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Chao Ma
Yuguang Huang *Editors*

Translational Research in Pain and Itch

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Preface

The past decade has witnessed exciting advances in the basic research of pain and itch – both are our most basic yet still mysterious sensations. Clinically, the treatment of prolonged and intractable pain (chronic pain) and itch (pruritus) cost billions of dollars every year worldwide while the results are often unsatisfying or accompanied with serious side effects. Despite the slow progress in drug discovery, scientists all over the world have recently acquired more insights into the mechanisms underlying pain and itch in both physiological and pathological conditions. The gaps between basic research and clinical application are eagerly waiting to be filled by translational research.

This book provides a comprehensive review of recent advances in the translational research on pain and itch. The contributing authors are world-renowned scientists and have made important discoveries in the relevant field of research. Their findings not only shed light on the mechanisms but also pave the way for developing novel strategies for the effective and safe treatment of chronic pain and pruritus. Hopefully not long from now, medical practitioners can be more confident and patients can be more optimistic when facing these annoying (and often terrible) conditions.

We sincerely appreciate all the contributing authors, our editorial team from the Joint Laboratory of Anesthesiology and Pain in Peking Union Medical College, and the Springer publisher. This book would not be possible without their time and effort.

Beijing, China

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Chapter 1
Assessment of Itch and Pain in Animal Models and Human Subjects

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Abstract For the past century, scientists have developed a variety of methods to evaluate itch and pain in both animal models and human subjects to throw light on some of the most important pathways mediating these unpleasant sensations. Discoveries in the mechanisms underlying itch and pain in both physiological and pathological conditions relied greatly upon these studies and may eventually lead to the discovery of new therapeutics. However, it was a much more complicated job to access itch and pain in animal models than in human subjects due to the subjective nature of these sensations. The results could be contradictory or even misleading when applying different methodologies in animal models, especially under pathological conditions with a mixed sensation of itch and pain. This chapter introduces and evaluates some of the classical and newly designed methodologies to access the sensation of itch and pain in animal models as well as human subjects.

Keywords Itch • Pain • Animal model • Human subject

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1.1 Introduction

Itch and pain are both unpleasant sensations that may indicate actual or potential tissue damage. Despite the ability to clearly discriminate between itch and pain in human subjects, it has never been an easy job to access such information in animal models. Itch, often defined as a "desire to scratch," is actually a multifaceted sensation. Although the general discourse mainly deals with histaminergic and nonhistaminergic itch (Davidson and Giesler 2010; Johanek et al. 2007), more sub-classifications could be beneficial. Pain faces a similar situation. In 1979, the International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Iasp 1979). This definition also clearly indicates that pain is a multidimensional experience. This chapter provides an overview of the methods used to assess experimentally induced itch and pain and analytically outlines the recently introduced animal models and human study protocols for itch and pain that have been reported in the research literature (Andersen et al. 2015).

1.2 Assessment of Itch in Animal Models and Human Subjects

Itch, also known as pruritus, is an unpleasant sensation that may prompt the sufferer to scratch the affected area that is aimed at alleviating or eliminating the effects of the stimulus and the on-going irritation or discomfort (Frese et al. 2011; Patel and Yosipovitch 2010; Shim and Oh 2008).

1.2.1 Assessment of Itch in Animal Models

1.2.1.1 Assessment of Itch in the Nape of Mice

An intradermal injection of histamine and capsaicin each elicited hind limb scratching behavior when injected into the nape of the neck of the mouse, indicated that there may be only one type of behavior toward an injection into the nape of the neck (Shimada and LaMotte 2008).

1.2.1.2 Assessment of Itch in the Cheek of Mice

In 2008, LaMotte's study modified Kuraishi model (Kuraishi et al. 1995) by administering intradermal injection of histamine and capsaicin, known to evoke predominantly itch and pain, respectively, in humans; each elicited hind limb scratching

behavior when injected into the nape of the neck of the mouse. When the same chemicals were injected into the cheek of the mouse, there were two site-directed behaviors: histamine elicited scratching with the hind limb, capsaicin evoked wiping with the forelimb, no crossover any more as happened in the nape intradermal injection (Shimada and Lamotte 2008).

Other pruritic chemicals, such as chloroquine, and cowhage spicules evoked both scratching and rubbing of the face, indicating a mixture of itch and nociceptive sensations after the application of these stimuli (Akiyama et al. 2010; Kim et al. 2011). Thus, the "cheek model" allows the animal to report differential responses to the application of a stimulus similar to the multiple choices available to humans. This could be advantageous in evaluating whether candidate therapeutic drugs applied to mice will be selective for blocking itch or pain in humans.

The cheek model might also be useful in determining whether an agonist selective for a specific isoform of a receptor elicits one type of site-directed behavior rather than a mixture of behaviors that might be evoked by a less selective chemical that activates multiple isoforms. For example, scratching the site of a histamine injection (Dunford et al. 2007) or an allergic contact dermatitis (Rossbach et al. 2009) on the rostral back of the mouse was reduced but not eliminated by either an H1 or an H4 antagonist. If the experiment were repeated on the cheek, it might be possible to determine whether the reduction produced by each antagonist was more related to pain, to itch, or to both.

1.2.1.3 Assessment of Itch in the Legs of Mice

In 2011 LaMotte's study, when different doses of histamine or capsaicin were injected into the calf of the mouse, there were two site-directed behaviors: capsaicin produced mainly licking, whereas histamine elicited more of a mixture of responses with more biting than licking for most animals (Lamotte et al. 2011), in which biting was characterized by contact of the incisors with the skin in a fairly high-frequency and low-excursion motion of the head. In contrast, licking was characterized by repeated protrusions of the tongue toward the skin over a longer excursion and lower frequency that could be readily distinguished from biting.

1.2.1.4 Assessment of Itch in the Eyes of Mice

Compared to other models described above, the eye model is relatively new and less used for itching research. However, it shows great potential for its obvious advantage: easy for experimenter to establish and measure. In fact, the eye model is mainly used in allergic conjunctivitis studies. The allergic conjunctivitis eye model was first established in guinea pig and then developed in mice (Laidlaw et al. 2002). Acute or chronic allergic conjunctivitis is induced by the instillation of histamine and other contact sensitizers (Nakano et al. 2009). Like many other models, scratching behavior is still the indication of an itch sensation in eye model. It has been

reported that ICR mice show the most marked scratching behavior in response to histamine; therefore, ICR mice are considered the most suitable strain for studying mediators and/or mechanisms for itching (Inagaki et al. 2001). A bout of eye scratching was defined as when a mouse stretched its hind-paw on the treated side toward its eye, leaned its head toward the paw, rapidly scratched its eye several times for approximately 1 s, and then lowered its hind-paw (Andoh et al. 2012).

The allergic conjunctivitis model might also be used to find candidate therapeutic drugs because it shows different symptoms by inhibiting specific receptors by selective antagonists. For example, histamine H1 receptor antagonists inhibited not only eye scratching behavior but also allergic-like symptoms such as edema and hyperaemia, while the histamine H4 receptor antagonist inhibited only scratching behavior induced by histamine (Nakano et al. 2009).

1.2.1.5 Assessment of Itch in the Rats

Similar methods have been applied to assess itch sensing in rats, although with fewer studies up to date (Table 1.1). In the cheek model, rats present the same behavioral responses to pruritogens and algogens as mice, that is, hind limb scratching and fore limb wiping, respectively (Klein et al. 2011). However, rats have a different pruritogen and algogen pattern compared with mice (Akiyama and Carstens 2013). For example, intradermal injection of histamine evoked pain-related behavior instead of itch sensing in rats. When injected intradermally in the rostral back, 5-HT and formalin stimulated hind limb scratching. In addition, cowhage spicules failed to elicit significant itch or pain behavior when injected into the cheek of rats (Klein et al. 2011). Other investigators (Minami and Kamei 2004) attempted to evaluate itch behavior in rat model in the eye, by applying eye drops containing histamine locally and creating a conjunctivitis model. However,

Table 1.1 Assessment methods and chemicals applied to evaluate itch in rats

Assessment methods	Application site	Behavioral response	Sense implication	Chemicals
Intradermal injection	Cheek	Hind limb scratching	Itch	5-HT, formalin, chloroquine, SLIGRL-NH2, capsaicin
		Fore limb wiping	Pain	Histamine, SLIGRL-NH2[a], capsaicin[a], AITC
	Rostral back	Hind limb scratching	Itch	5-HT, formalin
Cowhage spicules insertion	Cheek	Not significant	–	–
Eye drops dripping	Eye	Fore limb movements	Itch	Histamine

[a]SLIGRL-NH2 and capsaicin causes both itch- and pain-related behaviors in rats, therefore occurring in both boxes

"forelimb movements directed to the ocular surface" was regarded as implication of itch, which seems to contradict the cheek model and requires further evidence.

1.2.2 Assessment of Itch in Human Subjects

In humans, a pruritic stimulus elicits two types of response: one related to the sensations such as a verbal report ("I have a weak itch") and the other, a reaction to the sensation, such as a feeling of discomfort and behavior directed toward the stimulus site to reduce or eliminate the sensation and the source of irritation (e.g., scratching) (Lamotte et al. 2011).

1.2.2.1 Assessment of Itch Intensity and Quality

With the exception of mechanically and electrically evoked itch, most human surrogate models produce itch lasting 5–15 min with a peak intensity rating elicited between 1 and 3 min after induction. In the case of clinically, as well as experimentally, induced itch, the sensation frequently presents with one or more associated sensations, such as pricking or burning (Papoiu et al. 2011; Sikand et al. 2009). The most common approach is to instruct the participating subject to separately rate the sensory qualities of itch, pricking, and burning on a generalized labeled magnitude scale (gLMS), a visual analogue scale (VAS), or a numerical rating scale (NRS), frequently (every 10–30 s) upon itch induction. This allows for a temporal overview of the itch and other sensory qualities and reporting of itch latency, peak, area under the curve, etc. (Andersen et al. 2015).

1.2.2.2 Defining Histamine-Dependent Itch

Histamine is by far the most studied pruritogen, having been widely used as the prototypical experimental proxy of itch, and to induce itch, histamine can be applied epicutaneously in combination with iontophoresis, by epidermal penetration with a lancet or functionally inert cowhage spicules coated with histamine or as an intradermal injection (Hagermark 1973; Papoiu et al. 2011; Shim and Oh 2008). All routes of administration are shown to produce a moderate to strong sensation of spontaneous itch, with slight differences in the reported presence of nociceptive sensations, alloknesis, and hyperknesis (Sikand et al. 2011; Simone et al. 1991).

Histamine-dependent itch has some disadvantages in particular, when injecting histamine the induced response ratio between nociception and itch appears to shift away from itch toward a more nociceptive sensation characterized by burning and pricking (Sikand et al. 2011). Lastly, the use of histamine is accompanied by a significant wheal and flare reaction regardless of the route of administration (Bickford 1938; Bromm et al. 1995; Schmelz et al. 2000; Sikand et al. 2011).

1.2.2.3 Defining Histamine-Independent Itch

Unlike histamine-dependent itch, histamine-independent itch is thought to rely mainly on a subpopulation of mechano-heat-sensitive/polymodal c-fibers (CMH) incapable of producing the extensive flare that is characteristic for histamine-induced itch (Johanek et al. 2007; Simone et al. 1991).

In the nonhistaminergic pathways, the key second messenger role is played by transient receptor potential cation channel, subfamily A, member 1 (TRPA1), a downstream target of proteinase-associated receptor 2 (PAR) and Mas-related G-protein-coupled receptor (Mrgpr) member G signalling (Terada et al. 2013; Wilson et al. 2013; Wilson et al. 2011).

For practical purposes, in the experimental setting, a distinction between histamine-dependent and histamine-independent itch can be determined by showing that preadministration of topical antihistamine, such as doxepin, reduces the itch intensity (Johanek et al. 2007; Sikand et al. 2011). Since the terms "histamine-independent" and "nonhistaminergic" are essentially negative definitions, it is necessary to recapitulate on histamine as an itch inducer. Hence, despite the focus of this review being histamine-independent itch modalities, histamine-induced itch deserves a brief mentioning.

1.2.2.4 Human Surrogate Models of Itch

Electrically Evoked Itch

A few studies have explored the opportunity of using transcutaneous electrical stimulation to induce itch, with varying success (Ikoma et al. 2005; Tuckett 1982; van Laarhoven et al. 2010). Ikoma *et al*. (2005) explored numerous electrical stimuli paradigms designed to produce itch and found that a 2 ms, 50 Hz, 0.05 mA stimulation with a 0.1×7 mm electrode induced a highly selective sensation of moderate itch rated ≈ 3 on a NRS (VAS 0–10), while increasing the current intensity to 0.12 mA produced the most intense itch sensation, 4.5 (VAS 0–10). At this higher intensity level, itch occurred alongside a modest level of pain at 2.2 (VAS 0–10).

Mechanically Evoked Itch

Apart from the above-mentioned electrical approach, itch can also be induced non-chemically with the use of mechanical stimulation. In a recent study, microvibration of the facial vellus hairs in a stimulus paradigm of 0–1 mm probe amplitude, at 1–50 Hz for 90 s, resulted in a mean peak itch intensity at 5 (VAS 0–10). The chin was by far the most sensitive location, while the cheek and the forehead were considerably less responsive (both ~ 2.5, VAS 0–10), and stimulation on the forearm did not produce any itch. The mechanically evoked itch was unresponsive to antihistamine and did not entail flare or nociceptive sensations at any stimuli intensity, making the itch model unique (Andersen et al. 2015).

Proteinase-Activated Receptor 2/4 (PAR)-Mediated Itch

Cowhage spicules. The spicules found on the pod of the leguminous plant cowhage (Mucuna pruriens) and, more importantly, the sensory effects that these induce when inserted into the epidermis were described by Broadbent, who wrongfully concluded their itch inducing properties to be a consequence of an unknown substance causing histamine release (Broadbent 1953). A few years later, Shelley and Arthur isolated mucunain, identified it as a proteinase, suggested it to be the principal itch-inducing compound in cowhage, and reported that the itch sensation it induced was "very unlike that of histamine" (Reddy and Lerner 2010; Shelley and Arthur 1955). Cowhage spicules are 1–3 mm in length, with a diameter of 1–3 μm at their tip. Inserted into the epidermis the spicules evoke a moderate-to-intense sensation of itch and, to a lesser extent, sensations of burning and stinging pain (Johanek et al. 2007; Sikand et al. 2009).

Other proteinases. The use of various proteinases, such as papain and tryptase, has been attempted to mimic nonhistaminergic itch (Reddy and Lerner 2010). The results are relatively sparse and variable.

Mas-Related G-Protein-Coupled Receptor-Mediated Itch

Mrgprs are a family of approximately 50 receptors, of which several are exclusively expressed on small diameter neurons of dorsal root ganglia. In humans these include MrgprX1, a receptor for chloroquine and bovine adrenal medulla 8–22 peptide (BAM8-22), and MrgprD, which is restricted to axons innervating the epidermis and is responsive to the itch-inducing amino acid, that is, β-alanine (Dong et al. 2001; Lembo et al. 2002; Zylka et al. 2005). Itch is induced by algogens: serotonin, bradykinin, and substance P.

1.3 Assessment of Pain in Animal Models and Human Subjects

For patients with pain, based on their verbal report, diagrammatical representation of cutaneous spread, completion of pain questionnaires such as the McGill Pain Questionnaire, and pain scales such as the visual analogue scale and neuropathic pain scale, provide health specialists with information about the intensity, duration, and location of the pain. While we cannot ask an animal directly about the ongoing nature of its pain experience, many of the behaviors have been reported in different animal models of temporary, persistent, inflammatory, and neuropathic pain (Xie 2011).

1.3.1 Assessment of Pain in Animal Models

This part highlights several types of nociceptive stimuli (thermal, mechanical, or chemical), which have been used in different pain models such as acute pain, chronic pain, inflammatory, and visceral pain (Xie 2011).

1.3.1.1 Tests Based on Thermal Stimuli

The Tail-Flick Test

There are two variants of the tail-flick test. One consists of applying radiant heat to a small surface of the tail. The other involves immersing the tail in water at a predetermined temperature. This test has proved particularly sensitive for studying the analgesic properties of pharmacological substances. It can also be used to evaluate basal thermal pain sensitivity or to study putative genetic differences among animals without drug ("naïve") (Carstens and Wilson 1993; D'Amore et al. 1992; Hardy et al. 1940).

The Paw Withdrawal Test Using Radiant Heat

Radiant heat was applied to a paw that had already been inflamed by a subcutaneous injection of carrageenan. Basically, the animal moves freely on a glass surface. A focused infrared source is moved under the animal when the animal is not moving, and a button press applies the heat to the plantar surfaces of the foot. When the animal feels the pain and moves the paw, a photosensor stops the clock and shows the latency from heat onset to paw withdrawal. In each test session, each animal is tested in three to four sequential trials at approximately 5-min intervals to avoid sensitization of the response (Hargreaves et al. 1988; Randall and Selitto 1957).

The Hot Plate Test

This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid up to 65 °C. Animals are brought to the testing room and allowed to acclimatize for 10 min before the test begins. Pain reflexes in response to a thermal stimulus are measured using a hot plate analgesia meter (Ankier 1974).

Tests Using Cold Stimuli

Cold is very rarely used to test acute pain. On the other hand, it is more common to test cold allodynia in animal models of neuropathies. The techniques are directly inspired by those that use heat by contact: immersion of the tail or a limb (Attal

et al. 1990), or placing the animal on a cold surface (Bennett and Xie 1988), a cold plate cooled by cold water circulating under it. The temperature (−5 to 25 °C) of the cold plate, which is equipped with a Plexiglas box to contain test animals, is set and allowed to stabilize for 5 min (ambient temperature of testing room 21 ± 1 °C). The animal is then placed onto the cold plate, and the time taken for the first brisk lift or stamp of the ipsilateral hind paw to occur is recorded.

1.3.1.2 Tests Based on Mechanical Stimuli

Randall and Selitto Test

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. A common way to assess acute mechanical sensitivity is using withdrawal threshold to paw/tail pressure using the Randall–Selitto test (Randall and Selitto 1957). The analgesy meter for the rat paw allows for the application of a steadily increasing pressure to the dorsal surface of the rat's hind paw, tail, or muscle via a blunt point (dome-shaped plastic tip) mounted on the top of a system of cogwheels with a cursor that can be displaced along the length of a graduated beam. These devices permit the application of increasing measurable pressures and the interruption of the test when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. The intensity of pressure causing an escape reaction is defined as the withdrawal threshold. The threshold (in g) for either paw/tail withdrawal or vocalization is recorded.

Pricking Pain Test

Another approach to test for mechanical sensitivity is to use a pinprick, applying painful pressure to the plantar surface of the hind paw. This is similar to the pricking pain test done during the neurological exam in patients and represents an alternative to the "Randall and Selitto" test. In practice, the animal is gently restrained and maintained in a natural position. The force is applied between the two tips of a rodent pincher and is independent of the movements of the limb. The rodent pincher displays the force, at which the animal reacts, and reports the mechanical nociception threshold. The behavior can be measured by the duration of paw lifting following the pinprick application or recorded as a frequency of withdrawal (% of response to the pinprick in ten trials) (Xie 2011).

Von Frey Test

Finally, mechanical hypersensitivity can also be tested with von Frey monofilaments. The von Frey filament test, developed more than 100 years ago, is still widely used today for the assessment of tactile allodynia. Von Frey monofilaments

are short calibrated filaments (nylon filaments are mainly used today) inserted into a holder that allows the investigator to exert a defined pressure on a punctiform area of the rodent paw. The animal is repeatedly stimulated with increasingly stronger filaments to determine the threshold where a nocifensive paw withdrawal response is reliably elicited. In this paradigm, testing is initiated with 2.0 g hair, in the middle of the series. Stimuli are always presented in a consecutive fashion, either ascending or descending. In the absence of a paw withdrawal response to the initially selected hair, a stronger stimulus is presented; in the event of paw withdrawal, the next weaker stimulus is chosen. According to Dixon, optimal threshold calculation by this method requires six responses in the immediate vicinity of the 50 % threshold (Chaplan et al. 1994; Dixon 1980).

Electronic Von Frey Hair

Based on the von Frey test, electronic von Frey hair (Electronic VFH) was first developed by Jensen (Jensen et al. 1986) and later adapted to Rodents research by Cunha and colleagues (Cunha et al. 2004). Electronic VFH is also called an electronic pressure-meter and has three components: a von Frey filament, a hand-held force transducer, and a display. The animal is stimulated with the von Frey filament similar to the classical von Frey test, and the pressure is processed by the force transducer and displayed simultaneously on the screen. The maximum applied pressure, which is the withdrawal threshold, is automatically recorded on a paw withdrawal response in one single test. This method requires three to four repeated tests to get optimal threshold calculation. Animals displaying paw withdrawal thresholds more than 2 standard deviation (SD) below the mean threshold of the un-operated are considered neuropathic (Chaplan et al. 1994). The electronic VFH has several advantages over the classical von Frey test. First, there is no need to change filaments, so stimulation areas have an equal size (the area varies with the diameter of the von Frey filaments). Second, the withdrawal thresholds are automatically recorded in every single test and have a higher level of resolution because pressure can be recorded continuously rather than in increments in the form of weights of manual filaments. Third, there is a reduction of the number of attempts required, so animals spend less time confined in the testing box and are therefore less stressed during an experiment (Cunha et al. 2004; Martinov et al. 2013). Recently, an automated von Frey equipment has been developed using a mechanically advancing probe as the stimulator can record time to withdrawal along with withdrawal thresholds (Bradman et al. 2015). This automated von Frey equipment inherited almost all the advantages from electronic VFH except the limitation by the position or placement of the hind paw (Nirogi et al. 2012).

Q-tip Test

The terms Q-tip, cotton wisp, or cotton swab test are often used interchangeably. It is a common approach to assess allodynia, especially tactile allodynia, in both animal models and human beings. A wisp of cotton pulled up but still attached to a cotton swab was lightly stroked the plantar surface of the rodent paw through the floor of a wire mesh cage (Song et al. 1999). The duration of each stroke is at least 1 s, and the inter-stroking interval is 10–15 s. A single, quick withdrawal response is considered to indicate the presence of tactile allodynia. At least three measurements are taken at each time point. The threshold is expressed as the percentage of withdrawals of the total strokes (Zhang et al. 2000).

1.3.1.3 Tests Based on Spontaneous Pain-Related Behavior

Spontaneous Foot Lifting, Biting, and Licking to Estimate the Spontaneous Pain of Rats

One of the most common measures of spontaneous pain behavior in models of neuropathic pain is the quantification of foot lifting, biting, and licking (Choi et al. 1994). Each rat is placed on a brass plate kept at a neutral temperature (30 ± 1 °C) and covered by a transparent plastic dome (8 × 8 × 18 cm) without apparent external stimulus. After 5 min adaptation, use a camera to capture the behavior of the rat for the next 5 min and quantify the cumulative duration of time that the rat lifts, bits and licks its paw.

Behavior of foot lifting, biting, and licking is interpreted as a kind of guarding action of the injured paw. Foot lifting is the behavior to increase weight on the intact hind limb and decrease the weight of injured hind paw, which indicates spontaneous pain in the injured hind paw.

However, paresthesia and dysesthesia (tingling and numbness), which are common sensory complaints of peripheral neuropathic patients, can also induce pain-like behavior as described above (Mogil 1999). Therefore, the observation of pain-like behavior may not only be implied as spontaneous pain but may also include paresthesia and dysesthesia.

Formalin Test

The formalin (37 % solution of formaldehyde) test was first conducted in rats to study the analgesic effects of morphine and meperidine (Dubuisson and Dennis 1977) and was later modified for use in mice (Hunskaar et al. 1985). Depending on the specific goal of the experiment, formalin can be injected into different body regions such as forepaw or hindpaw, either subcutaneously or intramuscularly. A number of other chemicals have also been used to induce pain, such as hypertonic saline, ethylene diamine tetra-acetic acid, Freund's adjuvant, capsaicin, and bee

venom (Xie 2011). Different experiments can adopt different doses, depending on the object of the experiment. Usually, the average dose is 10–20 μl for mice and 50 μl or 80–150 μl, occasionally 250 or 400 μl, for rats. Most commonly, the rats receive subcutaneous injection of 5 % to the plantar surface of the hind paw (Watson et al. 1997). Animals should be allowed to accommodate in the observation chamber 15 min before and recorded up to 60 min after injection. The first 10 min and the 20–40 min after injection are for early phase responses and late phase responses, respectively, with a quiescently period of 5–10 min in between.

A four-level pain rating scale can be used to evaluate formalin-evoked painful behaviors. The rating criteria are the following: 0, both paws are placed on the floor with even distribution of weight; 1, the injected paw rests lightly on the ground with little or no weight placed on; 2, the injected paw is obviously elevated; and 3, the injected paw is licked, bitten, or shaken, while the uninjured paw remained stable (Dubuisson and Dennis 1977). Additionally, the number of licks or twitches of the paw per unit of time or the cumulative time spent biting/licking the paw, or even a measure of the overall agitation of the animal obtained by a strain gauge coupled to the cage, can be used as a criterion to evaluate formalin-evoked pain.

A small necrotic area will produce after formalin injection, which requires 7–10 days to recover, and an analgesic drug should be injected after the test.

