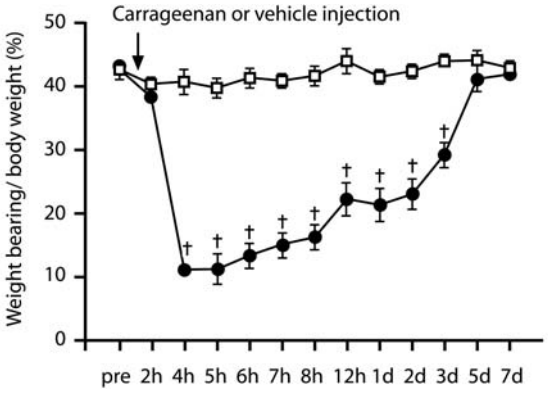
There are several animal models of muscle and joint pain utilized to study ► nocifensive behaviors. These include carrageenan (or kaolin/carrageenan) inflammation of the knee joint or the muscle (gastrocnemius, triceps). Carrageenan inflammation results in an initial acute phase of inflammation that converts to a chronic phase within 1 week after induction (Radhakrishnan et al. 2003). Alternatively, ► complete Freund's adjuvant (CFA) or capsaicin can be injected into the joint or muscle to produce inflammation and behavioral changes (Yu et al. 2002; Sluka 2002). A non-inflammatory model of muscle pain is induced by two injections of acidic saline (Sluka et al. 2001).

Joint Inflammation

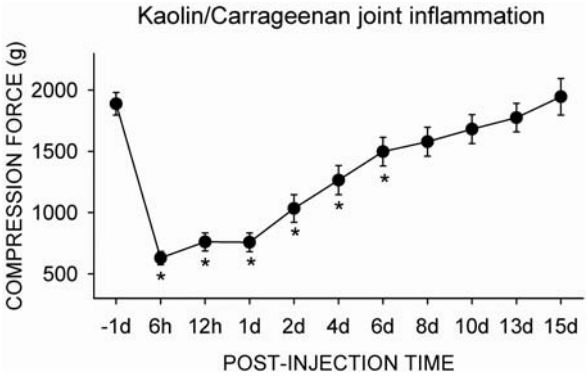
Nocifensive responses include spontaneous pain behaviors, ► primary and secondary hyperalgesia and ► allodynia. Spontaneous pain behaviors are observed following knee joint inflammation with carrageenan. These include decreased weight bearing and guarding of the limb, which are most pronounced during the acute phase of inflammation (Sluka and Westlund 1993; Scott et al. 1994; Min et al. 2001; Radhakrishnan et al. 2003) (Fig. 1). When the temporomandibular joint (TMJ) is inflamed with CFA, changes in meal pattern occur such that there is an increase in duration of the meal, and a decrease in food intake (Kerine et al. 2003).

Primary Hyperalgesia

Primary hyperalgesia of the knee joint is measured by the response to compression applied to the medial and lateral aspects of the knee joint with a pair of tweezers. This results in a lower threshold to vocalization within 4 hours after induction of inflammation, and lasts for several days (Yu et al. 2002) (Fig. 2). Similar responses are observed after injection of CFA into the knee joint (Yu et al. 2002).



Nocifensive Behaviors, Muscle and Joint, Figure 1 The time course for the decreased threshold to vocalization from compression of the knee joint before and after injection of kaolin and carrageenan into the knee joint. There is a decrease in vocalization threshold within 6 hours that lasts for approximately 1 wk. From Yu et al. 2002. Reprinted with permission of Elsevier Science.



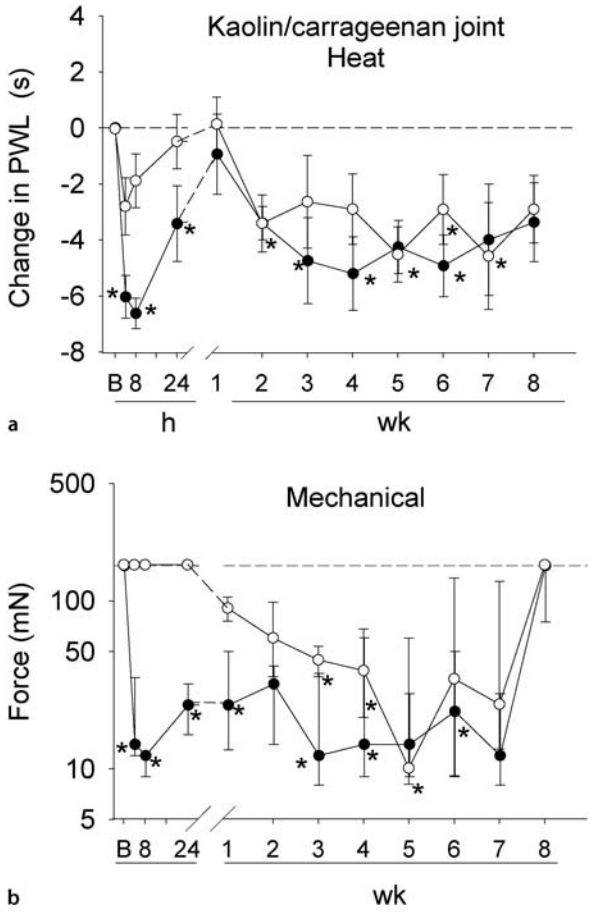
Nocifensive Behaviors, Muscle and Joint, Figure 2 Time course for the decrease in grip force that occurs after inflaming the triceps surae muscle with carrageenan. There is a decrease in grip force by 12h after induction that lasts for 36h. From Kehl et al. 2000. Reprinted with permission of Elsevier Science.

Secondary Hyperalgesia

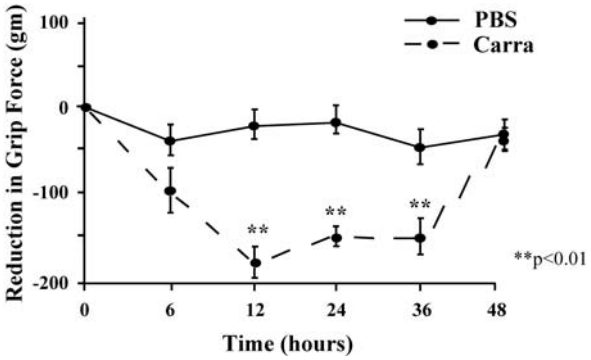
Secondary hyperalgesia to heat stimuli is measured with a radiant heat source applied to the paw following inflammation of the knee joint. Secondary mechanical hyperalgesia is measured by assessing the withdrawal threshold to von Frey filaments applied to the paw. Following carrageenan (3%) inflammation, there is decreased withdrawal latency to heat and decreased threshold to mechanical stimuli, ipsilaterally, that is long-lasting (weeks). Within 1–2 weeks, a time that corresponds to the conversion to chronic inflammation, the hyperalgesia to both mechanical and heat stimuli spreads to the contralateral limb (Radhakrishnan et al. 2003) (Fig. 3). The length of hyperalgesia and the contralateral spread are dose-dependent, such that lower concentrations of carrageenan produce shorter lasting hyperalgesia that remains unilateral (Radhakrishnan et al. 2003). Capsaicin is also utilized to inflame the joint and produces an acute inflammatory response that is associated with a decreased withdrawal threshold to mechanical stimuli, and an increased withdrawal latency to heat stimuli outside the site of injury (Sluka 2002). This secondary hyperalgesia to mechanical stimuli lasts for weeks and spreads to the contralateral limb. The time course and contralateral spread following joint inflammation are distinctly different from the hyperalgesia associated with injection of capsaicin into the skin. Injection of capsaicin into the skin produces a short lasting (hours) hyperalgesia and remains unilateral (Sluka 2002).

Muscle Pain

Two models of muscle pain, inflammatory and non-inflammatory, are utilized to study nocifensive behaviors resulting from muscle insult. Inflammation is induced by injection of carrageenan or capsaicin into



Nocifensive Behaviors, Muscle and Joint, Figure 3 Time course for the change in paw withdrawal latency to heat (a), and the withdrawal threshold to mechanical stimuli (b) following injection of 3% carrageenan into the knee joint. The hyperalgesia is initially unilateral and spreads to include the contralateral side within 1–2 weeks after induction of inflammation. Modified from Radhakrishnan et al. 2003. Reprinted with permission of Elsevier Science.



Nocifensive Behaviors, Muscle and Joint, Figure 4 Time course for the change in paw withdrawal latency to heat (a), and the withdrawal threshold to mechanical stimuli (b) following injection of 3% carrageenan into the gastrocnemius muscle. The hyperalgesia is initially unilateral and spreads to include the contralateral side within 1–2 weeks after induction of inflammation. Modified from Radhakrishnan et al. 2003. Reprinted with permission of Elsevier Science.

muscle tissue. Injection of carrageenan into the muscle results in an initial acute inflammatory response, which converts to a chronic inflammation by 1 week (Radhakrishnan et al. 2003). Spontaneous pain behaviors assessed by limb guarding occur during the first 24–48h, and are less severe than those following the same dose of carrageenan injected into the knee joint.

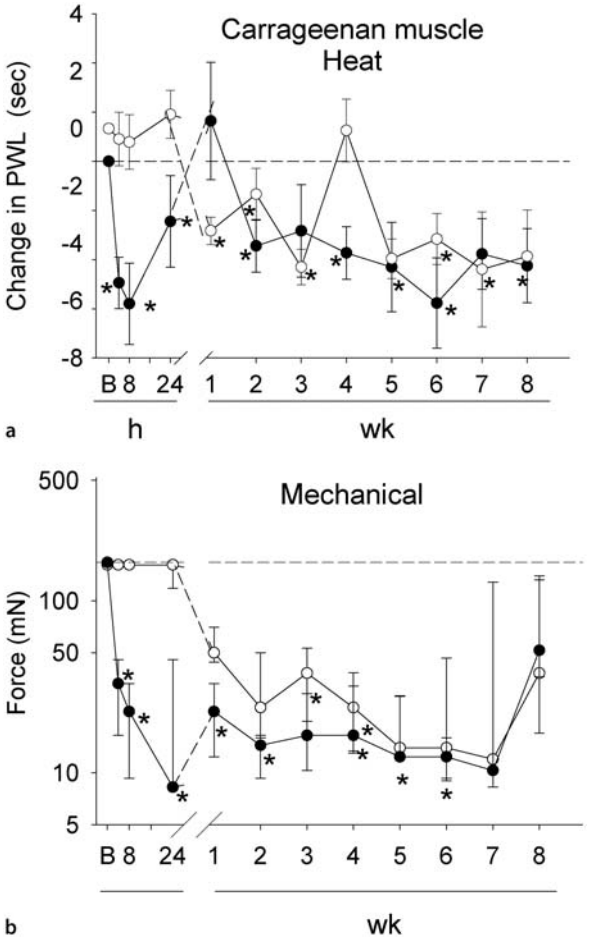
Primary Hyperalgesia

Primary hyperalgesia of the muscle is measured by examining grip force of the limb. There is a decrease in grip force after carrageenan inflammation of the triceps muscle within 12h that lasts for 36h (Fig. 4). Similarly, intramuscular injection of tumor necrosis factor alpha (TNF–α) and intramuscular formalin injection, reduced grip force for up to 1 day (Schäfers et al. 2003). Alternatively, measurement of withdrawal/vocalization threshold to pressure applied over the belly of the muscle has also been utilized to assess primary hyperalgesia (Schäfers et al. 2003). When TNF–α or formalin is injected into the muscle, decreases in the mechanical threshold applied to the muscle persist for at least 1 day.

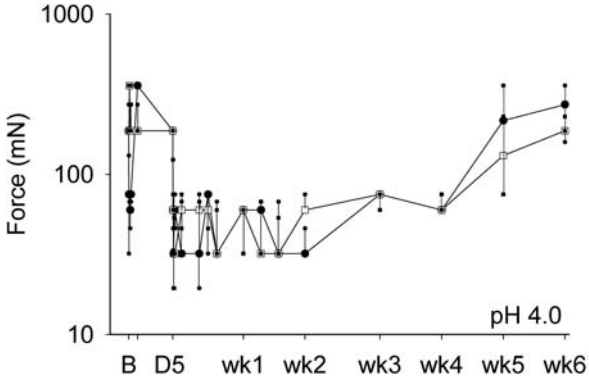
Secondary Hyperalgesia

Secondary hyperalgesia is measured in a manner similar to that described above for joint inflammation, but outside the site of injury such as on the paw of the rat. Specifically, after inflammation of the gastrocnemius muscle with carrageenan, there is a decrease in mechanical withdrawal threshold and decreased latency to radiant heat on the paw, ipsilateral to the inflamed knee joint, within 4h that lasts for weeks (Fig. 5) (Radhakrishnan et al. 2003). After 1–2 weeks, when the inflammation becomes chronic, this hyperalgesia to mechanical and heat stimuli will spread to the contralateral limb (Radhakrishnan et al. 2003). A similar long-lasting, bilateral mechanical hyperalgesia occurs when capsaicin is injected into the plantar muscles of the paw (Sluka 2002). The effect of carrageenan muscle injection, and particularly the bilateral spread of hyperalgesia, is dose-dependent. Lower doses of intramuscular carrageenan produce only unilateral hyperalgesia that is shorter in duration (Radhakrishnan et al. 2003). As a model of chronic non-inflammatory muscle pain, two injections of acidic saline injected 2–5 days apart, results in a bilateral, long-lasting mechanical, but not heat, hyperalgesia of the paw (Sluka et al. 2001). This hyperalgesia is not associated with muscle tissue damage or inflammation, and once developed the hyperalgesia is independent of continued primary afferent input (Sluka et al. 2001). In contrast, injection of TNF–α or formalin into the muscle does not produce secondary hyperalgesia to mechanical or heat stimuli, as observed with the other models listed above (i.e. carrageenan, acid) (Schäfers et al. 2003).

In summary, nocifensive behaviors after injury to muscle or joint include spontaneous pain behaviors, primary



Nocifensive Behaviors, Muscle and Joint, Figure 5 Time course for the decrease in mechanical withdrawal threshold following two acid injections (Day 0 and Day 5) into the gastrocnemius muscle. A bilateral, long-lasting decrease in mechanical withdrawal threshold occurs following repeated acid injection. From Sluka et al. 2001. Reprinted with permission of Wiley Publishing.



Nocifensive Behaviors, Muscle and Joint, Figure 6 Time course for the changes in weight bearing of the inflamed and contralateral hindlimb after unilateral injection of carrageenan into the knee joint. From Min et al. 2001. Reprinted with permission of Elsevier Science.

hyperalgesia, and secondary hyperalgesia. These behaviors are longer lasting than when the same stimulus is applied to the skin, and there is a bilateral spread of the secondary hyperalgesia. The development of hyperalgesia (primary vs. secondary OR mechanical vs. heat) and contralateral hyperalgesia is stimulus-dependent and dose-dependent.

Musculoskeletal Pain in Humans

Musculoskeletal pain can arise from a variety of disorders including ► myofascial pain, ► fibromyalgia, ► myositis, or ► arthritis. The quality of pain associated with injury to a muscle or joint differs from that associated with injury to the skin. Injury to deep structures results in diffuse, difficult to localize, aching pain (reviewed in Graven-Nielsen and Arendt-Neilsen 2002). In contrast, injury to skin usually produces well-localized, sharp, stabbing or burning pain. Using microneurography, intraneuronal stimulation of muscle nerve fascicles produces only the sensation of deep cramp-like pain (in contrast to stimulation of cutaneous C-fibers [Group IV] which produces burning pain). For muscle pain, the size of the area of ► referred pain correlates with the intensity and duration of the primary muscle pain (reviewed in Graven-Nielsen and Arendt-Nielsen 2002). In human subjects, painful intramuscular stimulation is rated as more unpleasant than painful cutaneous stimulation (Svensson et al. 1997), pain is longer lasting, and referred pain is more frequent (see Graven-Nielsen and Arendt-Nielsen 2002). In patients with fibromyalgia or knee osteoarthritis there is more pain, and a larger and more diffuse area of referred pain following infusion of hypertonic saline into the tibialis anterior muscle when compared to subjects without pain. Similarly, in patients with chronic whiplash pain, there is an extended area of referred pain following infusion of hypertonic saline into the infraspinatus muscle of the shoulder or a distal muscle of the leg (tibialis anterior muscle) suggesting changes in central processing. Thus, pain associated with injury to skin would be expected to differ in quality to pain associated with injury to deeper tissues such as muscle, and results in diffuse widespread increases in sensitivity to noxious stimuli.

Primary Hyperalgesia

In humans, muscle hyperalgesia is assessed by measuring threshold to pain from application of deep pressure, or by threshold to pain from intramuscular electrical stimulation (reviewed in Graven-Nielsen and Arendt-Nielsen 2002). Decreased pressure pain thresholds occur following intramuscular injection of capsaicin, hypertonic saline, serotonin and bradykinin. ► Delayed onset muscle soreness (DOMS) as a model of inflammatory muscle pain, results in decreased muscle function and increased pain to pressure.

Secondary Hyperalgesia

Secondary hyperalgesia in deep tissue and skin occurs following painful stimulation of deep somatic tissue, as

well as in patients with chronic musculoskeletal pain conditions (reviewed in Graven-Neilsen and Arendt-Neilsen 2002). Infusion of hypertonic saline in muscle results in increased sensitivity to electrical or mechanical stimulation of the skin, or to deep pressure outside the site of infusion, i.e. secondary hyperalgesia. In patients with chronic cervical injury, there is an increased sensitivity to intramuscular electrical stimulation of the anterior tibialis muscle of the leg, and an increased response to pressure in the referred pain area. Several studies show an increased heat and/or mechanical sensitivity both at the site of an arthritic joint and at a distance from the joint (O'Driscoll and Jayson 1974; Farrell et al. 2000; Kosek and Orderberg, 2000; reviewed in Graven-Nielsen and Arendt-Nielsen 2002; Hendiani et al. 2003). The increased sensitivity to mechanical pressure is found in patients with arthritic pain for greater than five years, but not those with arthritic pain for less than one year. However, people with arthritis have higher mechanical sensation thresholds (measured with Frey filaments) simultaneously with lower mechanical pain thresholds (measured with von Frey filaments), suggesting that there is an increased activation of descending inhibitory processes in addition to central sensitization.

Thus, secondary hyperalgesia to heat and mechanical stimuli are found in patients with deep tissue pain, similar to that found in animal models of pain.

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Nocifensive Behaviors of the Urinary Bladder

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Synonyms

Pseudaffective response; pseudoaffective response; Urinary Bladder Nocifensive Behaviors

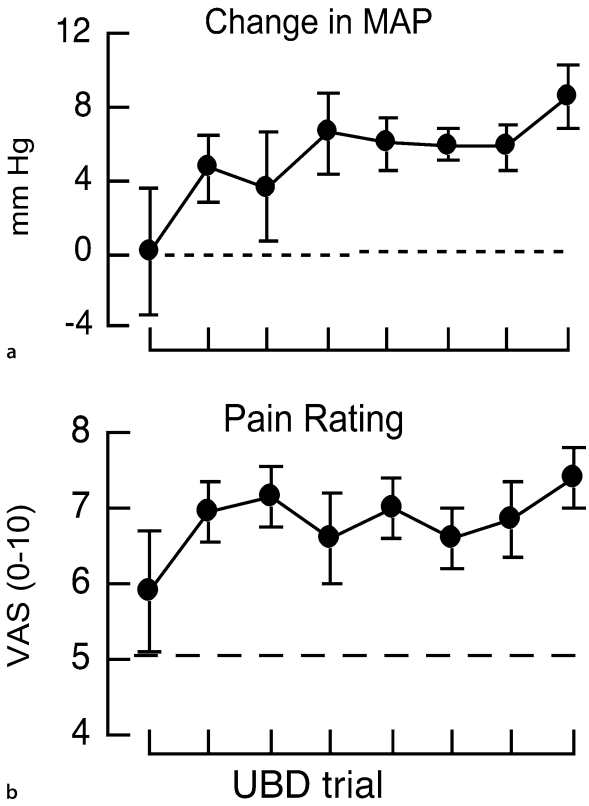
Definition

► Nocifensive reflexes are those reflexes evoked by noxious stimuli. Most acute motor responses to noxious cutaneous stimuli are nocifensive. For visceral systems in general, and the urinary bladder-related systems in particular, similar reflexes are not obvious. Micturition reflexes enhanced by local inflammation can expel toxic urinary contents and so may serve a protective function, but may also increase pain (unlike cutaneous nocifensive reflexes). Many responses to urinary bladder stimulation in non-human animals correlate with nociception in humans, and in this way are similar to nocifensive reflexes. These include biting/scratching behaviors, changes in heart rate, blood pressure, respiration and abdominal muscular tone. These behaviors/reflexes are collectively called pseud(o)affective responses.

Characteristics

Clinical Observations and Psychophysical Studies

The urinary bladder is a common site of visceral pain generation in humans. Urinary tract infections, inflammation produced by radiation, the action of irritating constituents of urine or acute obstruction of outflow from the bladder can all lead to severe pain localized to the lower abdomen, suprapubic region and perineum. At the same time, there is typically a sense of urgency (a need to void) and increased frequency of urination. When the bladder is inflamed, the act of urinating may



Nocifensive Behaviors of the Urinary Bladder, Figure 1 (a) Pressor responses to urinary bladder distension (UBD) in humans, an example of a nociceptive reflex response. In non-human animals this would be termed a pseudaffective response. Repeated presentation of a UBD stimulus that produced progressively vigorous increases in mean arterial blood pressure (MAP). (b) Reports of pain to the same stimulus paralleled the pressor response with increased vigor of responses with repeated presentation of the UBD stimulus. Data from Ness et al. 1998.

become painful and frequently there is a sensation of incomplete emptying. Autonomic responses such as heart rate changes, sweating and increases or decreases in blood pressure may precede, accompany and outlast the discomforting sensations. Psychophysical studies using fluid distension of the bladder via a urethral catheter have evoked reports of pain in humans as well as increases in blood pressure (e.g. Fig. 1), increases in abdominal tone and respiratory changes (Ness et al. 1998). Functional imaging has also demonstrated activation of numerous cortical sites of sensory processing, including sensory and motor cortices, the anterior cingulate cortex and the insular cortex (Athwal et al. 2001). Chemical stimulation of the bladder using ► intravesical capsaicin has similarly produced reports of pain and autonomic responses (e.g. Giannantoni et al. 2002). Similar physiological responses have been noted in non-human animals.

Micturition Reflexes

Micturition reflexes are reflexes involved in the emptying of the urinary bladder when full. They consist of a reflex contraction of the bladder itself, coupled with a relaxation of the sphincters and associated pelvic floor musculature which block outflow from the bladder. A lack of coordination of these two components can lead to painful contractions of the bladder and incomplete bladder emptying. When the urinary bladder becomes inflamed by local infection or other irritant action, micturition reflexes become enhanced with lower volume/pressure thresholds for activation. Micturition reflexes enhanced by noxious bladder stimulation could be viewed as "nocifensive", in that they may remove the irritating stimulus causing the pain by emptying the bladder. However, micturition reflexes normally occur in the absence of pain-producing stimuli, and the presence of irritating factors may paradoxically increase pain by increasing mechanical stimulation of the bladder through enhanced contractile activity. In this way, micturition reflexes may enhance nociception unlike cutaneous nocifensive reflexes.

As an assessment of micturition reflexes, distension of the bladder may be allowed to occur secondary to urine production and measures made of spontaneous voiding frequency and volume. Evoked micturition reflexes can be produced by the introduction of a catheter, which infuses fluid directly into the bladder at a rate higher than that typically produced by spontaneous urine formation. This catheter may be placed in a relatively non-invasive fashion via the urethra, or in non-human animals may also be surgically placed via an abdominal incision (Abelli et al. 1989.) A cystometrogram can then be performed by slow infusion of fluid into the bladder with simultaneous measurement of intravesical pressure. Micturition reflexes are identified as sharp increases in intravesical pressure coupled with the appearance of urine.

Pseud(o)Affective Reflexes

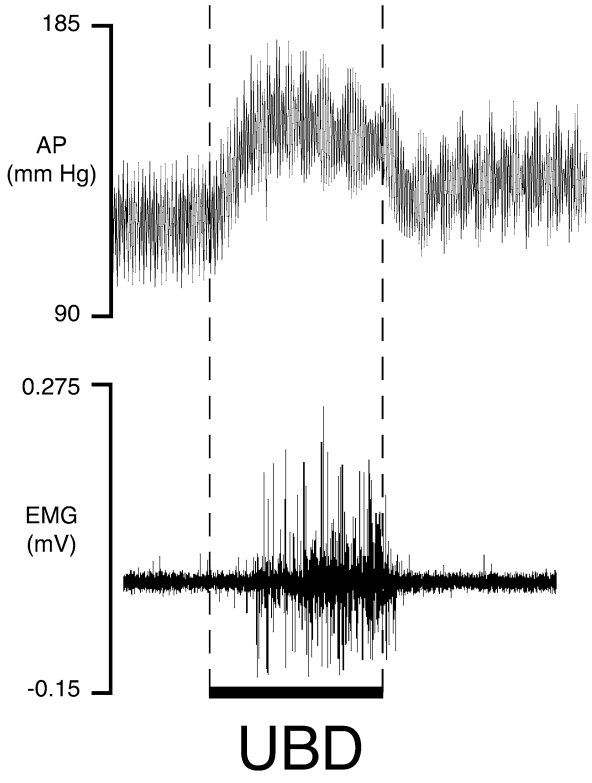
Highly localized motor reflexes such as a flexion-withdrawal response to a pinprick of the foot are nocifensive. The limb is reflexively pulled away from the damaging stimulus, the threat to the body is removed and pain generation is minimized. In visceral systems, a similar, direct link between tissue damage and protective behaviors is not obvious. There are typical behaviors in non-human animals evoked by visceral stimuli that would be pain-producing in humans, but these responses do not generally remove the threat to the organism. Abdominal licking, contractions of abdominal muscles and changes in heart rate, blood pressure, and respiratory pattern can all be evoked by stimulation of the urinary bladder of rats. These types of responses were called pseudaffective responses by Sherrington, in that they appeared to be reflex correlates to emotional responses to painful stimuli (Woodworth and Sherrington 1904). Pseudaffective responses are not specific to pain-producing stimuli, but when it can be demonstrated that they are inhibited by pharma-

cological manipulations known to be analgesic (i.e. morphine) the case for their correlation with accepted nocifensive reflexes becomes stronger.

There have been numerous characterizations of responses to noxious urinary bladder stimulation in non-human animals, many of which have been utilized as models of urinary bladder pain. The stimuli for these models can be broadly stratified into those that use mechanical stimuli to activate afferents arising in the bladder (typically by air or fluid distension using a catheter), and those which chemically activate/sensitize afferents arising in the bladder using irritants. These irritants may be administered directly into the bladder using a catheter, or indirectly by the renal excretion of irritating urinary constituents.

Responses to Urinary Bladder Distension (UBD)

Pseudaffective responses to UBD (vigorous cardiovascular and visceromotor reflexes) have been demonstrated in numerous species including rats, mice, guinea pigs, rabbits, cats, dogs and monkeys. Such studies related to nociception have commonly used a progressively increasing UBD stimulus, similar to that used in studies of micturition but at a much faster rate of filling, or have produced a phasic (on-off) stimulus through the use of a rapid, near instantaneous, infusion of air or saline. In anesthetized animals, vigorous neuronal and reflex responses can be evoked (e.g. Westropp and Buffington 2002). These responses can be inhibited by analgesics. Reliable and reproducible pressor responses and contractions of the abdominal musculature (► visceromotor responses measured by electromyogram) evoked by UBDs have been characterized (e.g. Ness et al. 2001) (Fig. 2) with gender differences (see ► Gender and Pain) and gonadal hormonal influences apparent. Genetic models of painful bladder disorders such as the feline interstitial cystitis model have utilized such responses to bladder stimulation as endpoints (Westropp and Buffington 2002.) Sensitizing chemicals associated with inflammation when administered into the urinary bladder lead to more vigorous responses to UBD, particularly at low intensities of stimulation (Dmitrieva and McMahon 1996).



Nocifensive Behaviors of the Urinary Bladder, Figure 2 Examples of cardiovascular (arterial pressure AP; above) and distension (UBD) in a halothane-anesthetized rat. Visceromotor responses can be measured by direct visualization or electromyographically (EMGs), as in the figure. Responses are graded in response to graded UBD.

Responses to the Intravesical Instillation of Proinflammatory Compounds

Inflammation of the bladder commonly produces reports of pain and urgency in patients suffering from a urinary tract infection. Enhanced micturition reflexes lead to spasms of pain. Experiments in non-human animals have artificially inflamed or sensitized the bladder with the intravesical administration of inflammation-inducing compounds such as turpentine, mustard oil, croton oil and neurotrophic agents. Such irritation has been demonstrated to produce alterations in the spontaneous activity of primary afferent neurons encoding for bladder stimuli (e.g. Dmitrieva and McMahon 1996) and spontaneous behavioral responses. Most reflex studies have been performed in rats, although behavioral and neurophysiological studies have been performed in primates and cats.

McMahon and Abel (1987) characterized visceromotor and altered micturition reflexes in chronically decerebrate rats, and demonstrated that following the administration of an irritant, the magnitude of responses to UBD correlated with measures of inflammation such as tissue edema, plasma extravasation and leukocyte infiltration. While becoming more sensitive to bladder-related stimuli, the rats at the same time became hypersensitive to noxious stimuli applied to the lower abdomen, perineum and tail, as measured by the number of "kicks" evoked by a given stimulus. The model of McMahon and Abel (1987) has been modified to examine pharmacologically novel mechanisms related to visceral hyper-reflexia (altered cystometrograms) or secondary hyperalgesia (decreased thresholds to heat stimuli in hindlimbs). Modulatory effects of glutamate-receptor antagonists, nitric oxide synthase inhibitors, neurotrophic antagonists, cannabinoids and bradykinin receptor antagonists have all been noted (e.g. Jagger et al. 1999).

Responses to the Direct Application of Irritants

Administration of intravesical irritants via a chronically implanted intravesical catheter was described by Abelli et al. (1989) and modified by Craft et al. (1993). An injection of xylene, capsaicin or its related compound resiniferatoxin is performed via an intravesical cannula placed a day earlier. Immediate responses include abdominal/perineal licking, headturns, hindlimb hyperextension, head grooming, biting, vocalization, defecation, scratching and salivation, all of which can be independently measured and graded. The time to onset, incidence and number of individual behavioral responses are recorded for fifteen minutes or more following intravesical drug administration. Baclofen, mu/kappa/delta opioid receptor agonists and intravesical tetracaine have all been demonstrated to inhibit these behavioral responses. Bladder denervation abolishes all behavioral responses.

Responses to Cyclophosphamide (Chemotherapy-Induced Cystitis)

Subacute, irritant-induced urinary bladder inflammation occurs secondary to antineoplastic regimens that include the chemotherapeutic agent cyclophosphamide (CP). In humans, pain and increased urgency/frequency are sequelae of this treatment, although use of sodium 2-mercaptoethane sulfonate reduces the metabolite of CP that is the actual irritant, acrolein. In non-human animal models, CP is administered intraperitoneally, which evokes behavioral responses such as hypolocomotion and alterations in position (e.g. Lanteri-Minet et al. 1995). Chronic (2 week) treatments with CP have also been described with numerous physiological and biochemical alterations (e.g. Vizzard et al. 1996). Olivar and Laird (1999) have characterized similar responses to acutely administered CP in mice.

Conclusions

Numerous responses to urinary bladder stimulation have been noted. Other than enhanced micturition reflexes, none of these responses fit the precise criteria of nocifensive reflexes, in that they are not generally protective reflexes that remove the noxious stimulus. They are nociceptive reflexes in that they do appear to be representative of the sensory processing related to pain from the bladder, and should be expected to be predictive of pharmacological or procedural manipulations intended to treat urinary bladder pain.

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Nodes of Ranvier

Definition

Axonal zones within gaps in myelin in which excitation occurs.

► Trafficking and Localization of Ion Channels

Nodose Ganglia

Definition

Vagal ganglia containing cell bodies of vagal afferent fibers.

► Visceral Pain Model, Esophageal Pain

Nomogenic Symptoms and Signs

► Non-Organic Symptoms and Signs

Non-Anatomic Symptoms and Signs

► Non-Organic Symptoms and Signs

Non-Cardiac Chest Pain

► NCCP

Non-Competitive Antagonist

Definition

Non-competitive antagonist is a compound that inhibits receptor function without directly acting at the endogenous ligand binding site.

► Metabotropic Glutamate Receptors in Spinal Nociceptive Processing

Non-Corpuscular Sensory Endings

Synonyms

Free nerve endings

Definition

The peripheral end structures of unmyelinated (C or Group IV) and thinly myelinated (Aδ or Group III) sensory fibers that encode noxious, cold or warm stimuli, that consist of either single fibers or bundles of sensory axons associated with peripheral glia (Schwann cells), and that are characterized by their lack of perineurium and the loss of myelination in Aδ endings. Typically, the sensory axons of nociceptive sensory endings have exposed membrane areas that are not covered by Schwann cell processes and can be regarded as chemoreceptive sites.

► Nociceptors in the Orofacial Region (Meningeal/Cerebrovascular)
► Toxic Neuropathies

Non-Drug Treatment

► Psychological Treatment of Headache

Nonexertional Capability

Definition

Capability to perform any of the nonexertional activities, which are activities other than any of the seven strength demands of work.

► Disability Evaluation in the Social Security Administration

Nonexertional Limitations and Restrictions

Definition

Limitations or restrictions that affect the capability to perform a work-related function that is not exertional, i.e. a limitation or restriction of mental abilities, vision, hearing, speech, climbing, balancing, stooping, kneeling, crouching, crawling, reaching, handling, fingering, and feeling, or an environmental restriction.

► Disability Evaluation in the Social Security Administration

Non-Neurogenic Inflammation

Definition

Inflammatory processes that are not specifically initiated by activity in primary afferent C-fibers; typically this includes inflammatory processes initiated by mediators released from resident or circulating monocytes and leukocytes, including prostaglandins, prostacyclins, leukotrienes, and cytokines, to name a few.

► Formalin Test

Non-Nutritive Sucking

Definition

Placing an object (e.g. pacifier, non-lactating nipple) into an infant's mouth to stimulate sucking behaviors during a painful event.

► Acute Pain Management in Infants

Nonopioid Analgesia

Definition

Type of pain inhibition NOT meeting any of the criteria for opiate mediated effects (including blockade by opiate antagonists and cross-tolerance with opiate agonists).

► Pain Modulatory Systems, History of Discovery

Non-Migraine Headaches

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► Migraine is the best investigated and understood headache entity. About 50% of all headache publications deal with migraine. The International Headache Society created a headache classification in 1988 (Headache Classification Committee 1998), which was updated in 2004 (Olesen et al. 2004). This classification contains all other headaches in addition to migraine and provides operational criteria for diagnosis. Headaches are grouped into primary and secondary headaches. Primary headaches are migraine, ► tension type headache and the trigemino-autonomic headaches.

Tension type headache is the most frequent headache. The new classification distinguishes infrequent from frequent episodic tension type headache. ► Chronic tension type headache is diagnosed when >180 headache days/year are occurring. The group of trigemino-autonomic headaches is characterised by headaches with autonomic features, e.g. conjunctival injection, lacrimation, nasal congestion, rhinorrhoea, forehead and facial sweating, miosis, ptosis or eyelid oedema (Goadsby and Lipton 1997). This group of headaches includes paroxysmal and chronic cluster headaches, episodic and ► chronic paroxysmal hemicrania (Sjaastad and Dale 1976) and short lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing (► SUNCT) (Sjaastad et al. 1991). Other primary headaches include primary stabbing headache (Pareja et al. 1996), ► primary cough headache (Symonds 1956), ► primary exertional headache, primary headache associated with sexual activity (Pascual et al. 1996) and ► hypnic headache. In all headaches with provocation by physical activity, a secondary headache such as subarachnoidal haemorrhage has to be excluded. Hypnic headache occurs in elderly women during the nighttime and responds to treatment with lithium (Raskin 1988; Newman et al. 1990). New entities in this group are ► hemicrania continua (Sjaastad and Spierings 1984), a chronic headache and new daily persistent headache (Li and Rozen 2001). ► New daily persistent headache has similar characteristics to chronic tension headache but an abrupt onset without improvement.

Secondary headaches are related to physical changes to the brain, the head or the neck. Headache attributed to head and/or neck trauma is divided into acute and chronic headache. This group includes acute and chronic post-traumatic headache (Young and Packard 1997), acute headache attributed to whiplash injury, headache attributed to intracranial haematomas (subdural, epidural), headache attributed to other head and/or neck trauma and postcraniectomy headache.

A large group of secondary headaches are headaches attributed to cranial or cervical vascular disorders. Headache in these cases can be due to cerebral ischaemia, cerebral bleedings or an unruptured AV malformation or aneurysm. Other reasons for headache are arteriitis (giant cell and intracranial arteriitis), cerebral venous thrombosis and dissections of the carotid or vertebral arteries. Migraine is a risk factor for stroke and may lead to an ischaemic stroke during the aura phase (Diener et al. 2004).

The next group in the classification defines headaches attributed to intracranial non-vascular causes. High (Wall 1990) as well as low (Lay et al. 1997) intracranial pressure causes headache. ► Headache attributed to low cerebrospinal fluid pressure can occur spontaneously or be the result of lumbar puncture. Increased intracranial pressure is due in most cases to intracranial neoplasms or hydrocephalus. Headache attributed to non-infectious inflammatory disease can be due to neurosarcoidosis, aseptic meningitis or lymphocytic hypophysitis. Another interesting association exists between epilepsy and migraine (Leniger et al. 2003). Migraine auras can lead to a seizure and a tonic–clonic seizures may trigger a migraine attack.

A major revision took place in the classification of ► headaches attributed to substance use or withdrawal. This section includes acute headache provoked by drugs or chemical substances such as nitric oxide donors, phosphodiesterase inhibitors or carbon monoxide, but also food components, e.g. alcohol or monosodium glutamate. Recreational drugs such as heroin, cocaine and cannabis can cause headache. A new concept is medication overuse headache (Diener and Limmroth 2004). The frequent or regular use of drugs to treat acute headaches can lead to a chronic headache or deterioration of a pre-existing headache. Medication overuse headache can be due to analgesics, combination drugs, ergots, triptans or opioids. Withdrawal of substances, e.g. caffeine, oestrogen or opioids can also result in headache.

Headache attributed to infections is associated with bacterial meningitis, lymphocytic meningitis, encephalitis, brain abscess or subdural empyema. Headache can also be due to systemic infection with bacteria, viruses (including HIV) or other systemic infections.

Group 10 summarizes headaches associated with disorders of homeostasis. In this group are headaches due to hypoxia and/or hypercapnia, to arterial hypertension, hypothyroidism and fasting. Headache and facial

pain attributed to disorders of cranium, neck, eye, ears nose, sinuses, teeth, mouth or other facial or cranial structures will be covered in another part of the encyclopaedia.

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Nonopioid Analgesic Drug

Definition

Drugs that relieve pain by other mechanisms than interacting with opioid receptors, e.g. paracetamol and NSAIDs.

- ► Cancer Pain Management
- ► NSAIDs, Survey
- ► Postoperative Pain, Acute Pain Management, Principles

Nonorganic Physical Findings (Waddell Signs)

Definition

A group of low back pain physical signs that were identified in the past as being nonorganic, and therefore the presence of which has historically indicated that the patient is either suffering from conversion disorder or malingering.

Non-Organic Symptoms and Signs

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Synonyms

Behavioral Responses to Examination; Behavioral Descriptions of Symptoms; Inappropriate Symptoms and Signs; Inconsistent Symptoms and Signs; Medically Incongruent Symptoms and Signs; Non-Anatomic Symptoms and Signs; Nomogenic Symptoms and Signs; Waddell Signs

Definition

Descriptions of symptoms and responses to clinical examination in patients with low back pain, which relate more to cognitive-behavioral processes than to physical pathology.

Characteristics

Clinical assessment and diagnosis are based on the recognition of patterns of symptoms and signs, which in most patients fit to a greater or lesser degree with anatomy, mechanics and pathology. Occasional patients, however, present symptoms and signs that are not only vague and ill-localized, lack the normal relationship with time and activity, and appear out of proportion to the physical injury or pathology, but positively contradict normal anatomic boundaries and biomechanics.

Non-organic signs in low back pain have been recognized since the beginning of the 20th century, initially in the context of compensation assessment, where any

Non-Organic Symptoms and Signs, Table 1 Standardized battery of non-organic signs in low back pain

tenderness	superficial
	non-anatomic
simulation	axial loading
	simulated rotation
distraction	straight leg raising
regional	weakness
	sensory disturbance
over-reaction to examination	Subsequently replaced by overt pain behavior (Keefe and Block 1982)
	Grimacing, sighing, guarding, bracing, rubbing

clinical findings that were judged excessive or not entirely consistent with physical injury, were interpreted as evidence of malingering. That over-simplification gradually became discredited. The modern description by Waddell et al. (1980) standardized a battery of non-organic signs (Table 1), and re-interpreted them in the light of modern understanding of illness behavior.
Waddell et al. (1984) subsequently developed a corresponding battery of non-organic or behavioral descriptions of symptoms in low back pain (see list below), though these have never received as much attention or use as the non-organic signs.
Non-organic or behavioral descriptions of symptoms in low back pain:

- Pain at the tip of the tailbone
- Whole leg pain
- Whole leg numbness
- Whole leg giving way
- Complete absence of any spells with very little pain in the past year
- Intolerance of, or reactions to, many treatments
- Emergency admission(s) to hospital with nonspecific low back pain.

These groups of non-organic symptoms and signs were demonstrated to be reliable, internally consistent, separable from the standard symptoms and signs of physical pathology, and related to psychological distress and other measures of illness behavior. Independent studies (reviewed in detail in Waddell 2004) have since confirmed that they relate to: measures of physical impairment, severity of pain and poorer physical performance (though there is debate about the extent to which these reflect physical impairment or illness behavior); affective measures of pain and psychological distress; other measures of illness behavior (the pain drawing, overt pain behavior, UAB pain behavior scale, and various scales of the Illness Behavior Questionnaire); disability and incapacity; and the prediction of clinical outcomes (but not occupational outcomes) of conservative and surgical treatment and rehabilitation. Non-organic symptoms and signs were initially observed in patients with chronic low back pain, and illness behavior was considered to be the consequence of chronicity. It is now clear that they can occur at a much earlier stage, reflecting the involvement of illness behavior in the process of chronification. Illness behavior is learned and a form of communication between patients and health professionals.
Non-organic symptoms and signs can help to clarify clinical assessment by:

- Distinguishing and hence permitting separate assessment of physical and behavioral elements in the clinical presentation
- Providing a simple clinical screen for psychological and behavioral issues in the clinical presentation, indicating the need for more detailed psychological assessment
- Clarifying clinical decision making - helping to direct physical treatment to treatable pathology, and reducing inappropriate physical treatment; helping to identify patients who may need more psychological support

However, there are a number of important caveats to their use (Main and Waddell 1998; Waddell 2004):

1. Diagnostic triage should be performed first, to exclude serious spinal pathology (such as tumor or infection), widespread neurological disorders (involving multiple nerve roots) or major psychiatric disorders (e.g. psychosis or major depression) before assessing non-organic symptoms and signs.
2. Clinical observation of illness behavior depends on careful technique and it is important to minimize observer bias.
3. Isolated behavioral symptoms and signs are common in normal patients and of no clinical, psychological or behavioral significance. Only multiple positive findings (preferably of several different dimensions of illness behavior) are clinically significant.
4. Behavioral symptoms and signs do not provide any information about the original cause of the pain: they do not mean that the patient's pain is 'not real', 'psychogenic' or 'hysteric'.
5. It is not a differential diagnosis between physical disease and illness behavior: most patients have both a physical problem in their backs and varying degrees of illness behavior. Inability to demonstrate the physical basis of the pain does not mean that the pain is psychogenic, any more than the presence of illness behavior excludes a treatable physical problem. Diagnosis of psychological dysfunction and illness behavior depends on positive psychological and behavioral findings.

6. Non-organic symptoms and signs are only a preliminary clinical screening test, indicating the need for more detailed clinical and psychological assessment. Clinical observations of illness behavior do not provide a complete psychological assessment or a psychological or psychiatric diagnosis. They are less sensitive than psychometric measures for detecting distress, so there is a significant false negative rate.
7. Non-organic symptoms and signs are not lie-detectors, but observations of normal human behavior in illness. They do not necessarily mean that the patient is acting, faking or malingering. Most illness behavior occurs in pain patients who have no claim for compensation or any question of secondary gain.

This was summarized in the original article (Waddell et al. 1980):
It is safer to assume that all patients complaining of back pain have a physical source of pain in their back. Equally, all patients with pain show some emotional and behavioral reaction. Physical pathology and non-organic reactions are discrete and yet frequently interacting dimensions; they are not alternative diagnoses but should each be assessed separately.
Non-organic signs are widely used – and misused – in surgical, compensation and medico-legal practice, but there is continuing controversy about their value and interpretation. There are two main criticisms (which are mutually exclusive):

1. Pain specialists have argued that modern neurophysiologic and clinical understanding of chronic pain provides a purely biologic explanation for these findings (Merskey 1988; Margoles 1990; Fishbain et al. 2003). Neurophysiologic mechanisms can produce spread of pain and tenderness, hypersensitivity, altered sensation and inhibition of motor activity, in the absence of other evidence of illness behavior. That is why non-organic symptoms and signs must be elicited and interpreted with care, and isolated findings should not be over-interpreted. However, non-organic symptoms and signs often spread far beyond any likely neurophysiologic mechanism and fit better with body image patterns, they form part of a constellation of other illness behaviors and correlate with other psychological findings.
2. On the contrary, many medicolegal experts claim that non-organic symptoms and signs demonstrate conscious and deliberate attempts to deceive the examiner, and evidence of faking or malingering. The scientific evidence in this area is weak (Fishbain et al. 1999; Waddell 2004) but this is a legal rather than a clinical matter. Case law leaves no doubt that non-organic symptoms and signs can occur in patients with other evidence of lack of credibility and represent conscious attempts to exaggerate the severity of pain and disability. However, there is a wealth of clinical and legal evidence that illness behavior can also be produced by unconscious, psychological mechanisms. Thus, non-organic signs per se do not provide sufficient evidence to prove malingering. As in the clinical setting, non-organic signs are a screening tool: they may raise the question of credibility, but they do not provide an answer - that depends on thorough assessment and judgment of all the clinical, psychological and legal evidence.

Non-organic symptoms and signs have only been standardized in white Anglo-Saxon patients of working age with chronic low back pain. Further research would be required before they can be used in younger or older patients, in non-white patients, or in different cultures. In principle, it appears likely that the concept of non-organic symptoms and signs may be equally applicable to other pain conditions, although there have only been a few preliminary studies in neck pain, other musculoskeletal conditions and cardiac pain and their use without low back pain is not established.
Non-organic symptoms and signs are a powerful tool to extend the scope of routine clinical assessment of patients with low back pain, provided care is taken to define what they are and what they are not, and to recognize their strengths and their limitations. Like all clinical tools, they must be used with care and compassion.

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Non-Pharmacologic Pain Management

Definition

Non-pharmacologic pain management includes all approaches to pain control that don't involve drug therapies, such as distraction (e.g. music), psychological

interventions (e.g. imaging/visualization, relaxation, meditation), biofeedback, massage, aromatherapy, physical modalities (electrical stimulation, heat, cold), etc.
► Opioids in Geriatric Application

Non-Pharmacological Interventions

Definition

Non-pharmacological interventions are used to prevent and manage newborn pain and distress. It refers to the use of comfort measures and the modification of environmental factors. Comfort measures may include flexed positioning with limb containment, non-nutritive sucking, skin-to-skin contact, rocking, holding, breast feeding and sucrose. Environment and caregiving (provided by health professionals) strategies include the use of light touch and reducing ambient sound and light levels.
► Pain Assessment in Neonates

Non-Pharmacological Treatment

Definition

Nonmedical, psychologically-based treatments that teach individuals skills so that they may handle pain in a more optimal manner.
► Biofeedback in the Treatment of Pain
► Psychological Treatment of Headache

Non-Selective COX-Inhibitors

► NSAIDs, Mode of Action

Non-Specific Low Back Pain

Definition

Low back pain that is not due to specific pathology such as tumor, fracture, inflammation, osteoporosis, or rheumatoid arthritis.
► Back Pain in the Workplace
► Lumbar Traction

Non-Steroidal Anti-Rheumatic Drugs

► Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)
► NSAIDs, Adverse Effects

Non-Structural Disorders

► Visceral Pain Model, Irritable Bowel Syndrome Model

Non-Systemic (Isolated) Vasculitic Neuropathy

Synonyms

NVNP

Definition

Neuropathy caused by vasculitis confined to the peripheral nervous system.
► Vascular Neuropathies

Nonthermal Effects

Definition

Effects produced by nominally thermal agents that do not directly result from heating or cooling. Examples of nonthermal effects include cavitation (the production of bubbles in liquids and tissues) in ultrasound, as well as the proposed and established benefits of low intensity pulsed delivery of the diathermies.
► Therapeutic Heat, Microwaves and Cold

Non-Topographic

Definition

Non-topographic organization contrasts with topographic/somatotopic organization and favors the notion that the region is involved in affect-motivation. Khanna and Sinclair (1992) reported that a noxious heat stimulus, whether applied to the hind paw or the tail, suppressed CA3 stimulation-elicited CA1 pyramidal cell excitation at a given site in the hippocampus.
► Nociceptive Processing in the Hippocampus and Entorhinal Cortex, Neurophysiology and Pharmacology

Non-Traditional Medicine

► Alternative Medicine in Neuropathic Pain

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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Synonyms

NSAIDs; non-steroidal anti-inflammatory drugs; Non-Steroidal Anti-Rheumatic Drugs; NSAR; Aspirin-Like Drugs; non-opioid analgesics

Definition

Non-opioid analgesic agents can be divided into two groups. The first group contains substances having anti-inflammatory effects in addition to their analgesic and antipyretic activity and are called non-steroidal anti-inflammatory drugs (NSAIDs). The members of this group, and one substance (lumiracoxib) within the group of selective inhibitors of cyclooxygenase 2 (COX-2), are acids. Acidic NSAIDs, which include salicylates, derivatives of acetic acid and propionic acid and oxicams among others, comprise molecules containing a lipophilic and a hydrophilic region and are more than 99% bound to plasma proteins. The second group of non-opioid analgesics, which are not classified as NSAIDs, consists of substances that lack anti-inflammatory properties, such as phenazones, metamizole (= dipyrone) and paracetamol. Their molecules are neutral or weakly basic, have no hydrophilic polarity and are much less strongly bound to plasma proteins than NSAIDs (see ► NSAIDs, chemical structure and molecular mode of action).

Mechanism of Action

In the 1970s, NSAIDs were shown to interfere with the biosynthesis of prostaglandins (Vane 1971). NSAIDs block cyclooxygenases (COX) that catalyze the formation of cyclic endoperoxides from arachidonic acid. Cyclic endoperoxides are precursors of the prostaglandins, thromboxane A2 and prostacyclin. Prostaglandins have a major role in the pathogenesis of pain, fever and inflammation. Inhibition of their biosynthesis would therefore be expected to result in analgesic, antipyretic and anti-inflammatory activity. However, since prostaglandins are synthesised in most tissues and have a variety of physiological functions, inhibition of their biosynthesis also causes unwanted effects. The clinically most important of these are gastrointestinal erosion and ulceration with bleeding and perforation and kidney disorders with retention of sodium ions and water. About 30 years ago the principle mode of the antinociceptive action of NSAIDs was related to their anti-inflammatory activity and attributed to the inhibition of the production of prostaglandins at the peripheral site of inflammation. The traditional belief in the exclusively peripheral action of NSAIDs, however, has been challenged by the growing evidence that prostaglandins are key players in spinal nociceptive processing. Thus, NSAIDs have a peripheral and central component of antinociceptive activity (see essays on ► history of analgesics; ► COX-1 and COX-2 in pain; ► NSAIDs and their indications; ► prostaglandins, spinal effects; ► NSAIDs, adverse effects).

Moreover, there are cyclooxygenase independent effects of NSAIDs potentially contributing to the activity of NSAIDs (for review, see Tegeder et al. 2001) (see also ► NSAIDs, COX-independent actions; ► NSAIDs and cancer).

The identification of two distinct types of cyclooxygenases in 1990 (Fu et al. 1990) encouraged the search for NSAIDs devoid of the side effects associated with COX-1 inhibition. The cyclooxygenase isoform COX-1 is physiologically expressed in the stomach, platelets and the kidney and is there responsible for the synthesis of prostaglandins needed for normal organ function. Its inhibition by conventional NSAIDs causes side effects e.g. inhibition of prostaglandin synthesis in the gastrointestinal tract results in a loss of protection in the gastrointestinal mucosa and ulcerations. The cyclooxygenase isoform, COX-2 is rapidly induced by various factors including cytokines and its expression is triggered by inflammation, pain or tissue damage. It is clear from this division of cyclooxygenases into COX-1 and an inducible COX-2 that the anti-inflammatory, analgesic and antipyretic effects of the NSAIDs are mainly attributable to inhibition of COX-2 whereas inhibition of COX-1 is associated with most of the unwanted effects of the NSAIDs. It follows that drugs that selectively inhibit COX-2 should cause fewer side effects than those that inhibit both COX-1 and COX-2. At therapeutic doses, all currently available NSAIDs, with the exception of coxibs, are nonselective and inhibit both COX isoforms (see ► NSAIDs, chemical structure and molecular mode of action).

Newer research has shown that the assignment of physiological activity exclusively to COX-1 and pathophysiological activity exclusively to COX-2 is not strictly valid, since COX-2 is expressed constitutively in organs such as spinal cord, kidney and uterus. Furthermore, COX-2 is formed during various physiological adaptation processes, such as the healing of wounds and ulcers.

Clinical Use and Side Effects

NSAIDs are indicated in the treatment of:

- various pain states (e.g. headache, toothache and migraine), primarily pathophysiological pain involving nociceptors e.g. rheumatic pain and pain caused by bone metastases,
- defects of the ductus arteriosus Botalli (short circuit connection between arteria pulmonalis and aorta; non-closure after birth)
- fever (see ► NSAIDs and their indications)

Side effects of NSAIDs include:

- gastrointestinal disorders (e.g. dyspepsia), gastrointestinal erosion with bleeding, ulceration and perforation,
- kidney malfunctions with retention of sodium and water, hypertension
- inhibition of platelet aggregation,
- central nervous symptoms such as dizziness and headache,
- disturbance of uterine motility,
- skin reactions,
- triggering of asthma attacks in asthmatics. This side effect is a pseudo-allergic reaction where COX-inhibition increases the availability of substrates for lipoxygenase that are converted to bronchoconstrictive leukotrienes (see ► NSAIDs, adverse effects and ► NSAID-Induced Lesions of the Gastrointestinal Tract).

Non-selective inhibitors of prostaglandin synthesis are contraindicated:

- in gastric and duodenal ulcer,
- in asthma,
- in bleeding disorders,
- during the last few weeks of pregnancy because of the danger of early sealing of the ductus Botalli.

Glucocorticoids increase the risk of gastrointestinal complications. Considerable caution is necessary when using NSAIDs in patients with severe liver and kidney damage and they should not be combined with coumatins. Owing to the limited experience obtained, these precautions and contraindications also apply to COX-2 selective inhibitors.
The following drug interactions are the most important that can occur when conventional NSAIDs are co-administered with other agents:

- the uricosuric effect of probenecid is reduced
- the diuretic effect of saluretics is weakened,
- the blood glucose lowering effect of oral antidiabetics is increased,
- the elimination of methotrexate is delayed and its toxicity is increased,
- the elimination of lithium ions is delayed,
- the anti-coagulation effect of coumatin derivatives is enhanced and
- the antihypertensive effect of ACE-inhibitors is reduced (see ► NSAIDs, pharmacokinetics).

Due to the short period of clinical use, the interaction profile of COX-2 selective inhibitors cannot be described at the present time.

Derivatives of Salicylic Acid

Salicylic acid for systemic use has been replaced by acetylsalicylic acid, amides of salicylic acid (salicylamide, ethenzamide, salacetamide), salsalate and diflunisal.

Acetylsalicylic Acid (Aspirin)

The esterification of the phenolic hydroxyl group in salicylic acid with acetic acid results not only in an agent with improved local tolerability, but also with greater antipyretic and anti-inflammatory activity and, in particular, more marked inhibitory effects on platelet aggregation (inhibition of thromboxane-A2 synthesis). Because of these qualities, acetylsalicylic acid is one of the most frequently used non-opioid analgesics and the most important inhibitor of platelet aggregation.
Acetylsalicylic acid irreversibly inhibits both COX-1 and COX-2 by acetylating the enzymes. Since mature platelets lack a nucleus, they are unable to synthesize new enzyme. The anti-platelet effects of acetylsalicylic acid therefore persist throughout the lifetime of the platelet and the half-life of this effect is thus much longer than the elimination half-life of acetylsalicylic acid (15 min). Since new platelets are continuously launched into the circulation, the clinically relevant anti-platelet effect of aspirin lasts for up to 5 days. This is the reason why low doses of acetylsalicylic acid (ca. 100 mg per day) are sufficient in the prophylaxis of heart attacks.
After oral administration, acetylsalicylic acid is rapidly and almost completely absorbed, but in the intestinal mucosa it is partly deacetylated to salicylic acid, which also exhibits analgesic activity. The plasma half-life of acetylsalicylic acid is approximately 15 min, whereas that of salicylic acid at therapeutic dosages of acetylsalicylic acid is 2–3 h. Salicylic acid is eliminated more slowly when acetylsalicylic acid is administered at high dose rates because of saturation of the liver enzymes. The metabolites are mainly excreted via the kidney. The dosage of acetylsalicylic acid in the treatment of pain and fever is 1.5–3 g daily and in the prophylaxis of heart attacks 30–100 mg daily.
Side effects of acetylsalicylic acid administration include buzzing in the ears, loss of hearing, dizziness, nausea, vomiting and most importantly gastrointesti-

nal bleeding and gastrointestinal ulcerations including gastric perforation. The administration of acetylsalicylic acid in children with viral infections can, in rare cases, produce Reye's syndrome, involving liver damage, encephalopathy and a mortality rate exceeding 50%. Acute salicylate poisoning results in hyperventilation, marked sweating and irritability followed by respiratory paralysis, unconsciousness, hyperthermia and dehydration.

Derivatives of Acetic Acid

Indomethacin

Indomethacin is a strong inhibitor of both cyclooxygenase isoforms with a slight stronger effect in the case of COX-1. It is rapidly and almost completely absorbed from the gastrointestinal tract and has high plasma protein binding. The plasma half-life of indomethacin varies from 3 to 11 h due to intense enterohepatic cycling. Only about 15% of the substance is eliminated unchanged in the urine, the remainder being eliminated in urine and bile as inactive metabolites (O-demethylation, glucuronidation, N-deacylation). The daily oral dose of indomethacin is 50–150 mg (up to 200 mg).

Indomethacin treatment is associated with a high incidence (30%) of side effects typical for those seen with other NSAIDs (see above). Gastrointestinal side effects, in particular, are more frequently observed after indomethacin than after administration of other NSAIDs. The market share of indomethacin (approximately 5%) is therefore low as compared to that for other non-steroidal antirheumatic agents.

Diclofenac

Diclofenac is an exceedingly potent cyclooxygenase inhibitor slightly more efficacious against COX-2 than COX-1. Its absorption from the gastrointestinal tract varies according to the type of pharmaceutical formulation used. The oral bioavailability is only 30–80% due to a first-pass effect. Diclofenac is rapidly metabolised (hydroxylation and conjugation) and has a plasma half-life of 1.5 h. The metabolites are excreted renally and via the bile.

Epidemiological studies have demonstrated that diclofenac causes less serious gastrointestinal complications than indomethacin. However, a rise in plasma liver enzymes occurs more frequently with diclofenac than with other NSAIDs. The daily oral dose of diclofenac is 50–150 mg. Diclofenac is also available as eye-drops for the treatment of non-specific inflammation of the eye and for the local therapy of eye pain (see ► NSAIDs, pharmacokinetics).

Derivatives of Arylpropionic Acid

2-arylpropionic acid derivatives possess an asymmetrical carbon atom, giving rise to S- and R-enantiomers. The S-enantiomer inhibits cyclooxygenases 2–3 orders of magnitude more potently than the corresponding R-enantiomer. This finding has led to the marketing of pure S-enantiomers (e.g. S-ibuprofen and S-ketoprofen) in some countries in addition to the racemates where the R-enantiomer is considered as "ballast". However, it is not yet proven whether 2-arylpropionic acids are better tolerated when given as S-enantiomer than as the racemate. Naproxen, for example, which is clinically available only as the S-enantiomer, does not cause less serious gastrointestinal side effects than, e.g. ibuprofen racemate.

Ibuprofen is the most thoroughly researched 2-arylpropionic acid. It is a relatively weak, nonselective inhibitor of COX. In epidemiological studies, ibuprofen compared to all other conventional NSAIDs, has the lowest relative risk of causing severe gastrointestinal side effects. Because of this, ibuprofen is the most frequently used OTC ("over the counter", sale available without prescription) analgesic. Ibuprofen is highly bound to plasma proteins and has a relatively short elimination half-life (approximately 2 h). It is mainly glucuronidated to inactive metabolites that are eliminated via the kidney. The typical single oral dose of ibuprofen as an OTC analgesic is 200–400 mg and 400–800 mg when used in anti-rheumatic therapy. The corresponding maximum daily doses are 1200 or 2400 mg, respectively but the dose in anti-rheumatic therapy in some countries can be as high as 3200 mg daily.

Other arylpropionic acids include naproxen, ketoprofen and flurbiprofen. They share most of the properties of ibuprofen. The daily oral dose of ketoprofen is 50–150 mg, 150–200 mg for flurbiprofen and 250–1000 mg for naproxen. Whereas the plasma elimination half-life of ketoprofen and flurbiprofen are similar to that of ibuprofen (1.5–2.5 h and 2.4–4 h, respectively), naproxen is eliminated much more slowly with a half-life of 13–15 h (see ► NSAIDs, pharmacokinetics).