1.3.1.4 Tests Based on Limb Function

Weight-Bearing Analysis Using Incapacitance Tester or CatWalk Setup

Normal rats and mice distribute weight on the paws equally. However, when one limb is injured, the weight distribution between injured and noninjured paw changed. Thus, by measuring the weight distribution, we can easily estimate the level of discomfort caused by pain. Incapacitance tester is an ideal instrument for automatically measuring the weight distribution on the two hind paws of small animals, especially in the osteoarthritis models, neuropathy, peripheral nerve injury models, cartilage degeneration, and inflammation models. By detecting the force exerted by each limb, it indicates the tendency for animal shifting its weight from one side to the other, hence facilitating a quantitative measurement of incapacitance.

During the static weight bearing test, the animal is placed into a holder with its hind paws rest on two separate sensor plates. If one of the limbs or paws injured, it will adjust its weight distribution on both hind paws according to the level of pain.

Moreover, with the application of an automated quantitative gait analysis system, CatWalk, it is possible to quantify several gait parameters, including the duration of each phase of the step cycle and pressure applied during locomotion (Gabriel et al. 2007). Because the parameters in the CatWalk method show great correlation with those determined by von Frey filament, the CatWalk method serves as an additional tool in the investigation of mechanical allodynia. In CatWalk, the animal traverses a walkway with a glass floor located in a darkened room. Light from a fluorescent bulb enters the distal end of the glass floor. It strikes the surface and entirely internally reflects. When the animal's paw touches the glass, light exits the

floor and scatters at the paw. Images are reflected by a mirror and recorded by a CCD video camera. The intensity of the signal is relevant to the depth of paw floor contact and pressure applied (Vrinten and Hamers 2003).

Posture and Gait Analysis with Stainless Steel Cylinder

It is a computer-assisted device for analyzing the abnormal posture of the hind paw and gait, which is used for rating pain-related spontaneous behavior especially in knee joint arthritis models (Tonussi and Ferreira 1992).

The animal is placed on a stainless steel cylinder of 30 cm in diameter, rotating at 4 rpm. Then the animal is forced to walk in the stainless steel cylinder. When the electrode on the animal's paw contacts the floor, the circuit is closed. The period during which the circuit is closed is recorded. Gait disturbance is detected by paw elevation time, which is defined as the period during which animal's hind paw fails to touch the surface for 1 min. Pain score is calculated by comparing static (standing) and dynamic (walking) behaviors, including complete touch of foot pad, partial touch, or one foot stand (standing) and slight limping, severe limping, or one foot gait (walking).

Its quantitation is independent of the observer and is sensitive to all kinds of analgesics.

Assessment of Spontaneous Mobility with Biotelemetry System or Activity Boxes

In animals with knee joint arthritis, loss of spontaneous mobility is detected. Biotelemetry system is a biological technology evaluating the spontaneous activity and body temperatures in rodents. It comprises a transmitter in the peritoneal cavity of the rodent and a receiver beneath the cage. The transmitter sends signals including locomotion activity and temperatures to the intermediated processor. Then the receiver detects the signal and interprets it in the computer system (Gegout-Pottie et al. 1999). Moreover, activity box is another way for detecting spontaneous mobility. It is divided into several zones by photobeams consisting of infrared light emitting diodes (LEDs) and phototransistors. When the animal has spontaneous mobility, the pattern of photobeam will be disrupted, which will be recorded on the computer.

1.3.1.5 Tests Based on Pain Emotion and Memory

Conditioned Place Paradigm

Conditioned place paradigm (CPP) has been regarded as the most classic model for assessing the motivational effects of drug rewards and addictions. In the recent years, it is increasingly used to study the affective components of pain, the

mechanism of spontaneous pain, and the selection of analgesic drugs. It has several advantages over the traditional animal models. First, since the traditional animal model is based on evoked pain, it cannot reflect the drugs' effects on the persistent spontaneous pain. So it is no wonder that many drugs selected by traditional animal model are finally proved to be useless on releasing chronic pain. Second, the reflex behavior measured by traditional way only indicates the sensory discriminative of pain, but not any negative affective components.

The main principle for CPP is to regard specific locus or environmental signals as conditioned stimulus (CS) and reward/punitive stimulus as unconditioned stimulus (UCS). CS pairs up with UCS to form conditioned reflex, which promotes the approaching to or avoidance of similar situation. CPP is an ideal tool for studying pain emotional component and spontaneous pain, which plays a significant role in uncovering the mechanism of pain and evaluating new analgesic drugs. It consists of conditioned place aversion (CPA) and conditioned place preference (CPP).

Conditioned Place Aversion (CPA, Fear Based)

In conditioned place aversion (CPA), two distinct neural compartments are paired with distinct unconditioned stimulus, such as drug vs. saline. Animals have the same opportunities to enter each compartment. The time they spent in each compartment is used as the index of reinforcing value of each UCS. Animals tend to spend less time in compartments with aversive reinforcing stimulus compared with those with neutral stimulus. As a result, the previous compartment cues become the secondary negative reinforcers (Swerdlow 2000).

Johansen et al. are the first to apply CPA to study the negative affective component of pain. On preconditioning day, each rat was allowed to move freely between each compartment. The time they spent in each compartment was recorded as the "baseline" preference. On conditioning day, distinct treatment is paired with conditioning compartment. Rats received an injection of aversive reinforcing stimulus (hind-paw injection of formalin) in one compartment (A) or control treatment (no drug) in another compartment (B). They are allowed to enter each conditioning compartment freely. The result shows the rats tend to spend less time in compartment A, which is paired with aversive reinforcing stimulus. It indicates the successful establishment of formalin-induced conditioned place aversion (F-CPA), which provides great opportunities to study the negative affective components of pain (Johansen et al. 2001).

Conditioned Place Preference, CPP (Award Based)

Since the CPA can reflect animal's negative affective components of pain and avoidance motivation, it can be used as indicator of spontaneous pain. Conversely, if the spontaneous pain can be controlled, the avoidance motivation to the previous environment can be reversed. Because the relief of pain is rewarding, it sheds light on the idea of conditioned place preference (CPP).

Chronic neuropathic pain model was established in rats with spinal nerve ligation (SNL). Different drugs are given in different compartments to see whether it can reverse SNL-evoked tactile allodynia or not. In one compartment (A), the rats with SNL are given analgesic agents such as clonidine or conotoxin, while in the other compartment (B), no drugs are given. As a result, the rats developed preference to compartment A, which indicates the establishment of CPP (King et al. 2009).

We used CPP to concomitantly demonstrate the presence of automatic spontaneous pain and evaluate the efficacy of analgesic drugs.

1.3.2 Assessment of Pain in Human Subjects

Experimental human pain models have improved our understanding of the physiology and pathophysiology of clinical nociception, inflammation, and analgesia (Bingel and Tracey 2008; Handwerker and Kobal 1993). They represent sophisticated tools to assess the efficacy of analgesic drugs in humans. They also have the potential to limit the costs of analgesic drug development by predicting clinical success with fewer resources than are needed for large clinical trials.

1.3.2.1 Requirements for Human Subjects for the Measurement of Pain

In human experimental pain models, subjects can be selected for age, sex, body measures, ethnicity, genetic and epigenetic background, health, or disease. The assay by which pain is assessed involves the pain stimulus, which can be electrical, thermal, mechanical, and chemical. This can be applied to different body parts to evoke superficial, muscle, or visceral pain.

Common criteria apply to the use of the stimuli (Beecher 1957). These include administration to body parts exhibiting minimal individual variation in terms of neuronal histological characteristics, ability to provoke minimal or no tissue damage, correlation between stimulus strength and perceived pain, and differential discrimination between strong stimuli with high resolution. In addition, the responses to stimuli should be largely time-invariant to allow for repeated measurements. The stimuli should evoke responses that can be measured by a variety of readouts.

The measure of pain involves surrogate markers, as pain cannot be measured directly, being a subjective phenomenon defined as “unpleasant sensory and emotional experience associated with actual or potential tissue damage.” The measures by which pain is quantitatively determined (Barrett 2015) range from psychophysical responses, obtained by questionnaires during most experimental pain studies or by measuring the length of visual rating scales or the number of items describing pain (Melzack 1975), to cortical evoked potentials (Chapman 1986), magnetoencephalographic, positron emission, and functional magnetic resonance tomographic assessments of the brain representation of pain (Price 2000).

1.3.2.2 Assessment of Pain in Human Subjects Using Capsaicin

In humans, intradermal injections of capsaicin always evoked only pain, typically described as burning or stinging. The localized pain began immediately upon injection, peaked within a minute later, then gradually declined. The duration of sensations produced by the highest doses was 10–15 min (Shimada and Lamotte 2008). In the capsaicin study, the subjects were not asked to judge the intensity of any itch they may have felt. It is well known that some chemical stimuli applied to the skin can evoke nociceptive sensations such as pricking, stinging, or burning that are not rated as painful, that is, does not hurt. However, capsaicin can produce significant itch if applied topically by soaked filter paper or by capsaicin soaked, inactivated cowhage spicules. Thus, the quality of a chemically evoked sensation may depend in part on how the chemical is delivered to the skin (Andersen et al. 2015).

1.4 Relationship Between Animal Models and Human Subjects

1.4.1 Similarities Between Animal Models and Human Subjects

The basic question at hand is whether these "site-directed behaviors" differ in relation to whether the chemical evokes predominantly itch or nociceptive sensations in humans (Lamotte et al. 2011).

Although differences probably do exist in comparison with humans, notably with respect to certain cerebral structures, generally, the most reliable signs of pain are physical ones (Xie 2011). Thus, if the models are well designed and conditioned are well controlled. Results of the animal models can have significant accordance with the human subjects. For example, when histamine or capsaicin was injected into to the cheek, mice behaved in an appropriate manner that was consistent with the respective sensations reported by humans. The mice wiped their cheeks to a substance that produces pain in humans and scratched to a chemical that evokes itch. The present finding that mice emit different behaviors in response to capsaicin and histamine applied to the cheek is in agreement with human observations that the former is nociceptive and the latter pruritic (Shimada and Lamotte 2008).

1.4.2 Differences Between Animal Models and Human Subjects

We must always bear these factors in mind because they can influence the pharmacokinetics and pharmacodynamics of administered substances just as much as the physiological mechanisms that underlie the recorded responses.

Variability can also relate to the anatomy of the nervous system: noradrenergic neurons from the locus coeruleus project toward the dorsal or ventral horn, depending on whether Sprague–Dawley rats belong to the Harlan or the Sasco stock (Cizza and Sternberg 1994). At a pharmacological level, the effects of morphine are also genetically determined, at least in the mouse. There is another problem that itch and pain cannot be monitored directly in animals but can only be estimated by examining their responses to nociceptive stimuli; however, sometimes such responses do not necessarily mean that there is a concomitant sensation (Iasp 1979).

Interspecies variability is undoubtedly even greater. Like in the hot plate test, the behavior is relatively stereotyped in the mouse but is more complex in the rat, including sniffing, licking its forepaws or hind paws, straightening up, stamping its feet, and starting and stopping washing itself. Because so many of these behaviors exist, observation of them is difficult. All these factors make this test a very delicate one to use (Bardo and Hughes 1979; Van Ree and Leys 1985). Another example is that NK1 receptors in humans are identical to those in the guinea pig but different from those in the rat and mouse (Watling et al. 1994).

Recent advances in neuroimaging technology have reinforced the concept that the recognition of pain in humans is a multifaceted process that involves the parallel integration of sensory, emotional, and noxious perceptual information by multiple brain structures. The absence of verbal communication in animals is undoubtedly an obstacle to the evaluation of pain (Rainville 2002). Humans can be tested on psychophysical measurements, while animals cannot. This makes human itch and pain models more diverse and complicated than animal models.

1.5 Limitations of Animal Models and Human Subjects

1.5.1 Limitations of Animal Models

Animal models, no matter how carefully designed and assessed, will never be able to 100 % accurately simulate human conditions. Unlike human subjects, animals cannot speak a language to accurately describe the sensations of itch and pain and the related qualities (burning, pricking etc.). Therefore, one may never understand the actual feelings of an experimental animal. In addition, animals are genetically different from human in terms of itch- or pain-related receptors, cellular pathways, and anatomical structures. These limitations may partially underlie the difficulty of translational medical research and drug development in pain and itch. However, under proper control and training, the margin of error could be minimized for the assessment of itch or pain when applying the above testing methods in certain animal models.

Mice and rats are easily disturbed and have to adapt to the experimental condition, especially in the assessment of itch-related behaviors. Animals should be handled, restrained, and placed in containers several times on different days before the

experiments began. It is worth noting that training the animal for at least 3 consecutive days prior the operation will help to obtain a more stable response and increase the sensitivity of the assay (Xie 2011). Efforts should be made to reduce distractions to a minimum. For example, to achieve a relatively accurate assessment for the itch- or pain-related behaviors, experiments should be conducted inside a sound proof room (Shimada and Lamotte 2008). Pseudo-white noise was delivered from a radio tuned in between stations to mask extraneous laboratory noises. When monitoring behavior in a closed test chamber, vacuum lines could be used to allow ambient air through the containers at a rate of about 300–500 ml/min so that mouse odors did not circulate between containers. Animals should be tested individually or effectively isolated so that they could not see each other during an experiment. A small amount of bedding was placed in each container to absorb any urine voided by the animals. Ambient temperature was maintained between 23 and 27 °C (Shimada and Lamotte 2008).

1.5.2 Limitations of Human Subjects

Experimental human pain models, like all models, provide a limited reflection of reality (Fioravanti et al. 2008). This reality is clinical pain, which is the most frequent reason for visits to a doctor and chronically affects one-fifth of adults in Europe, North America, and Australia (http://www.iasp-pain.org). Why, then, should analgesic efficacy be studied with models and not directly? In contrast to spontaneous clinical pain, experimental pain is controllable with regard to its spatial (localization), temporal (duration), quantitative (intensity), and qualitative (e.g., "pricking" or "pressing") properties.

Major confounders, such as analgesic therapy, can be avoided and placebo-controlled cross-over designs can be applied to healthy subjects. Withholding analgesic therapy would be unethical in pain patients. However, models capture not all attributes of the original pain but only those considered as relevant, and these obviously vary in their ability to reflect clinical pain. This is the background to the present comparative analysis that made use of a further characteristic of models, which can itself be subject to modeling (Trentin et al. 2006), namely, the agreement between analgesic efficacy under experimental and clinical conditions.

1.6 Conclusion

Experimental methodologies for the measurement of itch and pain have been widely used in animal models and human subjects under controlled conditions. Efforts to improve the reliability and feasibility of these approaches have been encouraging and largely facilitated our understanding of the underlying mechanisms for itch and pain. Further development of methodologies to assess itch and pain in both animal

models and human subjects will be required to overcome the gaps between the bench and the bedside.

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Chapter 2
Allergic Contact Dermatitis: A Model of Inflammatory Itch and Pain in Human and Mouse

Robert H. LaMotte

Abstract This chapter is an overview of published observations from our laboratory on the psychophysics and neurobiology of the persistent itch and pain of allergic contact dermatitis (ACD). ACD is a clinically significant problem with many features characteristic of other pruritic disorders. Our approach was to produce ACD experimentally in humans and in the mouse. The goal was to use the mouse as an animal model for investigating the peripheral neural mechanisms of itch and pain of ACD in humans. Humans and mice were each sensitized by cutaneous topical application of squaric acid dibutyl ester, a hapten not encountered in the environment. Subsequent challenge at another cutaneous site produced local inflammation ("ACD") with humans reporting persistent itch (lasting up to a week) and mice exhibiting persistent itch- and pain-like behaviors directed toward the ACD site. Enhanced mechanically evoked itch and pain in surrounding skin in humans were reversibly blocked by numbing the ACD site with cold, suggesting dependence on ongoing activity from the site. In mice, in vivo recordings revealed spontaneous activity in a subset of pruriceptive, mechanoheat-sensitive nociceptors with unmyelinated axons innervating the ACD site. These and a larger subpopulation of acutely dissociated small-diameter neurons innervating the ACD site exhibited an upregulation of the receptor CXCR3 and excitatory responses to one of its ligands, the chemokine CXCL10 (IP-10) that contributes to the pathogenesis of ACD. Preliminary findings point to possible therapeutic targets that could be investigated in inflammatory itch disorders in humans.

Keywords Pain • Itch • Dermatitis • Nociceptor

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2.1 Introduction

Persistent itch accompanying diseases of the skin and other organs can significantly impair the quality of life. The action potential activity in cutaneous nociceptors signals the presence, magnitude, and time course of chemical stimuli that elicit itch in humans and itch-like behavior in animals (LaMotte et al. 2014 for review). Nociceptors differ in their capacity to respond to different pruritic chemicals. For example, histamine elicits higher discharge rates in nociceptors that do not respond to noxious mechanical stimuli than those that do, whereas the reverse is true for the protease of cowhage (*Mucuna pruriens*) that evokes a histamine-independent itch (Schmelz et al. 1997; Johanek et al. 2008; Namer et al. 2008; LaMotte et al. 2009). In these experiments, the chemically evoked itch and nociceptor responses are typically transient (e.g. lasting less than half an hour). Less is known of nociceptor activity accompanying pathological itch that persists for days or longer.

One type of persistent itch is produced by allergic contact dermatitis (ACD), a disorder that affects a significant percentage of the population (Alikhan and Maibach 2014). ACD is a type IV delayed hypersensitivity reaction mediated by T lymphocytes specific for a substance, typically a chemical called a hapten, to which the individual has become sensitized. Immunological mechanisms of ACD have been intensively investigated (Christensen and Haase 2012), but studies of itch and nociceptive sensations accompanying ACD and the activity of cutaneous nociceptors within the area of inflammation are lacking. The present studies were designed to characterize the inflammatory itch and pain sensations accompanying ACD that is experimentally produced in humans using the contact sensitizer squaric acid dibutyl ester (SADBE). This hapten is not encountered in the environment but has been safely used in the clinic as an immunotherapeutic agent (Micali et al. 1996; Silverberg et al. 2000). Using the same model in mice, we observed that ACD evoked site-directed spontaneous itch- and pain-like behaviors and enhanced the excitability of certain cutaneous nociceptors.

2.2 ACD Produced a Persistent Itch and Enhanced Stimulus-Evoked Itch and Nociceptive Sensations

Eight healthy volunteers, four males and four females, participated in the study as described (Pall et al. 2015). Each was trained to use the generalized Labeled Magnitude Scale (gLMS) (Bartoshuk et al. 2004; Green et al. 1996; LaMotte et al. 2009) and then tested for their ratings of the perceived intensities of itch, pricking/stinging, and burning in response to cowhage spicules and to one or more other pruritic chemicals. Then each subject was sensitized to the hapten squaric acid dibutyl ester (SADBE) in acetone vehicle to filter paper lined Finn chamber, 1.2 cm, applied to the lower back for 48 h. Other than a slight erythema, the skin site was asymptomatic. Two weeks later, a Finn chamber was applied to each volar forearm

for a duration of 6 h, one chamber containing SADBE in acetone and the other only acetone as a vehicle control. The subjects took home copies of the gLMS scale and were instructed to mark on the scale their ratings of the perceived intensity of the greatest magnitude of itch, pricking/stinging, and burning occurring at the site of each Finn chamber for successive intervals of time starting after chamber removal and continuing up to 144 h. The subjects were instructed not to scratch or rub the affected skin.

All subjects experienced a skin reaction at the application site for SADBE but not vehicle alone. The reaction was characterized by erythema starting at 6 h and subsequently edema, vesiculations, and an increase in skin thickness, which reached a peak at 72 h. Similarly, there developed spontaneous itch at the SADBE challenge but not at the vehicle site. The itch reached a peak mean magnitude at 48 h and gradually disappeared within a week. Three subjects reported occasional nociceptive sensations, and these were of lesser magnitude than the itch.

Within and surrounding the site of ACD, the skin exhibited abnormal sensations (dysesthesias) to mechanical stimuli manifested as itch to lightly stroking with a cotton swab (alloknesis) and greater than normal pain and itch (hyperalgesia and hyperkinesis) to indentations with von Frey filaments with tip diameters of 200 and 50 um, respectively. The borders of each area of dysesthesia were marked on the skin. The subjects then rated the magnitude of pain and itch evoked by the two types of von Frey filaments applied to multiple sites within the areas of hyperalgesia.

An ice-cold probe (1 cm diameter) was applied to the ACD site. When the skin under the probe became numb to mechanically evoked pricking, any ongoing spontaneous itch disappeared, the areas of dysesthesia were significantly reduced or eliminated, and the pricking evoked itch and pain within these former areas reduced to normal values. Upon rewarming the ACD site to normal skin temperature, the areas of dysesthesias and enhanced ratings of pricking-evoked itch and pain returned to former values. We hypothesized that the dysesthesias were dependent on ongoing neuronal activity that a) originated at the ACD site and b) was reduced or eliminated when the skin was anesthetized by cooling.

In three additional experiments, we tested whether ACD altered stimulus-evoked itch or nociceptive sensations in response to stimuli delivered to the site of ACD (vs. vehicle-treated site). In the first experiment, subjects were asked to judge the maximal perceived intensity of pricking pain evoked by von Frey stimuli, each having the same tip diameter of 200 um but differing in bending forces (5 to 180 mN). All but the lowest force elicited a significantly greater mean magnitude of pricking pain on the ACD vs. the control site.

In a second experiment, subjects rated the maximal itch, pricking, and burning elicited by noxious heat stimuli of 41 to 51 °C, each of 6 s duration and delivered on a base temperature of 38 °C. Not only did stimulus temperatures of 45 °C or greater evoke greater pricking pain on the ACD (vs control-) site, but each heat stimulus elicited itch as well. In contrast, itch was rarely reported during heat stimulation of the control site.

In the third experiment, subjects were asked to rate the perceived intensity of itch, pricking, and burning every 30 s after injection of a pruritic chemical into

either the ACD or control sites: Histamine, bovine adrenal medulla (BAM8-22) peptide, or beta-alanine. Each pruritic chemical normally elicited a dominant sensation of itch that was accompanied by sensations (typically weaker) of pricking/stinging and burning. But in comparison with ratings obtained from the control site, the peak magnitude of itch and the area under the curve plotting itch rating vs. time were each significantly greater in response to each pruritogen injected in the site of ACD. In contrast, there were no significant differences in the ratings of pricking or burning for the ACD vs. control sites.

Taken together, these psychophysical findings provide a preliminary characterization of the persistent abnormal itch and pain sensations that accompany ACD in humans. The findings are useful in cross-species comparisons between sensations in humans and both sensory behavior and underlying neural mechanisms observed in animals.

2.3 ACD Enhanced Itch- and Pain-Like Behaviors in Mice

The following procedures and findings are described in Qu et al. (2014). Wild-type C57BL/6 mice were sensitized to SADBE with a daily topical application of the hapten in a vehicle of acetone to abdominal skin for three consecutive days. Five days later, for different groups of mice, either the cheek or the calf of the hind paw was challenged with a topical application of SADBE ("ACD mice") or acetone alone (control mice) on two consecutive days. Behaviors directed toward the site of chemical application were video recorded for a 2-h period before the first challenge and again 24 h after the first and again 24 h after the second challenge. Similar to the spontaneous sensations reported by humans during ACD, spontaneous itch-like and pain-like behaviors of mice were directed toward the site of SADBE challenge and not toward the site of the application of vehicle alone. In comparison with control values obtained either in the prechallenge phase or on the vehicle site, the number of bouts of scratching the cheek with the hind limb (itch-like behavior) and wiping the check with the forelimb (pain-like behavior) significantly increased 24 h after the first or the second challenge. Similarly, for the calf, there was a significant increase in itch-like "biting" behavior (scraping or "scratching" the skin with the teeth) and also licking (pain-like behavior) directed toward the SADBE but not vehicle-application sites. Thus, both mice and humans exhibited spontaneous itch and nociceptive sensations or behaviors directed toward the ACD site. We hypothesize that one reason why mice exhibited more spontaneous pain (behavior) than humans (sensory reports) is because only the mice were allowed to scratch the ACD site thereby exacerbating the injury.

The effects of ACD on itch- and pain-like behaviors evoked by intradermal injection of a pruritic or algesic chemical into the cheek were tested by Fu et al. 2014. When injected into the vehicle (acetone-treated) site in control mice, histamine and BAM8-22 each elicited significantly more scratching in comparison with the effects of a saline injection but not more wiping. In contrast, the algesic chemical bradykinin

evoked significantly more wiping but not scratching in comparison with saline. These findings are consistent with the sensory reports of humans, that is, histamine and BAM8-22 are primarily pruritic (Sikand et al. 2011) and bradykinin algesic (Hosogi et al. 2006). The similarities between sensory reports of humans and site-directed responses of mice support the validity of using the mouse as an animal model for studying neural mechanisms of itch and pain in humans. On the other hand, the minor nociceptive sensations of pricking/stinging and burning that humans report as accompanying an itch to histamine or BAM8-22 did not cause site-directed nociceptive behaviors in mice. Thus, the present sensory measures in mice do not reflect a one-to-one correspondence with all the qualities of sensation reported by humans in response to a pruritic chemical applied to normal skin.

When injected into the site of ACD, BAM8-22 evoked significantly more scratching (but not more wiping) than it did after injection into a vehicle-challenged site in accordance with findings we obtained from humans. However, ACD had no effect on either scratching or wiping responses to histamine, in contrast to our finding of the increased itch reported by humans. Again, while some behavioral findings translate from mice to humans, others may not.

Bradykinin elicited significantly more scratching (but not more wiping) when injected into the ACD (vs. acetone-control) site. Although we have yet to try bradykinin after ACD in humans, the findings of scratching accompanying wiping after ACD in mice are reminiscent of the reports of experimental studies of patients with atopic dermatitis. These patients report that mildly painful stimuli such as bradykinin applied to lesion sites elicit itch in addition to pain sensations (Hosogi et al. 2006).

2.4 ACD Enhanced the Excitability of Cutaneous Mechanosensitive C-nociceptors in Mice

Chemical stimuli that elicit itch sensation in humans or itch-like behavior in animals elicit action potential activity in certain types of cutaneous nociceptors (for review, LaMotte et al. 2014). These sensory neurons typically also encode the intensities of noxious cutaneous stimuli that elicit different ratings of pain in humans, suggesting that the same types of nociceptor may encode both pruritic and algesic stimuli. For example, unmyelinated peripheral nerve fibers (C-fibers) with cutaneous nociceptors are activated by certain histamine-independent pruritic agents such as cowhage, beta-alanine, or BAM8-22 (Han et al. 2013; Johanek et al. 2008; Liu et al. 2012; Ma et al. 2012; Namer et al. 2008; Wooten et al. 2014). This type of C-nociceptor also encodes with graded frequencies of discharge to the temperature of noxious heat or the force of punctate mechanical indentation that can elicit different incidences and magnitude of pain when applied to the human skin (Torebjork et al. 1984; Wang et al. 2015; Ziegler et al. 1999). In general, it appears that a smaller proportion of nociceptors are activated and/or the discharge frequencies are lower in response to

a pruritic vs. a painful stimulus (e.g. Ma et al. 2012; Wooten et al. 2014). These differences in encoding of pruritic vs. algesic stimuli appear to hold as well in the responses of projection neurons in the spinal dorsal horn (Davidson et al. 2012).

Experiments were designed to test whether mechanosensitive C-nociceptive neurons innervating an area of ACD (neurons of "ACD mice") were more excitable than those terminating in healthy, vehicle-treated skin (neurons of "control mice") (for details, refer to Qu et al. 2014). Enhanced excitability after ACD might contribute to increased discharges to external stimulation or to spontaneous activity in the absence of stimulation and therefore increase the likelihood of generating site-directed itch- or pain-like behaviors or sensation. Electrophysiological recordings were obtained from the cell bodies of cutaneous mechanosensitive C-nociceptors, visually identified in transgenic mice by their expression of a green fluorescent protein (GFP) marker. In one type of mouse, the GFP was present in neurons that expressed the MrgprA3 receptor for chloroquine and also typically expressed receptors for capsaicin (TRPV1), histamine (H1), and BAM8-22 (MrgprC11) (Han et al. 2013). In the other type of transgenic mouse, the GFP was present in neurons that expressed the MrgprD receptor for beta-alanine (Zylka et al. 2005) and that do not normally express TRPV1, H1, or MrgprC11 (Han et al. 2013). These two types of neurons each innervate the stratum granulosum of the epidermis (Han et al. 2013; Zylka et al. 2005) and constitute the majority of mechanosensitive C-nociceptors innervating the hairy skin of the mouse (Imamachi et al. 2009).

GFP-labeled neurons of ACD and control mice were electrophysiologically recorded in vitro and in vivo (Qu et al. 2014). In the in vitro studies, whole-cell patch-clamp recordings were obtained from acutely dissociated cell bodies of GFP-labeled neurons that, for separate groups of each type of transgenic mouse, innervated either skin with ACD ("ACD mice) or vehicle-treated skin ("control mice"). In comparison with neurons from control mice and under current-clamp recording, both MrgprA3+ neurons and MrgprD+ neurons exhibited significant signs of increased membrane excitability. These signs included a more depolarized resting membrane potential, a decreased threshold current for an action potential (rheobase), and more action potentials evoked by a stimulus current that was twice rheobase. Thus, ACD made these neurons more likely to respond to a near-threshold stimulus and to fire more action potentials to a suprathreshold stimulus.

As no significant differences were observed in input resistance between ACD and control neurons, the next experiment examined the possibility that increased neuronal excitability after ACD might be accompanied by an increased expression of voltage-gated sodium currents. Using different voltage stimulus protocols under voltage clamp recording, it was found that in comparison with responses of control neurons, both MrgprA3+ and MrgprD+ neurons from ACD mice exhibited significant increases in the peak amplitudes of both tetrodotoxin-sensitive and tetrodotoxin-resistant sodium currents (Qu et al. 2014). If this increased magnitude of sodium current is present at the cutaneous nerve endings of these C-nociceptors, then action potentials might be more easily evoked by natural stimulation or might even occur spontaneously thereby eliciting spontaneous itch- and pain-related behaviors directed toward the ACD site.

To examine the possibility that these Mrgpr mechanosensitive C-nociceptors at the ACD site might exhibit signs of hyperexcitability such as spontaneous activity in the intact animal, action potentials were extracellularly from their cell bodies in the intact DRG (Qu et al. 2014). For ACD or control transgenic mice, the functional properties of the cutaneous receptive fields were characterized for MrgprD+ or MrgprA3+ neurons innervating the chemically treated skin 24 h after the second challenge. Each type of neuron was identified as a mechanosensitive C-nociceptor. Some of the MrgprD+ neurons and all of the MrgprA3+ neurons were also responsive to noxious heat. In control mice, none of these C-nociceptors exhibited any ongoing (spontaneous) activity in the absence of applied cutaneous stimuli. But for neurons in ACD mice with receptive fields in the area of dermatitis, there was a low rate of ongoing activity in 43 % of the MrgprA3+ neurons (vs. only 5 % of MrgprD+ types) and some neurons of either Mrg type exhibited abnormally high and long discharges to heat or to punctate mechanical noxious stimulation.

Taken together, these electrophysiological findings support the hypothesis that ACD causes an increase in the incidence of spontaneous activity in a subpopulation of cutaneous C-nociceptors, secondary to enhanced sodium current and membrane excitability, which, in turn, may trigger spontaneous itch- and pain-like behaviors directed toward the site of inflammation.

2.5 ACD Upregulates CXCR3 Chemokine Receptor Signaling in Cutaneous C-nociceptors

Some of the chemical mediators that orchestrate the inflammation during the challenge or elicitation phase of ACD might also act to increase nociceptor excitability. When humans and mice are exposed to the hapten to which they were previously sensitized, keratinocytes produce cytokines including tumor necrosis factor alpha (TNF-alpha) and the chemokine CXCL10 (IP10), a ligand for CXCR3 which is expressed on activated T-helper type 1 (Th1) cells. These chemical stimuli facilitate the migration of cytotoxic CD8+ Th1 cells that are specific for the antigen (a hapten-protein complex) and that cause apoptosis of the antigen presenting cells in the challenged skin.

Based on the capacity of TNF-alpha to be retrogradely transported to the DRG (Shubayev and Myers 2001) and its effect of increasing TTX-resistant sodium currents when applied to dissociated DRG neurons (Jin and Gereau 2006), it is possible that TNF-alpha could act to increase voltage-gated sodium current both at the nerve terminals and at the somas of nociceptive neurons innervating the area of ACD. This hypothesis remains to be tested for the neuronal effects of ACD.

After SADBE challenge, acutely dissociated small-diameter DRG neurons that innervated the area of ACD upregulated the expression of mRNA and protein for CXCR3 and exhibited responses to CXCL10 – responses rarely and weakly seen in control neurons (for full details see Qu et al. 2015). The neurons innervating the

inflamed skin (ACD neurons) exhibited a calcium response to CXCL10 that was blocked by prior delivery of an antagonist for the ligand's receptor, CXCR3. These neurons included but were not confined to those that expressed MrgprA3 or MrgprD. During electrophysiological recordings, CXCL10 evoked a membrane depolarization and action potentials in ACD neurons but not in control neurons. In behavioral studies, systemic delivery of a selective antagonist of CXCR3 decreased the incidence of itch- but not pain-like behaviors directed toward the ACD site. Also CXCL10 elicited itch-like site-directed behaviors when injected into the ACD site but evoked no significant itch- or pain-like behaviors when injected into vehicle-challenged skin (Qu et al. 2015).

In other studies, CXCL10 was upregulated in DRG neurons in rats after an experimentally induced inflammation of the ganglion (Strong et al. 2012) or after a demyelination of the nerve (Bhangoo et al. 2007) and was upregulated in DRG neurons from human after infection of the ganglion with varicella-zoster virus (Steain et al. 2011). It remains to be determined whether the activation of neurons expressing message or protein for CXC10 would release CXCL10 from DRG neurons. If so, the chemokine might facilitate the attraction of CXCR3-expressing lymphocytes and activate these cells as well as itch- and pain-mediating nociceptive neurons that express CXCR3 after ACD. Clearly there is much work to be done to fully understand the role of this chemokine receptor in nociceptor physiology during ACD given the complexities of chemokine biology (Van Raemdonck et al. 2015). In addition, there are probably multiple inflammatory mediators that may modulate the excitability of cutaneous nociceptors with C-fibers and probably also certain nociceptors with myelinated axons. The advantage of the ACD model is that it can be experimentally applied in humans and animals. Behavioral and cellular physiological findings in animals can be related more easily to sensory measurements in humans using similar experimental stimuli and protocols. This interspecies comparison should facilitate the discovery of molecular targets for treating persistent itch and pain in humans.

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Chapter 3
Modulation of C-nociceptive Activities by Inputs from Myelinated Fibers

Wan-Ru Duan and Yi-Kuan Xie

Abstract To understand the mechanisms of neuropathic pain caused by demyelination, a rapid-onset, completed but reversible demyelination of peripheral A-fibers and neuropathic pain behaviors in adult rats by single injection of cobra venom into the sciatic nerve, was created. Microfilament recording revealed that cobra venom selectively blocked A-fibers, but not C-fibers. Selective blockade of A-fibers may result from A-fiber demyelination at the site of venom injection as demonstrated by microscope examination. Neuropathic pain behaviors including inflammatory response appeared almost immediately after venom injection and lasted about 3 weeks. Electrophysiological studies indicated that venom injection induced loss of conduction in A-fibers, increased sensitivity of C-polymodal nociceptors to innocuous stimuli, and triggered spontaneous activity from peripheral and central terminals of C-fiber nociceptors. Neurogenic inflammatory responses were also observed in the affected skin via Evans blue extravasation experiments. Both antidromic C-fiber spontaneous activity and neurogenic inflammation were substantially decreased by continuous A-fiber threshold electric stimuli applied proximally to the venom injection site. The data suggest that normal activity of peripheral A-fibers may produce inhibitory modulation of C-polymodal nociceptors. Removal of inhibition to C-fiber polymodal nociceptors following demyelination of A-fibers may result in pain and neurogenic inflammation in the affected receptive field.

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Keywords A-fiber demyelination • Neurogenic inflammatory pain • C-polymodal nociceptors • Dorsal root reflexes • Antidromic discharges of C-fiber

3.1 Introduction

In clinical settings, some types of peripheral neuropathic pain such as multiple sclerosis, Guillain-Barre syndrome (Pentland and Donald 1994), and diabetic neuropathy (Reeh et al. 1986) are associated with damage to myelin rather than to axons of primary sensory neurons, suggesting that the myelinated fibers may modulate pain sensation. Studies performed in most neuropathic models also indicated that sensory disorders following peripheral nerve injury are partially or completely associated with deafferentation of peripheral nerve (Bennett and Xie 1988; Chung et al. 1993; Coderre et al. 1993; Dubner and Ruda 1992; Kim and Chung 1992; Koltzenburg 1998; Seltzer et al. 1990; Wall and Gutnick 1974). These models, such as chronic constriction injury (CCI) of the nerve (Bennett and Xie 1988), and lysolecithin-induced selectively demyelination of A-fibers with damage to both axons and myelin (Wallace et al. 2003), produced neuropathic pain including hyperalgesia, allodynia and spontaneous pain. The injured nerve presented selective A-fiber demyelination, but the C-fibers were intact (Basbaum et al. 1991). It is generally believed that the sensitized low-intensity stimulation-activated A_{β} induced by normally innocuous stimulus is responsible for allodynia (Amir and Devor 1996; Devor and Wall 1990; Dubner and Ruda 1992; Gillespie et al. 2000; Koltzenburg and Mcmahon 1986; Wall and Gutnick 1974; Wallace et al. 2003). Nonetheless, the exact role of A-fibers in nerve injury-induced neuropathic pain, especially in allodynia behavior, was not to be evidenced conclusively. Here is the apparent paradox that in the neuropathic patients and neuropathic animal models, the conductive function of demyelinated A-fibers has lost, the action potentials of low-intensity stimulation-activated Aβ failed to propagate along their axons to the spinal cord, but the allodynia behavior response to low-intensity stimulation-activated Aβ-fibers could be evoked. For the understanding of neuropathic pain caused by demyelination, a study by Zhu et al. (2012) focused on the role of A-fiber inputs in the generation of peripheral inflammatory pain will be discussed in detail.

3.2 A Rapid-Onset of Selective Demyelination of A-fibers by Cobra Venom Injection

Based on the composition of the myelin of the A-fibers, which consists mainly in phospholipids, cobra venom with cartiotoxin and phospholipase was injected underneath the epineurium (0.15 mg/5 μl) to induce degradation of the phospholipids of

the myelin. Transmission electron microscopic (TEM) studies of myelin of A-fibers found that the epineurium injection induced the compact myelin sheathes to be totally broken down, the myelin damage was limited only to the site of injection, about 2–3 mm long, and TEM examinations showed that the axonal filaments of A-fibers or unmyelinated C-fibers were generally otherwise normal, and the samples were collected as early as 1 h after injection. Light microscopic examination of sections stained with osmic acid also demonstrated that early demyelination only occurred locally at the site of venom injection where no myelin sheath was observed in any section, but the myelin sheath structure was rather normal in the nerve 5 mm proximal to or 5 mm distal to the site of venom injection 24 h post venom injection. The results obtained from the EM and light microscopic examination indicate that A-fibers were selectively demyelination by cobra venom at the site of injection (Zhu et al. 2012).

Along with A-fiber demyelination, electrophysiological studies also found that compound action potential (CAP) of A-fiber was progressively attenuated and eliminated within 5–10 min post intra-sciatic nerve injection of cobra venom. However, CAP of C-fibers was completely remained. Moreover, microfilaments containing at least one or two A-fibers and one C-fiber were recorded for testing the result of CAP, and the results showed that almost all of the tested A-fibers ($n = 128$), but none of the C-fibers ($n = 71$), lost conductivity at the site of cobra venom injection. Saline injected did not affect the conductivity of either A- or C-type fibers, indicating injecting cobra venom into sciatic nerve selectively interrupted A-fiber conduction (Zhu et al. 2012).

Electromyography (EMG) evoked by stimulating the sciatic nerve and recorded from the tibialis anterior also gradually diminished within 5–10 min post injection following disappearance of the muscle twisting, showing the conductions of afferent A-fibers and efferent A-fibers all were blocked by venom injection (Zhu et al. 2012).

3.3 A-fiber Demyelination Induced Neuropathic Pain and Inflammatory Responses

A direct measured index of inflammatory response is elevated the temperature of the affected skin area innervated by demyelinated nerve with 5–10 min latencies (Fig. 3.1). And the behavior abnormality including thermal hyperalgesia and tactile and cold allodynia also appeared within 10–21 min after cobra venom injection, measured in conscious rats plasma extravasation of Evans blue dye, as a method to measure local inflammatory response (Koltzenburg and Mcmahon 1986; Mcmahon and Abel 1987; Saria and Lundberg 1983) that was observed in the affected skin area within 10 min after cobra venom injection of nerve (Zhu et al. 2012). These responses evoked by selective demyelination indicated that interruption of A-fiber inputs is responsible for generating inflammatory pain.

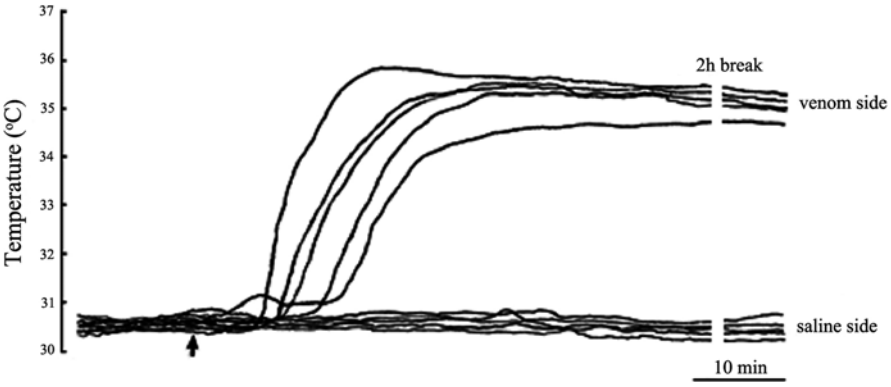

Fig. 3.1 Temperature shift triggered by cobra venom injection into the sciatic nerve. Following venom-induced A-fiber deafferentation, temperature of the hind paw ipsilateral to venom injection into the sciatic nerve increased rapidly from 30.5 ± 0.31 °C to 35.3 ± 0.45 °C (at 2 h after venom injection). The temperature of the contralateral paw (sciatic nerve injected with saline) showed no significant change

3.4 Cobra Venom Intra-Nerve Injection Induced Hyperexcitability of C-fiber Poly-Modal Nociceptors

Although normal conductivity of C-fibers was preserved in cobra-venom-injected nerve, the excitability of C-polymodal nociceptors was increased, about 90 % recorded C-fibers presented spontaneous discharges at an average frequency of 3.85 ± 0.5Hz following the blockage of A-fiber activity, whereas spontaneous activity in C-fibers was not observed in the saline injection of the sciatic nerve. There were three types of activity patterns observed in C-fibers spontaneous orthodromic discharges, in which the bursting firings recorded in this study were never seen in normal nerve (Zhu et al. 2012). Injecting lidocaine into the nerve 20 mm distal to the venom injection site, all recorded spontaneous activity was completely stopped in 10–30 s, indicating that the orthodromic spontaneous activity was generated from the nociceptors peripheral terminals, rather than from the site of venom injection. No A-fiber spontaneous activity was observed after applying cobra venom. Unexpectedly and strikingly, lidocaine applied 20 mm proximal to the fiber recording site also gradually reduced the spontaneous activity for more than 1 h. The activity frequency reduced gradually from 4.37 ± 0.39 Hz to 0.42 ± 0.1 Hz after injecting lidocaine, suggesting that a descending modulation from central nervous system may be involved in generating and maintaining C-fiber orthodromic spontaneous activity. Saline injected into the sciatic nerve either distal or proximal to the cobra venom injection site did not change the spontaneous activity in C-fibers.

Cobra venom intra-nerve injection triggered spontaneous activity from quiescent C-fibers that resulted in hyperexcitability from the subset of C-fiber nociceptors. Innocuous stimuli such as thermal (43 °C water), mechanical (7.37 g von Frey Hair), and cold (6 °C water), which could not induce any discharges from identified

normal quiescent C-fibers before cobra venom injection, however, evoked tonic activity after cobra venom injection. However, injecting saline instead of cobra venom failed to change the sensibility of C-fiber nociceptors. This result suggested the rat's allodynia may result from hyperexcitability of C-fiber polymodal nociceptors after venom injection (Zhu et al. 2012).

3.5 Interruption of A-fiber Conductivity Evoked Antidromic Activity in C-fibers

The antidromic spontaneous activities of identified, previously quiescent C-fibers were recorded from central terminals in the dorsal root within 15 min following venom injection. This tonic or irregular antidromic activity presented as low frequency at about 5 Hz. There was no spontaneous antidromic activity recorded from A-fiber. Furthermore, antidromic activity in C-fibers was gradually decreased and even eliminated by continually stimulating the sciatic nerve proximal to the site of venom injection at A-fiber threshold (10 Hz) to mimic intensive A-fiber input, which had been interrupted by cobra-venom-induced selective demyelination in A-fibers; the antidromic activity eventually silenced during stimulation and appeared again after stopping the stimulation (Zhu et al. 2012). This result indicated that the generation of antidromic activity in C-fibers may be a result interruption of A-fiber inputs. Another result similar to that seen in the antidromic activity of C-fiber, following venom injection, is that a distinct Evans blue extravasation was observed in the affected skin. Continually stimulating the sciatic nerve proximal to the site of venom injection at A-fiber threshold (10 Hz) or blocking the antidromic activity with lidocaine, or aminooxyacetic acid (AOAA), an inhibitor of gamma-aminobutyric-alpha-ketoglutaric-acid transaminase (Baxter and Roberts 1961), administered intrathecally at spinal lumbar 4-5, prevented strongly Evans blue extravasation (Zhu et al. 2012), suggesting antidromic activity originating from C-fiber central terminals may activate C-fiber nociceptor-mediated inflammatory response and A-fiber inputs may inhibit C-fiber nociceptors at their central terminals.

3.6 Dorsal Root Reflexes (DRRs) Involve in Hyperexcitability of C-Fiber Nociceptors Induced by Demyelination of A-fibers

Selective demyelination of A-fibers evoked spontaneous pain, hypersensitivity in C-fiber polymodal nociceptors, and abnormal pain behaviors in this model may be the result of abnormal hypersensitivity in C-fiber polymodal nociceptors. Unlike other peripheral nerve injury neuropathic pain models (Devor and Wall 1990;

Wallace et al. 2003), abnormal expression of ion channels cannot be the major reason for ectopic activity observed in this model, because changes in protein expression would take hours or days. The spontaneous activity in C-polymodal nociceptors induced by venom injection was generated in minutes, was parallel with the rapid disappearance of A-fiber inputs, and inhibited by continually stimulating sciatic nerve at A-fiber strength to compensate for lost A-fiber inputs. Taken together, these findings suggest DDRs, which has been suggested as a potential neuropathic pain mechanism (Cervero and Laird 1996; Willis 1999), may more likely be the key reason for hyperexcitability in C-fiber polymodal nociceptors. As first hypothesized in the gate-control theory (Melzack and Wall 1965), there are central interactions between low-threshold mechanoreceptors and nociceptors in the spinal cord. Input from low-threshold mechanoreceptors may activate gamma-aminobutyric acid (GABA)-ergic interneurons and inhibit nociceptive inputs (Powell and Todd 1992; Sivilotti and Woolf 1994). This hypothesis has been supported by mounting evidence that the critical inhibitory tone in dorsal horn of spinal cord for maintaining normal sensory signaling was induced by GABA-ergic and glycinergic inhibitory interneurons, which were activated by low-threshold input from A-fibers (Takahashi et al. 2006; Takazawa and Macdermott 2010). In the present study, the data also suggested there may be a local circuitry as shown in Fig. 3.2. I that modulates the

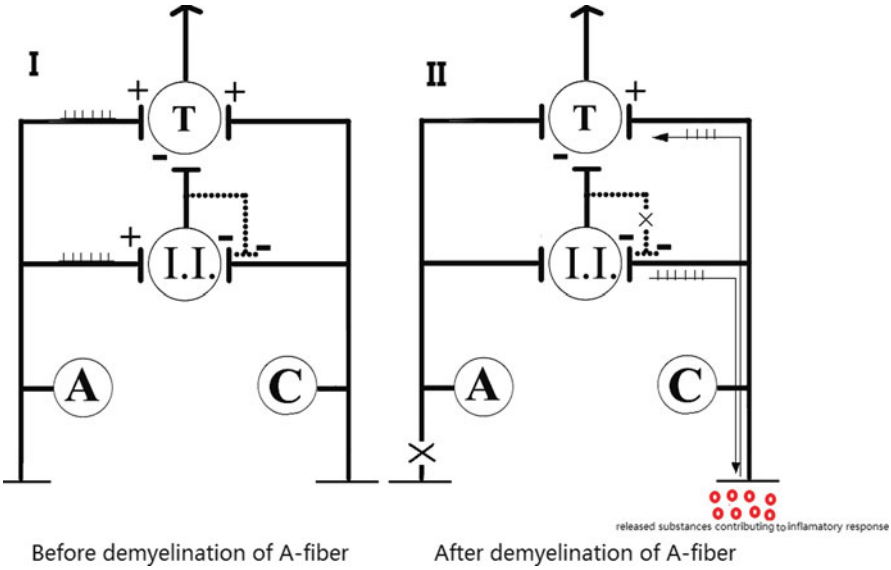

Fig. 3.2 Dorsal root reflex of C-fibers induced by interruption of A-Fiber inputs. (I) shows that A-fibers (A) were normal in both function and structure. The central terminals of C-fiber (C) were tonically inhibited by A-fiber-activated inhibitory interneurons (I.I.), without dorsal root reflex in the (C). (II) shows that interruption of A-fiber inputs by cobra venom-demyelination causes interneurons (I.I.) silent, the inhibitory effect on the central terminals of the C-fiber is removed, bounce influence of the excitability at the C-fiber terminals triggers dorsal root reflex, and the antidromic discharges lead to releasing substances contributing inflammatory response

excitability of C-fiber central nociceptors via the activity of A-fiber in the spinal cord. In normal physiological state, A-fiber inputs activate inhibitory interneurons, which not only inhibit projection neurons but also depress presynaptically central terminals of C-fibers, with the result that no antidromic activity in central terminals of C-fibers can be generated. However, once the input from A-fibers was interrupted, as seen in some neuropathies (Pentland and Donald 1994; Reeh et al. 1986) and in present study, disinhibition of C-fiber central terminals in the spinal cord may occur and result in their hyperexcitability and generating spontaneous activity, which is conducted antidromically to the C-fiber peripheral terminals, increasing their excitability and even evoking orthodromic activity. As a result, spontaneous activity generated in both central and peripheral terminals of C-fibers following A-fiber demyelination and loss of conductivity may form a positive feedback cycle to generate and maintain neuropathic pain. A-fiber may play important roles in modulating sensitivity of C-fibers not only at their central terminals in spinal cord but also at their peripheral terminals. The circuit of modulating excitability of peripheral terminals contains A-fibers, inhibitory interneurons, and central terminals of C-fibers. Selective demyelination of A-fibers by cobra venom not only caused the loss of the conductivity in A-fibers but also interrupted the balance between A-fiber and C-fiber inputs and reduced inhibitory tone on C-fiber central terminals. As a result, a rebound effect of C-fiber central terminal active may generate discharges and conduct antidromically to C-polymodal nociceptors via DRRs (Fig. 3.2.II).