Oxicams

Oxicams e.g. piroxicam, tenoxicam, meloxicam and lornoxicam are non-selective inhibitors of cyclooxygenases. Like diclofenac, meloxicam inhibits COX-2 slightly more potently than COX-1. This property can be exploited clinically with doses up to 7.5 mg per day, but at higher doses COX-1 inhibition becomes clinically relevant. Since the dose of meloxicam commonly used is 15 mg daily, this agent cannot be regarded as a COX-2 selective NSAID and considerable caution needs to be exercised when making comparisons between the actions of meloxicam and those of other conventional NSAIDs. The average daily dose in anti-rheumatic therapy is 20 mg for pi-

roxicam and tenoxicam, 7.5–15 mg for meloxicam and 12–16 mg for lornoxicam. Some oxicams have long elimination half-lives (lornoxicam 3–5 h, meloxicam approximately 20 h, piroxicam approximately 40 h and tenoxicam approximately 70 h).

COX-2 Selective NSAIDs (COXIBs)

The development of the COXIBs has been based on the hypothesis that COX-1 is the physiological COX and COX-2 the pathophysiological isoenzyme. Inhibition of the pathophysiological COX-2 only is assumed to result in fewer side effects as compared to non-selective inhibition of both COX isoenzymes. Rofecoxib and celecoxib were the first substances approved that inhibit only COX-2 at therapeutic doses. Substances with higher COX-2-selectivity than rofecoxib and celecoxib have been recently approved or will shortly be approved (e.g. etoricoxib, parecoxib, lumiracoxib).

Unlike conventional NSAIDs, with the exception of lumiracoxib the "COXIBs" have no functional acidic group. The indications for these agents are in principle identical to those of the non-selective NSAIDs, although they have not yet received approval for the whole spectrum of indications of the conventional NSAIDs. Because they lack $COX\text{-}^{1}$-inhibiting properties, COX-2-selective inhibitors show fewer side effects than conventional NSAIDs. However, they are not free of side effects because COX-2 has physiological functions that are blocked by the COX-2-inhibitors. The most frequently observed side effects are infections of the upper respiratory tract, diarrhoea, dyspepsia, abdominal discomfort and headache. Peripheral oedema is as frequent as with conventional NSAIDs. The frequency of gastrointestinal complications is approximately half that observed with conventional NSAIDs. The precise side effect profile of the selective COX-2-inhibitors, however, will only be known after several years of clinical use (see ► NSAID induced lesions of the gastrointestinal tract).

On September 30, 2004, MSD voluntarily withdrew rofecoxib from the market because of a colon cancer prevention study (APPROVe) suggesting that rofecoxib nearly doubled the rate of myocardial infarction and strokes as compared to placebo. Celecoxib, a less potent selective COX-2-inhibitor with a shorter half life showed a similar dose related problem in another colon cancer prevention trial (APC), but did not show an increased risk in an Alzheimer prevention trial (ADAPT) or in another colon cancer study (PreSAP). The reasons for these discrepancies are a matter of scientific debate (Tegeder, Geisslinger 2006). Many scientists favour a group effect of all coxibs, because the balance between two fatty acids, prostacyclin and thromboxane A2 that control blood clotting and vasodilation may be disturbed after intake of selective COX-2-inhibitors. As a result of this possible imbalance, the risk for cardiovascular events may increase. However, there are also data suggesting that nonselective NSAIDs are also not safe with respect to thromboembolic events. In the aforementioned ADAPT trial, the nonselective NSAID naproxen significantly increased the risk of heart attacks and stroke (see ► NSAIDs, chemical structure and molecular mode of action; ► NSAIDs and their indications; ► NSAIDs, pharmacokinetics; ► NSAIDs, adverse effects; ► NSAIDs and cardio-vascular effects).

More than 20 years ago it was shown for the first time that chronic use of NSAIDs reduces the risk of colon cancer. The molecular mechanisms of the anticarcinogenic effects of NSAIDs are still not fully understood. Predominantly, these effects have been suggested to be due to their COX-inhibiting activity. This notion is based on the observation that COX-2 is overexpressed in more than 80% of colon carcinomas and other cancer types and that an enhanced production of prostaglandins plays a crucial role in cell proliferation and angiogenesis. However, several *in vitro* and *in vivo* results cannot be explained by an enhanced COX-2 expression and PG-synthesis indicating that COX-2 independent mechanisms must also be involved. Until now five selective COX-2 inhibitors have been developed and partly introduced into clinical practice. Of these, only celecoxib has been approved by the FDA for adjuvant treatment of patients with familial adenomatous polyposis.

It has been shown that the antiproliferative effects of celecoxib are at least in part mediated through induction of a cell-cycle block (in G_1-phase) and apoptosis. These effects occurred in COX-2 expressing as well as in COX-2 deficient colon carcinoma cells (see ► NSAIDs, COX-independent actions and ► NSAIDs and cancer).

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NOP Receptor

Definition

The term NOP or ORL1 receptor (N for ► Nociceptin or ► orphanin-FQ N/OFQ) represents the G-protein coupled receptor that is closely related to MOP, DOP and KOP receptors, but responds to the peptide N/OFQ rather than any of the classical opioid drugs or peptides. It is expressed in many areas of the nervous system and has effects that may be analgesic or hyperalgesic depending on the anatomical region. The NOP receptor protein is produced by a single gene. When activated, the NOP receptor predominantly transduces cellular actions via inhibitory G-proteins. The electrophysiological consequences of NOP receptor activation are usually inhibitory.

► Opioid Electrophysiology in PAG

Noradrenaline

Definition

Noradrenaline is a catecholamine that acts as a neurotransmitter both centrally and peripherally by binding to adrenergic receptors. It is also known as norepinephrine.

► Descending Circuitry, Transmitters and Receptors

Noradrenergic

Definition

Neurons containing norepinephrine.

► Stimulation-Produced Analgesia

Noradrenergic and Serotonergic Inhibitory Pathways

Definition

Noradrenaline (acting via α2 receptors) and Serotonin (acting mainly via 5-HT_1 receptors) both exert inhibitory effects on nociception in the dorsal horn.

► Postoperative Pain, Transition from Parenteral to Oral Drugs

NOS

► Nitric Oxide Synthase

Noxious Cold Receptor

► Nociceptors, Cold Thermotransduction

Noxious Stimulus

A noxious stimulus is one that is painful and potentially damaging to normal tissues. Stimuli that are painful can be thermal, mechanical or chemical.

► Acute Pain Mechanisms
► Arthritis Model, Kaolin-Carrageenan Induced Arthritis (Knee)
► Descending Modulation and Persistent Pain
► Functional Imaging of Cutaneous Pain
► Gynecological Pain, Neural Mechanisms
► Human Thalamic Response to Experimental Pain (Neuroimaging)
► Metabotropic Glutamate Receptors in Spinal Nociceptive Processing
► Nociceptive Processing in the Amygdala, Neurophysiology and Neuropharmacology
► Polymodal Nociceptors, Heat Transduction
► Postoperative Pain, Acute Pain Management, Principles
► Postoperative Pain, Acute Pain Team
► Postoperative Pain, Pre-Emptive or Preventive Analgesia
► Psychological Aspects of Pain in Women
► Referred Muscle Pain, Assessment
► Somatic Pain
► Thalamic Nuclei Involved in Pain, Cat and Rat

Noxious Stimulus Intensity

Definition

The physical magnitude of a potentially injurious stimulus that is being applied to the body. This could be the amount of energy deposited from a mechanical or thermal stimulus, or a concentration of chemicals.

► Encoding of Noxious Information in the Spinal Cord

Noxious Stimulus Location

Definition

The body region that is being affected by a potentially injurious stimulus.

► Encoding of Noxious Information in the Spinal Cord

NPY

► Neuropeptide Y

NRS

► Numerical Rating Scale

NRSF

► Neuron Restrictive Silencer Factor

NSAID-Induced Lesions of the Gastrointestinal Tract

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Definition

Definition of gastrointestinal complications due to application of non-steroidal antiinflammatory drugs (► NSAIDs): ersosions, ulcers, bleeding erosions or bleeding ulcers, ulcer perforations. These lesions are mostly located in the stomach, less common in the upper duodenum. However, lesions are possible along the entire gastrointestinal tract, i.e. small gut and colon.

Characteristics

Pathogenesis of NSAID Induced Peptic Ulcers

Inhibition of prostaglandin synthesis in the stomach by NSAIDs seems to be the key pathogenetic factor. Prostaglandins of the E-type stimulate gastric blood circulation, mucus secretion and cell regeneration and inhibit acid secretion. Furthermore, NSAIDs exert direct side effects at the gastric mucosa besides this systemic effect. NSAIDs are weak acids. Within an acidic environment NSAIDs are not dissociated. As lipophilic substances they may penetrate the mucus and exert direct damaging effects.

Gastrointestinal Side Effects Due to Therapy with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Treatment with NSAIDs may cause life-threatening complications in the gastrointestinal tract such as bleeding or perforation. In many cases no symptoms precede. In a study by Sing et al. including 1,921 patients, 81% had no preceding symptoms (Sing et al. 1996). Very often complications affect rather old patients with comorbidities. Thus, a rather high mortality may be a consequence. In patients on NSAIDs accessory risk factors increase the odds ratio of risk of peptic ulcer bleeding (Weil et al. 2000). In a case control study on 1,121 patients with bleeding ulcers, therapy with anticoagulants increased the risk by 7.8, history of peptic ulcer by 3.8, heart insufficiency by 5.9, oral glucocorticosteroids by 3.1 and smoking by 1.6. The odds ratio increased multiplicatively when in addition to these risk factors NSAIDs were administered (Weil et al. 2000).

Primary Prophylaxis, Therapy, Secondary Prophylaxis of NSAID Induced Gastrointestinal Lesions

In primary prophylaxis of NSAID induced gastrointestinal lesions, one has to discuss the roles of coxibs, ► Helicobacter pylori eradication and prophylactic therapy with either misoprostol, histamine-2-receptor-antagonists (H2-blockers) or proton pump inhibitors (PPIs). In acute treatment of bleeding ulcers, endoscopic therapy is one of the most important mainstays. For treatment of NSAID induced gastrointestinal lesions, one has to compare the effectiveness of H2-blockers with that of misoprostol or PPIs. In secondary prophylaxis, H2-blockers have again to be compared with misoprostol, PPIs and *H. pylori* eradication. 15–20% of ulcers rebleed after successful endoscopic therapy. In primary prevention of NSAID induced gastrointestinal lesions one has to discuss the role of coxibes, -antagonists, and proton pump inhibitors (PPIs).

Intravenous omeprazole reduced this risk of recurrent bleeding after successful endoscopic treatment of bleeding peptic ulcers (Lau et al. 2000).

240 patients were randomly assigned to either placebo or omeprazole (80 mg bolus intravenously followed by 8 mg / h for 72 h). Thereafter, both groups received omeprazole 20 mg orally for 4 weeks. In the PPI group, 8 patients rebled (6.7%) within 30 days *versus* 27 (22.5%) ($p< 0{,}001$) in the placebo group. 5 patients died in the PPI group (4.2%) as compared to 12 (10%) in the placebo group. This difference did not reach statistical significance ($p = 0.13$).

In primary prevention of diclofenac associated ulcers and dyspepsia, omeprazole was compared with triple therapy in *H. pylori* positive patients (Labenz et al. 2002). Patients had no history of ulcer disease. Patients were on continuous NSAID therapy (diclofenac 2×50 mg / day). They received either French triple therapy for *H. pylori* eradication (PPI plus clarithromycin plus amoxicillin) followed by placebo or omeprazole or omeprazole alone or placebo alone. Ulcer incidence after 5 weeks was 1.2% *vs* 1.2% *vs* 0% *vs* 5.8% respectively. Thus, persistence of *H. pylori* gastritis and no inhibition of acid secretion were associated with an increased risk of ulcer development due to diclofenac therapy. In another study from China, *H. pylori* eradication decreased the risk of ulcer development when NSAID naive patients received long term NSAID therapy (Chan et al. 2002a).

A key question in prophylaxis is not only prevention of gastric or duodenal ulcers in patients on NSAID therapy but also prevention of serious ulcer complications such as bleeding or perforation. For misoprostol it has been clearly demonstrated that this substance reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving nonsteroidal anti-inflammatory drugs (Silverstein et al. 1995). 8,843 patients on NSAIDs were randomly assigned to either misoprostol (4 × 200 µg / day) versus placebo. 25 out of 4,404 patients on misoprostol developed ulcer complications as compared to 42 out of 4,439 in the placebo group (risk reduction around 40%; odds ratio 0.598 (95% CI, 0.364–0.982; P <0.049). However, 20% of patients on misoprostol terminated the therapy due to side effects such as severe diarrhea, as compared to 15% on placebo.

Therapy of NSAID Induced Peptic Ulcers

H2-antagonists, misoprostol and PPIs have been shown to be effective in healing gastric and duodenal ulcers. Omeprazole has been compared with the prostaglandin analogue misoprostol for ulcers associated with nonsteroidal anti-inflammatory drugs (Hawkey et al. 1998). Omeprazole was superior to misoprostol with regard to the percentage of healed ulcers after 4 weeks. A concomitant *H. pylori* gastritis favors the healing rates with a PPI. This effect could be due to endogenous prostaglandin synthesis induced by the gastritis and / or to the gastric acid buffering by ammonia produced by *H. pylori*.

When omeprazole was compared with ranitidine, the PPI therapy was significantly superior to the H2-receptor antagonist therapy in healing NSAID induced gastric or duodenal ulcers (Yeomans et al. 1998).

Secondary Prophylaxis of Gastrointestinal Complications Due to NSAIDs

The most efficient prophylaxis is certainly termination of any NSAID administration. However, this is not possible in many cases due to the underlying diseases. Further options are *H. pylori* eradication since an underlying *H. pylori* gastritis may increase the risk of NSAID induced lesions. Other options are administration of inhibitors of acid secretion such as PPIs or H2-blockers, treatment with misoprostol or switching from non-selective NSAIDs to coxibs. The choice of the most efficient prophylaxis can be based on the results of several controlled prospective studies but the reduction of risk by a switch to coxibs and additional acid inhibition by PPIs has not been studied.

H. pylori Eradication versus PPI

400 patients with a history of a bleeding ulcer due to NSAIDs or aspirin and concomitant *H. pylori* gastritis were randomly assigned to either *H. pylori* eradication or acid inhibition by omeprazole (20 mg per day). NSAID therapy (250 patients, naproxen 2 × 500 mg per day) or aspirin (150 patients, aspirin 80 mg per day) was continued for 6 months (Chan et al. 2001). The bleeding probability within 6 months in the aspirin group was 0.9% when omeprazole was used and 1.9% after *H. pylori* eradication. The difference was not statistically significant. Thus, *H. pylori* eradication is as efficient as inhibition of acid secretion when low dose aspirin is used. However, in the naproxen group, 18.8% rebled when *H. pylori* was eradicated as compared to 4.4% when omeprazole was used as prophylaxis. Thus, *H. pylori* eradication does not protect against ulcer recurrences when NSAID therapy is continued. In another study, patients with *H. pylori* gastritis who developed an ulcer complication under long-term therapy with aspirin (<325 mg per day) were enrolled (Lai et al. 2002). *H. pylori* was eradicated and after healing of the ulcers aspirin (100 mg per day) was continued. The PPI lansoprazole (30 mg per day) was compared to placebo for up to 12 months or until the occurrence of the next complication. 9 out of 61 (14.8%) patients developed an ulcer complication in the placebo group *versus* 1 out of 62 (1.2%) in the PPI group. 4 out of 10 patients had a re-infection by *H. pylori*, 2 out of 10 used further NSAIDs. Thus, in countries with a rather high risk of *H. pylori* re-infection or in cases of uncontrolled concomitant NSAID use, acid inhibition by PPIs seems to be more effective than *H. pylori* eradication alone when aspirin is continued. In a recent study from Hong Kong, patients who experienced ulcer bleeding after aspirin were switched to either clopidogrel (75 mg per day) or aspirin (80 mg per day) together with the pure enantiomer of omeprazole esomeprazole (2 × 20 mg per day). Both groups were *H. pylori* negative. 13 out of 161 rebled in the clopidogrel group *versus* 1 out of 159 in the aspirin plus esomeprazole group. Thus, a switch to clopidogrel does not offer any protection in contrast to efficient acid inhibition by esomeprazole (Chan et al. 2005).

Misoprostol versus PPI

When omeprazole was compared with misoprostol for ulcers associated with nonsteroidal anti-inflammatory drugs, misoprostol was slightly less effective than omeprazole in healing gastric and duodenal ulcers. In secondary prevention, omeprazole was superior. Furthermore, patients given omeprazole experienced fewer side effects (Hawkey et al. 1998).

Selective Cyclooxygenase-2 (COX-2) Inhibitors, So-called Coxibs, Solution of the Problem?

Coxibs do not inhibit aggregation of platelets. The gastrointestinal bleeding risk seems to be decreased. However, these drugs may increase the risk of cardiovascular diseases such as myocardial infarction

and stroke. Prostaglandins of the E-type are gastroprotective. They stimulate gastrointestinal blood flow, cell regeneration and mucus secretion and are weak inhibitors of gastric acid secretion. Selective COX-2 inhibitors, so-called coxibs, are supposed mainly to inhibit prostaglandin synthesis in inflammation but not constitutive prostaglandin synthesis by cyclooxygenase-1. Thus, coxibs should offer less gastrointestinal toxicity. Celecoxib was compared with diclofenac in long-term management of rheumatoid arthritis (Emery et al. 1999). 655 patients with rheumatoid arthritis received either celecoxib (2 × 200 mg per day) or diclofenac (slow release 2 × 75 mg) for 24 weeks. Gastroduodenal ulcers developed in 4% in the celecoxib group and in 15% given diclofenac. The CLASS study evaluated gastrointestinal toxicity with celecoxib *versus* nonsteroidal anti-inflammatory drugs in patients suffering from osteoarthritis and rheumatoid arthritis. 4,573 patients received either celecoxib (2 × 400 mg per day) or ibuprofen (3 × 800 mg per day) or diclofenac (2 × 75 mg per day) for 6 months. Aspirin (<375 mg per day) was permitted. Ulcer complications were seen in the celecoxib group in 0.76% *vs* 1.45% in the NSAID group. The difference was not significant (p <0.09)). Symptomatic ulcers were fewer in the celecoxib group, 2.08% *versus* 3.54% (p <0.02). Less gastrointestinal toxicity was only seen in patients who did not take aspirin in addition (complicated ulcers, no aspirin 0.44% *versus* 1.27% (p <0.04); symptomatic ulcers, 1.40% *versus* 2.91% (p <0.02); with aspirin 2.01% *versus* 2.12% (p <0.92) and 4.70% *versus* 6.00% (p <0.49)) (Silverstein et al. 2000). The data after 1 year of treatment (not published but available *via* internet) are less impressive with regard to less gastrointestinal toxicity with celecoxib. Moreover, celecoxib was evaluated in a meta-analysis (Goldstein et al. 2000). 14 multi center studies on 11,008 patients who had been treated for 2–24 weeks and 5,155 patients on therapy for up to 2 years demonstrated ulcer complications such as bleeding, perforation and obstruction in the placebo group in none out of 1,864 patients, in the celecoxib group in 2 out of 6,376 and in the NSAID group in 9 out of 2,768 patients. Thus, the incidence of ulcer complications is reduced by a factor of 8 by celecoxib. Rofecoxib (25 mg and 50 mg per day) was compared with ibuprofen (3 × 800 mg per day) and placebo in 775 patients with osteoarthritis (Hawkey et al. 2000). The endpoint of this prospective study was gastric or duodenal ulcers. In the ibuprofen group, 29.2% of patients developed ulcers after 12 weeks and 46.8% after 24 weeks *versus* 5.3 and 8.8% in the low dose rofecoxib group and 9.9 and 12.4% in the 50 mg group. Thus, rofecoxib is clearly much safer as regards gastroduodenal ulcers than ibuprofen. However, not all endoscopically demonstrated ulcers may be clinically relevant.

In another study rofecoxib (50 mg per day) was compared with naproxen (2 × 500 mg per day) with special emphasis on ulcer complications such as bleeding, stenosis, perforation or symptomatic ulcers in patients with rheumatoid arthritis (Bombardier et al. 2000). 8,076 patients older than 50 years, or 40 years when on steroids in addition, were enrolled. In the rofecoxib group, 2.1 ulcers (0.6 life threatening) developed within 9 months in 100 patient years and 4.1 (1.4 threatening) with naproxen. However, myocardial infarction was observed in 0.4% *versus* 0.1 % with naproxen. When valdecoxib (2 × 40 mg per day), another coxib, was compared with naproxen (2 × 500 mg per day) and placebo in a multicenter study with 62 patients per group, gastroduodenal ulcerations were seen in 3% with placebo, 0% with valdecoxib and 18% with naproxen (Goldstein et al. 2003). Thus, the risk of developing gastroduodenal ulcerations is clearly lower during treatment with coxibs as compared to non-selective NSAIDs. However, a larger prospective trial is still warranted to clarify the key question as to whether the rate of life threatening ulcer complications is really markedly decreased when treatment is with coxibs *versus* non-selective NSAIDs. The risk reduction for gastroduodenal ulcers has to be compared with the risk elevation of cardiovascular side effects attributed to treatment with coxibs. This risk has led to the withdrawal of rofecoxib from the market. The elevated risk of cardiovascular side effects is possibly a class effect of coxibs and not solely seen under rofecoxib. A trial to reduce the incidence of new colon adenomas by celecoxib after endoscopic polypectomy had to be stopped. In this trial (*ca*. 2,000 patients), with placebo 6 cardiovascular events were seen *versus* 15 in the low dose celecoxib group (2 × 200 mg per day) and 20 in the high dose celecoxib group (2 × 400 mg per day). In another trial in which celecoxib was used to treat Alzheimer disease, no elevated cardiovascular risk was seen. In a population-based observation, 1,005 patients used COX-2 inhibitors and 5,245 patients used a non-naproxen NSAID. Of the 6,250 patients, 70% were female, 50% were African American and 30% were older than 50 years. Overall, 12% of the patients had at least 1 cardiovascular thrombotic event after treatment within the follow-up period. The propensity adjusted odds ratio showed no significant effect of COX-2 inhibitor use on this percentage of patients (odds ratio, 1.09; 95% confidence interval, 0.90–1.33). The authors conclude that coxibs do not increase cardiovascular risk over non-naproxen NSAIDs in a high-risk Medicaid population (Shaya et al. 2005).

Several questions are still not solved. NSAIDs may lead to lesions in the jejunum and ileum that cannot be prevented by the addition of proton pump inhibitors. In a small study on 21 chronic NSAID users and 20 controls, a small video capsule was used to look for lesions in the small intestine. Small-bowel injury was seen in 71% of

NSAID users compared with 10% of controls (P <001). The injury was mild (few or no erosions, absence of large erosions / ulcers) in 10 NSAID users compared with 2 controls. 5 NSAID users had major (>4 erosions or large ulcers) damage compared with none in the control group. The authors conclude that endoscopically evident small intestinal mucosal injury is very common among chronic NSAID users (Graham et al. 2005). However, the clinical significance and quantitative risk of lesions in the small intestine due to non-selective NSAIDs is still not known.

Co-treatment with aspirin to overcome the coxibst' possible cardiovascular risk does not solve the problem since the gastroprotective effect is lost (Laine et al. 2004). Low dose aspirin plus rofecoxib resulted in ulcers within 12 weeks in 16%, similar to aspirin plus ibuprofen. Furthermore, it is not clear whether the elevated cardiovascular risk with rofecoxib can be decreased by aspirin.

Addition of proton pump inhibitors to non-selective NSAIDs causes a similar risk reduction to that seen under treatment with coxibs. This has been demonstrated in a double-blinded randomized comparison of celecoxib *versus* omeprazole and diclofenac for secondary prevention of ulcer bleeding in chronic NSAID users (Chan et al. 2002b). Rebleeding occurred in the celecoxib group in 4.9% (7 / 144 patients) versus 6.4% (9 / 143 patients) in the omeprazole plus diclofenac group. The difference was not significant. Finally, one has to be aware that prostaglandins are needed for ulcer healing. Thus, coxibs may delay gastric ulcer healing when one switches from non selective NSAIDs to coxibs due to abdominal pain, not knowing whether an ulcer is present or not.

In summary, coxibs decrease the risk of gastroduodenal ulcers when compared with non-selective NSAIDs. However, this risk reduction has to be balanced against the possible elevated risk of severe cardiovascular side effects. Addition of proton pump inhibitors to NSAIDs causes a similar risk reduction to that of coxibs. However, lesions in the small intestine due to NSAIDs cannot be prevented by PPIs. The overall risk and clinical relevance of lesions in the small intestine has still to be clarified in larger trials.

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NSAIDs

- ► COX-2 Inhibitor
- ► Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)
- ► NSAIDs, Adverse Effects
- ► NSAIDs and Cancer
- ► NSAIDs and Cardio-Vascular Effects
- ► NSAIDs, COX-Independent Actions
- ► NSAIDs, Mode of Action
- ► NSAIDs, Survey
- ► Postoperative Pain, Non Steroidal Anti-Inflammatory Drugs

NSAIDs and Cancer

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Synonyms

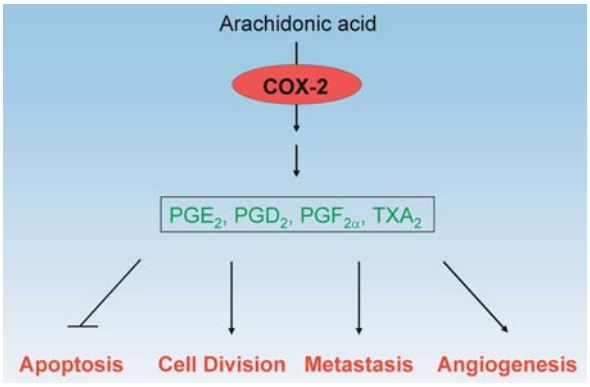
Cyclooxygenase Inhibitors; NSAIDs; non-steroidal anti-inflammatory drugs

Definition

NSAIDs are inhibitors of the ► Cyclooxygenases-1 (COX-1) and/or -2 (COX-2). While COX-1 is constitutively expressed in a wide range of tissues, COX-2 is predominantly cytokine and stress-inducible. Interestingly, in numerous cancer types the regulatory mechanisms of COX-2 expression are abrogated, leading to an overexpression of the COX-2 protein and enhanced prostaglandin production. Especially in the case of colon cancer, an upregulation of COX-2 has been shown to occur already in the early ► adenoma stage, and is therefore one of the first deregulation mechanisms in tumor development (Shiff and Rigas 1999). Enhanced COX-2 expression has been attributed a key role in the development of cancer by promoting cell division, inhibiting ► apoptosis, altering ► cell adhesion, enhancing ► metastasis and neo ► vascularization (Fig. 1) (Trifan and Hla 2003). Therefore, inhibition of COX-2 activity by NSAIDs thwarts all these effects, and accounts for the anticarcinogenic effect of these drugs.

Characteristics

Publications in recent years, investigating the molecular pathway of the anticarcinogenic effects of NSAIDs, revealed that next to COX-2 inhibition, COX-2-independent mechanisms are also responsible for the anti-neoplastic effects of these substances. The main arguments for this hypothesis are:

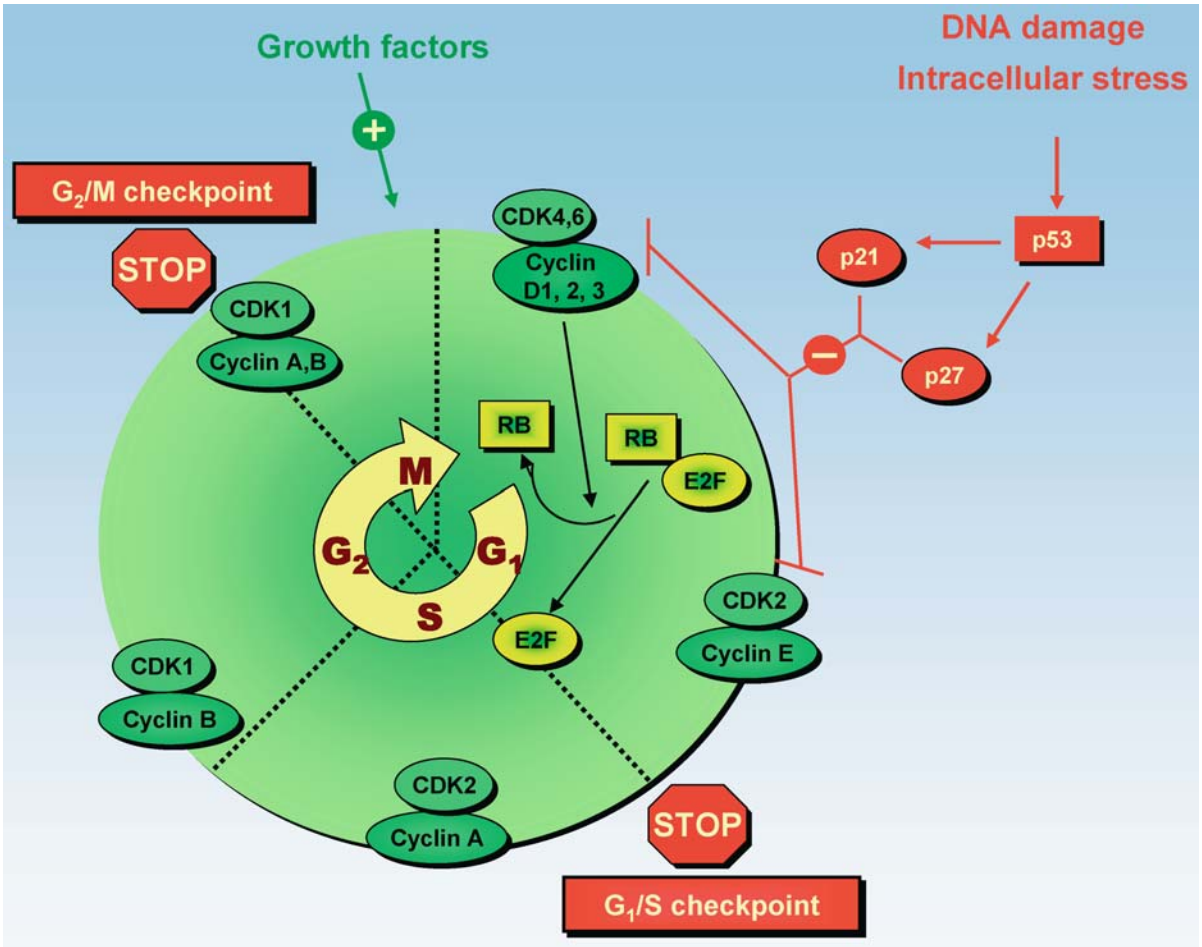


NSAIDs and Cancer, Figure 1 The COX-2 pathway. The cyclooxygenase-2 catalyses the conversion of arachidonic acid to prostaglandin H_2 (PGH_2) which is further converted by different prostaglandin synthases to PGE_2, PGD_2, PGF_2 α and TXA_2. These prostaglandins promote cell division, metastasis and angiogenesis and inhibit apoptosis leading to an enhanced tumor growth.