In the nerve-injury-induced neuropathic pain models, chronic neuropathic pain such as hyperalgesia and mechanical allodynia was believed to be mediated by spontaneous activity in A-fibers (Amir and Devor 1996; Devor and Wall 1990; Gillespie et al. 2000; Koltzenburg 1998; Wall and Gutnick 1974; Wallace et al. 2003). However, in the present study, the venom-induced demyelination of A-fiber in the sciatic nerve rapidly caused spontaneous pain, hyperalgesia, and allodynia via hyperexcitability in C-polymodal nociceptors. The results suggest that inputs of A-fibers, in the normal physiological conditions, can modulate the sensitivity of nociceptors of peripheral terminals of C-fiber and that removal of inhibition to C-fibers may result in pain and neurogenic inflammination in the affected receptive field.

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Chapter 4
New Mechanism of Bone Cancer Pain: Tumor Tissue-Derived Endogenous Formaldehyde Induced Bone Cancer Pain via TRPV1 Activation

You Wan

Abstract In recent years, our serial investigations focused on the role of cancer cells-derived endogenous formaldehyde in bone cancer pain. We found that cancer cells produced formaldehyde through demethylation process by serine hydroxymethyltransferase (SHMT1 and SHMT2) and lysine-specific histone demethylase 1 (LSD1). When the cancer cells metastasized into bone marrow, the elevated endogenous formaldehyde induced bone cancer pain through activation on the transient receptor potential vanilloid subfamily member 1 (TRPV1) in the peripheral nerve fibers. More interestingly, TRPV1 expressions in the peripheral fibers were upregulated by the local insulin-like growth factor I (IGF-I) produced by the activated osteoblasts. In conclusion, tumor tissue-derived endogenous formaldehyde induced bone cancer pain via TRPV1 activation.

Keywords Bone cancer pain • Formaldehyde • Transient receptor potential vanilloid subfamily member 1 (TRPV1) • Insulin-like growth factor I (IGF-I)

4.1 Introduction

Chronic pain is common in clinic and a hot point in neuroscience research field. According to causes, chronic pain is usually classified into neuropathic, inflammatory and cancer pain. Relative to neuropathic and inflammatory pain, much less has been known about cancer pain.

With the advances of cancer diagnosis and treatment technologies, the survival time of cancer patients was extended and cancer pain has become a serious problem affecting the quality of life of cancer patients. Early reports on the prevalence of

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pain in cancer patients draw attention to high figures that ranged from 52 to 77 % in patients with advanced cancer (van den Beuken-van et al. 2007). Malignant bone tumors occur in patients with primary bone cancer, but are far more commonly found to be distant metastases from other primary cancers, notably breast, lung and prostate cancers (Ghilardi et al. 2005). As such, bone is the most common site of origin of chronic pain in patients with metastatic lung, breast and prostate cancers and myeloma (Coleman 2001).

There are two reasons considered to cause abnormal primary sensory neuron excitability enhancement by bone cancer. One is the stimulation of peripheral nerve by endothelin-1 (ET-1) (Lassiter and Carducci 2003), prostaglandins (Sabino et al. 2002), tumor necrosis factor α (TNFα), and interleukin-1 (IL-1) secreted from cancer cells, and the other is the activation of acid-sensing ion channels such as TRPV1 and acid-sensitive ion channel 3 (ASIC3) in the peripheral nerve endings, which results from acid microenvironment formed by osteoclasts (Rousselle and Heymann 2002) and cancer cells (Griffiths 1991). And this is commonly referred to as the peripheral sensitization. Besides, there has the central sensitization taken place in the spinal cord and the brain (Honore et al. 2000).

Transient receptor potential vanilloid subfamily member1 (TRPV1) is a nociceptive receptor in unmyelinated (C-fibers) or thinly myelinated (Aδ-fibers) peripheral sensory neurons (Pei et al. 2007). TRPV1 is a ligand-gated nonselective cation channel, which can be activated by capsaicin and other stimuli such as noxious heat and low pH 7.0 (Yu et al. 2008). TRPV1 also contains consensus sites for protein kinases A and C and Src tyrosine kinases that regulate its properties through phosphorylation (Bhave et al. 2003). TRPV1 can therefore be viewed as a complex, highly modulatable sensory switch (Prager-Khoutorsky et al. 2014). TRPV1 also plays a pivotal role in the development of cancer pain (Luo et al. 2004). The expression of TRPV1 receptors is upregulated in inflammation- or nerve injury-induced thermal hyperalgesia (Huang et al. 2006) and in diabetic neuropathy (Rashid et al. 2003). TRPV1 is also upregulated and involved in cancer pain (Niiyama et al. 2007).

A research found that formaldehyde could elicit currents via TRPV1, and this current could be blocked by the specific TRPV1 antagonist capsazepine in dorsal root ganglion (DRG) neurons (Tian et al. 2009). This indicates that formaldehyde may participate in nociception via TRPV1.

Formaldehyde is ubiquitous in nature, and it is an endogenous chemical in most organisms including human (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006). Formaldehyde presents in all tissues, body fluids and blood with a concentration about 0.1 mM (Kalasz 2003). It is produced as a by-product from N-, O- and S-demethylation reactions in cells [8] and usually detoxified by L-glutathione (GSH) (Ho et al. 2007). Enzymes in formaldehyde production mainly include histone demethylase 1 (LSD1) (Metzger et al. 2005), serine hydroxymethyltransferase (SHMT) (Schirch et al. 1986), dimethylglycine dehydrogenase (DMGDH), and sarcosine dehydrogenase (SDH) (Binzak et al. 2001). Clinical data have shown that formaldehyde concentration is elevated (2–8 folds) in the urine of patients with prostate and bladder cancer (Spanel et al. 1999) and in the expired air from tumor-bearing mice and breast cancer patients (Ebeler et al. 1997),

and these patients frequently suffer from bone cancer pain (Sabino and Mantyh 2005). Formaldehyde is considered to be a risk factor of cancer development (Thorndike and Beck 1977). Therefore, whether does excessive endogenous formaldehyde induce cancer pain? And if so, does endogenous formaldehyde induces cancer pain via TRPV1?

The role of TRPV1 receptors in cancer pain is of great interest. Cancer cells metastasized to bone marrow grow rapidly, and bone tissue is progressively destroyed. At the same time, bone tissue reconstruction is initiated as a coordinated process of bone formation and absorption. Many growth factors have been reported to participate in bone formation by activating or increasing the number of bone-formation cells (Canalis 2009). In osseous metabolism processes, insulin-like growth factor-1 (IGF-1) promotes mitosis, osteoblast differentiation and bone construction (Bogdanos et al. 2003). It has been reported that local administration of IGF-1 induces thermal hyperalgesia and mechanical allodynia through the activation of IGF-1 receptors (Spencer 1987). The fact that insulin plays a role in diabetic pain through its interaction with TRPV1 may suggest a role of IGF-1 in pain. Diabetic patients with reduced insulin sensitivity experience hypoalgesia and TRPV1 downregulation (Hong and Wiley 2005), while type II diabetic patients in early stage with high insulin levels and insulin resistance often experience hyperalgesia and TRPV1 overexpression (Kamei et al. 2001). These research studies suggest us a possibility that IGF-1 is responsible for the upregulation of TRPV1 expression after bone cancer metastasis and therefore induces bone cancer pain.

To find out the above-mentioned questions, a series of research have been done in our laboratory in the past 10 years. Here, we would like to have a review on these researches.

4.2 Formaldehyde Concentration Increased in Cancer Cells and Tissues

4.2.1 Formaldehyde Concentration Increased in Cultured MRMT-1 Cells

It has been reported that high formaldehyde concentration was measured in cultured human prostate cancer cell line PC-3 (Szende et al. 1995) and human breast cancer cell line MCF-7 (Kato et al. 2001). We first investigated whether formaldehyde concentration was elevated in cultured tumor cell lines. We use high-performance liquid chromatography (HPLC) method to measure the concentration of formaldehyde. In Tong's research, formaldehyde concentrations in rat breast cancer cell line MRMT-1 cells were significantly higher at day 2 than those of controls; at the first day, cell density reached 105 cells/ml and 8×10^5 cells/ml but decreased at day 3. Formaldehyde concentration was also significantly increased in human lung cancer cell line H1299 cells and SY5Y cells at day 3 (Tong et al. 2010).

Liu et al. also measured formaldehyde concentration in cultured rat breast MRMT-1 cells. And it was increased significantly at 48 h and 72 h compared with that 24 h after inoculation. These results indicated that formaldehyde concentration was elevated in cultured tumor cell lines (Han et al. 2012).

4.2.2 *Formaldehyde Concentration Rose in Tumor Tissues from Cancer Patients*

Clinical data have shown that formaldehyde concentration increased (8–10 folds) in the serum of cancer patients (Trezl et al. 1983). It is elevated (2–8 folds) in the urine of patients with prostate and bladder cancer (Ghilardi et al. 2005) and in the expired air from tumor-bearing mice and breast cancer patients (Coleman 2001).

Formaldehyde in tumor tissues from cancer patients was measured by HPLC method. In preparations from lung cancer patients, the average formaldehyde concentration was 0.72 ± 0.06 mM ($n = 10$) with the highest concentration of 1.01 mM. This was significantly higher than that in the normal tissues adjacent to the cancer (0.19 ± 0.06 mM). In breast cancer tissues from patients, the formaldehyde concentration was 0.75 ± 0.12 mM with the highest concentration 2.35 mM. Although the breast tumor adjacent tissues (as controls) were not gained, levels of formaldehyde in human tissues were approximately 0.1–0.2 mM as previously reported (Heck and Casanova 2004). These levels are similar to the average level (0.19 ± 0.06 mM) found in human lung cancer adjacent tissues in the present experiment.

Taken together, these data show that the tumor-derived formaldehyde concentration is elevated in cancer tissues, strongly suggesting that tumor tissues secrete formaldehyde (Tong et al. 2010).

4.2.3 *Formaldehyde Concentration Was Elevated in Tissues from Rats with Bone Cancer Pain*

To clarify the change of formaldehyde concentration in bone cancer pain model, formaldehyde was detected in all of the collected tissues and blood plasma. Compared with the control group, formaldehyde concentrations significantly increased in the bone cancer pain group at day 14 in plasma, ipsilateral spinal cord, bone marrow, pancreas, liver, spleen kidney and especially in bone marrow and ipsilateral spinal cord (Han et al. 2012).

With the injection of MRMT-1 cell suspension into the bone marrow cavity, the formaldehyde concentration increased significantly in bone marrow and sera of rats with bone cancer pain at days 7, 14 and 21 compared with day 0 after inoculation. Compared with PBS group, formaldehyde concentration in bone marrows and sera

also increased significantly in bone cancer pain group at day 7 after inoculation (Han et al. 2012).

In addition, we measured the formaldehyde concentration in the spinal cord of bone cancer pain rats. In MRMT-1 bone cancer pain rats, the formaldehyde concentration increased from 0.27 ± 0.03 mM to 0.40 ± 0.01 mM. Similarly, the formaldehyde concentration was elevated (2–3 folds) in Wistar 256 bone cancer pain rats. These results indicated that formaldehyde concentration also elevated in the spinal cord from rats with bone cancer pain (Tong et al. 2010).

4.2.4 Formaldehyde Concentration Increased in Tumors and Sera of the MRMT-1 Subcutaneous Vaccination Rats

To measure the formaldehyde concentration in solid MRMT-1 tumors, rats were transplanted by subcutaneous injection of 10 μl MRMT-1cell suspension to the right side of the back (Harada 1976). Tumors and sera of these rats were taken out for the formaldehyde measurement. The results show that formaldehyde concentration increased significantly in tumors of the MRMT-1 subcutaneous vaccination model at day 7 and in sera at day 14 compared with that at day 0 after inoculation (Han et al. 2012).

A significant increase was observed not only in the MRMT-1 subcutaneous vaccination rats, but also in the Syngeneic Walker 256 intraperitoneal injection rats. Syngeneic Walker 256 mammary gland carcinoma cells were cultured by seeding into the abdominal cavity. A significant increase in the formaldehyde concentration was found in 0.5 ml ascitic fluid at day 6 and day 12 after inoculation. Formaldehyde concentration was elevated twofolds at day 6 after inoculation (from 0.04 mM to 0.08 mM) and decreased at day 12 when tumor cells grew into terminal phase (Tong et al. 2010).

Our study shows that formaldehyde concentration increased in cultured cancer cells, cancer patients' tumor tissues and tissues of rats with bone cancer pain. It is considered to be closely related to the high proliferation of tumor cells. We wondered what are the biochemical mechanisms in the increase of formaldehyde concentration in cancer cells.

4.2.5 LSD1 in MRMT-1 Cells Participated in the Production of Endogenous Formaldehyde

Lysine-specific demethylase 1 (LSD1) is an amine oxidase that removes mono- and dimethyl moieties from Lys4 of histone H3 and generates the demethylated H3 tail and formaldehyde (Shi et al. 2004; Shi et al. 2005). LSD1 regulates epigenetic gene expression that does not involve changes in the underlying DNA sequence and plays

key roles in such biological functions as embryonic development and homeostasis (Shi 2007). The expression level of LSD1 and concentration of formaldehyde were upregulated in certain high-risk tumors, such as the prostate cancer (Spanel et al. 1999; Willmann et al. 2012; Metzger et al. 2005), bladder carcinomas (Hayami et al. 2011; Kauffman et al. 2011), lung cancer (Hayami et al. 2011; Lv et al. 2012) and breast cancer (Wysocka et al. 2005; Itoh et al. 2014; Serce et al. 2012).

4.2.5.1 LSD1 Protein Expression in Cancer Cells and Tissues

From the above data, we could speculate that LSD1 might play an important role in endogenous formaldehyde production. Accordingly, expression of LSD1 was detected by Western blotting analysis in bone marrows of MRMT-1 bone cancer pain rats, cultured MRMT-1 cells, subcutaneous vaccination tumors of MRMT-1 in rats and breast tissues of normal rats. The expression of LSD1 was detected by Western blotting analysis in bone marrows of MRMT-1 bone cancer pain rats, cultured MRMT-1 cells, subcutaneous vaccination tumors of MRMT-1 in rats and breast tissues of normal rats. LSD1 expression was found in these samples. Furthermore, expression of LSD1 in the bone marrow was analyzed in MRMT-1 bone cancer pain model. Compared with that at day 0, the level of LSD1 was significantly increased at days 14 and 21 after MRMT-1 inoculation.

The location of LSD1 was shown by immunofluorescence in cultured rat breast cancer cell line MRMT-1 cells, in bone marrows of MRMT-1 bone cancer pain rats and in bone marrows of normal rats. Pan-cytokeratin is a marker for epithelium-derived tumor-like MRMT-1 breast cancer cells. It was found that LSD1 expression mainly located in nuclei of cytokeratin-positive MRMT-1 cells (cultured MRMT-1 tumor cells and bone marrow cells of MRMT-1 bone cancer pain rats), not in bone marrow cells of normal rats. Few LSD1 located in the cytoplasm of bone marrow cells of MRMT-1 bone cancer pain rats. These data suggested that LSD1 mainly expressed in the nucleus of the MRMT-1 cancer cell after inoculation into bone marrow in vivo as well as in the cultured one in vitro (Liu et al. 2013).

4.2.5.2 Inhibition of LSD1 Function Decreased Formaldehyde Concentration and Bone Cancer Pain

We would like to know whether this increased LSD1 in inoculated MRMT-1 cancer cells mediated the endogenous formaldehyde production to induce subsequent cancer pain. Cultured MRMT-1 cells were treated with pargyline, an LSD1 inhibitor. The cell viability assay results showed that pargyline at 1–10 mM had no effect, while at 20 mM inhibited the cell growth, so the pargyline at 1–2 mM was used in further experiments.

Exposure of MRMT-1 cells to pargyline (1, 1.5 or 2 mM) for 30 h significantly increased monomethyl-H3K4 and dimethyl-H3K4. The results indicated that pargyline inhibited the LSD1 function in cultured MRMT-1 cells without any

influence on the cell viability. The treatment of pargyline (2 mM) also decreased formaldehyde concentration in cultured MRMT-1 cells, suggesting that this increased LSD1 expression contributed mainly to the formaldehyde production in MRMT-1 cells.

Furthermore, we analyzed the effects of pargyline on bone cancer pain. In the bone cancer pain model of rats, thermal hyperalgesia was attenuated by intraperitoneal injection of pargyline at day 14 after MRMT-1 inoculation, and mechanical allodynia was inhibited by pargyline at days 7, 14 and 21. These results in vitro and in vivo suggest that the inhibition of LSD1 could decrease the formaldehyde production and then inhibit the subsequent bone cancer pain (Liu et al. 2013).

4.3 Formaldehyde Induced Bone Cancer Pain via TRPV1 Activation

4.3.1 Formaldehyde-Induced Bone Cancer Pain

The previous studies and the above data give direct evidence that formaldehyde can be secreted from both the cultured cancer cell lines and tumor tissues from certain cancer pain patients and cancer pain rats with its concentration reaching abnormally high levels. It has been reported that formaldehyde is considered as a cause of cancer (Wang et al. 2009) and formaldehyde at a concentration 0.1–2.5 % (33–834 mM) could induce acute pain responses (Aloisi et al. 1995a, b), while the pathological concentration of formaldehyde in tumor tissues ranged from 0.3 to 3.0 mM in both rats and patients suffering from cancer pain. From these data, we have a question here: What is the relationship of formaldehyde with cancer pain? To figure out this question, we carried out the following researches.

4.3.1.1 Formaldehyde at Low Concentration Induced Acute Pain Behaviors

It was mentioned that high concentration of formaldehyde (2.5–5 %, equal to 883–1666 mM) as a chemical irritant induced inflammatory pain (Yi et al. 2011; Su et al. 2010). However, in our previous studies, formaldehyde concentration in bone marrows of bone cancer pain models and solid tumors of cancer patients was relatively low (<3 mM). Therefore, we would like to know whether formaldehyde at such a low concentration could induce pain responses. After the intraplantar injection of formaldehyde at doses of 1 mM and 3 mM to the right hind paw of normal rats, pain behaviors within 5 min was recorded. Compared with the NS control group, 3 mM formaldehyde increased the pain response time. Preapplication of glutathione (GSH, a formaldehyde scavenger) decreased the formaldehyde-induced pain responses. These data suggested that formaldehyde at a pathological concentration as low as in the cancer tissues could induce pain behavior (Liu et al. 2013).

4.3.1.2 Formaldehyde Secreted by Cancer Tissues Induced Bone Destruction

Cancer cell metastasis to bone marrow increases osteolysis and osteoclastic activity and induces an acidic microenvironment (Nagae et al. 2007). A recent research report demonstrated that formaldehyde, gradually released by root canal sealers, elicited bone necrosis (Tortorici et al. 2007). Elevated formaldehyde was also observed in patients with dental caries (Rozylo et al. 2000). Cytotoxicity resulting from excessive formaldehyde in human osteoblastic cells has been considered to be an important factor in bone destruction (Ho et al. 2007; Huang et al. 2005). Formaldehyde can accumulate in bone marrow (Gronvall et al. 1998). In our present study, formaldehyde concentration was elevated to about 0.6 mM in bone marrow of MRMT-1 bone cancer pain model in rats. This level is high enough to be toxic to osteoblastic cells. Bone destruction was found in the MRMT-1 bone cancer pain model in rats. Formaldehyde scavengers, resveratrol and glutathione obviously decreased bone destruction. Therefore, excessive formaldehyde secreted by cancer tissues may play a role in bone destruction. This bone destruction then contributes to cancer pain, because nerve fiber endings innervating bone is more easily exposed to tumor tissue-derived factors (Tong et al. 2010).

4.3.1.3 Formaldehyde Enhanced Neural Excitatory

One report has indicated that formaldehyde (0.013 mM) can elicit currents in DRG neurons (Tian et al. 2009). To find more evidence that formaldehyde induced bone cancer pain, we further used calcium imaging to test whether low-concentration formaldehyde can directly excite DRG neurons. As expected, formaldehyde at concentrations of 1 mM to 100 mM induced a concentration-dependent increase of [Ca2+]i in acutely isolated rat DRG neurons (Tong et al. 2010).

4.3.2 Formaldehyde Induced Pain Responses via TRPV1

From above results, we could reach a conclusion that formaldehyde induces cancer pain. But by which target can formaldehyde induce pain? Recent researches have shown that both TRPA1 and TRPV1 are possible targets of endogenous formaldehyde in vitro and in vivo (Macpherson et al. 2007). In the report of Macpherson et al., formaldehyde-evoked calcium responses in DRG neurons and nocifensive behaviors were almost abolished in TRPA1-/- mice. At the same time, formaldehyde could still evoke pain responses in the TRPA1-/- mice. In addition, a recent study also showed that TRPV1 participates in nociception especially under acidic conditions (Ugawa et al. 2002). And other researches reported that the selective TRPV1 antagonists, such as iodo-resiniferatoxin (Kanai et al. 2006) and capsazepine, and the nonselective antagonist ruthenium red (Santos and Calixto 1997)

inhibited formalin-induced pain behaviors. These findings suggest that TRPV1 may participate in the formaldehyde-evoked pain.

4.3.2.1 Formaldehyde Increased TRPV1 Expression in Primary Cultured DRG Neurons

The expression of TRPV1 protein in primary cultured DRG neurons treated with formaldehyde (30, 100 and 300 μmol/L) was measured with Western blot at 4, 12, 24, 48 and 72 h of formaldehyde incubation. Compared with the control group, TRPV1 protein increased significantly at 48 h and further increased at 72 h of incubation in the 100 μmol/L formaldehyde group. In addition, TRPV1 mRNA levels detected by RT-PCR showed changes consistent with those of protein expression. In the 100 μmol/L formaldehyde group, TRPV1 mRNA increased at 48 h and further increased at 72 h. Thus, it is suggested that endogenous formaldehyde at very low concentrations upregulates TRPV1 expression (Han et al. 2012).

4.3.2.2 Inhibitory Effects of MAPK and PI3K Inhibitors on Formaldehyde-Induced TRPV1 Upregulation in Primary Cultured DRG Neurons

MAPKs, PI3K and PKC play critical roles in cell signaling. MAPKs have three major family members: ERK, p38 and JNK, which represent three different signaling cascades (Cheng and Ji 2008). TRPV1 expression can result from ERK and p38 signaling pathways (Bron et al. 2003; Chen et al. 2008). PI3K is another key mediator of central pain sensitization and of inflammatory heat hyperalgesia through TRPV1 sensitization (Pezet et al. 2008; Zhuang et al. 2004).

To figure out which pathway participated in formaldehyde-induced TRPV1 upregulation, we used Western blot to detect the TRPV1 protein. After 72-h incubation of cultured DRG neurons with 100 μmol/L formaldehyde alone, TRPV1 protein expression increased significantly. Compared with the formaldehyde-only group, the TRPV1 expression decreased after preincubation with the signal transduction pathway inhibitors PD98059 (an ERK inhibitor), SB203580 (a p38 inhibitor), SP600125 (a JNK inhibitor) or LY294002 (a PI3K inhibitor). However, TRPV1 protein expression did not change after addition of BIM (BIM, a PKC inhibitor).

Furthermore, changes in TRPV1 mRNA expression detected by RT-PCR in DRG neurons were consistent with those of TRPV1 protein expression. TRPV1 mRNA increased significantly 72 h after 100 μmol/L formaldehyde treatment. Compared with the formaldehyde-only group, TRPV1 mRNA expression decreased in the formaldehyde groups with preaddition of the inhibitors PD98059, SB203580, SP600125 or LY294002, but did not change after addition of BIM (Han et al. 2012).

So, the present study gives preliminary evidence that formaldehyde upregulates TRPV1 expression through MAPK and PI3K signal pathways, but not through the PKC signal pathway.

4.3.2.3 Formaldehyde Induced Ca^{2+} Influx and Elicited Currents in TRPV1-CHO Cells with pH of 6.0

To verify whether formaldehyde directly activates TRPV1, the effect of formaldehyde on TRPV1-transfected CHO (TRPV1-CHO) cells was examined by measuring the fluorescent intensity of Ca2+. Formaldehyde (>0.1 mM) induced an increase of cytosolic [Ca2+]i in a concentration-dependent manner. As a control, in the untransfected CHO cells, formaldehyde at 100 mM elicited only slight Ca2+ influx. Formaldehyde-induced Ca2+ influx in the TRPV1-CHO cells was significantly inhibited by the TRPV1 antagonists capsazepine and melatonin.

Furthermore, the TRPV1 current induced by capsaicin, a TRPV1 antagonist, and formaldehyde (with or without pH 6.0) using patch clamp recording in TRPV1-CHO cells was recorded. Capsaicin at 10 mM induced an inward current with voltage clamped at −60 mV. Capsazepine strongly suppressed the capsaicin-induced current. Similarly, formaldehyde at 3 mM (concentration detected in human tumor tissues) induced an inward current in TRPV1-CHO cells in a concentration-dependent manner, and 10 mM capsazepine blocked the formaldehyde-induced current. As controls, neither 3 mM formaldehyde, nor 10 mM capsaicin, nor formaldehyde plus capsaicin induced any current in the untransfected CHO cells. Although low pH of 6.0 in extracellular solution had little effect on TRPV1-CHO cells, currents induced by formaldehyde at 1–10 mM were significantly potentiated by pH 6.0. This result indicates that there is a synergistic effect between formaldehyde and an acidic environment. As a positive control, formaldehyde at 1 and 3 mM also markedly potentiated the capsaicin (1 mM)-induced current in the TRPV1-CHO cells. These data suggest that formaldehyde directly activates TRPV1 with more efficiency at low pH (Tong et al. 2010).

4.3.2.4 Formaldehyde Induced Pain Behaviors via TRPV1 Activation

The formalin test (5 % formalin, i.e. 1667 mM formaldehyde) is a commonly used classic pain model. We found that formaldehyde scavengers glutathione (GSH) and resveratrol (Res) and TRPV1 antagonists capsazepine (CPZ) and melatonin (MT) significantly decreased the number of flinchings in a dose-dependent manner in both acute and tonic phases, similar to that in previous reports (Ray et al. 2004). The solvent used for these regents, DMSO (final concentration 10 %), by itself did not show significant effect.

This in turn to see whether formaldehyde at pathologically low concentrations (1, 3 mM, based on the concentrations of formaldehyde detected in human tumor tissues) can induce pain responses and whether TRPV1 or TRPA1 is involved in the pain responses. The results show that capsazepine, melatonin and AP-18 (a TRPA1

antagonist) all attenuated the low concentration formaldehyde (5 mM)-induced pain responses. These indicate that formaldehyde at low pathological concentration can induce pain behavioral responses, possibly through activation of TRPV1 and TRPA1. Moreover, formaldehyde (3 mM) with a low pH of 5 or 6 (mimicking the acidic cancer microenvironment) induced more severe pain responses than formaldehyde in a neutral environment (pH 7.4). These responses were partially inhibited by AP-18, but almost completely inhibited by capsazepine (a TRPV1 antagonist). These data suggest that TRPV1 plays a key role in low concentration formaldehyde-induced pain behaviors under acidic environment (Tong et al. 2010).

4.4 IGF-1 Enhanced TRPV1 Function in Bone Cancer Pain (Li et al. 2014)

Insulin-like growth factor-1 (IGF-1), a highly conserved signaling molecule, is a multifunctional peptide that can promote mitosis, apoptosis, bone construction and enhance osteogenic differentiation of bone marrow mesenchymal stem cells (Bogdanos et al. 2003; Feng et al. 2014). On the other hand, IGF-1 has neurotrophic effects (Fernyhough et al. 1993) after nerve injury. When cancer cells grow in bone marrow after metastasis, nerve regeneration is concurrent with bone destruction and reconstruction (Spencer 1987). The regeneration of nerves may induce pain. For example, local administration of IGF-1 induces thermal hyperalgesia and mechanical allodynia through activation of IGF-1 receptors (IGF-1R) (Spencer 1987; Boucher et al. 2014). The fact that insulin plays a role in diabetic pain through its interaction with TRPV1 may suggest a role of IGF-1 in pain (Li et al. 2014). These indicated that IGF-1 may participate in pain via TRPV1.

4.4.1 IGF-1 Expression Increased in MRMT-1 Bone Cancer Pain Rats

The expression of IGF-1 in tibia bone marrow was investigated after cancer cell inoculation. The result showed the IGF-1 expression in tibia bone marrow with immunohistochemical staining. At days 7, 14 and 21 after MRMT-1 live cell inoculation, statistical analysis showed that IGF-1 expression increased significantly. At day 21, histological hemotoxylin and eosin (HE) staining of bone marrow tissues showed apparent bone regeneration.

4.4.2 TRPV1 Current Density and Protein Expression Increased in DRG Neurons in MRMT-1 Bone Cancer Pain Rats

The sensitivity of a DRG neuron to capsaicin was detected by capsaicin-induced current with patch clamp recordings. The membrane potential of the neuron was held at −60 mV and the neuron was perfused with capsaicin for 30 s. The peak amplitude of the capsaicin-induced current was used to calculate the current density. Perfusion with 1 μM capsaicin produced an inward current in a neuron. The result showed that the current density was significantly increased in DRG neurons from MRMT-1 bone cancer pain rats.

4.4.3 TRPV1 Expression Increased as Well as Functionally Enhanced in Bone Cancer Pain Rats

4.4.3.1 Co-localization of IGF-1 Receptor and TRPV1 in DRG Neurons

Existence of IGF-1Rs on the membrane of TRPV1-expressing DRG neurons is the basis for IGF-1 upregulation of TRPV1. Therefore, co-localization of TRPV1 receptors and IGF-1Rs was examined in one DRG neuron. As expected, immunofluorescent double staining showed that IGF-1R co-localized with TRPV1 in small DRG neurons.

4.4.3.2 IGF-1 Incubation Increased Total and Membrane TRPV1 Protein Expression in Primary Cultured DRG Neurons

The effect of IGF-1 was tested on the primary cultured DRG neurons. First, the time course of IGF-1-induced TRPV1 expression was analyzed. After incubation with IGF-1 at 30 ng/ml, the total TRPV1 expression increased significantly at 48 and 72 h in the IGF-1 incubation group as compared with that in the control group. This IGF-1-induced upregulation of TRPV1 protein expression could be observed even at 96 h.

The dose effect of IGF-1 was also analyzed. After incubation for 72 h with IGF-1 at 3, 30 and 100 ng/ml, DRG neurons were harvested to examine the TRPV1 protein expression. It was found that TRPV1 expression increased significantly at IGF-1 concentrations of 30 and 100 ng/ml compared with PBS control. Considering the functional importance of membrane TRPV1, membrane TRPV1 protein was further examined after incubation with IGF-1 at 30 ng/ml. As the result shown, membrane TRPV1 protein increased significantly at all-time points.

4.4.3.3 IGF-1 Incubation Increased TRPV1 Current Density in Primary Cultured DRG Neurons

We then tested whether the upregulated membrane TRPV1 by IGF-1 incubation in the primary cultured DRG neurons was functional. With whole-cell patch clamp recording technique, the capsaicin-induced currents were measured in primary cultured DRG neurons. Interestingly, incubation with IGF-1 at 30 ng/ml for 24 and 72 h significantly increased the capsaicin-induced currents, suggesting that the upregulated TRPV1 by IGF-1 is functional.

4.4.4 IGF-1R Inhibitor Reversed Pain Behavior in Bone Cancer Pain Rats

To detect the contribution of IGF-1 in bone cancer pain in vivo, the effect of picropodophyllotoxin (PPP, an IGF-1R inhibitor) was examined on pain behavior. PPP was injected intraperitoneally for three consecutive days from day 15 to day 17 after the MRMT-1 live cell inoculation when thermal hyperalgesia and mechanical allodynia were apparent. An equal volume of vehicle (PBS) was used as a control. As the result shown, before PPP application at day 14 after MRMT-1 live cell inoculation, both the PPP group and the PBS group showed thermal hyperalgesia and mechanical allodynia consistent with the establishment of pain behavior. During days 15–17 when PPP was administrated, pain behaviors were alleviated significantly, suggesting that IGF-1R inhibition could reverse bone cancer pain in rats.

4.5 Conclusion

As depicted in Fig. 4.1, cancer cells produced formaldehyde through demethylation by serine hydroxymethyltransferase (SHMT1 and SHMT2) and lysine-specific demethylase 1 (LSD1). When the cancer cells metastasized into bone marrow, the increased endogenous formaldehyde induced cancer pain through activation of the transient receptor potential vanilloid subfamily member 1 (TRPV1) in the peripheral nerve fibers. At the same time, TRPV1 expression in the peripheral fibers was upregulated by the local insulin-like growth factor I (IGF-I) produced by the activated osteoblasts. In conclusion, tumor tissue-derived endogenous formaldehyde induced bone cancer pain via the activation of TRPV1, which was upregulated by IGF-1.

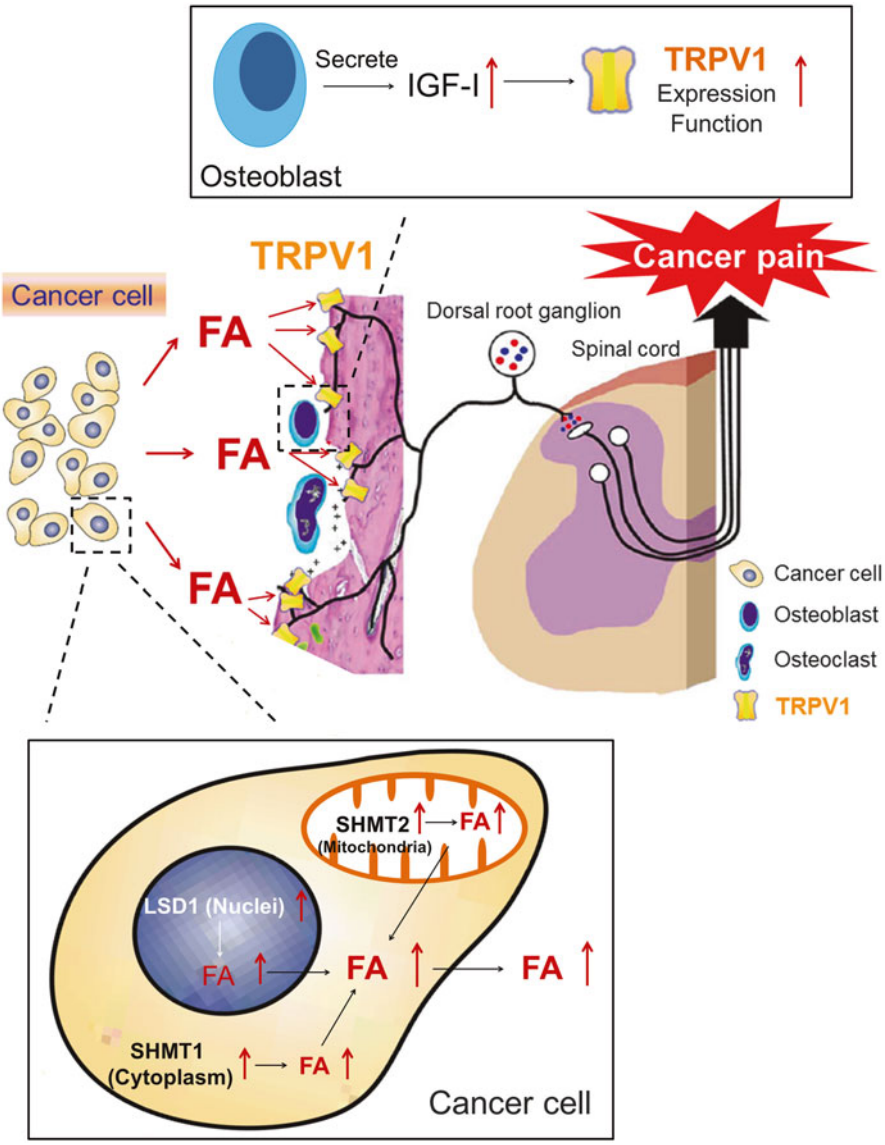

Fig. 4.1 Schematic illustration of tumor tissue-derived endogenous formaldehyde (FA) in the induction of bone cancer pain via TRPV1 after cancer cells metastasized into bone marrow (*FA* formaldehyde, *IGF-1* insulin-like growth factor 1, *LSD1* lysine-specific histone demethylase 1, *SHMT1* serine hydroxymethyltransferase 1, *TRPV1* transient receptor potential vanilloid receptor subtype 1)

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Chapter 5
Neuropathic Pain: Sensory Nerve Injury or Motor Nerve Injury?

Xian-Guo Liu, Rui-Ping Pang, Li-Jun Zhou, Xu-Hong Wei, and Ying Zang

Abstract Peripheral nerve injury often induces chronic neuropathic pain. Peripheral nerve is consisted of sensory fibers and motor fibers, it is questioned injury to which type of fibers is responsible for generation of neuropathic pain? Because neuropathic pain is sensory disorder, it is generally believed that the disease should be induced by injury to sensory fibers. In recent years, however, emergent evidence shows that motor fiber injury but not sensory fiber injury is necessary and sufficient for induction of neuropathic pain. Motor fiber injury leads to neuropathic pain by upregulating pro-inflammatory cytokines and brain-derived neurotrophic factor in pain pathway.

Keywords Neuropathic pain • Neuroinflammation • Ectopic discharge • Long-term potentiation • Motor fiber

5.1 Introduction

Peripheral nerve injury often induces chronic neuropathic pain that may persist for years, or even for life time. Neuropathic pain is considered as a disease of nervous system, as its underlying mechanisms are substantially different from those of physiological pain. In spite of intensive study for decades, prevention and treatment of the disease is still a big challenge for clinician and for pain researchers, because the mechanism still remains largely unknown. For example, peripheral nerve is consisted of sensory fibers and motor fibers, injury to which one is responsible for generation of neuropathic pain is still unclear. The answer to this basic question is important for determining the start point for investigation of neuropathic pain and for its clinical, especially surgical, treatment. Because neuropathic pain is sensory disorder, it appears reasonable to consider that it should be induced by injury to sensory fibers. In recent years, however, emergent experimental and clinical

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evidence shows that sensory fiber injury is neither necessary nor sufficient for induction of neuropathic pain. In contrast, motor fiber injury almost always leads to neuropathic pain. In this article, the mechanisms, by which motor fiber injury induces peripheral sensitization (ectopic discharges in primary sensory neurons) and central sensitization (long-term potentiation at C-fiber synapses in spinal dorsal horn), are reviewed with emphasis on the role of glial activation and subsequent overproduction of pro-inflammatory cytokines and of brain-derived neurotrophic factor.

5.2 Injury to Motor Fibers But Not to Sensory Fibers Often Induces Lasting Allodynia and Hyperalgesia

All the animal models used for neuropathic pain research, except for lumbar 5 ventral root transection (L5-VRT), are prepared by injury of peripheral nerves, such as transection, tight ligation or lose ligation, and so on. The data obtained from such kinds of animal models cannot tell injury to which type of fibers is responsible for generation of neuropathic pain, as peripheral nerve contains both sensory fibers and motor fibers. To answer the question experimentally, selective injury to either dorsal root (sensory) or ventral root (motor), or selective injury to the nerve branches that mainly contains sensory fibers or motor fibers is needed (Fig. 5.1a).

In majority of animal studies, L5 dorsal root transection (L5-DRT) fails to induce mechanical allodynia and thermal hyperalgesia, behavioral signs of neuropathic pain (Obata et al. 2006; Black et al. 1999; Sheen and Chung 1993; Luo et al. 2001; Xu et al. 2007), or leads to a short-lasting neuropathic pain behaviors, compared with L5 spinal nerve ligation (Sheth et al. 2002; Sekiguchi et al. 2009; Li et al. 2000). Similarly, transection of both C 7 and C8 dorsal roots only induces a short-lasting cold hyperalgesia (around 15d), but not mechanical or thermal allodynia in the ipsilateral forepaw, while transection of C8 dorsal root alone fails to induce any change in pain-related behaviors (Ramer et al. 2004). Only a few works showed that L5-DRT did lead to lasting neuropathic pain (Eschenfelder et al. 2000; Colburn et al. 1999). The discrepancy may be resulted from the differences in the accuracy of surgery (unintended injury to ventral root or spinal cord), in the environment, in which the experiments were performed, or in proficiency of behavioral tests. To rule out the possibility that factors other than nerve root injury may lead to the controversial results, we (Xu et al. 2007) re-examined the issue and found that L5-DRT did not induce mechanical allodynia and thermal hyperalgesia, while L5-VRT led to the behavioral signs of neuropathic pain in bilateral hind paws (Fig. 5.1a–d). Accordingly, injury to ventral root but not to dorsal root is necessary and sufficient for initiation of neuropathic pain. Consistently, a recent prospective cohort study on long-term effects of selective dorsal rhizotomy in patients with cerebral palsy shows that the surgery reduces pain but not induces pain (Tedroff et al. 2015). Furthermore, it has been shown that transection of the sural nerve, which contains only 3 % of

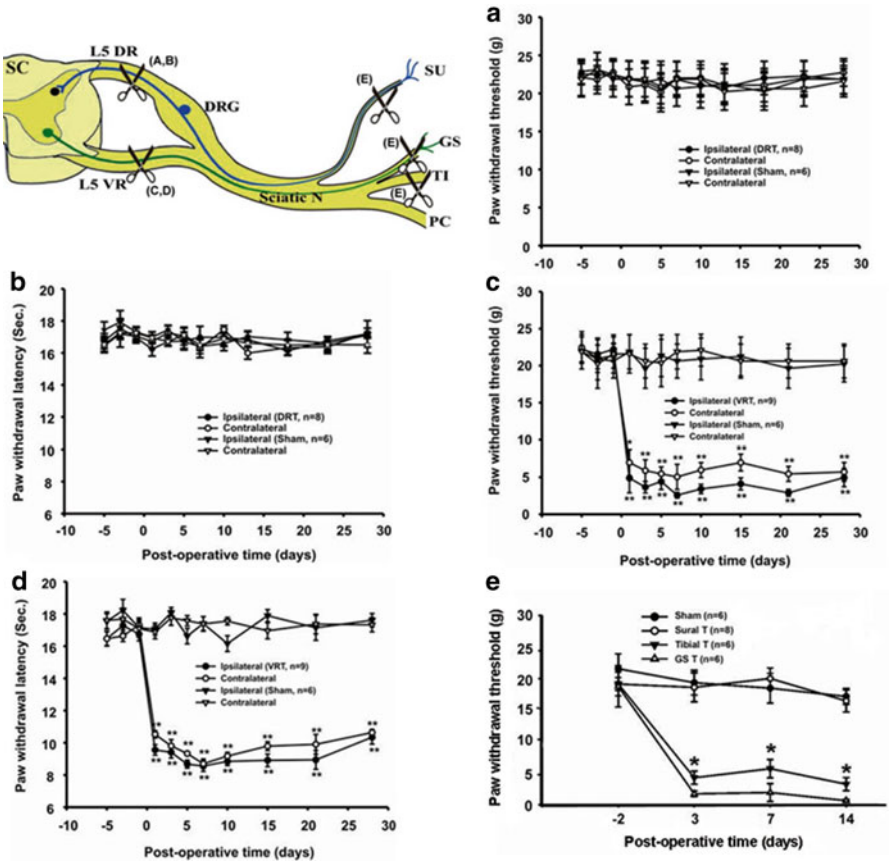

Fig. 5.1 Injury to motor fibers but not sensory fibers induces the behavioral signs of neuropathic pain

The branches of rat sciatic nerve and experiment designs are shown at top. (**a–b**): L5 dorsal root transection (L5-DRT) does not affect mechanical paw withdrawal threshold and paw withdrawal latency to heat. In contrast, L5-ventral root transection (L5-VRT) induces mechanical allodynia and thermal hyperalgesia bilaterally (**c**, **d**). (**e**): Transection of tibial nerve (TI) or gastrocnemius–soleus (GS) nerve but not the sural (SU) nerve decreased mechanical paw withdrawal threshold (**a–d** are adapted from Xu et al. (2007) and E from Zhou et al. (2010a) with permission)

motor neurons of the sciatic nerve (Swett et al. 1986), fails to induce the behavioral signs of neuropathic pain in rats (Zhou et al. 2010a) (Fig. 5.1f). This is in agreement with the clinical data that majority of patients with the transection of the sural nerve for nerve grafting do not suffer from postoperative donor site pain (Capek et al. 1996; IJpma et al. 2006; Martins et al. 2012).

In striking contrast, numerous animal studies from different groups have demonstrated that selective injury of motor fibers by L5-VRT leads to mechanical allodynia and thermal hyperalgesia in bilateral hind paws (Li et al. 2003; Xu et al. 2006;

He et al. 2010; Zang et al. 2014; Wei et al. 2013; Obata et al. 2006; Xiao et al. 2011; Li et al. 2002; Sheth et al. 2002; Wu et al. 2002). Consistently, injury to gastrocnemius–soleus (GS) nerve innervating muscles (Swett et al. 1986) also leads to lasting neuropathic pain in rats (Zhou et al. 2010a) (Fig. 5.1f).

The experimental and clinical evidence mentioned above demonstrates clearly that motor fiber injury but not sensory fiber injury is necessary and sufficient for generation of chronic neuropathic pain. Does damage of motor neurons produce chronic pain in human patients? Recent clinical data have shown that the patients with postpoliomyelitis and Guillain-Barré syndrome, both of which affect spinal motor neurons or motor axons (Kuwabara and Yuki 2013), suffer from chronic pain in low back and in legs (Stoelb et al. 2008; Rekand et al. 2009). This is in line with the finding that selective injury of motor fibers produces neuropathic pain in experimental animals, although the situations in patients with postpoliomyelitis and Guillain-Barré syndrome are more complicated.

5.2.1 The Differential Effects of Injury to Motor Fibers and Sensory Fibers on Peripheral Sensitization

The peripheral sensitization is mainly manifested as spontaneous discharge of action potentials in primary sensory neurons in neuropathic pain condition. The abnormal activity is also termed ectopic discharge or ongoing activity. In this section, we will discuss the differential effects of injury to sensory fibers and motor fibers on the generation of ectopic discharge in DRG neurons.

5.2.2 The Ectopic Discharges in Intact But Not in Injured Afferents Are Responsible for Neuropathic Pain

Since Wall et al. (1974) discovered the ongoing activity in primary afferents in injured peripheral nerve, the phenomenon has been intensively studied as a leading cause for neuropathic pain. The pathological significance of ectopic discharges is proposed to elicit spontaneous pain and to produce allodynia and hyperalgesia by induction and maintenance of central sensitization. It was once believed that the ectopic discharges in injured (axotomized) afferents contribute directly to neuropathic pain (Blumberg and Janig 1984; Govrin-Lippmann and Devor 1978). Later, the ectopic discharges are also recorded in spared intact afferents following peripheral nerve injury (Michaelis et al. 2000; Wu et al. 2001). Importantly, it has been demonstrated that neuropathic pain is independent on the inputs from injured afferents, as the mechanical hyperalgesia produced by L5 spinal nerve lesion cannot be prevented or reversed by transection of L5 dorsal root (Li et al. 2000). These data from different groups indicate that ectopic discharges from uninjured but not injured

afferents are important for the development of neuropathic pain. This notion is in complete agreement with the fact that selective injury to motor fibers, leaving the sensory fibers intact, by L5 VRT induces neuropathic pain.

5.2.3 *The Ectopic Discharge Is Produced by Injury to Motor Fibers But Not to Sensory Fibers*

As mentioned above, rat sciatic nerve has two special branches: the sural nerve consisting of sensory fibers innervating skin and the GS nerve containing both sensory and motor fibers innervating muscle. It has been shown (Michaelis et al. 2000) that the ectopic discharges can only be recorded in the injured or uninjured GS nerve but never in the injured sural nerve following transection of both sural nerve and GS nerve, transection of all branches of the sciatic nerve, or transection of all branches except for SG nerve. The results indicate that the ectopic discharges occur only in muscle afferents but not in skin afferents and also suggest that injury to motor fibers but not to sensory fibers may be responsible for generation of ectopic discharges. The notion is confirmed by Wu et al. (2001) demonstrating that selective injury to motor fibers by L5 VRT leads to the spontaneous activity in 25 % of C-fiber afferents in intact L4 spinal nerve.

5.2.4 *The Differential Effects of Motor Fiber Injury and Sensory Fiber Injury for the Expression of Voltage-Gated Sodium Channels in Dorsal Root Ganglion Neurons*

The expression of voltage-gated sodium channels (VGSCs), which are essential for the generation and propagation of action potentials, is altered substantially in dorsal root ganglion (DRG) neurons following peripheral nerve injury, and the change is proved to underlie the ectopic discharges (Rush et al. 2007). To date, at least nine subtypes (Nav1.1-Nav1.9) of VGSCs have been cloned and identified on mammalian cells. Among them Nav1.6 ~ 1.9 are highly expressed in DRG neurons, while Nav1.3 is expressed at a high level in embryonic nervous system but is barely detectable in the DRG neurons of adult rats (Beckh et al. 1989). As reviewed by Tao et al. (Wang et al. 2011), peripheral nerve injury only upregulates Nav1.3 but downregulates Nav1.6 ~ 1.9 in injured DRG neurons. Therefore, the role of VGSCs in neuropathic pain is questioned. As discussed above, the ectopic discharges in intact but not in injured afferents are responsible for neuropathic pain. Consistently, it has been shown that Nav1.6 ~ 1.9 mRNAs are upregulated in uninjured DRG neurons but downregulated in injured ones (Berta et al. 2008), and Nav1.3 mRNA is upregulated in both of them (Waxman et al. 1994; Boucher et al. 2000).