1. NSAIDs inhibit growth of tumor cells which do not express COX-2 *in vitro* and *in vivo* (Grösch et al. 2001)
2. NSAIDs which exhibit no COX-2 inhibitory efficacy nevertheless cause anticarcinogenic effects (Grösch et al. 2003)

In the literature, various molecular mechanisms were described to be involved in the anticarcinogenic effect of different NSAIDs, whereas each NSAID seems to have its specific COX-independent mode of action. An overview is given in a separate chapter of this issue (Tegeder and Niederberger) and also in Tegeder et al. 2001.

For cancer therapy we have to bear in mind that anticarcinogenic treatment regimes are usually carried out for a long time (months to years). Therefore, a reasonable benefit-risk ratio of the substances used is of vital importance. Unfortunately, long-term use of classical non-selective NSAIDs, which inhibit both COX-1 and COX-2, is associated with serious gastrointestinal side effects such as ► ulcerations of the gastric mucosa. These side effects are thought to arise from the inhibition of the constitutive cyclooxygenase isoform (COX-1), which is mainly responsible for the maintenance of the physiologically important prostaglandin synthesis in the gastrointestinal mucosa. To circumvent this problem, selective COX-2 inhibitors such as celecoxib, rofecoxib, etoricoxib, valdecoxib and lumiracoxib have been developed and introduced into clinical practice. However, due to the withdrawal of rofecoxib and valdecoxib from the pharmaceutical market in September 2004 and April 2005, respectively, the whole group of Coxibs is being reviewed regarding their potential risk to evoke cardiovascular side effects. Very recently, the European Medicines Agency (EMEA), as well as the Food and Drug Administration (FDA), scrutinized whether these side effects, which appeared with some



NSAIDs and Cancer, Figure 2 A simplified diagram of cell cycle control. The cell cycle is divided in four phases: the G_1-phase, S-phase, G_2-phase and M-phase. The cell cycle transition is controlled by different cyclins, cyclin-dependent kinases (CDKs) and cell cycle inhibitors such as p21 and p27.

Coxibs, should be regarded as a class effect. In February 2005 the EMEA and the FDA committee, evaluating the cardiovascular risk of Coxibs, arrived at the decision that despite the cardiovascular risk of these substances, Coxibs should not been withdrawn from the market, but should labeled with contraindications and warnings. Nevertheless, celecoxib is the only NSAID that was approved in December 1999 by the FDA for ► adjuvant treatment of patients with ► familial adenomatous polyposis and is, therefore, the only NSAID which is admitted for cancer therapy (Koehne and Dubois 2004). The anticarcinogenic properties of celecoxib are based on its ability to inhibit cell cycle progression and ► angiogenesis and to induce apoptosis. These effects are certainly on the one hand due to its COX-2-inhibiting potency according to the mechanism mentioned above, but on the other hand COX-2-independent mechanisms also seem to be responsible.

Inhibition of Cell Cycle Progression

The cell cycle transition is controlled by different regulatory proteins such as cyclins and cyclin-dependent kinases as well as by cell cycle inhibitors (Fig. 2). Treatment of different tumor cell lines with celecoxib caused downregulation of cyclins and cyclin-dependent kinases and an increase in the expression of various cell cycle inhibitors (Table 1), resulting in a cell cycle block in the G_1-phase. However, the precise target(s) responsible for these effects are still unknown.

Induction of Apoptosis

Apoptosis, or programmed cell death, can be induced either by the extrinsic pathway via activation of death receptors, or the intrinsic pathway via releasing of cytochrome c from the mitochondria (Green and Evan 2002). Both pathways finally lead to the activation of various ► caspases which, as the executors of apoptosis, cleave different cellular substrates and cause DNA-fragmentation (Fig. 3). After treatment of cancer cells with celecoxib, it has been shown that the expression of various anti-apoptotic proteins such as ► Bcl-2, ► Mcl-1 and ► survivin decreases, whereas the expression of the pro-apoptotic protein Bad is upregulated. This was further accompanied by a rapid

NSAIDs and Cancer, Table 1 Effect of celecoxib on proteins involved in apoptosis, cell cycle regulation and angiogenesis

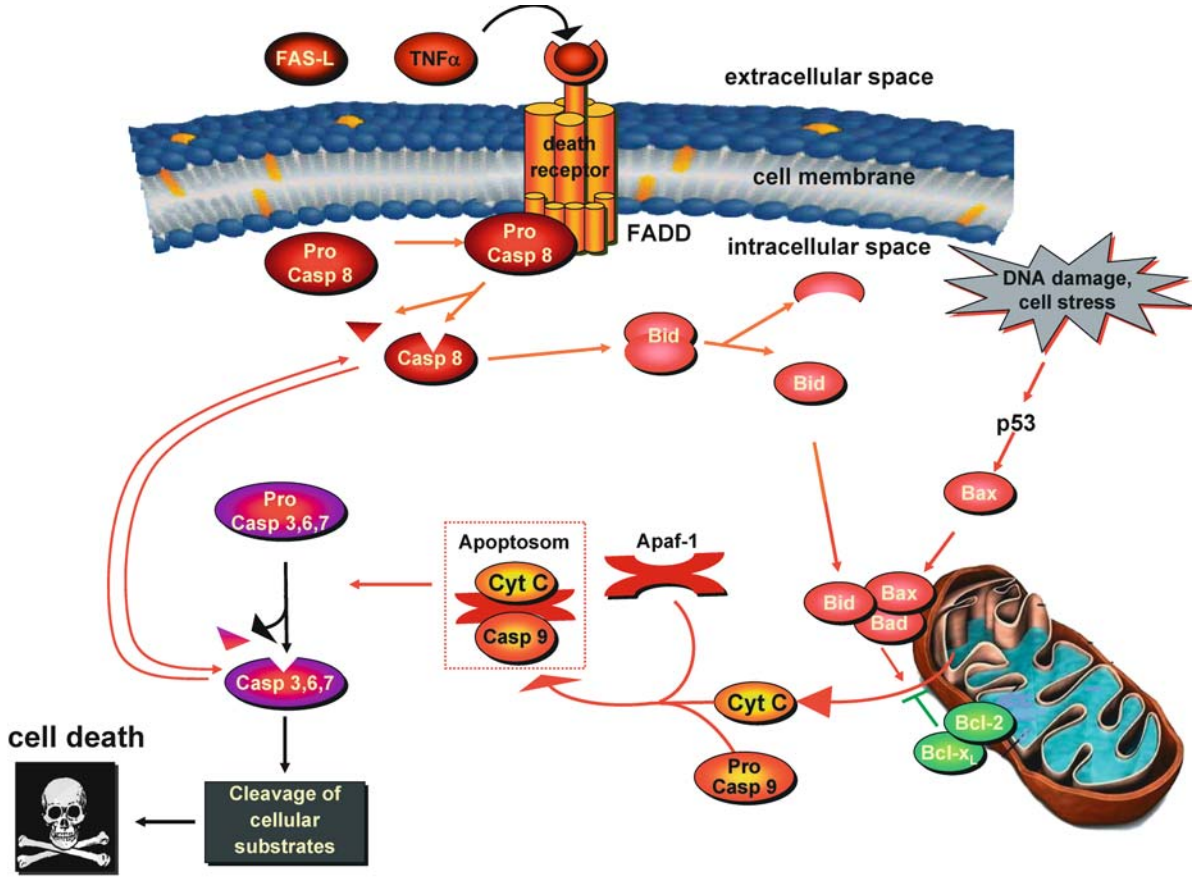
Apoptosis	Cell cycle	Angiogenesis/ Metastasis	Kinases	Transcription factors
Caspase 3 ↑	Cyclin D1 ↓	VEGF↓	PDK1 activity ↓	NF-κB ↓
Caspase 8 ↑	Cyclin A ↓	phospho EGFR ↓	AKT/PKB activity ↓	PPARγ ↑
Caspase 9 ↑	Cyclin B ↓	MMP-1 ↓	phospho SAPK ↓	SP-1 ↓
Bcl-2 ↓	p21 ↑	MMP-2 ↓		Egr-1 ↓
Bcl-xL ↓	p27 ↑	MMP-3 ↓		c-Fos ↓
Survivin ↓	Rb ↑	MMP-9 ↓		
Mcl-1 ↓	Rb ↑			
Bad ↑ Ceramide ↑	Ornithine decarboxylase ↓			
Apaf-1 ↑				

release of cytochrome c from mitochondria and subsequent activation of caspase-3, -8 and -9 (see also Table 1). These data provide evidence that after celecoxib treatment apoptosis is induced by activation of the intrinsic pathway. Currently, several cellular pathways have been identified which play a role for the apoptosis-inducing potency of celecoxib. It has been shown that celecoxib treatment leads to inhibition of the 3-phosphoinositide-dependent-kinase 1 (PDK1) or its downstream substrate protein kinase B (PKB/AKT). Due to the anti-apoptotic potency of these enzymes, inhibition of these kinases promotes apoptosis. Another recently identified target of celecoxib is the endoplasmatic reticulum Ca^{2+}-ATPase, which is strongly inhibited by celecoxib. As a consequence, Ca^{2+}-reuptake in the endoplasmatic reticulum is prevented, leading to an elevated cytoplasmatic Ca^{2+}-concentration. Ca^{2+} may then trigger the induction of apoptosis by activating Ca^{2+}-sensitive proteases, endonucleases and caspases, and by opening Ca^{2+}-sensitive mitochondrial permeability transition pores, resulting in cytochrome c release.

Furthermore, nanomolar concentrations of celecoxib and valdecoxib have been shown to inhibit members of the carbonic anhydrase (CA) family such as CA I, II, IV and IX. This effect is closely associated with the presence of an arylsulfonamide-group in both drugs, which seems to be responsible for this effect (Weber et al. 2004). CA II as well as CA IX have been described to play a pivotal role in tumor growth and development, and represent potential biomarkers for various tumor types. Carbonic anhydrases catalyze the reversible hydration of carbon dioxide, thereby buffering protons that protect the cell from undergoing intracellular acidification. Since the growth of tumor cells requires a high bicarbonate flux, inhibition of carbonic anhydrases by celecoxib could lead to an acidification, and subsequently to growth regression.

Inhibition of Angiogenesis

Early tumor growth is divided into several stages. In stage one the malignant cells form small tumors of limited size due to an inadequate supply of oxygen (► hypoxia). In stage two, hypoxia triggers a drastic change in gene expression leading to the formation of new blood vessels. Overexpression of COX-2 in tumor cells has a decisive impact on angiogenesis: a) The eicosanoid TXA_2 stimulates endothelial cell migration and b) Prostaglandin E_2 (PGE_2) causes a release of the vascular endothelial growth factor (VEGF), thereby promoting endothelial cell proliferation. Both mechanisms play a pivotal role in the formation of new blood vessels. Therefore, inhibition of COX-2-activity by celecoxib represses the effects mentioned above, and leads to inhibition of angiogenesis and diminished tumor growth (Gately and Li 2004). Nevertheless, COX-2-independent mechanisms also contribute to the anti-angiogenic effect of celecoxib. It has been shown that celecoxib inhibits the activation of the early growth response factor (Egr-1) (Table 1). Egr-1 is a ► transcription factor which is rapidly activated by multiple extracellular agonists (such as growth factors and cytokines) and environmental stresses (hypoxia, vascular injury). As a major activator of pro-angiogenic genes, such as fibroblast growth factor (FGF), cytokines and receptors, Egr-1 is essentially involved in angiogenesis and promotion of tumor development. Furthermore, it has been shown that inhibition of angiogenesis after celecoxib treatment is associated with suppression of VEGF expression due to the inhibition of the transcription factor Sp1. Celecoxib treatment reduced both Sp1 DNA binding activity and transactivating activity, which correlated with reduced Sp1 protein expression and its phosphorylation. The ► promoter region of VEGF contains a Sp^1-binding site that seems to be crucial for VEGF expression. Taken together, COX-2-independent mechanisms, such as the inhibition of



NSAIDs and Cancer, Figure 3 A simplified diagram of apoptosis. Apoptosis (programmed cell death) is a succession of controlled molecular events leading to the suicide of the cell. Apoptosis can be initiated from the cell surface membrane (extrinsic pathway) via activation of death-receptors and subsequent activation of caspase 8 (and others) or by intracellular stress (e.g. DNA-damage) leading to the release of cytochrome c from the mitochondria and activation of the apoptosom (intrinsic pathway). Both pathways finally lead to caspase-mediated cleavage of different cellular substrates (proteins, DNA) and subsequent cell death.

Sp1 and Erg-1 transcriptional activity, may explain the antiangiogenic effects of celecoxib.

Implications for Clinical Management

In light of the recent information on the cardiovascular risks associated with COX-2-selective inhibitors the view about the usage of these substances in clinical practice has changed dramatically. In the last few years a lot of studies were planned and performed to investigate the anticarcinogenic effects of rofecoxib or celecoxib in different tumor types. Owing to the withdrawal of rofecoxib and valdecoxib from the pharmaceutical market, the other Coxibs are also now under critical observation. In December 2004 the National Cancer Institute cancelled the "Adenoma Prevention with Celebrex" (APC) Study due to the occurrence of a statistically significant increase of cardiovascular side effects under celecoxib treatment. This discussion prompted Pfizer Inc. to delay the launch of Onsenal® (celecoxib) in the European Union (EU) for the treatment of intestinal polyps in FAP-patients. In contrast, two large ongoing trials with celecoxib, the "Prevention of Spontaneous Adenomatomous Polyps Trials" (PRESAP) and the „Alzheimer's Disease Anti-inflammatory Prevention Trial" (ADAPT), which were also evaluated by the Data Safety Monitoring Board (DSMB), revealed no increased risk of cardiovascular side effects for celecoxib. Therefore, these studies will contribute to appraise the potential cardiovascular risk of celecoxib, as well as its benefit for cancer and Alzheimer patients. Despite the current discussion, we have to consider that patients that have an increased risk of developing cancer probably benefit more from the chemopreventive potency of celecoxib than they have the risk to develop cardiovascular side effects. For cancer patients, the combination of celecoxib with standard chemo- or radiotherapy might also be advantageous, due to the possibility of reducing the dose of classical chemotherapeutics and drug-associated side effects.

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NSAIDs and Cardio-Vascular Effects

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Synonym

NSAIDS; Non-steroidal anti-inflammatory drugs; Cyclooxygenase Inhibitors

Definition

Non-steroidal anti-inflammatory drugs (► NSAIDs), alternatively called cyclooxygenase inhibitors, are the most widely used drugs worldwide. They comprise the mainstay of the management of pain associated with inflammation, as in e.g. rheumatoid diseases (Johnson 1997; Brune and Hinz 2004). NSAIDs exert their pharmacological actions through the inhibition of the enzyme cyclooxygenase, which exists as 2 isoforms, Cox1 and Cox2 (Hawkey 1999; Flower 2003). Cox1 is constitutively expressed in all tissues in the body, including platelets (Simmons et al. 2004). Cox2 is, as we now know, expressed at low levels in most normal tissues, but is induced rapidly if they are subjected to stress including trauma and inflammation (Simmons et al. 2004). The vascular system is a dominant source of vasoactive mediators that modulate and regulate the vascular tone as well as kidney and heart function (Francois and Coffman 2000; Simmons et al. 2004; Hennan et al. 2001). These regulatory mediators include prostacyclin (PGI_2), prostaglandins (PGE_2, $PGF_{2\alpha}$) and thromboxanes (TXA_2) – to name just a few.

Characteristics

Whilst thromboxane is almost exclusively produced in platelets by cyclooxygenase–1, prostacyclin is also a product of constitutively expressed Cox2 of the vascular endothelium (McAdam et al. 1999; FitzGerald 2002; Cipollone et al. 2004). Similarly, the adult kidney demonstrates a relatively high level of constitutively expressed Cox2 (Harris et al. 2004). From the theoretical point of view, non-selective and selective inhibition of Cox1 and –2, or Cox2 exclusively, is likely to affect the function of the cardio-vascular system. It is conceivable that selective, in contrast to non-selective, inhibitors, the latter interfering with the balance of e.g. the prothrombotic TXA_2 and antithrombotic PGI_2, may show different clinical effects (FitzGerald 2002). Since evidence-based consensus on the role of NSAIDs on the cardio-vascular system has not been reached, we shall concentrate on compiling the theoretical, experimental and clinical evidence of cardio-vascular effects of NSAIDs.

Theoretical Considerations

The cardio-vascular system is exposed to the prostaglandins PGI_2, E_2 and $F_{2\alpha}$, in addition to the thromboxane A_2 (TX) and the lipoxygenase products leucotrien (LT) B_4, D_4 and E_4 (the situation concerning endocannabinoids and lipoxins is as yet not clear enough for further discussion). Whilst thromboxane initiates vasoconstriction, platelet aggregation and possibly bradycardia (Wacker et al. 2002), prostaglandin E_2 and I_2 cause vasodilatation. Prostacyclin also inhibits platelet aggregation. $PGF_{2\alpha}$ is less important for the cardio-vascular system. Moreover, PGE_2 and $PGF_{2\alpha}$ may interfere with the release of norepinephrine from the sympathetic nervous system (Malic and Sehic 1990). Conversely, Cox2 expression in the macula densa is regulated by angiotensin 2, and Cox2 derived PGs reduce renin release from the macula densa (Harris and Breyer 2001). PGI_2 finally interferes with the activation of angotensin 2 and aldosterone (Harris et al. 2004). The situation becomes more complex due to the fact that there are different receptors for the eicosonoids, which may show a differential distribution in the cardio-vascular system and kidney. Nevertheless, it is obvious that the general inhibition of all prostaglandins, including TXA_2 and PGI_2, may have other consequences than the selective inhibition of the production of PGI_2 in the endothelium alone. Moreover, selective inhibition may shunt more substrate (arachidonic acid) into alternative pathways, i.e. Cox1 and 5–lipoxygenase.

In conclusion, from the theoretical point of view, the normal physiology of the cardiovascular system of the human body will be influenced by both selective and non-selective inhibitors. Selective inhibition, however, may have a different profile than non-selective inhi-

bition. For example, blood coagulation will remain unimpaired whilst blood pressure is influenced in a similar manner by selective inhibitors. On the other hand, the propensity for cardiac infarction or ischemic stroke may be furthered by selective inhibition (see below).

Experimental Pharmacology

A large number of experimental pharmacological data are available supporting the view that both selective and non-selective Cox–inhibitors may interfere with the cardio-vascular system (for review Simmons et al. 2004). Experiments in mice lacking either Cox1 or Cox2, as well as those lacking the corresponding receptors, demonstrate that:

Cox2 is necessary for the closure of the ductus arteriosus Botalli (Loftin et al. 2001)

Cox2 is needed for renal adaptation and protection from hyperosmolarity (Harris and Breyer 2001)

PGI_2 via IR receptors leads to vasodilatation, but also attenuation of ischemic injury and cardiac hypertrophy due to pressure overload (Hara et al. 2003)

PGE_2 via EP_3 and EP_4 receptors mediates cardioprotection during ischemic injury (Hara et al. 2003). It is also involved in thromboembolic events via (EP_2) receptors. EP_2 receptors also appear to mediate blood pressure control under high salt intake (Ushicubi et al. 2000)

PGI_2 and PGE_2 in the kidney tubules enhance renal blood flow and increase water and electrolyte excretion. (Cox2 appears more important than Cox1; Komhoff et al. 1997; Harris and Breyer, 2001). The latter effect may be sex-hormone regulated, and mediated via EP_1 receptors in males (Ushikubi et al. 2000).

Thromboxane activates platelet aggregation via TP receptors (Narumiya et al. 1999). In platelets TXA_2 production is almost exclusively Cox1 dependent (Baignent and Patrono, 2003).

TXA_2 appears to initiate bradycardia and reduction of blood pressure via a vagal reflex (Wacker et al. 2002).

Cox1 dependent prostaglandins support the development of atherosclerosis (Pratico et al. 2001).

These complex synergistic or antagonistic effects of prostanoids in the cardio-vascular system allow for the assumption that lack of inhibition of Cox1 along with blockade of Cox2 may reduce bleeding, but may have complex effects which support the development of hypertension, renal failure, thrombosis, myocardial infarction and ischemic stroke (FitzGerald 2002).

Clinical Pharmacology

Many occasional observations as well as clinical studies, support the view that selective and non-selective inhibition of cyclooxygenases in man may result in serious consequences in patients at specific risk. Three effects appear well documented and should be taken into consideration when administrating NSAIDs (non-selective or selective) to patients:

Non-Selective/Classical NSAIDs

It is widely accepted that all non-selective inhibitors of both cyclooxygenases may increase systolic and diastolic blood pressure. Long-term therapy with these compounds, particularly when given at high doses (2.4 g ibuprofen / day) for prolonged periods of time (in particular NSAIDs with long elimination half-life, e.g. piroxicam), may enhance the risk of stroke, but also cardiac infarction (Johnson 1997; Johnson et al. 2003). These drugs may also interfere with the activity of the blood pressure lowering diuretics, ACE inhibitors and AT-1 receptor blockers (Cipollone et al. 2004).

Aspirin, due to its permanent acetylation of Cox1 in blood platelets, interferes with blood coagulation for days, thus enhancing the risk of bleeds following surgical interventions (Merritt et al. 2004). Reversible inhibitors of platelet Cox1, such as ibuprofen, flurbiprofen and naproxen, may exert similar effects, however, for shorter periods of time.

Weak, non-selective or indirect inhibitors, such as acetaminophen, phenazone and propyphenazone are devoid of these bleeding enhancing activities. The literature, however, is scarce (see below).

Selective Inhibitors

These drugs, including celecoxib, etoricoxib, rofecoxib, valdecoxib and lumiracoxib do not interfere with blood coagulation. They can be used safely (with respect to bleeding) in the perioperative time period (Hegi et al. 2004). Recent observations suggest that compounds with slow elimination may be safely and advantageously given before surgical intervention in order to decrease the postinterventional need for opioids (Reuben and Connelly 2004). Contraindications, however, have to be obeyed.

Selective Cox2 inhibitors appear not to differ from non-selective cyclooxygenase blockers with respect to impairment of renal function. Many clinical investigations have equivocally shown that they do increase blood pressure (systolic and diastolic) as non-selective inhibitors (Schwartz et al. 2002), lead to fluid and water retention, and interfere with the activity of diuretics, ACE inhibitors and AT1-receptor antagonists (Brater 2002). These effects appear to be limited to the time of presence of the compounds in the blood and kidney (compare e.g. White et al. 2002; Fig. 3).

Recent evidence indicates that beyond effects on blood pressure and blood coagulation, selective Cox2 inhibitors may enhance the risk of cardiac infarctions, venous thromboses and ischemic stroke in patients at risk. A first report indicated that patients suffering from connective tissue diseases may be particularly prone to venous thrombosis following the administration of Cox2 inhibitors, as, for example, celecoxib (Crofford et al. 2000). The VIGOR study demonstrated a four times increase of cardiac infarctions in the group of patients receiving rofecoxib (rheumatoid arthritis)

(Bombardier et al. 2000). Valdecoxib, given to patients undergoing coronary bypass surgery, was associated with an increased frequency of cerebro-vascular and renal complications (Ott et al. 2003). Patients receiving high doses of rofecoxib appear to die from sudden cardiac death more frequently than those that have not been exposed to cyclooxygenase inhibition (Graham et al. 2004). Similarly, a recent outcome study signalled more cardiac infarctions during long-term treatment with a Cox2 inhibitor (lumiracoxib), as compared to the naproxen treated control group (Farkouh et al. 2004). Nested case control studies (e.g. Hippisley-Cox et al, BMJ 2005) indicate, however, that a similar increase in risk of CV-events is seen with widely used non-selective inhibitors such as diclofenac or ibuprofen.

Conclusion

Theoretical, clinical and experimental evidence shows that effects of non-steroidal, anti-inflammatory drugs on the cardio-vascular system are of major clinical importance. The impact of these drugs on blood coagulation is well defined. Moreover, selective and non-selective NSAIDs increase blood pressure and lead to water and fluid retention, at least in some patients. The importance of the effect of selective Cox2 inhibitors on thrombosis, cardiac infarction and stroke is not completely clear. Nevertheless, these effective drugs should be used in patients at risk with caution and at low doses, for short periods of time.

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NSAIDs and Coxibs

► Coxibs and Novel Compounds, Chemistry

NSAIDs and their Indications

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Synonyms

Non-Steroidal Anti-Inflammatory Drugs and their indications; Antipyretic Analgesics; Aspirin-Like Drugs; simple analgesics; COX–2 selective inhibitors

Definitions

The term Non-Steroidal Anti-Inflammatory Drugs, NSAIDs, refers to a group of drugs whose major therapeutic activities are the suppression of pain (analgesia), reduced body temperature in fever (antipyresis) and decreased signs of inflammation (anti-inflammatory activity).

Characteristics

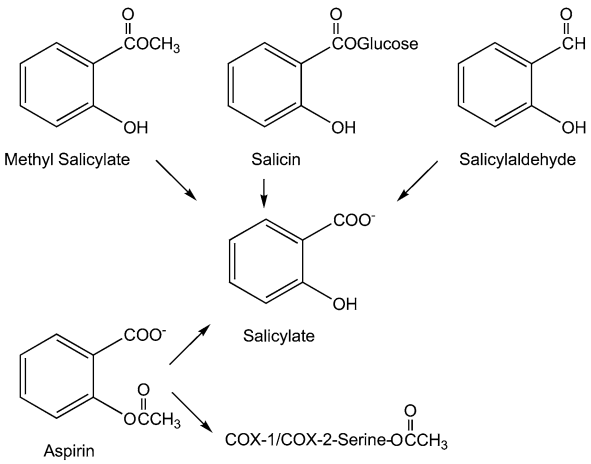
The NSAIDs can be separated into two major groups:

- non-selective NSAIDs, such as aspirin, ibuprofen and indomethacin, which also produce gastrointestinal damage, inhibit the aggregation of platelets, decrease kidney function in some patients and precipitate aspirin-induced asthma. The activity of these drugs is due to inhibition of two central enzymes involved in the synthesis of ► prostaglandins and related compounds. These central enzymes are cyclooxygenase–1 (COX–1) and COX–2.
- COX–2 selective inhibitors (coxibs or COX–1 sparing agents, CSIs) such as celecoxib, which have similar activities to the non-selective NSAIDs but have improved gastrointestinal tolerance, little or no effect on platelets and, from present studies, no tendency to produce asthma. The COX-2 selective inhibitors tend to increase the incidence of heart attack and stroke although the incidence of these reactions is low and may not be significant at analgesic doses of all the COX-2 selective inhibitors.

In addition, there is the unique drug, paracetamol (acetaminophen), which has similar activities to the COX–2 selective inhibitors but has weaker anti-inflammatory activity.

History

Three well-known plant compounds, salicin, salicylaldehyde and methyl salicylate, are active analgesics, antipyretics and anti-inflammatory agents. All three owe their pharmacological activity to their metabolism to salicylate (Fig. 1). Salicin is the most well known because it is present in the bark of the willow tree, and in several other plants which were used in the treatment of pain and fever. The modern use of willow bark started in 1763, although it had been used in earlier times. Methyl salicylate is still widely used in liniments while salicylaldehyde has little modern use although, like salicin, it is still used in herbal preparations.
In the nineteenth century, salicin was superseded by synthetic salicylic acid and its salt, sodium salicylate (Fig. 1). In turn, the purely synthetic compound, aspirin, largely replaced salicylic acid and its salts. However, sodium salicylate continued to be used for many years in the treatment of rheumatic fever and, until recently, sodium and other salicylate salts were used as anti-inflammatory drugs for ► rheumatoid arthri-



NSAIDs and their Indications, Figure 1 Structures of the naturally occurring salicylate derivatives and the synthetic drug, aspirin. All compounds are metabolized to the pharmacologically active salicylate (the ionised form of salicylic acid). The effect of aspirin is due to the metabolite, salicylate, and also to the reaction in which a serine at the active site of both COX–1 and COX–2 is acetylated. The effect of aspirin is prolonged because of this covalent binding to COX–1 and COX–2, and also because the half life of salicylate is longer than that of aspirin.

tis. Salicylate is still of interest because it is an active metabolite of aspirin.

Mechanism of Action

The older, non-selective NSAIDs inhibit both COX–1 and COX–2 and, therefore, decrease the synthesis of all prostaglandins and related compounds, such as ► Thromboxane A2 and ► prostacyclin. The prostaglandins and related compounds are factors which can be synthesized widely throughout the body and produce a variety of physiological effects. For example, they potentiate the actions of painful mediators, such as bradykinin. Thus, by inhibiting the synthesis of prostaglandins, the non-selective NSAIDs are analgesic. Inhibition of the synthesis of prostaglandin also explains the antipyretic and anti-inflammatory actions of NSAIDs, as well as their common adverse effects (see below).

The discovery of two COX isoenzymes led to the development of the COX–2 selective inhibitors which retain the analgesic, antipyretic and anti-inflammatory activities, but have a much reduced risk of gastrointestinal toxicity, and do not inhibit platelet function or precipitate aspirin-induced asthma.

Both salicylate and paracetamol are poor inhibitors of COX–1 and COX–2 in broken cell preparations, but both drugs inhibit the production of prostaglandins by intact cells when the levels of the precursor, arachidonic acid, are low (Graham and Scott 2003). Consequently, it now appears that salicylate and paracetamol both produce their pharmacological effects by inhibition of prostaglandin synthesis. The pharmacological activities of paracetamol and salicylate are generally similar to those of the COX–2 selective inhibitors, but the actions of paracetamol and salicylate at a molecular level are not known.

The activity of aspirin is due, in part, to its metabolism to salicylate, but aspirin also inhibits both COX–1 and COX–2. In this regard, the acetyl group of aspirin is transferred to COX–1 and COX–2, the result being irreversible inhibition of both enzymes (Fig. 1). Although aspirin is rapidly hydrolyzed to salicylate *in vivo*, the irreversible inhibition of COX–1 leads to prolonged inhibition of platelet aggregation, an initial step in the coagulation of blood.

Clinical Uses of the NSAIDs

Pain

The NSAIDs are indicated for a wide variety of painful states affecting all organ systems and all ages of patients, and are particularly indicated when inflammation is a significant contributor to the painful state. Alone, they are not useful for severe, acute pain, for example pain of bone fractures, surgical procedures or myocardial infarction. In these cases, the opioids, such as morphine, are more effective analgesics. The opioids are very useful for the treatment of the pain of cancer, although they may be used with the NSAIDs for this indication. Although very effective analgesics, the opioids depress the function of the central nervous system and have well known addictive properties, actions which are not shown by the NSAIDs.

Musculoskeletal pain is the major indication for NSAIDs. Surveys have shown that 15–20% of individuals over the age of 65 years take NSAIDs regularly, and this is largely for musculoskeletal pain. ► Osteoarthritis has a prevalence of about 10% in western populations, and afflicts elderly people. Although osteoarthritis is not primarily an inflammatory disorder, NSAIDs are moderately effective at relieving pain and the muscle stiffness associated with osteoarthritis.

Paracetamol is a widely used analgesic and antipyretic drug, but without significant anti-inflammatory actions in usual doses in rheumatoid arthritis, although it does reduce swelling after oral surgery. Although the NSAIDs are, on average, somewhat more efficacious than paracetamol (Pincus et al. 2004), paracetamol is still recommended as first line treatment for osteoarthritis, not only because it is effective, but also because it is better tolerated than the NSAIDs. Inflammation is a relatively minor component of the pathophysiology of osteoarthritis, and this might explain the small advantage that NSAIDs demonstrate compared to paracetamol. Although little work has been done to evaluate the utility of combining NSAIDs with paracetamol, this practice has no obvious disadvantages and may control pain better than either drug alone. NSAIDs are also indicated for spinal pain, particularly lumbar and cervical mechanical origin pain, prevalent in middle to old age. So called ‘soft tissue’ rheumatic problems are common and include muscle strains and aches, tennis elbow, and many others. NSAIDs can be helpful if paracetamol is insufficient, along with physical and other non-pharmacological therapies. There is some controversy about whether continuous therapy with NSAIDs slow or increase the loss of cartilage from weight bearing joints in patients with osteoarthritis (Rashad et al. 1989). This issue remains to be resolved.

Good pain relief has been achieved with NSAIDs in conditions of painful, often obstructed contraction, of smooth muscle. Examples are renal and biliary colic. This is because the smooth muscle contractions are dependent on prostaglandin synthesis. Again, NSAIDs outperform the opioids which have been traditionally used for these indications.

In a similar way, the pain associated with menstruation is dependent on prostaglandin synthesis and is therefore amenable to treatment with the non-selective NSAIDs. The COX–2 selective drugs relieve the pain of this condition and, because they have no significant anti-platelet effect, they do not increase menstruation related bleeding (Daniels et al. 2002).

A major, relatively new indication for the NSAIDs is perioperative pain. This has evolved with the availability of

the COX–2 selective inhibitors. In contrast to NSAIDs, including aspirin, the COX–2 selective inhibitors do not inhibit platelet aggregation so that blood clots normally. This property is particularly useful with the major shift to 'day only' surgery (Buvanendran et al. 2003). Renal function may, however, be decreased by NSAIDs after surgery (see below), and they should be used carefully in this situation.

Inflammation

Inflammatory rheumatic disorders exemplified by rheumatoid arthritis (RA) are an important indication for NSAIDs. Pain and stiffness especially in the morning are characteristic and debilitating. These symptoms are relieved by the non-selective NSAIDs or the COX–2 selective drugs, but there is no clear effect of either group on the progression of the condition to joint damage and loss of function. However, the NSAIDs have much to offer symptomatically to patients with rheumatoid arthritis. Consequently, the NSAIDs are very commonly used with the ► disease-modifying anti-rheumatic drugs (DMARDs). These are drugs which slow the progression of rheumatic arthritis in many patients. NSAIDs also have well defined roles in many other inflammatory, painful, arthritic states, including acute gout, ► ankylosing spondylitis and the arthritis often associated with psoriasis.

Migraine

The non-selective NSAIDs and the COX–2 selective inhibitors are good analgesics and, consequently, have an important place in the treatment of migraine (Goadsby et al. 2002). Their use soon after onset of an acute attack of migraine is most effective. Combination with an antiemetic is often required in order to suppress vomiting.

Other Clinical Uses

The non-selective NSAIDs have antithrombotic effects due to their inhibition of the formation of thromboxane A_2, a prostaglandin-like compound which leads to the aggregation of platelets and, therefore, the initiation of clotting of blood. Thromboxane A_2 is synthesized by a COX–1 dependent pathway in platelets and, therefore, all the non-selective NSAIDs have antithrombotic activity. Aspirin is, however, the preferred anti-platelet agent because of its long duration of action. As thromboxane A_2 is synthesized by a COX–^{1}-dependent pathway, the COX–2 selective inhibitors do not have significant antithrombotic activity. This lack of antithrombotic activity may, however, be a cause for the major adverse reactions of these new NSAIDs, as is outlined below.

Adverse Effects

Gastrointestinal

Prostaglandins are cytoprotective in the stomach and small intestine. Thus, they are important in developing mechanisms which protect the gastrointestinal tract from damage from the digestive enzymes and, in the stomach and duodenum, from the acidic contents. The non-selective NSAIDs commonly cause a variety of serious adverse reactions in the gastrointestinal tract, most importantly, perforations, ulceration and bleeding. Serious cases of gastrointestinal damage affect nearly 1% of chronic users of the older NSAIDs per year. Older, sicker patients, particularly those with previous peptic ulceration are most at risk.

Gastrointestinal tolerance is improved with ► enteric coating, co-prescription of antacids, ingestion with food, or rectal or parental routes of administration, but the risk of serious upper gastrointestinal bleeding remains. Another approach is to use the non-selective NSAIDs with prostaglandin analogues which are cytoprotective, or with drugs which decrease acid secretion by the stomach. A further approach is to use the COX–2 selective inhibitors or paracetamol. The COX–2 selective inhibitors were developed in order to decrease the gastrointestinal toxicity of the NSAIDs. This was successful and the use of the selective inhibitors, in preference to the non-selective NSAIDs, reduces the incidence of serious gastrointestinal damage (Silverstein et al. 2000).

A particular problem arises for patients who require long-term dosage with both low dose aspirin as an antithrombotic and a NSAID. It now appears that low doses of aspirin decrease the gastrointestinal sparing effects of the COX–2 selective inhibitors (Schmidt et al. 2004). Consequently, patients who require both an analgesic and low-dose aspirin are now often prescribed a non-selective NSAID and a cytoprotective drug, as well as aspirin (Barraclough et al. 2002).

Thrombosis

As discussed above, thromboxane A_2 is synthesized by a COX–1 dependent pathway in platelets, and therefore not affected by the COX–2 selective inhibitors. However, the selective COX–2 inhibitors may block the synthesis of prostacyclin, a vasodilator and antiplatelet factor which is largely synthesized through COX–2. Thus, there has been considerable concern that the selective COX–2 inhibitors may increase the incidence of ► thrombosis, causing myocardial infarction, for example. This concern has been confirmed by the withdrawal of one COX–2 selective inhibitor, rofecoxib, because of the increased occurrence of myocardial infarction during long term therapy (FitzGerald 2004). On the other hand, blood clotting often develops at atherosclerotic plaques in arteries. The development of atherosclerosis or reactions following thrombosis in the heart, in part, may be inflammatory processes, and the COX–2 selective inhibitors may usefully reduce this inflammation. Much research is presently directed at examining this possibility.

Renal Impairment and Hypertension

The NSAIDs and the COX–2 selective drugs both may precipitate renal failure. Risk factors include: age over 60, pre-existing renal impairment, dehydration, cirrhosis, congestive cardiac failure, salt restricted diets, or concomitant treatment with diuretics or inhibitors of angiotensin formation or action (Barraclough et al. 2002). The renal function of patients in these situations is considered to be more dependent on the function of prostaglandins than normal subjects and, therefore, inhibition of prostaglandin synthesis may produce marked depressant effects on renal function. Blood pressure may rise, in some cases quite substantially, during treatment with either the non-selective NSAIDs or the COX–2 selective agents (Barraclough et al. 2002; Whelton et al 2002). Consequently, it is now recommended that blood pressure should be monitored if dosage with the non-selective NSAIDs or the COX–2 selective drugs is commenced in patients taking antihypertensives, and the dosage of antihypertensives increased if blood pressure rises.

Asthma

Asthma is precipitated in up to 20% of asthmatics by aspirin and other non-selective NSAIDs. This reaction is produced by inhibition of COX–1 because asthma is not induced by the COX–2 selective inhibitors (West and Fernandez 2003). Paracetamol is also safer in aspirin-sensitive asthmatics, but does produce mild asthma in occasional patients (Jenkins 2000).

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NSAIDs, Adverse Effects

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Synonyms

NSAIDs; non-steroidal anti-inflammatory drugs; Non-Steroidal Anti-Rheumatic Drugs; cyclooxygenase inhibitors; NSAIDs, Side Effects

Definition

NSAIDs constitute a large group of chemically diverse substances that inhibit ► Cyclooxygenases activity and thereby prostaglandin synthesis. Traditional NSAIDs inhibit both COX-isoenzymes (COX-1 and COX-2). Novel NSAIDs ("coxibs") inhibit only COX-2. NSAIDs are mainly used as analgesics.

Characteristics

Overview

► Adverse effects of NSAIDs arise from the fact that it is impossible to inhibit exclusively the synthesis of ► prostaglandins that cause pain and inflammation. The inhibition of cyclooxygenases will always also affect the synthesis of prostaglandins and ► thromboxanes that are needed for physiological processes. In addition, COX inhibition may shift arachidonic acid metabolism to ► leukotriene synthesis because of the excess supply of substrate. This rule particularly applies to traditional NSAIDs that inhibit both COX-isoenzymes, but also holds true for COX-2 specific inhibitors.

COX-1 and COX-2 perform different tasks; this is allowed for by a different localization and regulation. COX-1 is expressed in all tissues and mainly produces prostaglandins and thromboxanes that are needed for the maintenance of physiological functions. COX-2 is not expressed in most healthy tissue but is upregulated after stimulation, which may be any kind of tissue damage. Hence, exclusively targeting COX-2 will not affect COX-1 derived physiological prostaglandins, and will therefore avoid many adverse effects that are typical for traditional NSAIDs. However, COX-2 is also constitutively expressed (see ► Constitutive Gene) in

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some tissues, so that COX-2 selective NSAIDs are not able to spare physiological prostaglandin production completely. In addition to common adverse effects, some substance specific side effects may occur, so the individual tolerability of NSAIDs may vary among patients.

Gastrointestinal Toxicity

Physiological prostaglandin E2 (PGE2) and prostacyclin (PGI2) in the stomach play an important role in the gastric defense mechanisms that protect the gastric epithelium from the acidic environment. PGE2 increases the production of gastric mucus, which builds a protective layer on the epithelium, while PGI2 maintains gastric blood flow. Inhibition of PG synthesis in the stomach causes serious adverse effects such as gastric erosions, bleeding, ulceration and perforation. A single dose of aspirin is sufficient to cause small erosions. The risk for serious GI toxicity increases considerably with long-term use of traditional NSAIDs and concomitant use of glucocorticoids.

Multiple clinical trials have demonstrated that COX-2 selective inhibitors cause less gastrointestinal toxicity than traditional NSAIDs. Particularly, serious side effects are reduced to the placebo level, suggesting that the physiological PG production in the stomach is mediated primarily by the COX-1 pathway. However, experimental studies have revealed that gastric damage only occurs if both COX-enzymes are inhibited collectively, but not with COX-1 or COX-2 inhibition alone. This indicates that COX-2 also is important for protection of the gastric mucosa (Halter et al. 2001). This idea is further supported by studies showing that COX-2 is ► upregulated in the stomach in the case of ulceration (Schmassmann et al. 1998) or other epithelial damage such as ► Helicobacter pylori infection (Seo et al. 2002), and contributes to the production of prostaglandins that are involved in healing processes such as PGJ2 (Gilroy et al. 1999). Hence, although COX-2 selective inhibitors are relatively safe for the healthy stomach, they may impair ulcer healing (Jones et al. 1999).

Renal Toxicity

COX-2 is constitutively expressed in the kidney and is highly regulated in response to alterations in intravascular volume (Harris et al. 1994). COX-2-derived PGs signal the release of ► renin from the renal ► juxtaglomerular apparatus, especially during volume depletion. PGs also maintain renal blood flow and regulate salt and water excretion.

COX-2 inhibition, both with traditional NSAIDs or selective COX-2 inhibitors, may transiently decrease urine sodium excretion in some subjects and induce mild to moderate elevation of blood pressure. Substance specific differences have been suggested. For example, rofecoxib users were at a significantly increased relative risk of new onset hypertension compared with patients taking celecoxib, nonspecific NSAIDs or no NSAID (Solomon et al. 2004). The risk for renal side effects is increased in patients with pre-existing renal or heart disease.

Platelet Aggregation and Cardiovascular System

Thrombocyte aggregation depends on thromboxane A2 (TXA2), which is produced by the COX-1 pathway in platelets. Hence, traditional NSAIDs inhibit platelet aggregation, and particularly aspirin, the irreversible unselective COX-inhibitor, causes a long-lasting prolongation of the bleeding time, which may increase the risk of bleeding e.g. during or after surgery. On the other hand, inhibition of thrombocyte aggregation may be the desired therapeutic effect of aspirin for patients with increased risk of thrombosis such as coronary heart disease, or it may be a welcome side-effect of traditional NSAIDs.

The activation of platelets by TXA2 is counterbalanced by vascular prostacyclin (PGI2) under physiological conditions. Systemic PGI2 is mainly produced through the COX-2 pathway (McAdam et al. 1999). Specific COX-2 inhibitors may therefore shift the balance between TXA2 and PGI2 towards TXA2 mediated thrombocyte aggregation. This may result in an increased risk of ► thrombotic events in predisposed patients. Recent large clinical trials have revealed an increased risk for thrombotic cardiovascular effects with rofecoxib and other COX-2 inhibitors. However, observational studies reported a similar risk with selective and unselective COX-inhibitors, suggesting that an inbalance between PGJ_2 and TXA_2 does not sufficiently explain the increased cardiovascular risk with these analgesics.

Bone Healing

PGs participate in inflammatory responses in the bone, increased ► osteoclast activity and subsequent bone resorption, and increased ► osteoblast activity and new bone formation.

Data from animal studies suggest that both non-specific and specific inhibitors of cyclooxygenases impair fracture healing. This is due to the inhibition of COX-2 (Zhang et al. 2002). Although these data raise concerns about the use of traditional NSAIDs and COX-2-specific inhibitors as anti-inflammatory or anti-analgesic drugs in patients undergoing bone repair, clinical reports have been inconclusive.

Aspirin Asthma

Inhibition of cyclooxygenase activity reduces arachidonic acid consumption for prostaglandin synthesis, and hence yields more substrate for leukotriene and endocannabinoid synthesis. While ► endocannabinoids may enhance the analgesic effects of certain NSAIDs (Ates et al. 2003), overproduction of cysteinyl leukotrienes may cause bronchial constriction and trigger asthma

attacks in "aspirin-sensitive" patients (Szczeklik et al. 2001). Aspirin asthma has also been observed with other non-selective NSAIDs but not with COX-2 selective drugs. Similar mechanisms also account for the NSAID-evoked ► urticaria (Mastalerz et al. 2004).

Ototoxicity

A recent study suggested that salicylate induced tinnitus is mediated through an indirect activation of ► NMDA receptors in the cochlea, causing an increase of spontaneous auditory nerve activity. NMDA receptor activation is probably mediated by inhibition of prostaglandin synthesis (Guitton et al. 2003), and hence is not specific for salicylate. ► Tinnitus occurs at high plasma concentrations.

Pregnancy

COX-2-derived prostaglandins play a prominent role at all stages of female reproduction, from ovulation to implantation, ► decidualization and delivery. The regulation of prostaglandin release is mediated by transcriptional control of COX-2 and microsomal prostaglandin E synthase. Elevated uterine PGs or the enhanced sensitivity of the myometrium to PGs leads to contractions and labor. Hence, traditional NSAIDs as well as COX-2 inhibitors may prolong parturition, or may be used in the treatment of preterm labor (McWhorter et al. 2004).
In addition, prostaglandins regulate the transition to pulmonary respiration following birth that requires closure and remodeling of the ► ductus arteriosus. The maintenance of the ductus arteriosus in the open, or patent, state is dependent on prostaglandin synthesis, and the neonatal drop in prostaglandin E2 that triggers ductal closure is sensed through the EP4 receptor (Nguyen et al. 1997). Hence, NSAIDs may cause a premature DA closure if taken during late pregnancy, or may be used to induce DA closure in preterm infants.

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NSAIDs, Chemical Structure and Molecular Mode of Action

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Definition

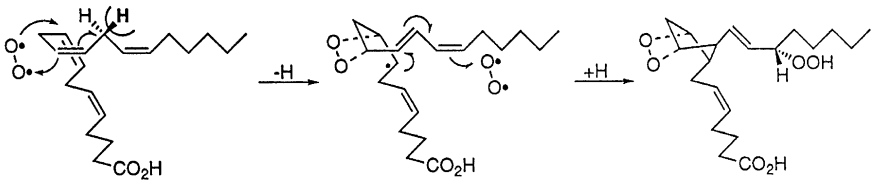
Structure and Metabolic Function of COX-1

COX-1 is a 70 kD enzyme, catalyzing the reaction of ► arachidonic acid to PGG_2 (cyclooxygenase reaction) and consecutively PGG_2 to PGH_2 (peroxidase reaction) as outlined in Fig. 1.
There are distinct active sites for the ► Cyclooxygenases (COX) and the peroxidase reactions (Fig. 2).

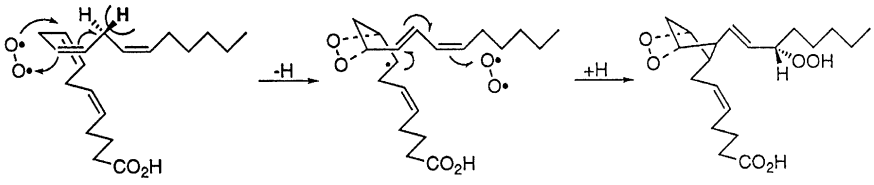
Characteristics

Inhibitors of Cyclooxygenases

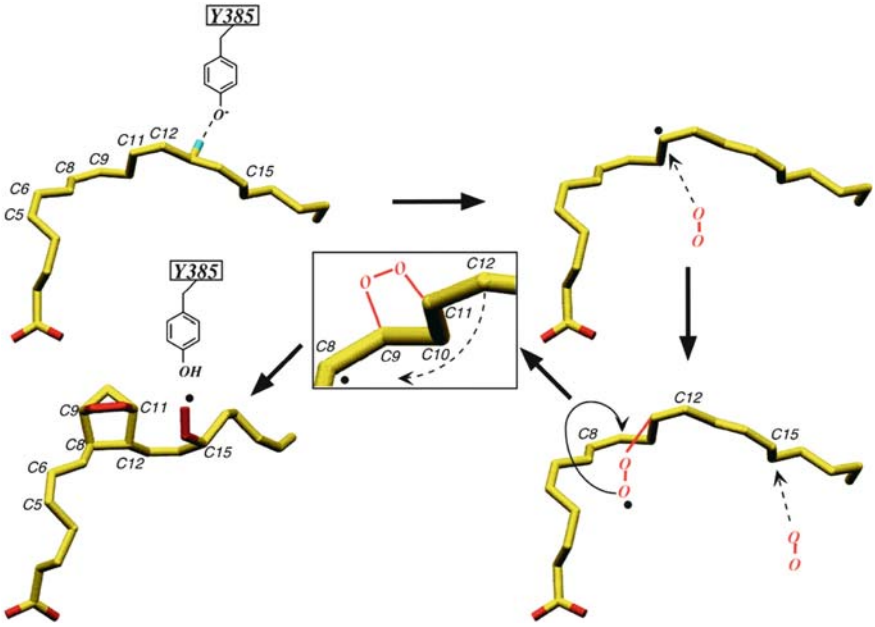
Different chemical classes can provide the structural features necessary to mimic arachidonic acid at the active site. The substrate, arachidonic acid is a C_{20} carboxylic acid with 4 isolated double bonds at positions 5, 8, 11 and 14. For the enzyme reaction, arachidonic acid must adapt to a "folded" conformation, allowing the oxygen to insert between C_9 and C_{11} and the ring closure between C_8 and C_{12} (Fig. 3).



NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 1 Reaction catalyzed by COX enzymes.



NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 2 A ribbon representation of the Co^{3+}-oPGHS-1 monomer with AA bound in the COX channel. The EGF domain, MBD, and catalytic domain are shown in green, orange and blue, respectively; Co^{3+}-protoporphyrin IX is depicted in red, disulfide bonds (Cys36-Cys47, Cys37-Cys159, $Cys4^{1-}$Cys57, Cys59-Cys69, and Cys569-Cys575) in dark blue and side chain atoms for COX channel residues Arg120, Tyr355 and Tyr385 in magenta (from Malkowski et al. (2000)).



NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 3 Mechanistic sequence for converting AA to PGG_2. Abstraction of the 13-proS hydrogen by the tyrosyl radical leads to the migration of the radical to C-11 on AA. The attack of molecular oxygen, coming from the base of the COX channel, occurs on the side interfacial to hydrogen abstraction. As the 11R-peroxyl radical swings over C-8 for an R-side attack on C-9 to form the endoperoxide bridge, C-12 is brought closer to C-8 *via* rotation about the C-10/C-11 bond, allowing the formation of the cyclopentane ring. The movement of C-12 also positions C-15 optimally for addition of a second molecule of oxygen, formation of PGG_2 and the migration of the radical back to Tyr385 (from Malkowski et al. (2000)).

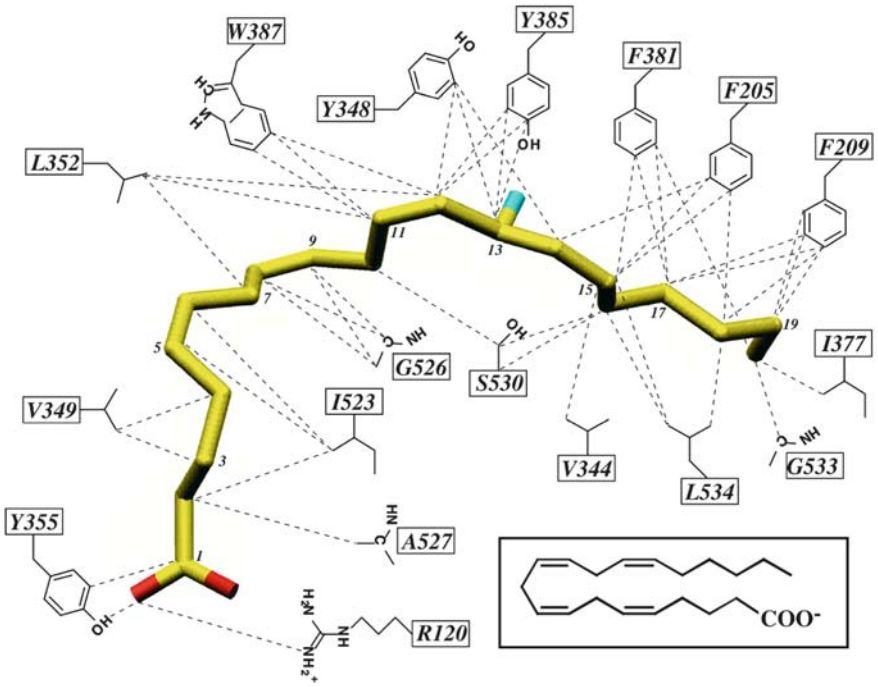
To fix arachidonic acid in such a conformation, several interactions with the active site of the enzyme are necessary, e.g. ionic interaction (a salt bridge) between the carboxylic group and arginine 120, π-π interactions between the double bonds of arachidonic acid and aromatic amino acids and numerous hydrophobic interactions (Fig. 4).

All these structural features can be identified in many ► NSAID s. Most acidic NSAIDs are therefore believed to mimic arachidonic acid in its folded conformation at the active site of COX. Structure activity relationships follow these structural constrictions closely.

The activity against COX-1 clearly correlates with torsion angles around the π-electron systems and the overall lipophilicity of the molecule (Moser et al. 1990).

Two classes of compounds however have a distinctly different molecular mode of action,

- ASS acetylates Ser 530 irreversibly at the active site of the enzyme
- The oxicames are believed to interfere with the peroxidase active site, which also explains their structural difference.



NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 4 A schematic of interactions between AA and COX channel residues. Carbon atoms of AA are yellow, oxygen atoms red and the 13proS hydrogen blue. All dashed lines represent interactions within 4.0 A between the specific side chain atom of the protein and AA (from Malkowski et al. (2000)).

N

Most of the currently used NSAIDs, including diclofenac, ibuprofen, naproxen, piroxicam and indomethacin for instance, may produce full inhibition of both COX-1 and COX-2 with relatively poor selectivity under therapeutic conditions (Warner et al. 1999).

Acidic NSAIDs like diclofenac accumulate particularly in blood, liver, milt and bone marrow, but also in tissue with acidic extracellular pH values. Such tissues are mainly inflamed tissues such as gastric tissue and the manifolds of the kidney. In inflamed tissue, NSAIDs inhibit the pathological overproduction of prostaglandins. In contrast, neutral NSAIDs (paracetamol) and weakly acidic NSAIDs (metamizol) distribute themselves quickly and homogeneously in the organism. They also penetrate the blood-brain-barrier.

Fenamate Group

The core structure is 2-aminobenzoic acid (anthranilic acid). The 2-amino group is substituted with aromatic residues.

- flufenamic acid
- mefenamic acid
- meclofenamic acid
- niflumic acid (core structure: 2-amino-pyridyl-3-carboxylic acid)

For topical application, the carboxylic acid group is esterified with diethyleneglycol

- etofenamate

Fenac Group

The core structure is 2-aminophenylacetic acid. The 2-amino group is substituted with aromatic residues.

- diclofenac
- felbinac (only used topically)

Heteroaryl Acetic Acid Group

- indomethacin
- acemetacine
- proglumetacine
- tolmetin (and its ring closed analog ketorolac)
- ionazolac

Profene Group

The core structure is 2-arylpropionic acid

- ibuprofen
- ketoprofen
- thiaprofen
- naproxen
- ketorolac (can be seen formally as a ring closed profene)

Oxicam Group

The core structure is 1,2-benzothiazine

- piroxicam
- tenoxicam
- lornoxicam
- droxicam

- cinoxicam
- sudoxicam
- meloxicam

Pyrazolone Group

The mode of action of the pyrazolones remains unclear. It is thought that they may not be involved in the inhibition of COX-1 or COX-2. The compounds of the pyrazol-3-on series at least are neutral molecules with no acidity. A central mode of action is suggested. They also act antispasmodically and are effective in visceral pain. In the past, pyrazolones were very frequently used nonsteroidal anti-inflammatory drugs. They show a high plasma protein binding and therefore have a high rate of interaction with other pharmaceuticals. Agranulocytosis is a rare but severe side effect.

The core structure is 3*H*-pyrazol-3-on

- propyphenazone
- metamizol-Na
- phenazone

Pyrazolidindione

The core structure is pyrazolidin-3,5-dion

- phenylbutazone
- mofebutazone

COX-2 Selective Inhibitors

The isoform 2 of the COX enzyme catalyzes the identical reaction AA to PGG_2, the active site however is slightly different from COX-1 (Fig. 5).

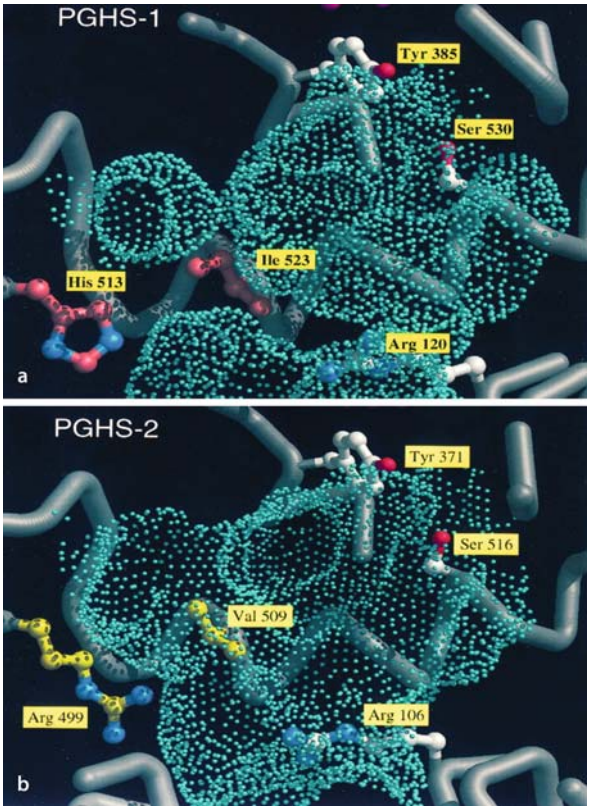
Isoleucine 523 is replaced by valine 509, making the active site of COX-2 more "spacious". This difference can be used to generate COX-2 selective inhibitors, as this active site tolerates more bulky molecules. Celecoxib is capable of producing full inhibition of COX-1 and COX-2. However it shows a preferential selectivity toward COX-2 (>5 fold). The newer coxibs like rofecoxib strongly inhibit COX-2 with only weak activity against COX-1 (Warner et al. 1999).

A common pharmacophore cannot be identified, however vicinal diaryl systems (celecoxib, rofecoxib, valdecoxib) and sulfone or sulphonamide groups seem to be advantageous (Laufer et al. 2000). Lumiracoxib however is an excellent example of the fact that spatial demanding substituents (bulky groups) alone are sufficient to generate selectivity, even with a diclofenac-like pharmacophore.

Structural Features of Selective COX-2 Inhibitors

Sulfonamide structure

- celecoxib
- valdecoxib



NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 5 Comparison of the active site of COX-1 (PGHS-1) and COX-2 (PGHS-2) (from Wong et al. 1997).