Importantly, silence of either Nav1.3 or Nav1.8 expression by specific antisense oligodeoxynucleotides or blockage of Nav1.8 with different kinds of specific antagonists reverses mechanical allodynia and thermal hyperalgesia produced by peripheral nerve injury (Hains et al. 2004; Lai et al. 2002; Jarvis et al. 2007; Ekberg et al. 2006). Clearly, the overexpression of VGSCs in uninjured DRG neurons is critical for the development of neuropathic pain.

In line with the notion that injury to motor fibers but not to sensory fibers induces neuropathic pain, it has been shown that selective injury of sensory neurons by L5 DRT does not affect the expression of Nav1.3 (Black et al. 1999), Nav1.8 and Nav1.9 in DRG neurons (Sleeper et al. 2000). In striking contrast, our recent works show that selective injury to motor fibers by L5-VRT leads to a long-lasting re-expression of Nav1.3 and upregulation of Nav1.8 in bilateral L4 and L5 DRG neurons (He et al. 2010; Chen et al. 2010). Taken together, motor fiber but not sensory fiber injury contributes to the upregulation of VGSCs in DRG neurons following peripheral nerve injury.

5.3 The Differential Effects of Injury to or Electrical Stimulation of Motor Fibers and Sensory Fibers on Central Sensitization

LTP, referring to a lasting increase in efficacy of synaptic transmission, was first discovered in hippocampus in 1973 (Bliss and Lomo 1973), since then it has been intensively studied as synaptic model of memory storage. LTP at C-fiber synapses in spinal dorsal horn was first reported in 1995 (Liu and Sandkuhler 1995). Based on the following experimental and clinical data, the spinal LTP is considered as a synaptic model of pathological pain (Liu and Zhou 2015). Afferent C-fibers that conduct nociceptive signals make synapses with second-order neurons in the superficial spinal dorsal horn (Light et al. 1979). The spinal LTP is induced by the events that lead to pathological pain, such as electrical stimulation of C-fibers but not of A-fibers (Liu and Sandkuhler 1997), peripheral nerve injury (Zhang et al. 2004; Zhou et al. 2010a), tissue inflammation (Ikeda et al. 2006), and opioid withdrawal (Drdla et al. 2009); LTP-inducible conditioning stimulation produces a long-lasting behavioral signs of pathological pain in human subjects (Klein et al. 2004). The spinal LTP at C-fiber synapses is a pathological plasticity in nature, and its pathological significance is to amplify pain signals in the first-order relay in pain pathway.

5.3.1 *Activation of Muscle Afferents But Not Skin Afferents Induces Late-Phase LTP in Spinal Dorsal Horn*

In general, spinal LTP can be induced directly by activation or injury of primary afferent C-fibers but not of efferents. LTP induced at the synapses that have been activated by the conditioning stimulation is defined as homosynaptic LTP, while that induced at synapses that have not been directly activated by the conditioning stimulation as heterosynaptic LTP (Engert and Bonhoeffer 1997). As in spinal dorsal horn afferents innervating skin and muscle convergence to the same neuron, LTP may be induced either homosynaptically or heterosynaptically, that is, the synaptic transmission at skin afferents may be potentiated by inputs from muscle afferents and vice versa. The strength (efficacy) of synaptic transmission at C-fiber synapses in spinal dorsal horn can be measured by recording C-fiber-evoked field potentials elicited by electrical stimulation of the sciatic nerve (test stimulus) in vivo, and LTP of the field potentials is generally induced by activation of the same nerve with conditioning stimulation, such as high-frequency stimulation (HFS), low-frequency stimulation (LFS) or injury of the nerve distal to stimulation electrode. With the use of such protocol, it is impossible to distinguish the activation of which afferents (muscle or skin) is responsible for LTP induction, as sciatic nerve contains both muscle and skin afferents. To solve the problem, the test stimuli for evoking C-fiber field potentials and conditioning stimulation for inducing LTP should be delivered to different nerves, such as to the sural nerve or GS nerve, respectively. As shown in Fig. 5.2a, b, the homosynaptic LTP of the sural nerve, that is, both the test stimuli and HFS or nerve injury are delivered to the sural nerve, persists less than 2 h, while homosynaptic LTP of tibial nerve innervating both skin and muscle persists until the end of the experiments (at least for 7 h). Consistently, the heterosynaptic LTP of the sural nerve-evoked C-fiber responses induced by HFS of the tibial nerve or injury of the GS nerve persists also until the end of the experiments, while HFS to the sural nerve at C-fiber strength does not affect C-fiber responses evoked by stimulation of the tibial nerve (Fig. 5.2c), suggesting that muscle nerve injury may lead to secondary hyperalgesia in skin (Zhou et al. 2010a). Both hippocampal LTP and spinal LTP are divided into two phases; the late-phase LTP (>3 h), but not early-phase LTP (<3 h), is dependent on de novo protein synthesis (Hu et al. 2003; Frey et al. 1988). Thus, the noxious inputs from muscle but not from skin afferents induce late-phase LTP, a long-lasting central sensitization.

5.3.2 *Injury to Motor Fibers May Induce Spinal LTP at C-Fiber Synapses, Indirectly*

Injury to motor fibers cannot directly induce the spinal LTP at C-fiber synapses because of lack of synaptic connection between motor fibers and dorsal horn neurons. Could injury to motor fibers induce the spinal LTP, indirectly? This has not been tested so far, because the time period for recording C-fiber-evoked field

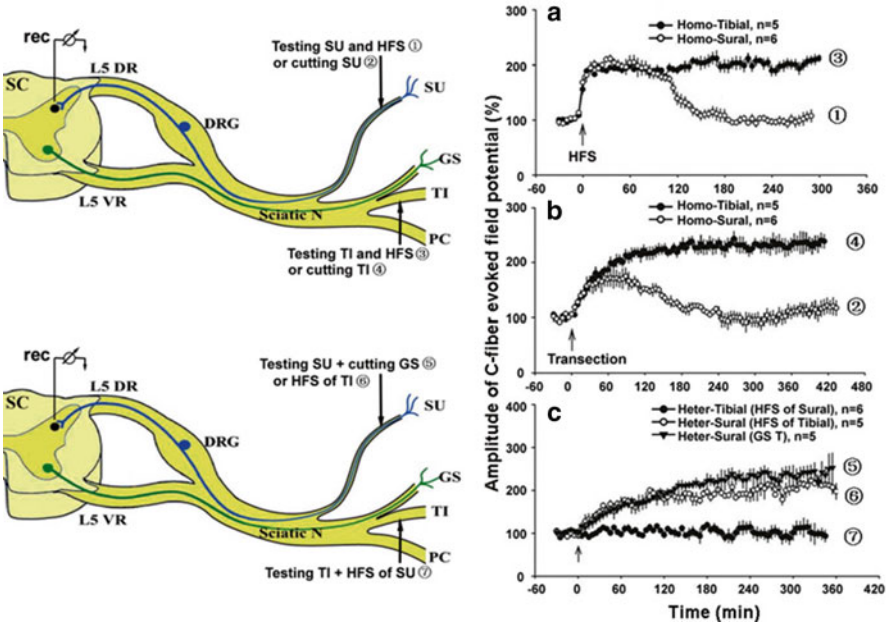

Fig. 5.2 Late-phase LTP of C-fiber-evoked field potentials is induced by electrical stimulation or injury to the nerve innervating muscle but not skin
Schematic drawings illustrate the experiment designs for investigating homosynaptic LTP and heterosynaptic LTP. (**a**): Homosynaptic LTP induced by high-frequency stimulation (HFS, 100 Hz, 40 V, 0.5 ms, 100 pulses given in four trains of 1-s duration at 10-s intervals) of either SU nerve or TI nerve. (**b**): Homosynaptic LTP induced by transection of either SU nerve or TI nerve distal to testing electrode. (**c**): Heterosynaptic LTP induced by electrical stimulation or transection of SU nerve or TI nerve. (**a**–**c**) are adapted from Zhou et al. (2010a) with permission

potentials in vivo is limited (<10 h). According to following data, we believed that possibility may exist. L5-VRT upregulates tumor necrosis factor-alpha (TNF-α) (Xu et al. 2006) and interleukin-1beta (IL-1β) (Winkelstein et al. 2001) in DRG and spinal dorsal horn and spinal application of TNF-α (100 pg/ml) or IL-1β (5 ng/ml) at recording segments is sufficient to induce spinal LTP of C-fiber-evoked field potentials in the rats with neuropathy (Liu et al. 2007; Zhong et al. 2009). In addition, intrathecal application of antibody against brain-derived neurotrophic factor (BDNF) markedly blocks the development of mechanical hyperalgesia produced by L5-VRT (Chen et al. 2013), and L5 spinal nerve ligation upregulates BDNF in uninjured L4 DRG neurons (Fukuoka et al. 2001). Importantly, spinal application of BDNF (1 ng/ml) is capable of inducing LTP of C-fiber-evoked field potentials in naive rats (Zhou et al. 2010b). Therefore, motor fiber injury may induce spinal LTP at C-fiber synapses indirectly via upregulation of pro-inflammatory cytokines and BDNF.

5.4 The Motor Fiber Injury Leads to the Neuropathic Pain by Upregulation of Pro-inflammatory in Pain Pathway

It has been shown that TNF-α and TNFR-1 are upregulated in DRG neurons and in neurons, microglia and astrocytes of spinal dorsal horn following L5-VRT, starting as early as one day and persisting for around 2 weeks after operation, and that pretreatment with TNF-α synthesis inhibitor, thalidomide (Xu et al. 2006) or genetic deletion of TNFR1 (Wu et al. 2014) prevents neuropathic pain. Local application of TNF-α in the sciatic nerve at physiological concentration is sufficient to induce lasting behavioral signs of neuropathic pain (Zelenka et al. 2005; Wei et al. 2007). Accordingly, it is no doubt that upregulation of TNF-α is not only necessary but also sufficient to induce neuropathic pain. Interestingly, crush of L5 dorsal root produces less TNF-α in DRG neurons and shorter-lasting mechanical allodynia, compared with crush of L5 spinal nerve (Sekiguchi et al. 2009). It has been well documented that nerve injury-induced nerve degeneration process, including macrophage invasion, activation of Schwann cells, as well as neurotrophin and cytokine upregulation, is critical for the development of neuropathic pain (Dubovy 2011). Considering that motor fiber has larger size of axon and thicker myelin sheath, compared to sensory fiber, it is not surprising that injury to motor fiber produces stronger degeneration response and more inflammatory molecules to induce and maintain neuropathic pain.

5.4.1 Nav1.3 and Nav1.8 in DRG Neurons Are Upregulated by TNF-α But Downregulated by IL-10

How could peripheral nerve injury upregulate VGSCs in DRG neurons? It has been proposed that the deprivation of neurotrophic factors from peripheral pools may lead to the re-expression of Nav1.3 and downregulation of Nav1.8 in injured DRG neurons, as both nerve growth factor and glial-derived neurotrophic factor are capable of reversing the changes in Nav1.3 and Nav1.8 produced by axotomy (Fjell et al. 1999; Leffler et al. 2002). However, the deprivation theory cannot explain the fact that selective damage of motor fibers, leaving sensory neuron intact, by L5-VRT leads to long-lasting re-expression of Nav1.3 and upregulation of Nav1.8 in intact DRG neurons (He et al. 2010). Because in L5 VRT model Nav1.3 and Nav1.8 are highly co-localized with TNF-α in uninjured DRG neurons, pretreatment with TNF-α synthesis inhibitor or genetic ablation of TNFR1 significantly attenuates the re-expression of Nav1.3 and the upregulation of Nav1.8; local application of TNF-α at the sciatic nerve without any nerve injury also upregulates the sodium channels in vivo and TNF-α upregulates Nav1.3 and Nav1.8 in cultured adult DRG neurons in a dose-dependent manner in vitro, it is concluded that the overproduction of TNF-α contributes to the upregulation of Nav1.3 and Nav1.8 in DRG neurons following nerve injury (He et al. 2010; Chen et al. 2010). Interestingly,

anti-inflammatory cytokine IL-10 exerts an opposite effect on sodium channel expression (Shen et al. 2013). In cultured DRG neurons, IL-10 (200 pg/ml) down-regulated Nav1.3, Nav1.6 and Nav1.8 in both mRNA and protein levels and reversed the upregulation of the channels by TNF-α. IL-10 also reduces the densities of TTX-sensitive and Nav1.8 currents in control DRG neurons and reverses the increase of the sodium currents induced by TNF-α, as revealed by patch-clamp recordings. Intrathecal administration of IL-10 attenuates mechanical allodynia in L5 spinal nerve ligation model and substantially reduces the excitability of DRG neurons. Therefore, imbalance of pro- and anti-inflammatory cytokines may lead to neuropathic pain by abnormal expression of ion channels in sensory neurons.

5.4.2 TNF-α and BDNF Are Essential for Induction of Spinal LTP at C-Fiber Synapses

Spinal LTP shares many common mechanisms with hippocampal LTP, such as activation of calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), PKC and extracellular signal-regulation of kinase/cAMP response-element binding protein (ERK/CREB), dopamine D1 receptor and TrkB receptor, are involved in the induction and maintenance of LTP in the similar manner. Accordingly, the drugs targeting at these molecules may impair the memory function of hippocampus. The striking difference between spinal LTP and hippocampal LTP is that the activation of microglia (Griffin et al. 2006) and astrocytes (Cowley et al. 2012) and subsequent overexpression of TNF-α (Ren et al. 2011) and IL-1β (Vereker et al. 2001) impair LTP in hippocampus but promote the spinal LTP at C-fiber synapses (Liu et al. 2007; Zhong et al. 2010; Zhou et al. 2010b; Zhong et al. 2009). Therefore, the drugs targeting at the neuroinflammatory processes may not only treat pathological pain but also improve the memory function of hippocampus.

In hippocampus, overproduction of TNF-α impairs LTP (Tancredi et al. 1992) by the activation of nuclear factor-kappaB (NF-kB), p38 MAPK and c-Jun N-terminal kinase (JNK) signaling pathways (Butler et al. 2004; Wang et al. 2004). In contrast, in spinal dorsal horn blockage of TNF-α with neutralized antibody or genetic deletion of either TNF receptor 1 (TNFR1) or TNFR2 prevents LTP induction (Zhong et al. 2010; Park et al. 2011). Furthermore, spinal application of TNF-α (10 pg/ml) at recording segment induces LTP of C-fiber-evoked field potentials in rats with neuropathy produced by either L5 VRT or spared nerve injury (Liu et al. 2007). Interestingly, the TNF-α-induced LTP is blocked by JNK inhibitor (SP600125), p38 MAPK inhibitor (SB203580) or NF-κB inhibitor (PDTC). Spinal application of IL-1β has same effect as TNF-α on the spinal LTP (Zhong et al. 2009), indicating that pro-inflammatory cytokines and their downstream molecules exert totally different effects on hippocampal LTP and spinal LTP. These results suggest that inflammatory cytokines are necessary for spinal LTP induction in intact animals and are sufficient to LTP in rats with neuropathy. This is in consistent with

the fact that the upregulation of TNF-α and TNFR1 in spinal dorsal horn is critical for the development of pathological pain.

TNF-α upregulates BDNF in DRG neurons (Lin et al. 2011), and BDNF is also essential for generation of neuropathic pain (Yajima et al. 2005). It has been shown (Zhou et al. 2008) that spinal application of BDNF induces LTP of C-fiber-evoked field potentials with a long latency in intact rats and the effect is blocked by protein synthesis inhibitor (anisomycin), suggesting that BDNF can directly induce a late-phase LTP. A recent work (Zhou et al. 2010a) shows that injury to the sural nerve induces a short-lasting (<2 h) homosynaptic spinal LTP and fails to produce neuropathic pain, while injury to GS nerve innervating muscle induces a late-phase LTP of C-fiber-evoked field potentials and a persistent mechanical allodynia. Immunohistochemical staining shows the expression of BDNF in the DRG neurons of sural nerve is much lower than that of GS nerve. Spinal application of low dose of BDNF, which does not affect baseline of C-fiber-evoked field potentials, enables HFS of the sural nerve to induce homosynaptic late-phase LTP. The results may explain why injury to the sural nerve fails to induce neuropathic pain.

5.4.3 The Direction of Synaptic Plasticity at C-Fiber in Spinal Dorsal Horn Is Decided by Microglia

It has been shown that microglia are activated following spinal LTP induction by HFS, and spinal application of minocycline, a specific microglia metabolism inhibitor, modulates the C-fiber synaptic plasticity in a dose-dependent manner (Zhong et al. 2010). The drug at 50 μM does not affect LTP induction, at 100 μM blocks LTP and at 200 μM HFS induces long-term depression (LTD), instead of LTP. Thus, the synaptic plasticity at spinal C-fibers may be tightly controlled by the functional states of microglia. Furthermore, Src-family kinases (SFKs) are activated exclusively in spinal microglia following injury or HFS of peripheral nerve, and spinal application of SFKs inhibitor has the same effect on spinal synaptic plasticity as minocycline. Activated microglia may promote LTP induction by release of TNF-α, as the pretreatment with low dose of TNF-α, which does not affect baseline of C-fiber-evoked field potentials, abolishes the inhibitory effect of minocycline or SFKs inhibitor on spinal LTP. Microglia is also indispensable for the spinal LTP induced by spinal application of either ATP or BDNF (Gong et al. 2009; Zhou et al. 2010b) without activation of presynaptic component. Both P2X4 and P2X7 receptors expressing exclusively in spinal microglia are reported to be involved in the spinal LTP induction. Spinal application of ATP induces LTP at C-fiber synapses by the activation of P2X4 receptors, and ATP-induced LTP is accompanied by phosphorylation of p38 MAPK and upregulation of P2X4 receptors in microglia. Inhibition of p38 MAPK prevents both ATP-induced LTP and the upregulation of P2X4 receptors (Gong et al. 2009). Blockage of spinal P2X7 receptor prevents spinal LTP and mechanical allodynia induced by HFS, and the effects are associated with the inhibition of p38 MAPK in microglia and the reduction of IL-1β (Chu et al. 2010).

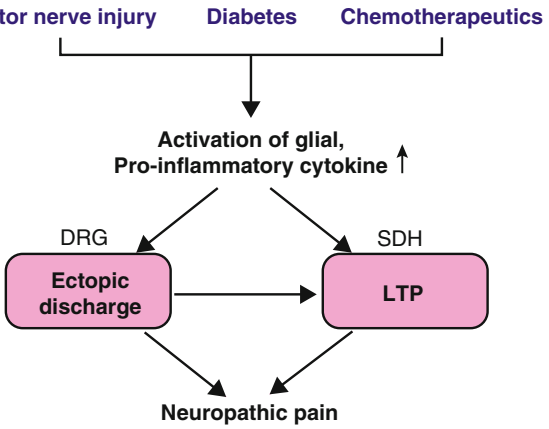

Fig. 5.3 Neuropathic pain induced by different pathological processes may share common mechanisms

5.5 Concluding Remarks

Experimental and clinical evidence have clearly demonstrated that motor fiber injury almost always induces neuropathic pain, while sensory fiber injury is neither necessary nor sufficient to do so. Although the mechanisms underlying difference are still not fully understood, accumulated evidence shows that motor fiber injury is more effective to upregulate pro-inflammatory cytokines and neurotropic factors. The pathological molecular alteration (or neuroinflammation) induces neuropathic pain by generating ectopic discharges in primary afferents via abnormal regulation of ion channels and by induction of LTP in pain pathway via activation of glial cells. Because upregulation of TNF-α, a leading pro-inflammatory cytokine, is not only necessary but also sufficient to induce neuropathic pain, it is not astonishing that any pathological process or factor that is able to upregulate TNF-α, such as diabetes (Sharma et al. 2007; Yamashita et al. 2008) and anti-cancer agents paclitaxel (Ledeboer et al. 2007), induces neuropathic pain even without peripheral nerve injury (Fig. 5.3).

It is worth to note that TNF-α is critical for many physiological functions, such as immunity, cell proliferation, differentiation and apoptosis (Baud and Karin 2001). Therefore, the stratagem to treat the diseases produced by the overexpression of TNF-α should be normalization of its expression but not simple inhibition.

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Chapter 6
Peripheral Nociceptors as Immune Sensors in the Development of Pain and Itch

Tao Wang and Chao Ma

Abstract The peripheral nervous system and the immune system perform a series of similar functionalities such as recognizing, responding, and adapting to external or internal stimuli despite significant morphological differences. The peripheral nervous system actively communicates and coordinates with the immune system to function as a unified defense system. The peripheral nervous system is highly regulated by the immune system, especially under inflammatory conditions. On the other hand, the nervous system can modulate the immune system via neurotransmitters and chemokines released by the peripheral nerve endings, particularly from nociceptors. In both physiological and pathological conditions, peripheral nociceptive (including pruriceptive) neurons may express a variety of immune-related receptors, such as chemokine receptors and immunoglobulin (Fc) receptors that are usually found on immune cells. Certain ligands such as chemokines and immune complexes may induce abnormal neuronal hyperexcitability and even ectopic action potential discharges, therefore producing the sensation of pain and/or itch in immune-related diseases. The immune-sensing mechanisms of peripheral nociceptors may play an important role in the development of chronic pain and pruritus and may indicate novel therapeutic strategies for these pathological conditions.

Keywords Pain • Itch • Peripheral nociceptor • Immune sensor

6.1 Introduction

The nervous system and the immune system seem to share little in common on almost each morphological levels, that is, from macroscopic structures to microscopic cellular types or from molecular components to embryological development

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(Steinman 1993). In addition, functional communications between the nervous and immune system are largely blocked due to the blood-brain (BBB) or blood-nerve barriers (BNB) as part of the protective mechanisms for the nervous system in vertebrate animals (Kanda 2013). However, there is an increasing body of evidences revealing that the peripheral nervous system and the immune system are closely correlated with each other, both morphologically and functionally, and share substantial similarities that will be discussed in the following sections in this chapter. Specifically, peripheral nociceptors play a vital role in such a correlation and may serve as immune sensors in the development of pain as well as itch in both physiological and pathological conditions (Qu et al. 2011, 2012).

In principle, the peripheral nervous system and the immune system perform a series of similar functionalities including recognizing, responding, and adapting of external or internal stimuli. The peripheral nervous system recognizes and responds to a variety of mechanical, thermal, and chemical stimuli applied to external or internal organs, while the immune system recognizes and responds to foreign- or self-immunogens. On the other hand, both systems demonstrate a high level of plasticity and adapt to environmental changes outside and inside the body, especially under pathological conditions. Systemically, the peripheral nervous system and the immune system are two components of a unified defense mechanism, coordinated by multiple layers of interactive pathways. Certain humoral or local factors such as inflammatory mediators, chemokines, and neurotransmitters may modulate the activities of both systems via acting on both the immune cells and neurons, especially peripheral nociceptors (Steinman 1993).

Peripheral nociceptors, or primary nociceptive (and pruriceptive) neurons, are the first layer of nocifensive sensors innervating almost all the tissues and organs, especially the epithelial of body surface (Dubin and Patapoutian 2010). Transduction and responding of noxious external stimuli via the peripheral nervous system is much faster than via the immune system. Peripheral nociceptors possess many of the same molecular pathways as immune cells and, upon activation, may directly communicate with immune cells. The intensive innervations network of sensory and autonomic fibers in peripheral nervous systems and high speed of neural transduction allows rapid local and systemic neurogenic modulation of immunity. Peripheral nociceptors expressing a variety of immune-related receptors may directly and actively interact with the immune system in both physiological and pathological conditions and may play a critical role in pain and itch related to immune diseases.

6.2 Morphological Correlations Between the Peripheral Nervous System and the Immune System

The primary site of coupling between neurons and immune cells is the peripheral tissue where “naked” free terminals of peripheral nociceptive neurons innervate the epidermis of skin, cornea, or mucosa. These nerve endings express various membrane receptors that are accessible to chemical factors in the peripheral tissue

released by residential or recruited immune cells during inflammatory response. Upon activation, peripheral nociceptive terminals may also release immunogenic agents such as substance P and induce so-called neurogenic inflammation (DeLeo and Yezierski 2001).

Another location for potential interactions between the nervous system and immune system is peripheral sensory ganglion. Just like the Schwann cells surrounding peripheral axons, each neuronal cell body (or soma) in the sensory ganglion such as dorsal root ganglion (DRG) and trigeminal ganglion (TG) is tightly covered by satellite glial cells. It has been found that BBB or BNB is partially defect in the peripheral sensory ganglia (Bush et al. 1991), leaving a potential "window" for crosstalk between the immune system and peripheral nervous system. Macrophages and T cells from blood invade the DRG after nerve injury and then may gradually move through satellite cells and migrate closer to the neuronal soma. These macrophages eventually form perineuronal rings under the satellite cells around DRG neurons after chronic constriction injury of the sciatic nerve (Hu and McLachlan 2002). Increased communication between satellite glial cells and between neurons and satellite glial cells after injury to the peripheral nerve or DRG may increase neuronal excitability and contribute to the development of chronic pain (Hanani 2005; Zhang et al. 2009). In pathological conditions such as demyelination and neuritis, immune cells may infiltrate around and interact with neuronal axons. Another potential location for such an interaction is central terminals in the spinal dorsal horn or brain stem, where glial cells such as microglia serve as residential immune cells and may be activated by lesions or inflammation in the periphery nervous system (DeLeo and Yezierski 2001; Thacker et al. 2007).