Methylsulfone structure

- rofecoxib
- etoricoxib

Aryl acetic acid

- Lumiracoxib

Others:

- parecoxib (water soluble prodrug for parenteral application, rapidly metabolized to valdecoxib)

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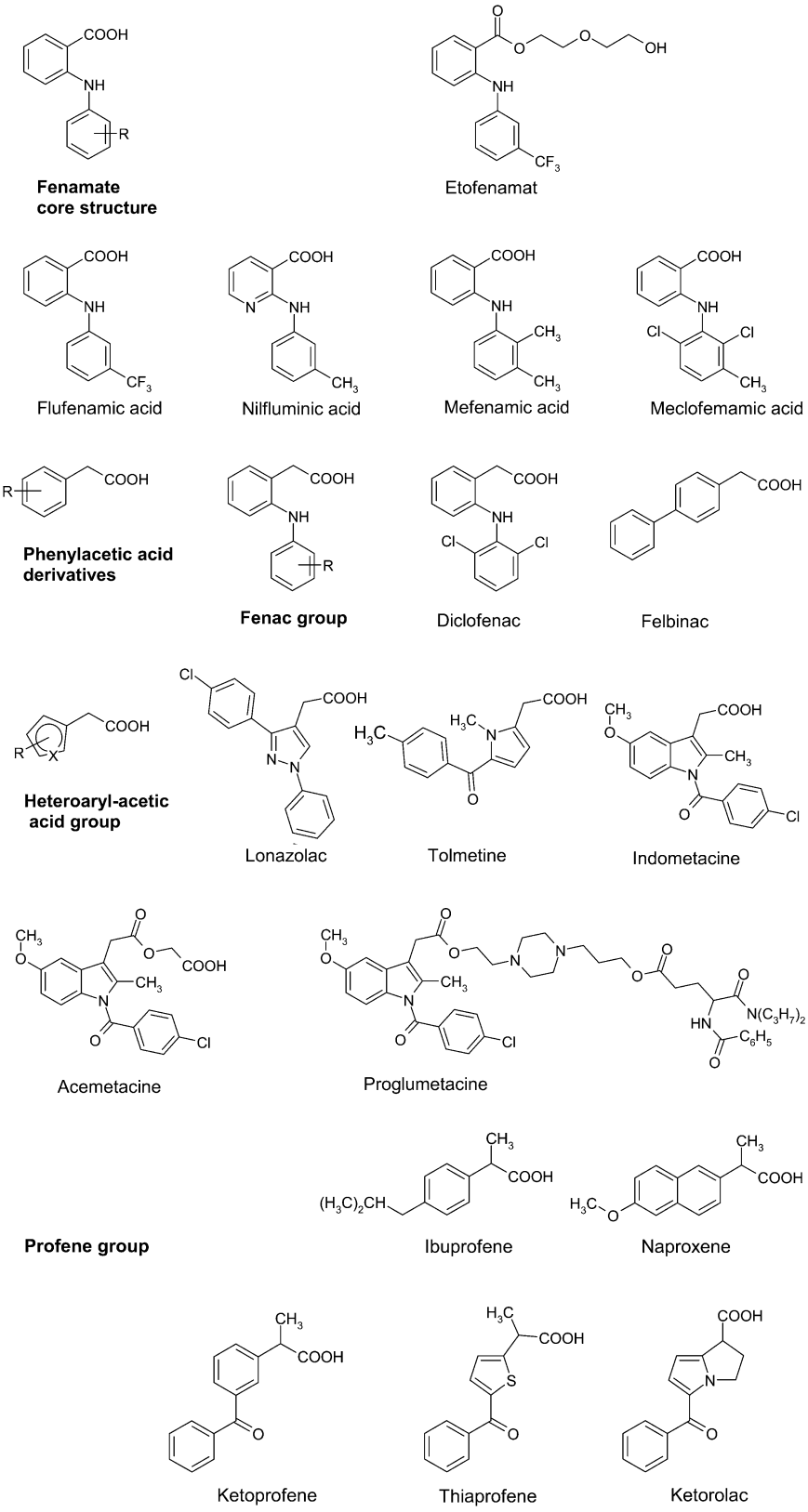

NSAIDs, Chemical Structure and Molecular Mode of Action, Figure 6 Chemical structures of NSAIDs.

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NSAIDs, COX-Independent Actions

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Synonyms

NSAIDs; non-steroidal anti-inflammatory drugs; COX; cyclooxygenase; PGH-Synthase; Prostaglandin H Synthase

Definition

NSAIDs are among the most commonly used ► analgesics and anti-inflammatory drugs. The major mechanism of action is supposed to be the inhibition of cyclooxygenase (COX) 1 and 2 enzymes, and thereby prostaglandin synthesis. However, since aspirin was reported to inhibit nuclear factor kappa B (NF-κB) activation (Kopp and Ghosh 1994), it is increasingly recognized that certain NSAIDs have various biological effects that are independent of cyclooxygenase activity and prostaglandin synthesis, and may account, at least in part, for their analgesic, anti-inflammatory and antiproliferative effects. These effects mainly occur at drug concentrations beyond the IC_{50} (► IC50 value) for COX-inhibition, and therefore probably occur primarily at high concentrations. Various mechanisms have been shown to be involved (Tegeder et al. 2001) and are summarized in this essay. A schematic overview over these mechanisms is shown in figure 1.

Characteristics

Effects on Transcription Factors

Nuclear Factor Kappa B (NF-κB)

NF-κB is an important mediator of the cellular response to a variety of extracellular stress stimuli. As homodimers and heterodimers, Rel/NF-κB proteins bind to DNA target sites and regulate gene transcription of pro-inflammatory mediators and proteins that are involved in cell death or survival. Various NSAIDs including salicylates, sulindac, ibuprofen and R- and S-flurbiprofen, inhibit NF-κB activation. While aspirin, ibuprofen and sulindac also inhibit COX-activity, R-flurbiprofen and salicylic acid are inactive in this regard, and therefore do not cause typical NSAID-evoked side effects that are due to COX-inhibition. Indomethacin, ketoprofen and ketorolac do not inhibit NF-κB. Hence, in contrast to COX-inhibition, NF-κB inhibition is not a "class-effect" The COX–2 selective inhibitors rofecoxib and celecoxib have different effects on NF-κB. While rofecoxib inhibits its activation in RAW 264.7 macrophages (Niederberger et al. 2003), celecoxib further increases LPS-induced NF-κB-activation. The latter effect of celecoxib results in a loss of its anti-inflammatory efficacy at high doses in an experimental inflammatory model in rats (Niederberger et al. 2003), suggesting that effects on NF-κB are important for the anti-inflammatory efficacy of some COX-inhibitors.
As a stress signalling molecule, NF-κB is also involved in the regulation of cell death and survival, either as being essential for the induction of ► apoptosis or more commonly as an inhibitor of apoptosis. Whether NF-κB promotes or inhibits apoptosis appears to depend on the cell type and the type of inducer. NF-κB is persistently active in numerous human cancer cells. This is suggested to contribute to increased resistance towards chemo– or radiotherapy. NSAIDs that inhibit NF-κB may eliminate this resistance mechanism and thereby re-increase cancer cell sensitivity towards apoptosis inducing treatments. Hence, inhibition of NF-κB in tumour cells may contribute to the observed anti-tumour activity of various NSAIDs including S- and R-Flurbiprofen (Wechter et al. 1997), celecoxib (Grosch et al. 2001), sulindac and aspirin (Thun et al. 1991).

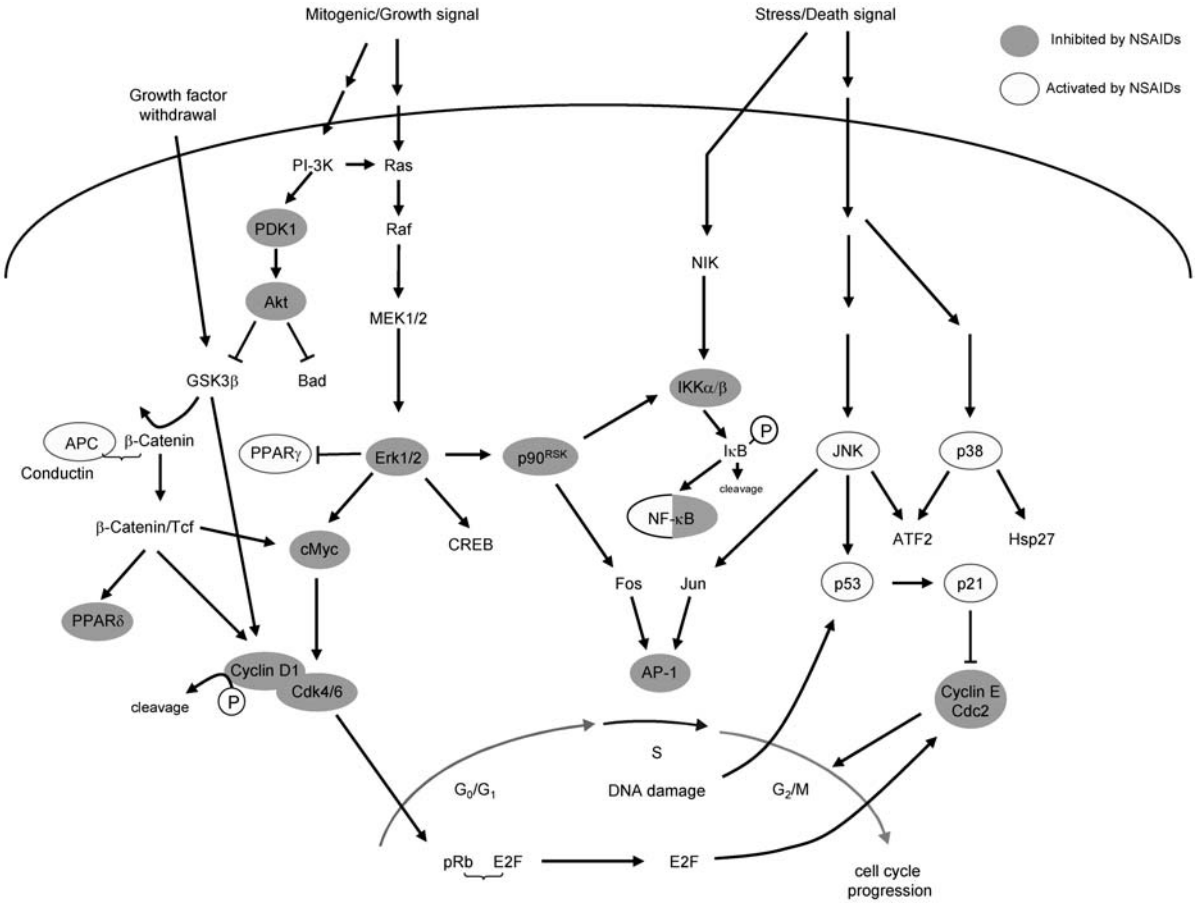
AP–1

The transcription factor AP–1 is a homo- or heterodimer of Jun, Fos and Fra oncogenes. AP–1 is activated by various stimuli including UV-irradiation, growth factors and inflammatory cytokines. Some of the genes known to be regulated by AP–1 are involved in the immune and inflammatory responses or tumour formation and progression. AP–1 regulated genes partially overlap with genes that are regulated by NF-κB. Inhibition of AP–1 has been shown for aspirin, sodium salicylate, piroxicam, R-flurbiprofen and the selective COX–2 inhibitors celecoxib and NS398 in various cell types. However, it is not presently known to what extent effects on AP–1 contribute to the anti-inflammatory or anti-proliferative effects of NSAIDs, because different AP–1 homo- and heterodimers may have either stimulating or inhibiting effects on gene transcription (Grosch et al. 2003), and the number of genes that are regulated by AP–1 is high. Therefore, the result of NSAID-induced AP–1 inhibition may greatly vary.

Effects on Cellular Kinases

Inhibitor Kappa B Kinase Complex (IKK)

In most cells NF-κB exists in the cytoplasm in an inactive complex bound to inhibitor IκB proteins. NF-κB is activated upon phosphorylation and subsequent proteasome-mediated proteolysis of IκB. The key regulatory step in this pathway is the activation of an IκB kinase (IKK) complex. IKK consists of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit (IKKγ) that regulates binding of activators. Liberated NF-κB translocates from the cytoplasm to the nucleus where it binds to the κB-sites of target genes and reg-



NSAIDs, COX-Independent Actions, Figure 1, Systematic overview of COX-independent NSAIDs effects. → activation or induction; ⊣ inhibition inhibited by NSAIDs; activated by NSAIDs Abbreviations: Akt/PKB, protein kinase B; APC adenomatous polyposis coli tumor suppressor gene; AP-1 activator protein-1; Cdk, cyclin dependent kinase; CREB, cAMP response element binding protein; Erk, extracellular signal regulated kinase; GSK3β, glycogen synthase kinase 3 beta; Hsp, heat shock protein; IKK, I-kappa kinase; JNK, Jun N-terminal kinase; MAPK, mitogen activated kinase; MEK/MKK, mitogen-activated protein kinase kinase; NIK, nuclear factor-kappaB inducing kinase; NF-κB, nuclear factor kappa B; PDK, phosphoinositol-dependent kinase; PI-3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PPAR, peroxisome proliferator activated receptor; pRb, retinoblastoma protein; p90RSK, ribosomal S6 kinase; Tcf, T cell factor.

ulates their transcription. Various NSAIDs including aspirin, sodium salicylate and sulindac inhibit the ATP binding to IKKβ, and thereby its catalytic activity (Yin et al. 1998). However, the IKK inhibitory potency of these NSAIDs is low, and the specificity of aspirin-induced IKK, and thereby NF-κB inhibition, has been doubted.

Mitogen Activated Protein (MAP)-Kinase Cascade

MAP-kinases (MAPK) play a central role in the differentiation and proliferation of several cell types, and can be activated by various extracellular stimuli. AP–1 is one downstream target of the MAP-kinase family members including extracellular signal regulated kinases (Erk-1 and -2; p42/p44 MAPK), c-Jun N-terminal kinases (JNK1-3) and p38 MAPK. Erk activation has been implicated in growth promotion and cell survival, whereas JNK and p38 MAPK activation are associated with stress responses and apoptosis. Recent results have shown that the effect of NSAIDs on MAP-kinases largely depends on the cellular context. In cancer cells, the ability of NSAIDs to modulate MAPK activities may play an important role in the cytotoxicity and induction of apoptosis.

Aspirin and sodium salicylate were shown to inhibit activation of Erk-1 and -2 under certain circumstances. Inhibition of angiogenesis by COX–2 selective and unselective NSAIDs has been shown to be mediated through inhibition of Erk-2 activity and interference with its nuclear translocation in vascular endothelial cells (Tsujii et al. 1998). p38 MAPK was reported to be activated by sodium salicylate in human fibroblasts leading to induction of apoptosis. TNFα-induced JNK activation was also inhibited by salicylate in human fibroblasts. Oppositely, in HT-29 colon cancer and COS-1 cells, salicylate treatment resulted in activation of JNK.

Protein Kinase B (PKB/Akt)

The protein kinase B (PKB/Akt) promotes cell proliferation and survival, thereby contributing to cancer

progression. Activation of Akt occurs by translocation of the kinase to the cell membrane and phosphorylation through phospho-inositide-dependent kinase 1 (PDK1). The COX–2 selective inhibitor celecoxib has been shown to induce apoptosis in prostate carcinoma (Hsu et al. 2000), hepatocarcinoma and colon carcinoma cells by inhibiting the phosphorylation of PKB/Akt, thereby blocking its anti-apoptotic activity. The effects of celecoxib on Akt depended in part on the inhibition of PDK1. Inhibition of PKB/Akt by celecoxib has also been observed in vascular smooth muscle cells leading to inhibition of neointima formation after ballon injury. Similarly, sulindac has been described to inhibit Akt-phosphorylation in lung adenocarcinoma cells. Aspirin has been shown to either activate or inhibit Akt-activation, dependent on the cell type.

Effects on Cell Cycle Proteins

The progression through the various phases of the cell cycle is regulated by cyclins and cyclin-dependent kinases (Cdks). The function of cyclins is primarily controlled by changes in cyclin transcription, while Cdks are regulated by phosphorylation. The activity of the Cdk/cyclin complex is inhibited by p21 and p27. Sodium salicylate inhibits the proliferation of vascular smooth muscle cells through up-regulation of these cell cycle inhibitors. This is probably caused by salicylate-induced up-regulation of p53, which is the primary regulator of p21 transcription. Similar to salicylates, sulindac, sulindac sulfide and celecoxib inhibited the proliferation of colon carcinoma cells and caused them to accumulate in the G_0/G_1 phase. This effect was attributed to inhibition of cyclin-dependent kinases and/or up-regulation of p27 and p21.

Modulation of the Activity of Nuclear Receptor Family Members

Activation of Peroxisome Proliferator Activated Receptor (PPAR)

PPARs α, δ and γ are nuclear hormone receptors that control the transcription of genes involved in energy metabolism, cell differentiation, apoptosis and inflammation. PPARs bind to sequence-specific DNA response elements as a heterodimer with the retinoic acid receptor (RXR). PPARγ is highly expressed in adipose tissue, and plays an important role in the regulation of genes involved in lipid utilization and storage, adipocyte differentiation, insulin action and inflammation. Indomethacin binds to PPARγ and induces the differentiation of mesenchymal stem cells into adipocytes ► in vitro. Some other NSAIDs including ibuprofen, fenoprofen and flufenamic acid also bind and activate PPARγ. However, they are less potent than indomethacin.

In addition to the role in adipogenesis and inflammation, PPARγ is highly expressed in normal large intestine and in breast, colon and prostate cancer. PPARγ-agonists such as troglitazone and 15deoxy-PGJ_2 were able to induce differentiation and apoptosis in tumour cells, suggesting that PPARγ suppresses tumour cell proliferation. Indomethacin was shown to reduce the colonogenic activity of prostate cancer cells and increased the antiproliferative effect of 5–fluorouracil in colon cancer cells. This effect was supposed to be mediated through activation of PPARγ.

PPARδ is a nuclear transcription factor that is activated by prostacyclin. Inhibition of COX-activity with aspirin or other NSAIDs causes inhibition of PPARδ, which has been identified as one of the downstream targets of the WNT-β-catenin pathway (He et al. 1999). This pathway plays a crucial role in embryonic development and carcinogenesis. PPARδ expression is normally controlled by the APC tumour suppressor. However, during colon carcinogenesis, APC function is almost always lost, leading to a dysfunction of β-catenin and uncontrolled PPARδ expression. This is considered as a crucial initiating step in tumour transformation. The suppression of PPARδ activity by various NSAIDs, including aspirin and sulindac, can compensate for the loss of APC or β-catenin dysfunction and thereby reduce colon carcinogenesis. Hence, the inhibition of PPARδ activity may contribute to the chemopreventive effects of some NSAIDs.

Other Targets

Intracellular Carbonic Anhydrase

Carbonic anhydrases play an important role in the extracellular acidification. Several studies suggest a possible involvement of these enzymes in tumour progression resulting from the acidic extracellular pH. Celecoxib and valdecoxib inhibit carbonic anhydrases. This effect is supposed to depend on their sulfonamide structure, and is therefore not shared by rofecoxib or etoricoxib.

Acid Sensing Ion Channels (ASICs)

H^+-gated currents mediated by acid sensing ion channels (ASICs) are involved in acidosis which occurs under inflammatory conditions and in tumours. Various NSAIDs including aspirin, salicylic acid, flurbiprofen, ibuprofen and diclofenac are inhibitors of these H^+-gated channels, thereby inhibiting acid induced pain reaction and inflammatory responses.

Ca^{2+}-Release

Treatment with some COX–2 inhibitors increased intracellular calcium levels in osteoblasts, PC–12 and HUVEC cells. This effect might be mediated by a block of endoplasmatic reticulum Ca^{2+}-ATPases, and may increase the risk of cardiovascular events in predisposed patients.

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NSAIDs, Mode of Action

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Synonyms

NSAIDs; non-steroidal anti-inflammatory drugs; Aspirin-Like Drugs; Non-Selective COX-Inhibitors; cyclooxygenase; COX; Prostaglandin H Synthase; PGHS

Definition

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed and used drugs for the management of pain, especially of pain in inflammatory conditions. Despite the wide use of NSAIDs over the last century, little was known on the mode of actions of these drugs for a long time. Initially, the principle mode of the antinociceptive action of NSAIDs was related to their anti-inflammatory activity, and attributed to the inhibition of the production of prostaglandins at the peripheral site of inflammation (peripheral mode of action) (Vane 1971). The traditional belief in the exclusively peripheral action of NSAIDs, however, has been challenged by the growing evidence showing the dissociation between the anti-inflammatory and antinociceptive effects of NSAIDs (McCormack and Brune 1991). This is the basis for the hypothesis of additional antinociceptive mechanisms existing with NSAIDs, where the inhibition of prostaglandin synthesis in CNS appears to be universally applicable for all NSAIDs (central mode of action).

While inhibiting prostanoid synthesis, NSAIDs do not typically elevate the normal pain threshold, but mainly normalize the increased pain threshold observed in ► inflammatory pain (► hyperalgesia), so that their antinociceptive effect should rather be defined as anti-hyperalgesic instead of analgesic.

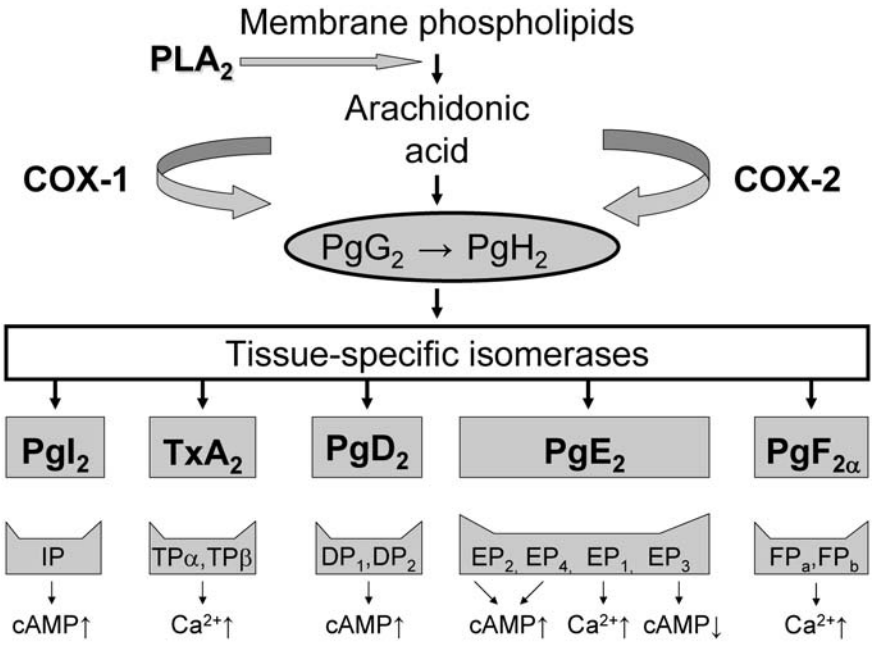
Characteristics

Prostanoid Synthesis

Despite the diverse chemical structure of aspirin-like drugs, all NSAIDs bear a common pharmacological property of inhibiting the formation of prostanoids. Prostanoids are formed by most cells and act as lipid mediators. They are synthesized from membrane-released arachidonic acid mobilized by phospholipases (PLA_2) when cells are activated by mechanical trauma, cytokines, growth factors, etc. (Fig. 1). Conversion of arachidonic acid to prostanoid end-products occurs by cyclooxygenases (COX), an enzyme also known as prostaglandin H synthase (PGHS), at two different sites of the enzyme. It is initially cyclized and oxidized to the endoperoxide PGG_2 at the cyclooxygenase site of the COX. This product is then reduced to a second endoperoxide, PGH_2, at the peroxidase site of the COX enzyme. Subsequent formation of prostaglandin end-products from PGH_2 depends on the presence of the specific synthase enzymes that produce functionally important prostanoids PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin) and TXA_2 (thromboxane), which mediate their effects through the specific receptors: PGD_2 (DP_1, DP_2 receptors), PGE_2 (EP_1, EP_2, EP_3, EP_4 receptors), $PGF_{2\alpha}$ (FP receptor), PGI_2 (IP receptors) and TXA_2 (TP_α and TP_β receptors).

Inhibition of Cyclooxygenases by NSAIDs

NSAIDs inhibit the formation of prostanoids by several different effects on COX, including irreversible inactivation of COX (e.g. aspirin) or reversible competitive inhibition (e.g. ibuprofen). COX is represented by two isoforms, COX-1 and COX-2, which are membrane-associated enzymes with a 63% amino acid sequence similarity. The identification of two isoforms of COX in the early 1990s offered a simple and attractive hypothesis: COX-1, being found in almost all cells, is the constitutive "house-keeping" enzyme responsible for production of basal "beneficial" PGs, which are vital for protecting the stomach through mucus production or maintenance of renal blood flow. In contrast, COX-2, in which expression is low or undetectable in most cells but increased dramatically in a variety of pathological



NSAIDs, Mode of Action, Figure 1 Prostanoids are synthesized from membrane-released arachidonic acid mobilized by phospholipases (PLA_2). Conversion of arachidonic acid to prostanoid end-products occurs by COX-1 and COX-2 at two different sites of the enzyme. It is initially cyclized and oxidized to the endoperoxide PGG_2 at the cyclooxygenase site of the COX and then reduced to a second endoperoxide, PGH_2 at the peroxidase site of the COX enzyme. Tissue-specific isomerases catalyze subsequent formation of prostaglandin end-products including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin) and TXA_2 (thromboxane). These prostanoids exert their effects by acting through the specific receptors: PGD_2 (DP_1, DP_2 receptors), PGE_2 (EP_1, EP_2, EP_3, EP_4 receptors), $PGF_{2\alpha}$ (FP receptor), PGI_2 (IP receptors) and TXA_2 (TP_α and TP_β receptors). The "relaxant" receptors IP, DP_1, EP_2 and EP_4 signal through Gs-mediated increases in intracellular cyclic adenosine monophosphate (cAMP). The "contractile" receptors EP_1, FP and TP signal through Gq-mediated increases in intracellular calcium. The EP_3 receptor is regarded as an "inhibitory" receptor that couples to Gi and decrease cAMP formation.

conditions, is the inducible enzyme responsible for "pathological" production of prostanoids in different conditions ranging from inflammation to cancer.

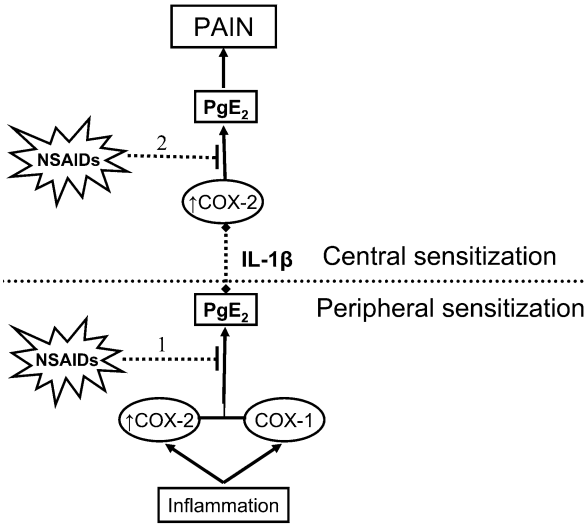
NSAIDs are non-selective inhibitors of COX (both COX-1 and COX-2). Initially, the favourable anti-inflammatory, anti-nociceptive and antipyretic effects of NSAIDs were solely attributed to the inhibition of COX-2, while the concomitant inhibition of COX-1 was supposed to lead to adverse reactions of the drugs (gastrointestinal and renal toxicities). With time, especially along with the introduction/gaining experience of selective COX-2 inhibitors (e.g. celecoxib, rofecoxib, etc.), this concept turned out to be more complicated than initially thought, indicating that both COX-1 and COX-2 have physiological and pathological roles, so that the inhibition of both isoforms may be responsible for favourable and unfavourable pharmacological effects of NSAIDs.

Cyclooxygenases and Prostanoids at the Peripheral Site

In injured tissue, COX-2 is the predominant isoform expressed and a main source of prostanoids during inflammation. The significant induction of COX-2 is found in activated polymorponuclear leucocytes, phagocytosing mononuclear cells and fibroblasts, which are abundantly present at the site of inflammation. However, COX-1 is also involved in the modulation of the inflammatory response, and is mainly increased in circulating monocytes and stimulated mast cells at the early inflammatory phase. Thus, both COX isoforms are involved in the inflammatory reaction in the periphery, and may contribute to the generation and maintenance of inflammatory pain. The earliest prostanoid response is dependent on COX-1, but COX-2 becomes a major source of prostanoids along with the progression of inflammation. NSAIDs-mediated inhibition of COX-1 and COX-2 at the site of inflammation results in the attenuation of peripheral ► sensitization associated with inflammatory pain (Fig. 2).

Cyclooxygenase and Prostanoids in CNS

In contrast to the periphery, in the CNS both COX-1 and COX-2 are expressed constitutively (Beiche et al. 1996). These isoforms are present in neurons and non-neuronal elements of the spinal cord and brain (Maihofner et al. 2000). The peripheral inflammatory reactions associated with tissue injury result in the release of pro-inflammatory cytokines, such as IL-1β, which may enhance the up-regulation of COX-2 in the CNS (Samad et al. 2001). This up-regulation is paralleled to the substantial elevation of prostanoids in the cerebrospinal fluid and typical nociceptive behaviour of the animals in experimental pain models. Therefore, COX-2 appears to be mainly responsible



NSAIDs, Mode of Action, Figure 2 The inhibition of prostaglandin synthesis by NSAIDs takes place at both the site of peripheral inflammation (1) and at the spinal level (2), indicating that peripheral and central mechanisms may be responsible for their antinociceptive action. NSAIDs-mediated inhibition of COX-1 and COX-2 at the peripheral site results in the attenuation of peripheral sensitization, whereas inhibition of the up-regulated COX-2 in CNS leads to the attenuation of central hyperalgesia. Peripheral and central hyperalgesia are hallmarks of inflammatory pain.

for the central processing of pain after peripheral inflammation. COX-1, however, can also become the source of spinal prostaglandins in response to peripheral inflammation under specific conditions, as has been particularly demonstrated in COX-2 deficient knockout mice. NSAIDs-mediated inhibition of COX-1 and COX-2 in CNS results in the attenuation of central sensitization associated with inflammatory pain (Fig. 2).

Mechanisms of Prostaglandin-Mediated Hyperalgesia

Prostaglandins are potent sensitizing agents, which are able to modulate multiple sites along the nociceptive pathway enhancing transduction (peripheral sensitization), as well as transmission (central sensitization), of the nociceptive information (Woolf 1983).

Peripheral Sensitization

Direct and indirect mechanisms of the peripheral sensitizing action of prostanoids have been suggested. The direct effects are mediated by their action upon prostaglandin receptors and modulation of ion channels in ► nociceptors. The indirect effects are produced through enhancing the sensitivity of nociceptors to noxious agents, including heat and bradykinin. Both direct and indirect sensitizing effects of prostanoids lead to the enhanced transduction of the nociceptive information and manifest as peripheral hyperalgesia. NSAIDs-mediated inhibition of prostanoid synthesis in the periphery results in the attenuation of peripheral hyperalgesia associated with inflammatory pain (peripheral ► antihyperalgesic effect).

Central Sensitization

The sensitizing effects of prostanoids in CNS are mediated by their action on presynaptic and postsynaptic membranes of the primary afferent synapse in the dorsal horn of the spinal cord. Acting via prostanoid receptors located on the presynaptic membrane, prostanoids may cause the enhancement of nociception via facilitation of the spinal release of the excitatory neurotransmitter ► glutamate and neuropeptides (► substance-P and/or ► calcitonin gene-related peptide). At the postsynaptic level, prostanoids can directly activate deep dorsal horn neurons and/or block the inhibitory glycinergic neurotransmission onto dorsal horn neurons. All this leads to the enhanced transmission of the nociceptive activity to the brain, and manifests as central hyperalgesia. NSAIDs-mediated inhibition of prostanoid synthesis in CNS results in the attenuation of central hyperalgesia associated with inflammatory pain (central antihyperalgesic effect).

Central and Peripheral Antihyperalgesic Effects of NSAIDs

From the pharmacological point of view, the contribution of the central versus peripheral mechanisms to the overall antihyperalgesic effects of NSAIDs depends on:

- the site of drug delivery (e.g. systemic, local-peripheral, epidural, spinal intracerebro-ventricular);
- uptake and distribution from the site of drug delivery, as determined by factors including the drug's physical and chemical properties, specific transport mechanisms, local and systemic blood flow, and tissue barriers to drug permeation, such as the blood-brain barrier.

One of the accepted approaches to prove that NSAIDs act upon CNS to alleviate pain is an assessment of their antinociceptive activity, following a direct application of NSAIDs to spinal or supraspinal structures. This approach has gained its particular importance in behavioural animal studies. The intrathecal delivery of various NSAIDs has been shown to be effective in the reduction of behavioural hyperalgesia in several animal models of acute, short-term and long-term inflammatory pain (Brune et al. 1991). The antihyperalgesic effects have been observed with doses that are significantly lower than those needed to produce the similar degree of antinociception by systemic administration. Clinical relevance of the central antinociceptive mechanisms of the antinociceptive action of NSAIDs has been demonstrated in patients with intractable pain due to various types of cancer conditions. In these patients, intrathecal administration of small doses of lysine-acetylsalicylate (equivalent to acetylsalicylic acid 500 μg/kg) has been shown to bring rapid and prolonged pain relief (Pellerin et al. 1987).

The evidence for a clinically relevant peripheral antinociceptive action has been obtained with locally applied NSAIDs, where the effective antinociceptive

effect of NSAIDs versus placebo has been established with intra-articular and topical applications of NSAIDs (Romsing et al. 2000).

The contribution of central versus peripheral mechanisms to the total antihyperalgesic effect of NSAIDs has been studied in the human experimental pain model of "freeze lesion". The experimental hyperalgesia in this model was produced by short-lasting freezing of volunteer's skin. The relative contribution of the central component of orally administered diclofenac has accounted for approximately 40% of the total antihyperalgesic efficacy of the drug (Burian et al. 2003).

Conclusion

NSAIDs are potent antinociceptive agents, whose efficacy in reducing pain is widely recognized in various pain conditions, including post-surgical pain and persistent pain states, such as arthritis and cancer. Although NSAIDs have long been used in clinical practice, the mechanism of their antihyperalgesic action remains controversial. It appears that the inhibition of prostaglandin synthesis by NSAIDs takes place both at the site of peripheral inflammation and at the spinal level, indicating that peripheral and central mechanisms are responsible for their antinociceptive action. The contribution of peripheral and central COX-dependent mechanisms to the overall antinociceptive action of NSAIDs is individual for each drug, and is dependent on the site of drug delivery, as well as pharmacokinetic characteristics of the drug determining its penetration to the sites of action (e.g. peripheral and spinal COX).

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NSAIDs, Pharmacogenetics

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Synonyms

Inherited Variability of Drug Response; Pharmacogenetics of NSAIDs

Definition

Pharmacogenetics seeks to explore how genetic variants influence the pharmacokinetic and pharmacodynamic properties of a given drug, by determining how mutations in the genes that encode drug metabolizing enzymes, drug targets and drug transporters influence drug response.

Characteristics

Nonsteroidal anti-inflammatory drugs (NSAID) block the formation of prostaglandins by inhibiting the rate-limiting cyclooxygenase (COX) enzymes, COX–1 and COX–2, also known as prostaglandin H_2 synthases (PGHS1 and PGHS2). Since prostaglandins participate in mediating the inflammatory response, the pharmacological activity of NSAIDs consists mainly of antinociceptive, anti-inflammatory and antipyretic properties. Variations of this pharmacological activity can arise as a basic principle from mutations in proteins, which (i) influence the bioavailability of a drug, (ii) vary the binding affinity to the drug target, or (iii) modify drug elimination. Since NSAIDs possess a high solubility and high permeability (Yazdanian et al. 2004), and were not found to be a substrate of drug efflux transporters, it is unlikely that the disposition varies on a genetic basis. Far more expected are mutations in the cyclooxygenase enzymes as drug targets and the metabolizing enzymes as determinants of drug elimination. The majority of such mutations are ► single nucleotide polymorphisms (SNP), which consist of an exchange of one nucleotide often, but not always, leading to an alteration in the amino-acid sequence of the resulting protein, provided that the SNP is located within the coding region of the ► gene.

Polymorphisms in the Cyclooxygenase-1 Gene

The COX–1 gene is located on ► chromosome 9 and consists of 11 exons. Several SNPs have been described (Halushka et al. 2003), of which so far only some single

nucleotide polymorphisms have drawn attention regarding a possible functional consequence leading to a decreased enzyme function or binding affinity. This was based on a computerized evaluation of the likelihood that a resulting amino acid exchange would lead to a phenotypic alteration (Ulrich et al. 2002). The SNPs 22C>T (arginine to tryptophan at position 8, R8W) and 50>T (proline to leucine at position 17, P17L) in ► exon 2 and the SNPs 688G>A (glycine to serine at position 230, G230S) and 709C>A (leucine to methionine at position 237, L237M) in exon 7 were considered as first-line candidates. Using human platelets as a system to study COX–1 activity, ► heterozygous carriers of the SNP 50C>T were determined to show a significantly greater inhibition of prostaglandin H_2 production after aspirin exposure than carriers of the CC50 ► genotype. The possible mechanism for the increased sensitivity to aspirin was seen in a decrease of COX–^{1}-enzyme levels. Since the 50C>T was found in complete ► linkage disequilibrium with -842A>G, a mutation in the COX–1 promoter, this SNP may also account for the functional impact, possibly because ► gene transcription is repressed (Halushka et al. 2003). However, with an allele frequency of 18% in Caucasians, a multitude of patients would be affected, but the overall contribution of these SNPs in explaining the interindividual variability of the pharmacological response to NSAIDs still remains to be determined.

Polymorphisms in the Cyclooxygenase-2 Gene

For the COX–2 gene on chromosome 1, a substantial number of SNPs was described for the promoter and the ten exons. However, so far, only the polymorphism -765G>C in the promoter was linked to a significantly lower promoter activity for carriers of the -765C- ► allele, displaying decreased plasma levels of the C-reactive protein in patients with coronary heart surgery (Papafili et al. 2002). How this polymorphism may eventually interfere with NSAID-induced effects still remains unclear, but because carriers of the CC-765 genotype presented with a more severe course of aspirin-induced asthma, reflected by an increased consummation of oral corticosteroids (Szczeklik et al. 2004), a possible relevance is under consideration.

Polymorphisms in Metabolizing Enzyme Genes

Many NSAIDs are hepatically metabolized by the cytochrome P450 (CYP) system. Diclofenac, ibuprofen, flurbiprofen, naproxen, piroxicam, tenoxicam, meloxicam, mefenamic acid and celecoxib are listed as substrates for one of the most important isoforms, CYP2C9. Polymorphisms in the CYP2C9 gene are recognized to account for variable NSAID pharmacokinetics. Among numerous mutations, the alleles CYP2C9*2 (Cys144, Ile359, Asp360) and CYP2C9*3 (Arg144, Leu359, Asp360) are of particular importance in the Caucasian population, due to the reduced intrinsic metabolic activity combined with a high allele frequency of 8–14% and 4–16%, respectively. For flurbiprofen, which is exclusively metabolized by CYP2C9, most of the pharmacokinetic variability could be explained by the CYP2C9 genotype, with most pronounced effects in carriers of the CYP2C9*3-allele (Lee et al. 2003). That the CYP2C9*3-allele is mostly responsible for the interindividual variability was also seen when the oral clearance of celecoxib was reduced more than two-fold in ► homozygous carriers of the CYP2C9*3 allele as compared to the non-mutated volunteers, while no significant influence was determined for the CYP2C9*2 allele (Kirchheiner et al. 2003). In the presence of non-functional CYP2C9-alleles, other cytochrome isoforms (CYP3A4) might compensate by increasing their contribution. This might be the reason why no evidence was seen that CYP2C9 mutations were a determinant for the diclofenac-induced hepatotoxicity (Aithal et al. 2000). Significant pharmacokinetic differences were also seen for racemic and S-(+)-ibuprofen between carriers of one or two CYP2C9*3 alleles and non-mutated alleles (Kirchheiner et al. 2002), while the CYP2C9*2 variant only displayed a compromised metabolic activity when found in combination with the CYP2C8*3 (K139, R399) mutation (Garcia-Martin et al. 2004). In fact, the two alleles CYP2C9*2 and CYP2C8*3 were shown to be in linkage disequilibrium (Yasar et al. 2002). Further information about CYP450 alleles is available at http://www.imm.ki.se/CYPalleles/.

The metabolism of NSAIDs further involves glucuronidation by uridine 5t'-diphosphoglucose glucuronosyl transferase (UGT) enzymes. Since most NSAIDs first undergo CYP450-mediated transformation to inactive metabolites, it is rather unlikely that UGT-alleles with a compromised catalytic activity cause a change in drug response due to drug accumulation. However, this perception may be challenged by rofecoxib, which after UGT2B15-mediated glucuronidation with minor contribution of UGT2B7 and UGT1A9 (Zhang et al. 2003), and deglucuronidation in the lower gastrointestinal tract, may cycle enterohepatically and reappear again in the plasma as rofecoxib. This explains the observed second maximum concentration peak in the concentration-time curves (Baillie et al. 2001), and serves as an example that mutations rendering the UGT less metabolically active could gain clinical importance in special circumstances. The current nomenclature can be accessed at http://som.flinders.edu.au/FUSA/ClinPharm/UGT/allele_table.html.

Substrates of the N-acetyltransferase 2 (NAT2) show three different acetylation phenotypes depending on the possession of two non-mutated alleles (fast), two mutated alleles (slow) or one non-mutated allele combined with a mutated allele (intermediate). The current knowledge that alleles are associated with a compromised catalytic function can be retrieved from

http://www.louisville.edu/medschool/pharmacology/NAT.html. NAT2 plays an important role in the detoxification of sulfasalazine metabolites; hence accumulation in slow acetylator genotypes was associated with the onset of adverse reactions such as infectious mononucleosis-like syndrome (Ohtani et al. 2003), acute pancreatitis (Tanigawara et al. 2002), discoid (Sabbagh et al. 1997) or systemic lupus erythematosus (Gunnarsson et al. 1997). Metamizol (dipyrone) also qualifies as an NAT2-substrate, but differences in drug response according to the acetylation ► phenotype have not yet been reported. The distribution of the polymorphic alleles in the NAT2 gene is an example of the relevance of the ethnic background, since frequency of genotypes associated with fast or intermediate acetylation ranges from approx. 40% in the European population to approx. 90% in the Japanese.

Impact of Non-Functional Alleles on Drug-Drug-Interaction

A patient genotyped as a compound carrier of non-functional CYP2C9*2/*3 alleles presented with normal INR values after therapeutic warfarin dosing. However, when a concomitant analgesic therapy was introduced with celecoxib, the INR rapidly increased (>10) with extensive ecchymosis (Malhi et al. 2004). The impaired warfarin-metabolizing capacity of the CYP2C9*2/*3 alleles had no clinically significant effect on the INR, but when these CYP2C9*2/*3 alleles were challenged by the second substrate celecoxib, which in addition exhibits a high affinity for CYP2C9, the metabolizing rate of (S)-warfarin via CYP2C9 rapidly decreased, while the metabolism of the 2–5 × less potent (R)-warfarin by CYP 1A2 and 2C19 was not affected. This observation should represent a possible mechanism for how a drug interaction may be elicited in the drug metabolizing system. However, in this particular observation this has to be further elucidated, since the warfarin-celecoxib interaction was not seen in healthy volunteers (Karim et al. 2000).

Celecoxib was shown to inhibit the CYP2D6-mediated metabolism of metoprolol in healthy volunteers, with a more pronounced rise in the plasma concentration-time profile in carriers of two functional alleles as compared to one allele. Such an effect is not anticipated with two mutated alleles leading to a minimum of CYP2D6 catalytic function (poor metabolizer). (Werner et al. 2003). Although little information is available of such induced drug interactions, further research may elucidate more, since over 40 drugs are listed as CYP2D6 substrates (see http://medicine.iupui.edu/flockhart/table.htm).

Aspirin-Induced Asthma

Patients with aspirin-induced asthma represent about 10% of all asthma-diseased adults. Inhibition of COX–1 leads to an excess supply of substrate for lipoxygenases which causes a surplus of bronchoconstrictory leukotrienes, of which cysteinyl-leukotrienes (Cys-LT) were determined as major mediators. The formation of Cys-LTs is regulated by the leukotriene (LT) C4-synthase gene, where a polymorphism in the promoter (-444A>C) was significantly more frequent in aspirin-induced asthma patients (Sanak et al. 1997; Sanak et al. 2000). However, since a relation of this polymorphism to disease severity or aspirin intolerance was not seen in a large cohort of asthma patients and healthy controls, the functional consequence of the C-444 genotype remains to be determined.

Conclusion

So far, little is known about the impact of pharmacogenetics on the therapeutic effect of NSAIDs. There is evidence that polymorphisms in the major drug metabolizing enzyme cytochrome P450 2C9 lead to a modified pharmacokinetic profile of its substrates, of which diclofenac, ibuprofen, naproxen and celecoxib are the most important ones. Polymorphisms in the drug targets COX–1 and COX–2 might alter drug response, but due to the preliminary character of such data, it is still too early to estimate the clinical relevance.

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NSAIDs, Pharmacokinetics

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Synonyms

Plasma Concentration Versus Time Profiles; Pharmacokinetics of the NSAIDs

Definition

The pharmacokinetics describes the journey of a drug molecule through the body. The journey includes its release from the drug product, its ► absorption into the body system, for some substances its bio-activation via ► metabolism, the ► distribution to its site of action and back into the blood, and its ► elimination from the body either via transformation into inactive metabolites, which are then excreted, or direct excretion of the active entity. The goal of pharmacokinetics is to describe the time course of the drug concentrations in the organism in order to derive dosing regimens that provide most effective clinical drug actions with least side effects.

Characteristics

NSAIDs are mainly administered orally. Formulations for intravenous, intramuscular, topical, rectal or intraocular administration are also available for some NSAIDs. The pharmacokinetics of the NSAIDs is best described by the LADME model, which describes the Liberation, Absorption, Distribution, Metabolism and Elimination of a drug. The concentration versus time profiles of the drug depends on these five processes.

The ► liberation of the active ingredient from a pharmaceutical product is mainly the result of the galenic engineering (tablet coating, tablet disintegration, particle size, etc.). Enteric- and sustained release-coatings are often used with NSAIDs to reduce their gastrointestinal toxicity. While the effectiveness of the coating for reduction of the toxicity is doubted, its influence on the absorption of the drug due to delayed release is clear. Once freed from the pharmaceutical formulation, the absorption of a drug is mainly defined by its physicochemical properties. Most of the NSAIDs are carbonic acids, or at least have an acidic function in their molecular structure. They pass the gastrointestinal wall by a passive ► diffusion process and are rapidly and extensively absorbed from the stomach and proximal small intestine, with peak plasma concentrations generally occurring within 2–3 h post-administration, or within 30 min in the case of fast release formulations. However, although absorption is extensive, some NSAIDs (e.g. diclofenac) have a low ► bioavailability because they are subject to a considerable ► First-Pass Metabolism that takes place in the intestinal wall and in the liver. Most NSAIDs are metabolised by ► phase-1 metabolic reactions such as oxidation, hydroxylation, demethylation, deacetylation, and hepatic conjunctions (► phase–2 metabolic reactions such as glucuronidation and sulphation), or both, with subsequent excretion into urine or bile. In addition, acetyl salicylic acid is deacetylated directly in the blood. The lower the bioavailability is, the fewer molecules reach the circulation and are available for transport to their site of action. Once the molecules of the NSAIDs have reached the blood, they are extensively bound to plasma proteins, especially to albumin. Their ► volume of distribution (Vd) is usually small, mainly between 0.1 and 0.3 l/kg body weight, which approximates plasma volume. After excretion into the bile, several NSAIDs undergo an ► enterohepatic recirculation. That is, they are re-absorbed from the intestine after cleavage of the phase–2-conjugates (i.e. glucuronides or sulphates) by intestinal human or bacterial glucuronidases or sulfatases. Depending on the elimination process (metabolism or excretion via the kidney or the bile), NSAIDs differ in their speed of elimination, which is usually numerically characterized by the ► elimination half-life values ($t_{½}$). The half-life of the NSAIDs can vary within and between individuals due to organ damage (i.e. kidney or liver

failure) or due to genetic polymorphisms of the enzymes involved in the metabolism of the NSAIDs. For example, some NSAIDs are metabolised via cytochrome P450 2C9 (CYP2C9), for which mutations resulting in a less-functional or non-functional enzyme have been described. Furthermore, pharmacokinetic drug-drug interactions can occur that results in inhibition of enzymes or of transporters involved in the elimination of the NSAIDs, with the consequence of altered, often decreased, rates of elimination, and thus increased half-lives of the NSAIDs (Davies and Skjodt 2000).

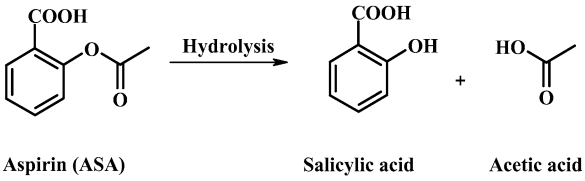
In the following, the pharmacokinetic properties will be described in more detail for the particular class of NSAIDs.

Salicylic Acid Derivatives

The salicylic acid derivatives are rapidly and completely absorbed after oral administration. The reduced bioavailability and very short elimination half-life of acetyl salicylic acid is the result of an extensive first-pass metabolism by hydrolysis of acetyl salicylic acid to salicylic acid and acetic acid (Fig. 1), which takes place while passing the gastrointestinal mucosa, in the liver and in the blood. Due to the irreversible acetylation of the amino acid serine at position 530 of the COX-1 protein in platelets, the half-life of the anti-thrombotic effect of acetyl salicylic acid is much longer than the half-life of acetyl salicylic acid in plasma. Thus, a clinically relevant aggregation of platelets will only be re-established after enough new platelets have been produced, which is several days after administration of acetyl salicylic acid, at a time when the drug has already completely been eliminated from plasma for some days.

Salicylic acid is an active metabolite of acetyl salicylic acid. It has a longer elimination half-life than acetyl salicylic acid. During treatment with high or repetitive doses of salicylic acid or diflunisal, the elimination half-life can increase due to saturation of liver enzymes involved in the metabolism (non-linear kinetic). Salicylic acid and its metabolites are excreted via the kidney. The renal elimination rate of salicylic acid is influenced by the urinary pH. Therefore, acidifying agents (e.g. ammonium chloride) decrease its excretion, while alkalinising agents such as sodium bicarbonate increase the urinary excretion. Salicylates can displace the anticonvulsants phenytoin and valproic acid from their plasma protein binding sites, and prevents the elimination of valproic acid due to inhibition of its main metabolic pathway, the β-oxidation. To prevent intoxication due to increased free plasma levels, combination of the drugs should be avoided (Brouwers and de Smet 1994; Needs and Brooks 1985).

In contrast to other salicylic acid derivatives, sulfasalazine and its active metabolite 5-aminosalicylic acid are only poorly absorbed, and therefore remain mainly within the gastrointestinal tract. Sulfasalazine and salsalat are prodrugs, and are mainly used for the treatment of ulcerative colitis and Crohnt's disease. While salsalat is an ester of two salicylic acid molecules, which is rapidly absorbed and then hydrolysed, the anti-inflammatory effect of sulfasalazine is probably attributed to its metabolite 5-aminosalicylic acid.

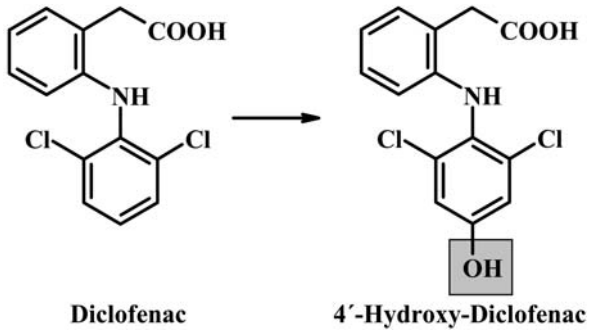


NSAIDs, Pharmacokinetics, Figure 1 Hydrolysis of acetyl salicylic acid.

Arylpropionic Acids

Chirality results when three-dimensional repositioning produces different forms (enantiomers) of the same molecule. A chiral drug exists as a pair of molecules that are each other's mirror image, called S-enantiomer and R-enantiomer. The most common example of chirality is a sp^3-hybridised tetrahydral carbon atom, to which 4 different atoms are attached (Fig. 2). Such a sp^3-hybridised tetrahedral chiral carbon atom is the common structural feature of the arylpropionic acids, and is located within their propionic acid side chain. Most of the arylpropionic acids are marketed as racemats, i.e. as mixtures of both enantiomers. In addition, pure S-enantiomers are available for ibuprofen and ketoprofen. Naproxen is available and clinically used only as S-enantiomer. This is because the S-enantiomers have been shown to possess almost the whole pharmacologic activity. However, more recent studies have also demonstrated some pharmacologic effects of the R-enantiomers.

Enantiomers of arylpropionic acids have different physical properties such as water solubility and differ in their pharmacokinetics. For example, a stereoselectivity, i.e., a different pharmacokinetic behaviour of the S- and R-enantiomers of ibuprofen and flurbiprofen has been reported (Davies and Skjodt 2000). Some arylpropionic



NSAIDs, Pharmacokinetics, Figure 2 Chirality of the arylpropionic acids.

NSAIDs, Pharmacokinetics, Table 1 Pharmacokinetics of the salicylic acid derivates

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L/kg]	CL [L /h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Salicylic acid (SA)	100	80–90	0.5–2	0.17	4.2	2–3 (–30)	No	Dose-dependent half-life due to saturation of liver enzymes involved in the metabolism
Aspirin (ASA)	68	85–95	0.4–0.5	0.15	0.6–3.6	14–20 min	Salicylic acid (SA)	Hydrolysis through non-specific esterases while passing the gut wall, in plasma and liver
Diflunisal	100	99.8	2-3	0.10	0.0066	5–20	No	Dose-dependent half-life due to saturation of liver enzymes involved in the metabolism
Sulfasalazine (Prodrug)	<15	>99.3	3–12	NR	1	4–11	5-Aminosalicylic acid (F = 10–30%, t_{Max} 10 h)	Bacteria mediated splitting in anti-inflammatory active 5-Aminosalicylic acid and sulfapyridine Half-life dependent on slow or fast acetylation by polymorphic enzyme
Salsalate (Prodrug)	100	NR	1.5	NR	NR	1.1(–16)	Salicylic acid (SA)	Rapid esterase hydrolysis in two molecules of salicylic acid in the small intestine and plasma

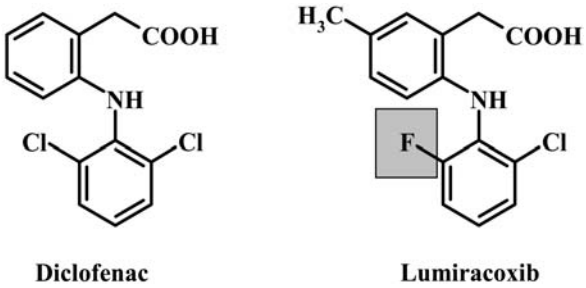
F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

NSAIDs, Pharmacokinetics, Table 2 Pharmacokinetics of the arylpropionic acid derivates

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Ibuprofen (Rac/S) *	100	98–99	1–2	0.15	0.045	1.5–3	No	Unidirectional inversion to S-(+)-enantiomer with an inversion-rate R:S=50-80% Metabolising enzymes (Phase 1): CYP2C9 Dose-dependent binding to plasma proteins
Flurbiprofen (Rac) *	100	>99	1.5	0.1	0.018	3–6	No	Unidirectional inversion to S-(+)-enantiomer Inversion-rate R:S=0-5%
Ketoprofen (Rac/S) *	81-84	98.7	0.5-3	0.11	0.072	2–4	No	Unidirectional inversion to S-(+)-enantiomer Inversion-rate R:S 10%
Naproxen (S) *	100	>99	2–4	0.10–0.16	0.0042	12–15	No	Dose-dependent binding to plasma proteins
Tiaprofenic acid (Rac) *	100	98	NR	0.4–1	0.036–0.084	3-6	No	Negligible R to S conversion upon oral administration
Fenoprofen (Rac) *	NR	>99	2	0.08–0.11	NR	1.5–3	NR	

* available as racemate and/or single S-(+)-enatiomer
F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

acids undergo a unidirectional inversion of the inactive R-enantiomer to the active S- enantiomer (Fig. 3). The extent of the inversion is variable from drug to drug and is most substantial for R-(-)-ibuprofen, while it is negligible for R-(-)-flurbiprofen (Table 2) (Geisslinger et al. 1994).



NSAIDs, Pharmacokinetics, Figure 3 Unidirectional inversion of the arylpropionic acids.

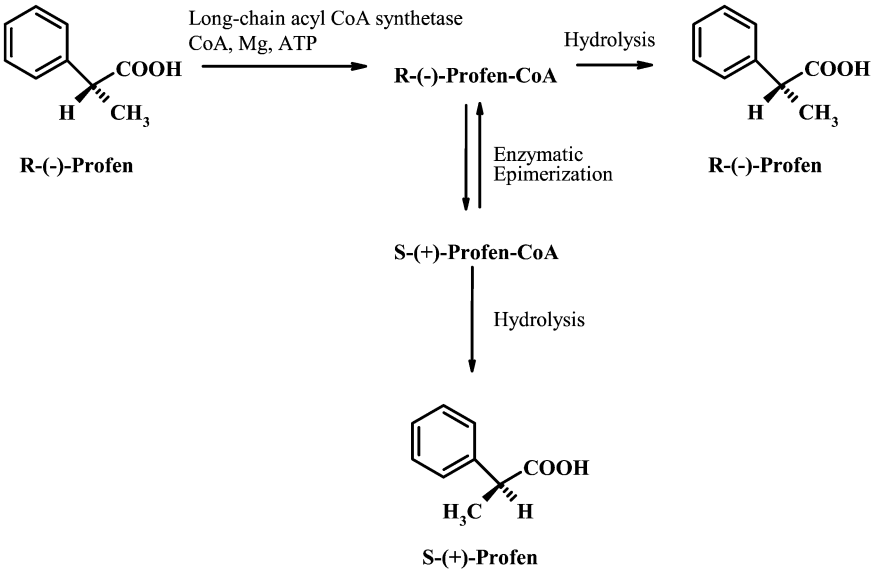
The arylpropionic acids get fast and near complete absorption. They have no extensive first-pass metabolism and their elimination is, with the exception of naproxen, quite fast. Ibuprofen and naproxen bind in a concentration-dependent manner to plasma proteins. There is an increase in the unbound fraction of the drug at doses greater than 600 mg and 500 mg, respectively, resulting in an increased ► clearance and reduced area under curve (AUC) of the total-drug. A decreased clearance of S-(+)-ibuprofen is reported in carriers of certain genetic polymorphisms, which results in a CYP2C9 enzyme with decreased or absent functionality (see ► NSAIDs, Pharmacogenetics).

Heteroaryl Acetic Acids

Diclofenac, aceclofenac, ketorolac and lumiracoxib are heteroaryl acetic acids. They are fast and nearly completely absorbed. Due to an extensive first pass metabolism, diclofenac has a decreased systemic availability. The primary metabolite of diclofenac, 4'-hydroxy-diclofenac (Fig. 4), is produced by the genetically polymorphic CYP2C9. The amount of 4'-hydroxy-diclofenac excreted in urine and bile accounts for 30% and 10–20%, respectively, of an oral dose of diclofenac (van der Marel et al. 2004). Data from experiments in laboratory animals suggest that 4'-hydroxy-diclofenac has 30% of the anti-inflammatory and antipyretic activity of diclofenac. Aceclofenac, an ester of diclofenac, appears to inhibit both COX isoforms through conversion into diclofenac and its metabolite 4'-hydroxy-diclofenac (Hinz et al. 2003). Ketorolac is a chiral NSAID and is marketed as a racemic mixture of the S-(-)- and the R-(+)-enantiomeric isoforms. The S-(-)-form possesses the analgesic and ulcerogenic activity. There is no evidence for an inversion of R-(+)-ketorolac to S-(-)-ketorolac in man, but the pharmacokinetics of ketorolac shows enantioselectivity. The S-(-)-form has a two times shorter plasma half-life and greater clearance in adults than the R-(+)-Form, and the elimination half-life of S-(-)-ketorolac seems to be further increased in children (Kauffman et al. 1999; Mroszczak et al. 1996). While the other heteroaryl acetic acids are classical NSAIDs, lumiracoxib is a selective inhibitor of COX–2. Its molecular structure is very similar to that of diclofenac (Fig. 5) and very different from the other COX–2 selective agents (diarylheterocycles). This difference is reflected in the pharmacokinetic properties of lumiracoxib, which are more similar to those of the classical acidic NSAIDs than to the diarylheterocycles.

Diarylheterocycles

The diarylheterocycles (celecoxib, rofecoxib, etoricoxib, and oxaprozin) have a higher selectivity to the COX–2 isoform compared to the classical NSAIDs. With the exception of oxaprozin, the diarylheterocycles do not possess an acidic function in their molecule. They are very lipophilic and poorly water-soluble. Since absorption of the drug molecules requires their



NSAIDs, Pharmacokinetics, Figure 4 Primary metabolite of diclofenac.

NSAIDs, Pharmacokinetics, Table 3 Pharmacokinetics of the Heteroaryl acetic acids

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Diclofenac	30–80	>99	0.37–89	0.1–0.2	0.26–0.45	1.1–1.7	4t'-Hydroxy-Diclofenac (30% activity of diclofenac in animal models)	Extensive first-pass metabolism Metabolising enzymes (Phase 1): CYP2C9, CYP3A4, CYP3A5 Prodrug of diclofenac: Aceclofenac
Tolmetin	100	99-	0.5–1	0.1	NR	2 (5)	No	Biphasic elimination with a rapid phase ($t_{1/2} = 2$ h), followed by a slow phase ($t_{1/2} = 5$ h)
Ketorolac (Rac)	80–100	>99	0.3–1	0.1–0.3	0.03	2.1–2.9(S) 3.3–6.7®	No	S-(-)-Ketorolac is the active enantiomer Stereoselective metabolism with increased clearance for the active S-(-)-enantiomer
Lumiracoxib	66–80.8	>98	1-4	7.3–10.7 (L)	NR	3-6	4'-Hydroxy-Lumiracoxib (Potency and selectivity is similar to Lumiracoxib)	Metabolising enzymes (Phase 1): CYP2C9

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

NSAIDs, Pharmacokinetics, Table 4 Pharmacokinetics of the Diarylheterocycles

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L]	CL [L/h]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Celecoxib	NR (22–40 in dogs)	>97	2–3	339–571	23.7–27.8	8–12	No	Metabolising enzymes (Phase 1): CYP2C9, CYP3A4
Rofecoxib	92–93	85	2–4	86–91	7.2–8.5	10–17	No	Metabolism: Cytosolic Reduction
Valdecoxib	83	>98	3	54.5	6	8-11	Yes (10% of the Valdecoxib dose with decreased anti-inflammatory activity)	Prodrug of Valdecoxib: Parecoxib (i.v.) Metabolising enzymes (Phase 1): CYP2C9, CYP3A4, Non-CYP450
Etoricoxib	100	NR	0.5–1.5	82–156	4.92–8.04	18.9–30.9	NR	-
Oxaprozin	95	99	2.4–3.1	0.16–0.24 (L/kg)	0.15–0.3 (L/h/kg)	41.4–54.9	No	-

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

dissolution in fluids, special formulations have had to be developed to enhance their water solubility, and thus to ensure their absorption. Compared to the classical NSAIDs, diarylheterocycles show very different pharmacokinetics, particularly the volume of distribution, the plasma clearance and the elimination half-life (Ahuja et al. 2003; Alsalameh et al. 2003).