In summary, the peripheral nervous system is closely correlated with the immune system on almost every location: the peripheral and central nerve terminals, the neuronal cell bodies, and the axons, as demonstrated in Fig. 6.1. Such morphological correlations may indicate multiple levels of functional interactions between these two systems.

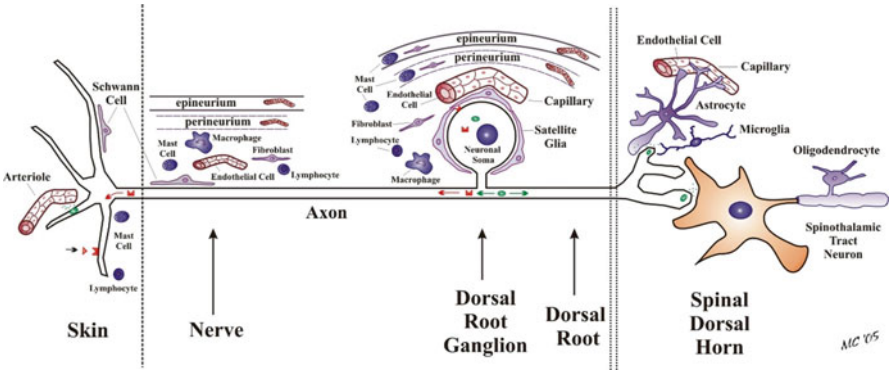

Fig. 6.1 Morphological correlations between the peripheral nervous system and the immune system

6.3 Interactions Between the Peripheral Nervous and the Immune System

The peripheral nervous system interacts with the immune system at multiple levels and has been implicated in the development and maintenance of both inflammatory and neuropathic pain (DeLeo and Yezierski 2001; Marchand et al. 2005; Thacker et al. 2007) as well as pruritus (F. Liu et al. 2014; Qu et al. 2014; LaMotte et al. 2014). There is now substantial evidence for bidirectional communications between the peripheral nervous and immune systems via chemical messengers such as hormones, neurotransmitters, and cytokines, while the detailed cellular signaling and regulatory mechanisms are still largely obscure.

Activation of immune system can affect the neurophysiological, neurochemical, and neuroendocrine activities of the nervous system. Cytokines, peptides, and other factors released by the immune cells may directly act on peripheral neurons, especially nociceptors. Cytokines may serve as "immunoneurotransmitters," that is, messengers from the immune system to the peripheral nervous system, and account for a variety of interactions between these two systems. A number of cytokines such as IL-1, IL-6, TNF-a, and the interferons are currently known to have the most relevance for the peripheral nervous system (Miller et al. 2009). So far most immune cells have been found to interact with peripheral nociceptors under different conditions as described below.

Mast cells are granulated resident immune cells and found closely located around peripheral nociceptors. Mast cells participate in innate immune response and allergic reactions via the release of histamine, bradykinin, and other mediators upon degranulation (Lawrence et al. 2002). These mediators released by mast cells may contribute to pain and itch sensitization in pathological conditions. It has also been found that degranulation of mast cells requires direct interaction between mast cells and peripheral nerve terminals, which is mediated by the calcium-dependent cell adhesion molecule N-cadherin expressed in both mast cells and primary sensory neurons (Folgueras et al. 2009; Suzuki et al. 2004).

Macrophages are derived from circulating monocytes and are recruited to the place of injury and mature in hours. Resident macrophages become phagocytic almost immediately after injury. Following recruitment and activation, macrophages contribute to nociceptor sensitization by releasing soluble mediators. The increased number of macrophages at the site of nerve injury correlates with the development of mechanical allodynia after nerve injury (Cui et al. 2000). Upregulation of the chemokine macrophage inflammatory protein-1α and its receptors CCR1 and CCR5 is observed in macrophages and Schwann cells after partial ligation of the sciatic nerve and may contribute to the development of neuropathic pain (Kiguchi et al. 2010). Clearance of circulating monocytes and macrophages by liposome-encapsulated clodronate partially alleviates hyperalgesia in animal models of neuropathic pain (Barclay et al. 2007). The recruitment of macrophages is mediated by many inflammatory cytokines including monocyte chemoattractant protein 1 (MCP-1 or CCL-2), which may be released by neurons and satellite glial cells after injury (White et al. 2005).

Lymphocytes contribute to the sensitization of peripheral nociceptors. T cells infiltrate the sciatic nerve and DRG after nerve injury. Hyperalgesia and allodynia induced by nerve injury are markedly attenuated in rodents deprived of T cells (Costigan et al. 2009). B cells produce immunoglobulins (antibodies) that may form immune complexes with antigens and act on peripheral nociceptors (Andoh and Kuraishi 2004a; Qu et al. 2012; F. Liu et al. 2015), as will be discussed in the following section.

Likewise, peripheral nociceptors may play a key role in modulating the activities of the immune system. Nociceptive neuron can release neurotransmitters such as glutamate, ATP, substance P, calcitonin gene related peptide (CGRP), brain-derived neurotrophic factor (BDNF), IL-6, and CCL2 that act on multiple immune cells (Guillot et al. 2012). Mediators released from the peripheral terminals of nociceptors not only induce vasodilation to facilitate inflammation, but also directly attract and activate immune cells (mast cells and dendritic cells) and adaptive immune cells (T lymphocytes) (Ansel et al. 1993; Ding et al. 2008; Hosoi et al. 1993; Mikami et al. 2011; Cyphert et al. 2009) (White et al. 2005).

6.4 The Immune-Related Receptors in Peripheral Nociceptors

It has been well known that primary nociceptive (including pruriceptive) neurons express a variety of mechanical, thermal, and chemical receptors to achieve specific sensory functional modalities. Subpopulations of polymodal nociceptive neurons and probably some mechanoinsensitive (chemo-sensitive) neurons responded to algesic and/or pruritic agents such as capsaicin and histamine that also induce inflammatory responses (Ma et al. 2012; LaMotte et al. 2014). In addition to their nocifensive functions, peripheral nociceptors can also serve as immune sensors and co-activated with immune cells by potential "warning signals" in the environment. Previous studies have indicated that peripheral nociceptors may express a variety of immune-related cellular receptors (i.e. those usually found in immune cells) in both physiological and pathological conditions and serve as immune sensors.

Unlike immune cells that perform a wide range of functions including phagocytosis, cell-mediated cytotoxicity, and the release of cytokines upon activation, activated nociceptors may depolarize firing action potentials and release neurotransmitters, therefore inducing the sensation of pain as well as itch. For example, a number of chemokine receptors including CCR2 and CX3CR1 are found upregulated in nociceptive neurons that became hyperexcitable in pathological conditions and may play a critical role in the development of chronic pain (Abbadie et al. 2009; White et al. 2005; Oh et al. 2001). Interestingly, primary nociceptors can express both the chemokine receptor CCR2 and its ligand CCL2 (MCP-1) after chronic compression to the DRG (White et al. 2005). Allergic contact dermatitis can upregulate certain chemokine receptors such as CXCR3 (Qu et al. 2015) in the pruriceptive neurons expressing MrgprA3 (Han et al. 2013) or

MrgprD (Q. Liu et al. 2012), which showed neuronal hyperexcitability and may contribute to pathological itch and pain in dermatitis (Qu et al. 2014).

Another intriguing story of nociceptors functioning as immune sensors was recently discovered for the antigen-specific immunity of DRG neurons that expressing Fc receptors (Andoh and Kuraishi 2004b; Qu et al. 2011). Chronic pain as well as pruritus can accompany antigen-specific autoimmune diseases, such as rheumatoid arthritis (Wolfe and Michaud 2007), multiple sclerosis (Kenner et al. 2007) and Guillain-Barre Syndrome (Moulin et al. 1997), allergic diseases such as atopic and allergic contact dermatitis (Valks and Conde-Salazar 2003; Wittkowski et al. 2007), and infectious diseases such as herpes zoster (Oaklander 2008) though there is insufficient information about the underlying neuronal mechanisms. One of the common features among these disorders is the elevated level of antigen-specific immunoglobulins, especially IgG in the serum and/or affected tissue. IgG is the major immunoglobulin in normal human serum and has a much longer half-life than other immunoglobulin isotypes. Fc-gamma receptor (FcγR) is the receptor that binds to the Fc portion of IgG. There are three activating receptors (FcγRI, III, and IV in the mouse; FcγRIa, IIa, IIc, and IIIa in humans) and one inhibitory receptor FcγRIIb. FcγRI (also known as CD64) is the only high-affinity activating receptor and has been found critically involved in a number of inflammatory and immune responses, including certain immune-related disorders in the central nervous system (Barnes et al. 2002; Ioan-Facsinay et al. 2002; Okun et al. 2010). Treatments such as intravenous immunoglobulin that potentially block FcγRI or reduce the IgG-IC were found to ameliorate symptoms including pain in a number of immune-related diseases. IC can induce cutaneous hyperalgesia after the injection of a foreign antigen to the hind paw of mice and rats immunized with the same antigen and exhibiting an elevated level of serum IgG (Verri et al. 2008). Recently it was discovered that FcγRI is expressed on the cell bodies (somata) and axons of a subpopulation of DRG neurons with nociceptive properties in mice (Andoh and Kuraishi 2004a; F. Liu et al. 2014, 2015) and rats (Qu et al. 2011). Some FcγRI-immunopositive DRG neurons with various sizes are also immunopositive for nociceptive neuronal markers IB4, TRPV1, substance P and CGRP, and a majority of FcγRI-immunopositive small-sized DRG neurons express TRPV1 (Qu et al. 2011). IgG and the corresponding antigen (forming the IgG-IC) can directly bind to the neuronal FcγRI and induce calcium influx in the dissociated, small- and medium-sized DRG neurons (Andoh and Kuraishi 2004a; Qu et al. 2011). These findings suggest that IgG-IC may directly excite nociceptive primary sensory neurons via Fc receptors and produce pain. Further studies revealed that FcγRI is functionally coupled with the transient receptor potential canonical channel type 3 (TRPC3) via intracellular calcium through the Syk-PLC-IP_3 signaling pathway (Qu et al. 2012). Opening of the nonselective cation channel TRPC3 induces influx of extracellular calcium and sodium ions that triggers membrane depolarization and action potential spikes in nociceptive DRG neurons; therefore pain (and maybe itch) is induced. In addition to FcγRI, other Fc receptors such as FcεR were also found in primary sensory neurons (Andoh and Kuraishi 2004b; F. Liu et al. 2014, 2015) and may play a role in

antigen-specific itch. Therefore, peripheral nociceptors expressing functional Fc receptors may serve as antigen-specific immune sensors and involve in the development of pain and itch.

6.5 Conclusion

In summary, the peripheral nervous system actively communicates with the immune system to perform the functions of recognizing, responding, and adapting. Peripheral nociceptors, including primary nociceptive and pruriceptive neurons, may serve as immune sensors and coordinate with immune cells to function as a unified defense system. A number of immune-related receptors, such as chemokine receptors and immunoglobulin (Fc) receptors, are expressed in peripheral nociceptors in physiological or pathological conditions and can be activated by certain ligands such as chemokines and immune complexes. The immune-sensing mechanisms of peripheral nociceptors may play an important role in the development of pain as well as itch in pathological conditions. Understanding the coordinated interaction of peripheral nociceptors with immune cells may advance therapeutic approaches for the treatment of chronic pain and pruritus.

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Chapter 7
Mas-Related G Protein-Coupled Receptors Offer Potential New Targets for Pain Therapy

Vineeta Tiwari*, Vinod Tiwari*, Shaoqiu He, Tong Zhang, Srinivasa N. Raja, Xinzhong Dong, and Yun Guan

Abstract The founding member of the Mas-related G-protein-coupled receptor (Mrgpr) family was discovered in 1986. Since then, many more members of this receptor family have been identified in multiple species, and their physiologic functions have been investigated widely. Because they are expressed exclusively in small-diameter primary sensory neurons, the roles of Mrgpr proteins in pain and itch have been best studied. This review will focus specifically on the current knowledge of their roles in pathological pain and the potential development of new pharmacotherapies targeted at some Mrgprs for the treatment of chronic pain. We will also discuss the limitations and future scope of this receptor family in pain treatment.

Keywords Mas-related G-protein-coupled receptors • Mrgpr • Pain • Dorsal root ganglion • Nerve injury

7.1 History of the Mas-Related G-Protein-Coupled Receptor (Mrgpr) Family

G-protein-coupled receptors (GPCRs) are widely expressed cell-surface receptors that have been successfully used as drug targets for a variety of human diseases. Currently, 46 GPCRs serve as drug targets for the treatment of disorders such as pain, hypertension, allergies, alcoholism, obesity, ulcers, glaucoma, HIV, and psychotic disorders. However, this leaves several hundreds of GPCRs as potential drug targets, approximately 150 of which are still considered orphan receptors (Lagerstrom and Schioth 2008) because we lack an understanding of their role in

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human physiology and their potential therapeutic value. The Mrgprs constitute a family of recently identified orphan GPCRs named for the mas-related genes (Dong et al. 2001). The family is also known as sensory neuron-specific receptors (SNSRs) (Lembo et al. 2002). It is a large gene family that consists of 32 murine and eight human genes (MrgprX1 to MrgprX4 and MrgprD to MrgprG) (Dong et al. 2001).

The founding member of the Mrgpr family was first isolated from DNA of a human epidermoid carcinoma cell line and was thought to be a proto-oncogene because of its ability to transform NIH3T3 cells (Young et al. 1986). Subsequent detailed analysis of the Mas amino acid sequence suggested that the protein had seven transmembrane domains and belonged to the class of GPCRs. This finding was the first direct evidence for oncogenic activity in a GPCR (Young et al. 1986). However, further studies challenged the oncogenic potential of Mas. In initial studies, the transfected cells or the tertiary tumor in nude mice contained amplified Mas sequences characterized by rearrangements in 5′- and 3′-noncoding regions (van Veer et al. 1993). However, the original tumor DNA used in the first round of transfection was neither amplified nor rearranged or mutated in the miscoding sequence. Thus, it could not be considered the driving cause of oncogenesis. This evidence proved that Mas was not an oncogene. It has never been found amplified in a primary tumor, but it can transform cells when artificially overexpressed.

The expansion of the Mrgpr family started in 1990 with the cloning of the rat thoracic aorta (RTA) gene and the Mas-related gene (mrg, now called Mas1L), which have 34 % and 35 % homology to Mas, respectively (Monnot et al. 1991; Ross et al. 1990). Ten years later, in 2001, Dong et al. conducted a detailed comparative analysis of the dorsal root ganglion (DRG) transcriptome in Ngn1-deficient mice, which lack a subclass of nociceptive neurons. This work enlarged the family by several genes that were expressed in such neurons and called Mas-related genes A, MrgprA (Dong et al. 2001). Subsequent bioinformatic analysis and screening of murine DRG cDNA libraries led to the identification of approximately 50 Mrgprs (also called SNSR) in mouse, rat, human, and macaque (Dong et al. 2001; Han et al. 2002; Lembo et al. 2002; Zhang et al. 2005). These receptors are now divided into several subfamilies and have been renamed according to a new nomenclature (http://www.guidetopharmacology.org): MrgprA, B, C, D, E, F, G, H, and a primate-specific MrgprX subfamily. Interestingly, different subfamilies consist of multiple duplicated genes in different species. Primates have several MrgprX genes, whereas rodents have multiple MrgprA, B, and C genes. Even different rodent species differ in the number of genes per subfamily (Dong et al. 2001; Zylka et al. 2003).

7.2 Distribution of Mrgpr

The Mrgpr family was originally discovered based on its expression in DRG (Dong et al. 2001; Lembo et al. 2002). In mice, all members of Mrgpr are expressed in small-diameter nociceptive neurons of DRG and trigeminal ganglia. However, Mas, as well as MrgprF, G, and H, are expressed at relatively low levels (Avula et al.

2011; Cox et al. 2008; Crozier et al. 2007; Zhang et al. 2005; Zylka et al. 2003). The initial discovery of the Mrgpr family points to the selective expression of Mrgpr in primary sensory neurons derived from the TrkA+ population (Dong et al. 2001). After birth, TrkA expression begins to terminate in almost half of the TrkA+ neurons, which then start to express c-ret, receptor for glial-derived neurotrophic factor (Molliver and Snider 1997). Notably, in adults, most Mrgpr subtypes are not coexpressed in the same c-ret+population; rather they are expressed in different compartments with a distinct subpopulation of neurons (Dong et al. 2001; Liu et al. 2008; Zylka et al. 2003). These expression patterns are not identical in mice and rats, suggesting different physiologic roles of distinct Mrgpr in rodents. The analysis of transcriptional pathways in distinct DRG populations of mice revealed that the expression of MrgprA–D initially depends on the activity of the runt-related transcription factor 1 (Liu et al. 2008). However, during the postnatal developmental period, runt-related transcription factor 1 leads to the differentiation of murine MrgprD and MrgprA–C expression by suppressing MrgprA–C through its inhibitory C-terminal domain. The distinct expression pattern of different Mrgprs in adults led to the assumption that neuronal subpopulations characterized by their Mrgpr expression pattern might have distinct functions. Accordingly, MrgprA3 expression specifies neurons that induce itch without transducing nociception in mice, whereas MrgprB4- or MrgprD-positive primary sensory neurons derive different somatosensory inputs from distinct skin areas (Han et al. 2013; Liu et al. 2007; Zylka et al. 2003; Zylka 2005).

Although they have highly selective expression in small-diameter primary sensory neurons, some Mrgprs have been found in other tissues as well. MrgprD mRNA has been detected in arteries, urinary bladder, uterus, and testis (Shinohara et al. 2004). MrgprE was found in medium- and large-diameter neurons of human DRG sections, as well as in a few areas of the central nervous system, including cerebral cortex, hippocampus, spinal cord, and cerebellum (Milasta et al. 2006; Zhang et al. 2005). MrgprE and F are also expressed in submucosal and myenteric neurons, indicating that they have a role in autonomic control of gastrointestinal functions (Avula et al. 2011). MrgprH transcript was found in heart, and human MrgprX2 was found in the adrenal glands and several brain areas (Kamohara et al. 2005; Robas et al. 2003). Recently, expression of MrgprX1 and 2 was also shown in human mast cells (Kashem et al. 2011; Solinski et al. 2010, 2012, 2013; Subramanian et al. 2011a; Tatemoto et al. 2006). Below, we will discuss the role of Mrgprs in pain modulation and the mechanisms involved.

7.3 Mrgpr Receptors: Potential Pain Modulators

Evolutionary studies point toward a strong positive correlation between Mrgprs and nociception (Dong et al. 2001; Zylka et al. 2003). As discussed above, Mrgprs are expressed predominantly in small-diameter sensory neurons (presumably nociceptive) that can be visualized by lectin IB4 labeling or by expression of the glial cell

line-derived neurotrophic factor co-receptor c-Ret27 (Solinski et al. 2014). Because of their restricted distribution in IB4+ nociceptive neurons, Mrgprs represent a compelling potential pain-specific target for pharmacologic therapy (Dong et al. 2001; Grazzini et al. 2004; Lembo et al. 2002). Among the Mrgprs, MrgprX1 (also called SNSR3) in humans is expressed exclusively in the DRG neurons and is activated by BAM22, a proenkephalin product (Dong et al. 2001; Lembo et al. 2002). Intriguingly, at its N-terminus, BAM22 exhibits the classical YGGFM (Met-enkephalin) motif and binds to both opioid and Mrgpr receptors (Boersma et al. 1994; Hollt et al. 1982; Lembo et al. 2002; Quirion and Weiss 1983). The C-terminal 15 amino acids of BAM22 activate MrgprX1. The N-terminal Met-enkephalin motif in the first 8 amino acids activates opioid receptors (Lembo et al. 2002) but is dispensable for the Mrgpr activity. Interestingly, the Mrgprs are insensitive to the classical opioid receptor antagonists. Thus, despite distinct structure–activity relationships and pharmacology with known ligands, the opioid receptor and Mrgprs may exhibit similar physiologic roles in nociception. In the next section, we will discuss the roles of individual Mrgprs in pain modulation.

7.4 Mrgpr A and D

MrgprA1–8 and MrgprD are all expressed by IB4+ and c-ret+sensory neurons (Choi and Lahn 2003; Molliver et al. 1997; Zylka et al. 2003, 2005). These neurons primarily send their central projections to lamina IIi (Snider and McMahon 1998; Stucky and Lewin 1999), which has been implicated in modulation of pain behavior (Basbaum et al. 2009; Cavanaugh et al. 2009; Malmberg et al. 1997). Accordingly, MrgprAs and D are potentially expressed in nociceptive sensory neurons. Interestingly, both MrgprAs and D are expressed by TRPV1− neurons within the IB4+ population. TRPV1 is activated by both chemical and noxious thermal stimuli, but in vivo is also required for the detection of noxious mechanical stimuli (Caterina et al. 2000; Eckert, III et al. 2006). TRPV1+ neurons may detect such mechanical stimuli via other receptors as well. There is a possibility that MrgprA+ and MrgprD+ neurons may detect stimuli of different modalities than are detected by TRPV1+ neurons. Silencing or genetic ablation of neurons that express MrgprA and MrgprD may confirm their involvement in specific sensory modalities. The expression of MrgprAs and D is highly diverse among IB4+ and TRPV1− sensory neurons, and currently it is unclear what aspect of cellular or functional diversity this molecular heterogeneity suggests. The MrgprD+ subpopulation coexpresses P2X3, whereas MrgprA+ neurons mostly do not, suggesting that they might have different physiologic and functional properties (Dong et al. 2001; Wang and Zylka 2009; Zylka et al. 2005). The IB4+ population is known to contain both unmyelinated (C-fibers) and small, thinly myelinated (Aδ) neurons (Jackman and Fitzgerald 2000). However, any physiologic correlation to Mrgpr expression remains to be determined.

Recent findings suggested that MrgprD+ neurons are necessary for behavioral hypersensitivity to mechanical, but not thermal, stimuli (Cavanaugh et al. 2009; Rau et al. 2009; Wang and Zylka 2009). The absence of a heat pain deficit in mice lacking MrgprD+ neurons is difficult to explain by either redundancy or compensation, because all heat pain sensitivity is lost in mice that lack TRPV1+ afferents but retain MrgprD+ neurons. However, it is possible that MrgprD+ neurons contribute to heat pain sensitivity in naive animals in a manner dependent on TRPV1+ neurons. Previous findings showed that use of a saporin conjugates to ablate IB4+ neurons, which includes all MrgprD+ neurons, transiently reduced both mechanical and heat pain sensitivity rats (Tarpley et al. 2004). This finding is in contrast to the selective and prolonged mechanosensitive deficit seen in mice lacking MrgprD+ neurons (Cavanaugh et al. 2009). However, IB4 labels a more heterogeneous population of neurons in rats than MrgprD does in mice (Price and Flores 2007). Moreover, IB4-saporin targets an epitope present on multiple cell types, whereas MrgprD is exclusively expressed in unmyelinated afferents (Dong et al. 2001; Zylka 2005; Zylka et al. 2005). Thus, cellular specificity achieved by targeted ablation of MrgprD+ neurons in mouse is much better than ablation of IB4+ neurons in rats. This insight raises an important question for future investigation if different species of rodents and humans exhibit different degrees of nociceptor specialization.

A recent study dissected the selective roles of MrgprD+ (nonpeptidergic) and TRPV1+ (peptidergic) neurons in mechanical and heat pain hypersensitivity, respectively (Zhang et al. 2013). The authors suggested that the nonpeptidergic population targets interneurons in lamina II, while the peptidergic population targets primarily dorsal horn projection neurons in lamina I and interneurons in superficial lamina II (Basbaum et al. 2009). More interestingly, these molecularly distinct nociceptor populations can be differentially activated by peripheral noxious stimuli and engage different ascending circuits (Braz et al. 2005; Braz and Basbaum 2010). Intrathecal injection of capsaicin, which ablates the TRPV1+ neurons, produced almost complete loss of responsiveness to noxious heat, with no change in response to noxious cold or mechanical stimuli (Zhang et al. 2013). These findings did not align with those reported by Mishra et al. (2011), who found that constitutive pharmacogenetic ablation of TRPV1+ neurons decreased responses to noxious cold as well as heat. However, Mishra et al. ablated TRPV1+ afferents in the embryo, when TRPV1 has a much broader distribution (Cavanaugh et al. 2011), potentially explaining the disparity in their findings. In another study, pharmacogenetic ablation of the MrgprD+ neurons by injection of diphtheria toxin into adult mice resulted in a selective reduction of mechanical hypersensitivity (Cavanaugh et al. 2009). Zhang et al. (2013) also reported that ablation of the MrgprD+ population selectively reduced neuronal activity of spinal cord dorsal horns to noxious mechanical stimulation. These findings suggest that despite the polymodal properties of the peptidergic and nonpeptidergic neurons, the influence of these populations on nociceptive processing of dorsal horn neurons appears to be modality specific.