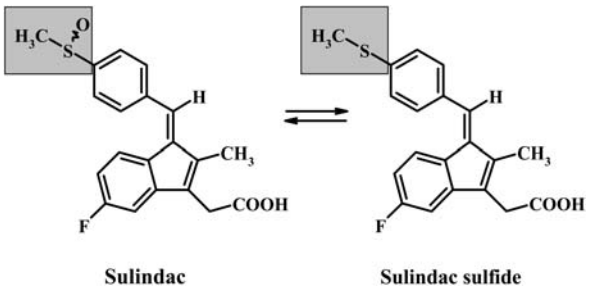
Enolic Acids

Members of the enolic acids family (piroxicam, meloxicam, tenoxicam, lornoxicam) are weakly acidic by virtue of the enolic 4-hydroxy substituent (Fig. 6). They are well absorbed and extensively bound to plasma proteins. Due to this plasma protein binding, their apparent volumes of distribution are small. They are

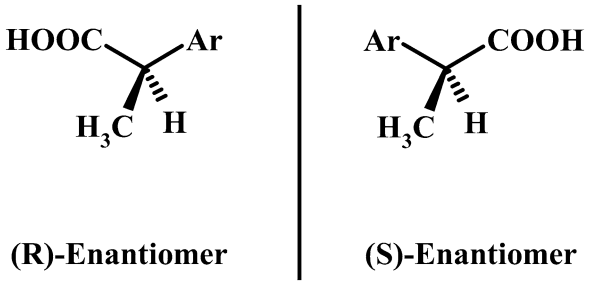
NSAIDs, Pharmacokinetics, Table 5 Pharmacokinetics of the Enolic acids

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Piroxicam	NR (100)	99	2–3	0.1–0.2	0.002–0.003	30–70	No	Prodrugs of piroxicam: Ampiroxicam, droxicam, pivoxicam
Meloxicam	100	>99.5	3–9	0.1–0.2	0.0066	20	No	Metabolising enzymes (Phase 1): CYP2C9
Tenoxicam	100	>98.5	2	0.15	0.001–0.002	49–81	No	Metabolising enzymes (Phase 1): CYP2C9
Isoxicam	100	98	10	0.1–0.2	0.3 (L/h)	30–50	No	Increased $t_{1/2}$ for about 10% of a population, eventually due to polymorphic enzyme
Lornoxicam	NR	99.7	0.5–2.5	0.1–0.2	1.5–3.4 (L/h)	3–5	NR	Metabolising enzymes (Phase 1): CYP2C9
Azapropazone	60-100	>99.5	3–6	8.4–15.4 (L)	0.48-0.73 (L/h)	11.5–17.1	NR	-
Phenylbutazone	90	>98	NR	0.02-0.15	0.09 (L/h)	29–175	Oxyphenbutazone γ-Hydroxyphenylbutazone	Dose-dependent half-life
Oxyphenbutazone	NR	97–98	NR	0.15	NR	27–64	NR	-

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculated the true Vd are usually not available and the volumes are derived from data after oral drug administration and have therefore be corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma



NSAIDs, Pharmacokinetics, Figure 5 Structural similarity of lumiracoxib to classical NSAIDs.



NSAIDs, Pharmacokinetics, Figure 6 Acidic nature of piroxicam.

mainly eliminated by hepatic metabolism (Olkkola et al. 1994). The polymorphic CYP2C9 provides the major catabolic pathway for tenoxicam and meloxicam, and are therefore candidates for an altered pharmacokinetic due to genetic polymorphisms (see ► NSAIDs, Pharmacogenetics). The elimination is usually slow. The elimination half-lives of the oxicams are long, with the exception of lornoxicam. Therefore, the oxicams have a tendency to accumulate in patients.

Phenylbutazone and oxyphenbutazone tend to accumulate due to the slow metabolism and renal elimination. In addition, they have a high potential to interact with other drugs, particularly with oral anticoagulants, anticonvulsants and oral antihyperglycaemic agents, by either inhibiting metabolic pathways or by displacement from plasma protein binding sites (Brouwers and de Smet 1994).

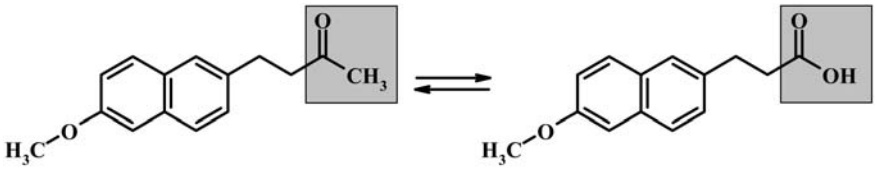
Indole and Indene Acetic Acids

Indomethacin, its prodrug acemetacin, sulindac and etodolac are all accounted to the indole and indene acetic acids. Their bioavailability is high and their binding to plasma proteins after absorption is extensive. They undergo extensive enterohepatic circulation, which results in a prolonged elimination half-life compared to the other NSAIDs because the already eliminated drug is re-absorbed. About 60% of an oral dose of indomethacine is excreted in the urine, while about 40% is excreted in the faeces after biliary secretion (Helleberg 1981).

NSAIDs, Pharmacokinetics, Table 6 Phramacokinetics of the Indole and indene acetic acids

Drug	F [%]	PB [%]	t_{Max} [h]	Vd [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Indomethacin	100	>90	1–2	0.12	0.044–0.11	3–11	No	Biphasic elimination due to Enterohepatic cycling Prodrug of Indomethacin: Acemetacin
Sulindac (Prodrug/Rac)	>90	93.1	1	NR	NR	1.7–7	Sulindac sulfide (PB=95.4, t_{Max} = 2–4, $t_{1/2}$ = 16–18)	Enterohepatic cycling Slower elimination in liver failure
Etodolac (Rac)	(100)	99	1–2	1.6(S) 0.21®	0.3(S) 0.02®	4.3(S) 6.6®	No	S-(+)-Etodolac is the active enantiomer Less binding to plasma proteins, higher volume of distribution and clearance for S-(+)-Etodolac

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma



Nabumeton 6-Methoxy-2-naphtylacetic acid (6-MNA)

NSAIDs, Pharmacokinetics, Figure 7 Bioactivation of sulindac.

NSAIDs, Pharmacokinetics, Table 7 Pharmacokinetics of the alkanones

Drug	F [%]	PB [%]	t_{Max} [h]	Vd/F [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Nabumetone[1] (Prodrug)	80 (35)*	>99	2.5–4	0.83	NR	20–30	6-methoxy–2-naphthylacetic acid (6-MNA)	Nabumetone is inactive, but is rapidly converted to the active 6-MNA 80% of a radiolabeled nabumetone dose can be found as metabolites in urine, 35% of nabumetone gets converted in the active 6-MNA

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

[1] Kinetic Parameters related to 6-MNA

Sulindac has a chiral sulphur atom and is marketed as racemat. It is a prodrug and is bioactivated in the kidney and liver to its active sulphide metabolite (Fig. 7), which has an increased elimination half-life compared to sulindac. It tends to accumulate after repeated administration, with an even greater tendency in elderly patients (Davies and Watson 1997). Etodolac is a chiral NSAID due to a chiral carbon atom and is marketed as the racemate. Similar to other chiral NSAIDs, it possesses some unique disposition features due to its stereoselective pharmacokinetics. The volume of distribution for the racemic etodolac is greater than that of other NSAIDs, mainly because of the extensive distribution of the active S-(+)-etodolac. Therefore, the concentrations of the inactive R-(-)-enantiomer are about 10-fold higher than the active S-(+)-enantiomer in plasma (Brocks and Jamali 1994).

Alkanones

After absorption from the gastrointestinal tract, the prodrug nabumetone undergoes an extensive hepatic metabolism to its active metabolite 6-methoxy–2-naphthylacetic acid (6-MNA) (Fig. 8), which is structurally related to naproxen. Approximately 35% of a single dose is converted into 6-MNA. Co-administration of food seems to increase the bioavailability of nabume-

NSAIDs, Pharmacokinetics, Table 8 Pharmacokinetics of the sulfanilides

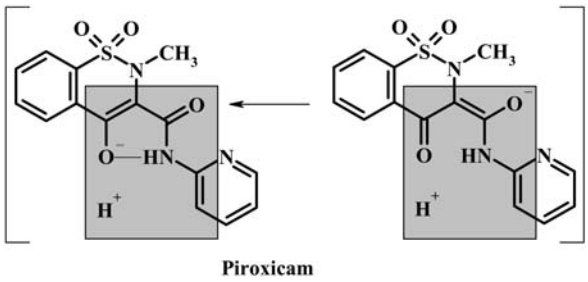
Drug	F [%]	PB [%]	t_{Max} [h]	Vd/F [L/kg]	CL [L/h/kg]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Nimesulide	NR	99	1.2–2.7	0.18–0.39	0.03–0.11	1.8–4.7	Yes, NR	Dose reduction (4–5 ×) in patients with hepatic impairment needed, due to increased elimination half-life

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma

NSAIDs, Pharmacokinetics, Table 9 Pharmacokinetics of the anthranilic acids

Drug	F [%]	PB [%]	t_{Max} [h]	Vd/F [L/kg]	CL [L/h]	$t_{1/2}$ [h]	Active Metabolites	Remarks
Mefenamic acid	NR	99	2-4	1.06	21.23	2–4	NR	Metabolising enzymes (Phase 1): CYP2C9
Meclofenamat sodium	NR	>99	0.5–2	0.13–0.62	7.6–20.5	0.8–5.3	3-Hydroxymethyl-MA (20% activity of MA, $t_{1/2}$ = 15 h)	Extensively metabolized to the still active metabolite 3-hydroxymethyl-MA
Flufenamic acid	NR	NR	1.5	NR	4.8–9	5–22	NR	Large interindividual variations in the pharmacokinetic parameters

F: oral bioavailability, PB: binding to plasma proteins, t_{Max}: time from administration until maximum plasma concentrations are reached, Vd/F: volume of distribution, divided by bioavailability (because data from systemic administration that are needed to calculate the true Vd are usually not available, and the volumes are derived from data after oral drug administration, and have therefore been corrected for bioavailability), CL. Drug clearance, describing the speed of its elimination from the body, $t_{½}$: half-life in plasma



NSAIDs, Pharmacokinetics, Figure 8 Hepatic activation of nabumetone.

tone and subsequent the appearance of 6-MNA. 6-MNA is highly bound to plasma proteins.

Sulfanilides

Nimesulide inhibits preferably the COX–2 enzyme. Its selectivity for the COX–2 enzyme seems to be as high as that of celecoxib. It is largely eliminated via the metabolism into several minor active metabolites with anti-inflammatory and analgesic actions. Hepatic insufficiency decreased the elimination of nimesulide pharmacokinetics, and therefore a dose-reduction (4–5 ×) for patients with hepatic impairment is required (Bernareggi 1998; Warner and Mitchell 2004).

Anthranilic Acids

The anthranilic acids are structurally related to the salicylic acids and heteroaryl acetic acids. Their physicochemical properties and their analgesic activities are very similar to the other NSAIDs. Since they do not show comparable anti-inflammatory activity, but have a similar occurrence of side-effects, their application in the clinic is restricted.

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NSAIDs, Side Effects

► NSAIDs, Adverse Effects

NSAIDs, Survey

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Synonyms

Non-steroidal anti-inflammatory drugs NSAIDs; Anti-Inflammatories

Definition

Non-Steroidal Anti-inflammatory Drugs (► NSAIDs) are a group of drugs derived from salicylates, which occur naturally in the bark of the willow tree. They encompass salicylic acid (aspirin) and a variety of more recent, synthesized agents.

Characteristics

NSAIDs have analgesic, anti-inflammatory, anti-pyretic properties, and inhibit the aggregation of thrombocytes. They have traditionally been used to treat pain and inflammation. They do not alter the disease process that is giving rise to pain, but interfere with the mechanisms that produce pain and inflammation.
The original NSAID was salicylic acid, marketed as aspirin. It has been synthesized by Bayer for over 100 years. As the mechanism of action of NSAIDs has been progressively elucidated, new agents have been developed, giving rise to different families of NSAIDs.

Mechanism of Action

The therapeutic and adverse effects of NSAIDs result from decreased production of prostaglandins from arachidonic acid, due to the inhibition of the cyclo-oxygenase (COX). The COX enzyme has two isotypes: COX 1 and COX 2. COX 1 is constitutively expressed by various tissues, including the gastrointestinal tract, the kidney and the platelet, where its functions include preserving the gastric mucosa, diminishing renal vascular resistance, and maintaining homeostasis respectively. COX 2 is an enzyme induced by injury, and produces large amounts of prostanoids involved in the pain and inflammation pathways (Lipsky 1999).
Non-selective, earlier NSAIDs (e.g. Diclofenac, Ibuprofen, Indomethacin, and Naproxen) inhibit both COX 1 and COX 2 isotypes. Newer agents (e.g. Celecoxib, Rofecoxib) specifically inhibit the COX 2 isotype.
Central analgesic effects, independent of COX inhibition, have also been described for NSAIDs, but their mechanisms are not well understood. Analgesic effects are a combination of central and peripheral actions.

Routes of Administration

Oral, rectal, intramuscular, intravenous, and topical routes of administration are available. The oral is the preferred route.

Applications

NSAIDs are commonly used for a variety of pain states, including somatic and visceral pain. They are used temporarily for immediate relief of acute pain, such as occurs after muscle sprains, gout, and dysmenorrhoea; for headache; and for post-operative pain. They are used long-term for persisting pain, such as occurs in osteoarthritis, rheumatoid arthritis, and other arthritides. Amongst individuals over the age of 65, the use of NSAIDs is in the order of 20%, due to the common occurrence of osteoarthritis (Day et al. 1999).

Side Effects

Side effects of NSAIDs are related to the inhibition of the constitutively expressed COX 1 enzyme, which in turn suppresses the synthesis of prostanoids that have several important homeostatic functions. Gastric ulceration, inhibition of platelet aggregation, and renal dysfunction are the most important side effects.
Prostaglandin E is known to protect the gastro-intestinal mucosa and limit gastric acid output (Raskin 1999).Gastroduodenal ulceration is the most common side effect of non-specific COX 1 and COX 2 inhibiting NSAIDs, with up to 2% of patients taking NSAIDs for 12 months developing a significant gastrointestinal bleed (Hawkey et al. 2000). The prevalence of gastric and duodenal ulcers varies from 9% to 22%, with 1 in 10 being complicated by obstruction, perforation or haemorrhage (Raskin 1999). In studies assessing gastroduodenal ulceration using endoscopy, approximately 45% of patients taking a non-specific NSAID developed an ulcer greater than 3 mm in length, compared to approximately 9% taking a specific COX 2 NSAID (Hawkey et al. 2000).
In Australia, with a population of 18 million, it is estimated that there are 4500 hospital admissions per year for serious upper gastro-intestinal side effects,

and it is estimated that 10% of these may die with between 200 and 400 deaths per annum (Day et al. 1999). Estimates in the United States of NSAID-induced gastroduodenal injury are: 107,000 hospitalisations and approximately 16,000 deaths per year (Singh and Rosen-Ramey 1998).

Prevention of gastroduodenal side-effects center on the use of proton pump inhibitors such as omeprazole, H2 receptor agonists such as ranitidine, and the PGE1 analogue misoprostol. Omeprazole seems the most effective in prophylaxis and treatment of NSAID-induced gastroduodenal ulceration (Raskin 1999). Prophylaxis is, however, not cost-effective for all patients, with the cost-effectiveness of misoprostol estimated to be £27,300 (sterling) per gastro-intestinal event avoided (Freemantle 2000).

NSAIDs can inhibit normal platelet function. Thromboxane A2 is a platelet activator that is suppressed by COX 1 inhibition. Aspirin irreversibly inactivates the COX enzyme and its action lasts for the lifespan of the platelet, 7–10 days. Other NSAIDs are reversible inhibitors of COX, and durations of action depend on clearance of drug from the circulation. Bleeding is not a significant side effect, as thromboxane A2 is only one of several mediators of platelet activation (Schafer 1999). The anti-thrombotic effect is important in the presence of coexisting bleeding disorders, and the simultaneous use of alcohol or anticoagulants.

The role of renal prostaglandin production for maintenance of stable renal haemodynamic function is limited. The prevalence of nephrotoxicity from NSAIDs is relatively low, but the risk is greater when renal perfusion is reduced, as in the aged population, in cardiovascular disease, and during dehydration. In these circumstances, a variety of clinical syndromes can include fluid and electrolyte imbalance, acute renal dysfunction, nephrotic syndrome, interstitial nephritis, and renal papillary necrosis (Whelton 1999).

NSAIDs adversely affect blood pressure control for those taking angiotensin converting enzyme inhibitors, diuretics and beta blockers. The risk of developing congestive cardiac failure also increases with NSAIDs in patients receiving diuretics.

Efficacy

Systematic reviews have found no important differences in analgesic effect between different NSAIDS (Gotzsche 2000), and the newer specific COX 2 inhibitors are of similar efficacy to older NSAIDs (Cannon et al. 2000). However, for specific conditions, the data on efficacy varies.

A Cochrane review for back pain (Van Tulder et al. 2003) collected evidence from 51 trials. It found that NSAIDs are more effective than placebo for short-term symptomatic relief in patients with acute low back pain. Sufficient evidence on chronic low back pain is still lacking. Qualitative analysis showed there is conflicting evidence that NSAIDs are more effective than paracetamol for acute low back pain.

For osteoarthritis of the knee, a review of randomised controlled trials (Towheed and Hochberg 1997) concluded that NSAIDs were superior to placebo in all short-term trials. Acetaminophen was also found to be superior to placebo, and comparably efficacious to low-dose naproxen and ibuprofen. Few studies have shown superiority of NSAIDs when compared to other analgesics (Watson et al. 2003).

A Cochrane review of lateral elbow pain concluded there is insufficient evidence to recommend or discourage the use of oral NSAIDs to relieve lateral elbow pain (Green et al. 2003). They found there was some evidence for the use of topical NSAIDs, and that a trial comparing oral administration with topical administration has not been performed.

Cost

The high comparative cost of newer COX 2 selective NSAIDs compared to non-selective older NSAIDs would significantly increase health care costs. The number needed to treat has been calculated as 133 for 6 months regular therapy with a COX 2 rather than a traditional NSAID to avoid a serious gastrointestinal event, and 1333 to avoid a death. It appears that it is not cost effective to change all patients from older non-selective medications to newer COX 1 selective agents.

Indications

In most conditions such as osteoarthritis, back pain, muscle strains and tendon strains it is well accepted that simple analgesics should be tried before trying NSAIDs. The American College Of Rheumatology guidelines (ACR subcommittee on Osteoarthritis Guidelines 2000) recommend acetaminophen as first-line therapy for the treatment of symptomatic osteoarthritis due to the significant side effects of NSAIDs, and the lack of data confirming the superior efficacy of NSAIDs over simple analgesics.

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NSAR

► Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NST

► Neurosensory Testing

NT-3 Neurotrophin 3

Definition

Neurotrophic factor that belongs to the Neurotrophin family.

► Spinal Cord Nociception, Neurotrophins

NTT

► Attributable Effect and Number Needed to Treat

Nuclear Magnetic Resonance

► Magnetic Resonance Imaging

Nucleoplasty

Definition

A non-heat driven process using Coblation technology, where a bipolar radiofrequency device is used to dissolve the disc nucleus, thus decreasing the disc volume and decompressing the disc.

► Discogenic Back Pain

Nucleotide

Definition

Originally a combination of a (nucleic acid) purine or pyrimidine, one sugar (usually ribose or deoxyribose), and a phosphoric group; by extension, any compound containing a heterocyclic compound bound by an N-glycosol line (e.g. adenosine monophosphate, NAD+).

► Headache Attributed to a Substance or its Withdrawal

Nucleotide Receptors

► Purine Receptor Targets in the Treatment of Neuropathic Pain

Nucleus Accumbens

► Nociceptive Processing in the Nucleus Accumbens, Neurophysiology and Behavioral Studies

Nucleus Caudalis DREZ

► DREZ Procedures

Nucleus Gelatinosus

► Nucleus Submedius (SM)
► Spinothalamocortical Projections from SM

Nucleus Gracilis

Definition

An area of cell bodies within the medulla that receive inputs from large afferent fibers; relays touch and vibration sensation.

► Peptides in Neuropathic Pain States

Nucleus Pulposus

Definition

The nucleus pulposus is a centrally located gelatinous mass that comprises of the central portion of the intervertebral disc. It serves to resist compressive forces and allows the spine to have increased mobility. The nucleus pulposus is encircled by layers of collagen referred to as the annulus fibrosis. Together the nucleus pulposus and the annulus fibrosis comprise the intervertebral disc.

- ► Cytokines as Targets in the Treatment of Neuropathic Pain
- ► Evoked and Movement-Related Neuropathic Pain

Nucleus Raphe Magnus

Definition

The nucleus raphe magnus is one of several midline structures in the brainstem that contain serotonergic neurons, among others. Neurons in this region are hypothesized to provide descending modulation of pain.

- ► Stimulation Produced Analgesia
- ► Vagal Input and Descending Modulation

Nucleus Submedius (SM)

Definition

A small oblong nucleus in the medial thalamus postulated to play a role in nociception, because trigeminal and spinal nociceptive neurons project to this thalamic region.

- ► Spinothalamocortical Projections from SM

Nucleus Tractus Solitarius

Definition

Bilateral sensory nuclei of the caudal medulla that receive input from several cranial nerves including the facial nerve (CN VII), glossopharyngeal nerve (CN IX), and the vagus nerve (CNX).

- ► Vagal Input and Descending Modulation

Number Needed to Treat

Synonyms

NNT

Definition

This is a measure of clinical meaningfulness in clinical trials, and is defined as the number of patients needed to be treated to obtain one patient with moderate or better improvement (50% or greater improvement) over and above placebo. This is a useful concept that allows the comparison, efficacy of analgesics that have different modes of action, as well as those from similar groups. It describes the magnitude of the difference between the active drug and the control. It can be calculated from:

$NNT = 1/((IMP_{act}/TOT_{act}) - (IMP_{con}/TOT_{con}))$,

Where:

IMP_{act}= number of patients given active treatment achieving target (e.g. 50% pain relief);

TOT_{act}= total number of patients given active treatment;

IMP_{con}= number of patients given control treatment achieving target;

TOT_{con}= total number of patients given control treatment.

The NNT of oxycodone 15 mg is 2.3.

- ► Antidepressants in Neuropathic Pain
- ► Attributable Effect and Number Needed to Treat
- ► Central Pain, Pharmacological Treatments
- ► Postherpetic Neuralgia, Etiology, Pathogenesis and Management
- ► Postoperative Pain, COX-2 Inhibitors
- ► Postoperative Pain, Oxycodone

Number Needed to Harm

Definition

The number of patients needed to treat with a certain drug before one patient will experience a defined degree of side effects, e.g. drop out of the drug trial due to side effects. It is calculated as the reciprocal value of the difference in drop-out rate on active treatment and placebo.

- ► Antidepressants in Neuropathic Pain

Numerical Rating Scale

Synonyms

NRS

Definition

A NRS allows a person to describe the intensity of his/her pain as a number usually ranging from 0 to 10, where "0" means "no pain" and "10" means pain as "bad as it could be".

- ► Central Pain, Outcome Measures in Clinical Trials

Nursing Home Residents

Definition

Chronic pain is more frequent in nursing home residents than in a community sample. Uncontrolled pain is a frequent cause of admission to nursing homes.

► Psychological Treatment of Pain in Older Populations

Nutraceuticals

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Synonyms

Glucosamine; chondroitin; Avocado-Soybean-Unsaponifiables

Definition

► Nutraceuticals are loosely defined as foods with a health benefit. They are naturally occurring substances that can be used as drugs in order to treat specific symptoms, or to modify disease processes. In the field of osteoarthritis, four nutraceuticals have been recognised and studied: ► glucosamine, ► chondroitin, glycosaminoglycan polysulfuric acid, and ► Avocado-Soybean-Unsaponifiables (ASU).

Glucosamine is a hexosamine sugar that is a component of almost all human tissues. It is one of the two molecules that form the repeating units of certain glycosaminoglycans, which in turn form the matrix of all connective tissues (Deal and Moskowitz 1999). Glycosaminoglycans, and therefore glucosamine, form a large component of articular cartilage, which is the tissue that is primarily damaged in osteoarthritis.

Chondroitin sulphate is the principal glycosaminoglycan found in articular cartilage. It is composed of a long unbranched polysaccharide chain, with a repeating disaccharide structure of N-acetyl galactosamine and glucuronic acid (Deal and Moskowitz 1999). Chondroitin sulphate is a strongly charged polyanion, which endows cartilage with water-binding properties. Functionally, this allows the cartilage matrix to absorb compression forces, and thereby protect the underlying bone from damage.

Glycosaminoglycan polysulfuric acid is an extract from bovine cartilage and bone marrow, which contains a variety of glycosaminoglycans, including chondroitin and chondroitin sulphate (Pavelka et al. 1995; Pavelka et al. 2000).

ASUs are unsaponifiable fractions of one-third avocado oil and two-thirds soybean oil (Maheu et al. 1998; Blotman et al. 1997; Appleboom et al. 2001).

Characteristics

Mechanism

Glucosamine has been shown to reach the articular cartilage after oral, intra-muscular and intravenous administration (Deal and Moskowitz 1999; McAlindon et al. 2000; Pavelka et al. 2003). It is preferentially incorporated by human chondrocytes into GAGS (Deal and Moskowitz 1999; Pavelka et al. 2003), and stimulates the synthesis of proteoglycans (Deal and Moskowitz 1999; McAlindon et al. 2000; Pavelka et al. 2003; Reginster et al. 2001).

Glucosamine and chondroitin sulphate have been shown to have anti-inflammatory effects (Deal and Moskowitz 1999; Pavelka et al. 2003; Reginster et al. 2001), positive effects on cartilage metabolism *in vitro*, and anti-arthritic effects in animal models (Deal and Moskowitz 1999; McAlindon et al. 2000; Towheed et al. 2002). These results suggest possible structure-modifying and disease-modifying roles for glucosamine and chondroitin in osteoarthritis.

In laboratory studies, glycosaminoglycan polysulfuric acid has been found to stimulate cartilage metabolism, and to inhibit the catabolic effects of interleukin-1 (Pavelka et al. 1995; Pavelka et al. 2000).

ASUs have also shown some anti-osteoarthritis properties both *in vitro* and *in vivo* (Maheu et al. 1998; Blotman et al. 1997; Appleboom et al. 2001).

Application

Nutraceuticals have been promoted for the treatment of osteoarthritis, on the grounds that they are natural substances that might promote healing or impede further deterioration of damaged cartilage.

Nutraceuticals are mainly taken by the oral route, as a tablet capsule or powder, and are also available as a cream. Although the majority of trials have focused on osteoarthritis of the knee, nutraceuticals are marketed for relief in a wide variety of conditions e.g. arthritis pain, fibromyalgia, and joint swelling.

Efficacy

One pragmatic (Deal and Moskowitz 1999) and four systematic reviews (McAlindon et al. 2000; Towheed et al. 2002; Richy et al. 2003; Leeb et al. 2000) in the last five years on the use of glucosamine and chondroitin in osteoarthritis reached similar conclusions. A number of randomised controlled trials showed benefits from these agents greater than that of placebo, in terms of reduction of pain and increased function. Others showed equivalent or better efficacy than treatment with ► non-steroidal anti-inflammatory drugs (NSAIDs). However, the reviews offer caution regarding the methodological problems associated with many of the studies, and advise that the treatment effects are probably exaggerated.

Important points raised by these reviews include:

- The ► effect-sizes in the larger and better quality studies are smaller than those of other studies.
- Many trials suffer from inadequate blinding and absence of intention-to-treat analysis of results.
- Publication bias may apply, in that studies with statistically significant and positive results are more likely to be published than studies with negative results.
- Manufacturer support was prevalent in many of the early trials.

These problems are not unique to trials of nutraceuticals, for they have also been noted in trials of drugs for osteoarthritis. Nevertheless, they constitute grounds for caution when interpreting or stating the results of trials. A further factor is that all of the early trials with positive results were of European origin. Later studies, conducted in the USA and in England, have found glucosamine to be no more effective than placebo for the relief of pain (Rindone et al. 2000; Hughes and Carr 2002).

Less contentious is the effect of glucosamine on the prevention of disease progression. Two randomised controlled trials have assessed the long-term effects of glucosamine sulphate on knee osteoarthritis (Pavelka et al. 2003; Reginster et al. 2001). Both studies compared the effects of placebo with that of 1500 mg glucosamine sulphate daily for three years. In both studies, patients treated with glucosamine showed greater reductions in pain, and greater improvements in function, as measured by the Western Ontario and McMaster Universities osteoarthritis index (WOMAC). Both studies also demonstrated significantly less loss of joint space width in those patients treated with glucosamine. The second study (Pavelka et al. 2003) showed that the ► NNT for preventing clinically substantial loss of joint space was 11.

For glycosaminoglycan polysulfuric acid the evidence has not been favourable. For the relief of pain it is no more effective than placebo (Pavelka et al. 1995). It does not protect against loss of joint space (Pavelka et al. 2000). Three studies of ASUs found greater reductions in pain and greater improvements in function than those following treatment with placebo ((Maheu et al. 1998; Blotman et al. 1997; Appleboom et al. 2001). These agents also had significant effects in reducing the need of patients to use NSAIDs.

Safety

No major toxicity problems have emerged with the use of these nutraceuticals. In particular, glucosamine has been shown to be safe in the two trials with three-year follow-up (Pavelka et al. 2003; Reginster et al. 2001). Side effects were similar to those of placebo, and no specifically adverse effects were uncovered.

Some chondroitin preparations are derived from bovine cartilage. Due to the recent European epidemic of bovine spongioform encephalopathy (BSE) and its transmission to humans (resulting in variant Creutzfeldt-Jakob disease), all bovine derived products are being re-evaluated for potential transmission risks. On the List of Tissues with Suspected Infectivity (World Health Organisation), bovine cartilage is listed as Category IV (no detectable infectivity). Most other commercially available chondroitin products are derived from shark cartilage.

Conclusions

The evidence for the use of nutraceuticals in the treatment of OA of the knee is growing. However, doubts remain about their effect-size when compared with placebo; and the efficacy of these agents has not been compared with other regimens of long-term treatment of osteoarthritis. It is still not evident if they are a cost–effective substitute for treatment with NSAIDs or exercise; or if they are a worthwhile addition to such treatment. Nor has their efficacy been determined for osteoarthritis of joints other than those studied to date, or for other painful conditions.

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Nutriceutical

Definition

The use of supplements and other substances like herbs and foods that are ingested or otherwise absorbed into the tissues to promote health.

► Alternative Medicine in Neuropathic Pain

Nutritional Neuropathies

► Metabolic and Nutritional Neuropathies

NVNP

► Non-Systemic (Isolated) Vasculitic Neuropathy