7.5 MrgprB

To date, no ligand has been identified for any of the rodent MrgprB members. However, in a reporter mouse carrying a gene for placental alkaline phosphatase under the control of the MrgprB4 promoter, a rare subset of primary sensory neurons that innervate only hairy skin showed high promoter activity (Liu et al. 2007). These neurons were nonpeptidergic and TRPV1-negative and were often associated with hair follicles. Most interestingly, MrgprB4+ neurons project to spinal lamina II, a spinal pain processing center that is part of the substantia gelatinosa. These findings indicate that MrgprB4 marks C-fiber tactile afferents, which are thought to detect gentle touch and stroking. In freely moving mice, pharmacogenetic activation of MrgprB4-expressing neurons resulted in conditioned place preference, suggesting that activation of these neurons is positively reinforcing and might be induced by interindividual social interactions such as allogrooming (Vrontou et al. 2013).

7.6 MrgprC

The rodent MrgprC subfamily consists of only one protein-encoding gene each in rats and mice, but the two receptor subtypes have been suggested to bind several ligands (Grazzini et al. 2004; Han et al. 2002; Solinski et al. 2010, 2013). Among the peptides thought to bind MrgprC are g2-melanocyte stimulating hormone (g2-MSH), BAM peptides, dynorphin-14, and proneuropeptide-FF-A peptides. These peptides originate from PENK (BAM), pro-opiomelanocortin (g2-MSH), prodynorphin (dynorphin-14), and NPPFA. Such a wide range in the binding profile of a distinct GPCR suggests that many different physiologic pathways rely on MrgprC signaling. The specific MrgprC agonist BAM8–22 was identified in vivo after microdialysis of exogenous BAM1–25 into the striatum of anesthetized rats. Although the proteolytic machinery to generate BAM8–22 in vivo is present in the brain, no one has yet shown that BAM8–22 or g2-MSH6–12 is produced endogenously in tissues adjacent to primary sensory neurons. Thus, it is still unknown whether, and under which circumstances, specific MrgprC ligands exist in vivo. MrgprC (mouse MrgprC11 and the rat homolog rMrgprC) shares marked homogeneity with human MrgprX1, but the role of MrgprC in sensory processing of pain is mixed as discussed below.

7.6.1 Facilitation of Pain by MrgprC in Rodents

Intraplantar injections of rats with MrgprC-specific peptide BAM8–22 or Tyr6-g2-MSH6–12 resulted in acute but dose-dependent, mechanical allodynia and thermal hyperalgesia (Grazzini et al. 2004; Ndong et al. 2009). Additionally, intrathecal

injection of MrgprC agonists into juvenile and adult rats and Kunming mice induced thermal hyperalgesia and acute pain-like behavior (Chang et al. 2009; Grazzini et al. 2004; Wei et al. 2010). Moreover, RNAi-mediated MrgprC knockdown attenuated complete Freund's adjuvant (CFA)-induced thermal hyperalgesia in rats, indicating a role for MrgprC in inflammatory pain (Ndong et al. 2009). Interestingly, all of the pain-enhancing effects of MrgprC were observed in a rigid time window to a maximum of 20 min postinjection (Wei et al. 2010).

Several studies have suggested that the heat-sensitive TRPV1 ion channel is the main downstream target of MrgprC responsible for its pain-enhancing effects (Hager et al. 2008; Honan and McNaughton 2007; Ndong et al. 2009; Wilson et al. 2011). In rat primary sensory neurons, BAM8–22 sensitized TRPV1 to its agonist capsaicin via a PKC-dependent pathway (Honan and McNaughton 2007). TRPV1 sensitization is one of the mechanisms responsible for an increase in capsaicin- or heat-induced calcitonin gene-related peptide (CGRP) release from rat or mouse paw skin, after preincubation with BAM1–22 in conjunction with naloxone, which blocks effects of BAM1–22 on opioid receptors but does not affect binding of BAM1–22 with MrgprC receptors (Hager et al. 2008). In another study, MrgprC-mediated thermal hyperalgesia was blocked by a specific TRPV1 inhibitor, and coexpression of TRPV1 with murine MrgprC in NG108 cells resulted in enhanced calcium signals by BAM8–22 (Ndong et al. 2009; Wilson et al. 2011). These findings suggest that TRPV1 might be a common target for cellular signaling induced by MrgprC and might be of high physiologic significance because it is frequently coexpressed with MrgprC in rodent primary sensory neurons (Hager et al. 2008; Lembo et al. 2002; Liu et al. 2009). Chang and colleagues (2009) identified the spinal N-methyl-D-aspartate (NMDA) receptor and neuronal nitric oxide synthase (nNOS) as other important players in MrgprC-mediated thermal hyperalgesia. They found that NMDA receptor antagonists D-APV [D-(2)-2-amino-5-phosphonopentanoic acid] and MK-801 [(5S,10R)- (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5, 10-imine] and the nNOS inhibitor NG-nitro-L-arginine methyl ester dose-dependently inhibit the pronociceptive effects of Tyr6-g2-MSH6–12 in mice (Chang et al. 2009). As nNOS inhibitors also reduce NMDA-induced pain-like behavior, it remains to be determined if both players are directly modulated by MrgprC in the same cell.

7.6.2 Role of MrgprC in Pain Inhibition in Rodents

In contrast to the pain-enhancing effects of MrgprC described above, a number of studies, including our own, have indicated that intrathecal administration of MrgprC-specific agonists has analgesic potential (Chen et al. 2006, 2008; Guan et al. 2010; He et al. 2014b; Hong et al. 2004; Jiang et al. 2013; Li et al. 2014; Zeng et al. 2004). BAM8-22, BAM1-22 plus naloxone, and Tyr6-g2-MSH6–12 each have been shown to dose-dependently attenuate acute nocifensive behavior induced by

intraplantar injection of formalin or intrathecal injection of NMDA (Chen et al. 2006, 2008; Hong et al. 2004; Zeng et al. 2004). Moreover, MrgprC agonists in these studies also reduced spinal c-Fos immune reactivity, a marker of nociception (Chen et al. 2006; Zeng et al. 2004). MrgprC agonists have also been shown to decrease thermal hyperalgesia induced by intraplantar formalin or CFA (Guan et al. 2010; Hong et al. 2004; Jiang et al. 2013). Conversely, we observed an augmentation of CFA-induced thermal hyperalgesia in Mrgpr-cluster knockout (KO) mice that are deficient in MrgprC (Guan et al. 2010). We and others also have shown that intrathecal administration of MrgprC agonists reduces mechanical hyperalgesia in both inflammatory (CFA) and neuropathic (spinal nerve ligation and chronic constriction injury) pain models (Cai et al. 2007; Guan et al. 2010; Jiang et al. 2013). BAM1–22 and MrgprC are known to be upregulated during the process of CFA-induced pain chronification (Cai et al. 2007; Jiang et al. 2013). After CFA injection, enhanced MrgprC signaling inhibited the induction of CGRP in primary sensory neurons and the induction of nNOS in spinal projection neurons (Jiang et al. 2013). Such nNOS induction is known to exert long-lasting effects on spinal sensitization (Latremoliere and Woolf 2009). Findings from our lab also suggest that BAM8–22 is an MrgprC/MrgprX1-selective agonist. Intrathecal injections of BAM8–22 attenuated nerve injury-induced neuropathic pain in rats and wild-type mice, but not in Mrgpr-cluster KO mice. These findings suggest that under neuropathic pain states, MrgprC ligands may function as anti-hyperalgesic agents at the spinal level and that this analgesic effect is mediated by Mrgprs.

In our recent study, spinal nerve ligation-induced nerve injury induced temporal changes in MrgprC expression that differed between injured and uninjured DRG neurons (He et al. 2014a). We also found that chronic constriction injury led to mechanical pain hypersensitivity in both wild-type and Mrgpr-cluster KO mice, but the duration of mechanical hypersensitivity was longer in the Mrgpr-cluster KO mice than in their wild-type littermates. These observations indicate that activation of Mrgprs may constitute an endogenous mechanism that inhibits the maintenance of neuropathic pain in mice (He et al. 2014a). Importantly, intrathecal injection of BAM8-22 and JHU58 (a novel dipeptide MrgprC agonist) inhibited both mechanical and heat hypersensitivity in rats that had undergone spinal nerve ligation (He et al. 2014b). The drug efficacy was lost in Mrgpr-cluster KO mice and was blocked by a selective MrgprC receptor antagonist or gene silencing with intrathecal MrgprC siRNA, suggesting that the drug action is MrgprC-dependent.

Our observations are in concordance with previous reports, suggesting that BAM8-22 inhibits persistent inflammatory pain, chemical pain, and spinal c-fos gene expression in an opioid-independent manner (Cai et al. 2007; Chen et al. 2006, 2008; Hong et al. 2004; Jiang et al. 2013; Zeng et al. 2004). However, they contradict the findings of other reports that suggest a pronociceptive effect of BAM8-22 (Grazzini et al. 2004; Ndong et al. 2009). The reasons for these contradictory findings remain unclear but may be related to differences in animal conditions and etiologies (physiologic condition vs nerve injury) (Julius and Basbaum 2001;

Pernia-Andrade et al. 2009), drug concentrations, and behavioral measures (spontaneous vs. reflex). The pronociceptive effect (e.g., scratching) observed after injection of BAM8-22 into skin can also be due to itch, as MrgprC may function in sensory processing of itch at peripheral terminals in skin (Ma et al. 2012; Wilson et al. 2011). However, intrathecal injection of MrgprC agonists at the doses we tested inhibited neuropathic pain manifestations without eliciting itch-like behavior or signs of discomfort. Further, intraplantar injection of MrgprC agonists produced no pain inhibition. Nevertheless, it is still unclear why peripheral injections of MrgprC agonists induce itch, whereas central administration inhibits pain. The different effects may relate in part to drug dose, local concentration, and the different sites of action and receptors involved. Other receptor ligands (e.g., serotonin, capsaicin) also produce different effects on pain and other sensations when applied at different locations. For example, capsaicin induces burning pain in the periphery, but it inhibits pain when applied centrally (Caterina et al. 2000). This central inhibition of pain by capsaicin may involve presynaptic inhibitory mechanisms (MacDermott et al. 1999). Finally, the varied effects of MrgprC agonists may also be caused by differential distribution and compartmentalization of MrgprC and by coupling of different downstream targets (e.g., Gi and Gq) at peripheral (e.g., TRP channels) and central (e.g., calcium channels) terminals (He et al. 2014b; Liu et al. 2008).

7.7 MrgprE-H

The subfamilies MrgprE to H consist of only one receptor per species and are conserved in rodents and primates. The exception is MrgprH, which is present only in rodents (Dong et al. 2001; Zylka et al. 2003). To date, no ligand has been identified for any member of these Mrgpr subfamilies, severely hampering their functional characterization. One study has pointed toward a possible role of MrgprE in pathological pain. MrgprE-deficient mice exhibited normal acute pain responses in a hot plate assay but showed a trend toward decreased nocifensive behavior in both phases of the formalin test. They also showed deficits in the induction, but not maintenance, of mechanical allodynia after chronic constriction injury (Cox et al. 2008).

7.8 MrgprX1

The human MrgprX1 was the first primate-specific Mrgpr, for which a ligand was identified, and several agonists and antagonists are now known. MrgprX1 expressed in HEK-293 cells binds BAM peptides with high affinity and was shown to elicit intracellular calcium release (Lembo et al. 2002). The agonist activity of BAM

peptide binding to rodent MrgprC was completely preserved in an N-terminal truncated form of BAM1–22 (BAM8–22). Despite sharing BAM peptides as a common agonist, human MrgprX1 and rodent MrgprC exhibit distinct pharmacologic profiles. Proneuropeptide-FF-A cleavage products and dynorphin-14, which are partial agonists of MrgprC, do not activate MrgprX1 at all. More interestingly, g2-MSH, the most potent and efficacious agonist of rodent MrgprC, only faintly activates MrgprX1 (Lembo et al. 2002; Solinski et al. 2014). Moreover, cyclic dimers of the C-terminal portion of g2-MSH antagonize BAM8–22-induced MrgprX1 activation, but do not bind to rodent MrgprC (Schmidt et al. 2009). Thus, both binding and pharmacologic properties of human MrgprX1 and rodent MrgprC are quite distinct. Indeed, MrgprC exhibits a high promiscuity toward many ligands, whereas MrgprX1 is much more restrictive and binds solely BAM peptides, a feature conserved to a certain degree in the MrgprX1 of rhesus monkeys (Burstein et al. 2006).

In addition to potential endogenous agonists, exogenous agonists of MrgprX1, such as tetracyclic benzimidazoles, have also been investigated (Malik et al. 2009). Furthermore, 2,4-diaminopyrimidine derivates and 2,3-disubstituted azabicyclooctanes antagonize BAM8–22-induced signaling via MrgprX1 (Bayrakdarian et al. 2011; Kunapuli et al. 2006). Interestingly, the MrgprA3 ligand chloroquine also activates MrgprX1, although the affinity is 1000-fold less and the efficacy 2.5-fold less than that of BAM8–22 (Liu et al. 2009). Hence, MrgprX1 shares some pharmacologic characteristics with rodent MrgprC and MrgprA subfamily members but also has unique features.

TRPV1 might contribute to MrgprX1-induced sensory neuron excitation via two distinct signaling pathways: one through PKC-mediated phosphorylation of the channel serine residues 502 and 800 and the other via direct TRPV1 activation mediated through diacylglycerol binding to channel regions (Solinski et al. 2012). This multifaceted modulation of TRPV1 activity is unique in the GPCR superfamily (Kim et al. 2009; Prescott and Julius 2003; Solinski et al. 2012; Woo et al. 2008) and points toward an important role of the TRPV1-MrgprX1 regulatory axis in pain and sensation.

MrgprX1 might either attenuate or increase neuronal activity, depending on the cell type. This possibility raises an important question about the mechanisms responsible for these opposing effects. Inhibition of neuronal activity apparently depends on Gi/o, whereas increased activity depends on Gq/11 signaling. Therefore, distinct G-protein coupling of the MrgprX1 in different cell types might be the most plausible explanation for its opposing effects on neuronal activity (Hermans 2003; Hur and Kim 2002; Kukkonen 2004). Because endogenous model systems are currently lacking, effects of MrgprX1 on neuronal activity have been analyzed only after MrgprX1 protein overexpression, which may also affect G-protein coupling (Chen and Ikeda 2004; Solinski et al. 2010). Hence, it is still not clear if MrgprX1 can act as an analgesic by decreasing neuronal activity or as an algesic by increasing neuronal activity in different circumstances. Another possibility is that receptors might induce both effects at the same time, in which case the net effect in a given cell would depend on which of the two signaling pathways dominates. It will be

very interesting to analyze whether and how MrgprX1 regulates the activity of primary sensory neurons of primates and thus nociception in vivo. These experiments are difficult to perform because functional primary sensory neurons from humans are not available and rodents do not harbor MrgprX1-encoding genes.

7.9 MrgprX2

The pharmacology of MrgprX2 subtype is completely different from that of MrgprX1 because MrgprX2 does not bind BAM peptides (Burstein et al. 2006). However, several other peptidergic ligands have been proposed in various heterologous and endogenous expression systems (Kamohara et al. 2005; Kashem et al. 2011; Liu et al. 2011; Malik et al. 2009; Robas et al. 2003; Subramanian et al. 2011a, b; Subramanian et al. 2013; Tatemoto et al. 2006). The best characterized MrgprX2 ligand so far is cortistatin-14 peptide, which activates MrgprX2 with potencies in the medium to high nanomole range (Kamohara et al. 2005; Malik et al. 2009; Robas et al. 2003; Subramanian et al. 2013). Like MrgprX1, the ligand binding profile of MrgprX2 is conserved in rhesus monkeys, as cortistatin-14 can activate MrgprX2 in this species (Burstein et al. 2006; Malik et al. 2009). However, MrgprX2 exhibits a much broader expression pattern than other MrgprXs, as it was detected in primary sensory neurons, several brain areas, mast cells, and the adrenal medulla (Kamohara et al. 2005). Proadrenomedullin peptides, which are produced endogenously as side products during adrenomedullin synthesis in the adrenal medulla, also have been shown to activate MrgprX2 (Kamohara et al. 2005). Importantly, MrgprX2 was shown to be involved in mast cell activation by a set of endogenous and exogenous basic secretagogues (Kashem et al. 2011; Subramanian et al. 2011a, 2013; Tatemoto et al. 2006). A recent study suggested that basic secretagogues activate mouse mast cells in vitro and in vivo through a single receptor, MrgprB2, the orthologue of the human MrgprX2 (McNeil et al. 2015). That study further demonstrated that most classes of US Food and Drug Administration-approved peptidergic drugs that are associated with allergic-type injection-site reactions also activate MrgprX1 and that drug-induced symptoms of anaphylactoid responses are significantly reduced in Mrgpr-cluster KO mice. Thus, MrgprX2 may be a potential therapeutic target for reducing secretagogue-induced histamine release, inflammation, and airway contraction.

7.10 MrgprX3 and 4

So far, no study has reported the activation of MrgprX3 and 4 by a given ligand. Therefore, no data are available regarding the signaling cascade induced by MrgprX3 and 4 or their biologic significance. It has been shown that the expression of MrgprX3 is under the control of the β-actin promoter in rats and that the

activation results in increased proliferation of lens fiber cells and keratinocytes in basal and suprabasal layers of the skin (Burstein et al. 2006; Kaisho et al. 2005). Additionally, a screening approach revealed that the MrgprX4 protein is one of 15 mutational hot spots in human colorectal cancer cells (Burstein et al. 2006). Thus, the roles of MrgprX3 and 4 in pain sensation are still unexplored.

7.11 Conclusions and Future Directions

The evidence collected to date suggests that some members of the Mrgpr family can play an important role in the initiation and maintenance of pathological pain conditions. In particular, MrgprC may act as an analgesic at spinal sites—depending on the conditions—and human MrgprX1 appears to modulate excitability of primary sensory neurons and gene expression. Thus, MrgprX could be a valuable drug target that produces few or even no side effects because its expression is restricted to primary sensory neurons and mast cells. However, a lack of specific research tools makes it challenging to study the roles of individual MrgprXs in different pathological pain conditions. Most of the available data on Mrgpr are based solely on experiments obtained after heterologous expression of the MrgprX1. Therefore, our knowledge of the Mrgprs is still incomplete. For example, what is the physiologic role of distinct Mrgprs in primary sensory neurons? Additionally, what are the differences between distinct species in this regard, how can the findings from rodent Mrgprs be correlated with those of humans, and who have a relatively different form of Mrgpr (e.g., X1). Because we still lack specific ligands, several subtypes of MrgprA and MrgprX subfamilies have not yet been analyzed. Progress in the field of MrgprE–H receptors is similarly hampered by the lack of specific agonists. Moreover, the signaling pathways involved in MrgprC/X1 agonist-induced antinociception are not yet fully understood.

In vivo model systems are needed that will allow us to test the effect of human MrgprXs on pain. Human MrgprX1 has been shown to enhance or inhibit signaling pathways that affect neuronal activity, suggesting that it might have an important role in human nociception. Thus, it is imperative that we analyze how data obtained from BAM8–22-sensitive rodent MrgprC can be extended to BAM8–22-sensitive human MrgprX1. It would also be very helpful if we can develop human-based DRG-derived cell models or humanized mice that endogenously express MrgprX1. Such models will help to provide us with a better understanding of the physiologic role of various Mrgprs and contribute to the development of novel therapeutic agents that can be used for pain treatment with minimal side effects.

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Chapter 8
Pain Modulation and the Transition from Acute to Chronic Pain

Mary M. Heinricher

Abstract There is now increasing evidence that pathological pain states are at least in part driven by changes in the brain itself. Descending modulatory pathways are known to mediate top-down regulation of nociceptive processing, transmitting cortical and limbic influences to the dorsal horn. However, these modulatory pathways are also intimately intertwined with ascending transmission pathways through positive and negative feedback loops. Models of persistent pain that fail to include descending modulatory pathways are thus incomplete. Although teasing out individual links in a recurrent network is never straightforward, it is imperative that understanding of pain modulation be fully integrated into how we think about pain.

Keywords Pain-modulation • Descending control • Rostral ventromedial medulla • Feedback • Plasticity

8.1 Introduction

It has been said that "pain demands attention." This observation encompasses the all-consuming nature of pain and resonates with most people's personal experience. Pain-related goals have high behavioral priority, and terminating or at least reducing pain usually takes precedence over other motivated behaviors. The high emotional and cognitive load imposed by pain reflects the fact that tissue damage engages a high-priority "automatic" pathway, a sensory pathway sufficiently strong to resist interference or distraction. One can easily imagine how this would be advantageous, since an immediate high-priority response to tissue damage would potentially limit injury and enhance behaviors that protect and promote healing.

Despite the automatic character of nociceptive transmission, the relationship between tissue damage and pain is complex, and pain is subject to contextual

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demands. At one end of the spectrum, pain threshold is somewhat elevated during feeding (Casey and Morrow 1989, 1988; LaGraize et al. 2004; Foo and Mason 2005), and at the extreme, severe stress can result in potent analgesia (Amit and Galina 1988; Watkins and Mayer 1982). Conversely, pain can be facilitated by mild or "psychological" stress, during illness, or by enhanced attention (Watkins et al. 1994; Wagner et al. 2013; Imbe et al. 2006; Willer et al. 1979). Although adjustments in intracortical and thalamocortical circuits undoubtedly have some role in how pain varies with behavioral context and task engagement, the key mechanism underlying top-down regulation of pain is thought to be *pain-modulating* systems. These defined brainstem circuits regulate nociceptive transmission and are sometimes referred to as "descending controls" because they are mediated by projections from the brainstem to nociceptive circuitry at the level of the spinal and trigeminal dorsal horn. This allows modulation of high-priority "automatic" nociceptive processing at the earliest central stages.

The critical links in brainstem pain-modulating circuitry have been identified as the midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). Anatomically, this system is well positioned to mediate cognitive and emotional effects on pain by altering spinal nociceptive processes: the periaqueductal gray receives massive inputs from forebrain areas implicated in mood, attention and executive control, stress, and arousal and relays this information to the RVM, which projects to the dorsal horn of the spinal cord via the dorsolateral funiculus as well as to the trigeminal dorsal horn. The RVM also receives direct input from some of the same areas, including the central nucleus of the amygdala, insula, and hypothalamus.

Understanding the physiology and function of this system is more challenging. This is due to a number of factors. First, the system exerts bidirectional control and can both inhibit and facilitate pain. In addition, neither the PAG nor the RVM is a "center," devoted exclusively to pain modulation. Finally, pain-modulating circuitry is closely intertwined with nociceptive transmission pathways, forming a recurrent network that can only be fully understood from an integrative perspective. The present chapter provides an overview of avenues toward understanding pain modulation that address these challenges, with an emphasis on how the system is altered in the transition from acute to persistent pain.

8.2 The PAG and RVM as a Pain-Modulating Circuit

The brain's ability to modulate somatosensory processing at the level of the dorsal horn has been recognized for over a century. However, the idea that specific brain circuits are dedicated to regulating transmission of pain-related sensory signals is usually traced back to the demonstration that electrical stimulation of the PAG can inhibit behavioral responses to noxious stimuli in rats (Reynolds 1969). Subsequent adoption of this concept by neurosurgeons for treatment of intractable pain in patients, although not without significant limitations and drawbacks, demonstrated

that electrical stimulation of the PAG region actually reduces pain and is not simply inhibiting motor responses (Barbaro 1988). Discovery of the opioid receptor and endogenous opioid peptides at about the same time heightened interest in pain-modulating circuits, with the ultimate demonstration that the analgesic actions of potent opioids acting at the μ-opioid receptor were through engagement of this system, with specific targets in the PAG and RVM (Heinricher and Ingram 2008; Heinricher and Fields 2013). More recently, imaging studies have confirmed engagement of the PAG-RVM system in pharmacological and behavioral modulation of pain (Lee et al. 2008; Tracey 2010; Bingel and Tracey 2008).

Because the dominant behavioral effect of electrical stimulation in either the PAG or RVM is analgesia, and because both sites support opioid analgesia, the system was initially viewed as an "analgesia" system. It later became clear, however, that this is true modulatory system, with the capacity to enhance or inhibit pain in different circumstances. This requires a more nuanced view and suggests that the system is best conceived as a regulatory node that modulates lower-level nociceptive sensory transmission mechanisms in accord with behavioral context. Bidirectional control is mediated by two distinct outputs from the RVM, a pain-facilitating population referred to as "ON-cells" and a pain-inhibiting population referred to as "OFF-cells." Top-down inputs to this regulatory node can therefore set the priority for nociceptive sensory processing. Pain-modulating circuits must also monitor the nociceptive transmission system. Indeed, the pain-modulating neurons of the RVM are most easily identified by changes in firing associated with behavioral responses to noxious stimulation (Heinricher and Fields 2013). The fact that pain-modulating neurons also "respond" to noxious input has, on occasion, led to conceptual and methodological confusion when viewed from a sensory, rather than modulatory, perspective.

An important question is whether descending pain-modulation systems are relevant to pain as a sensory experience or whether these systems simply modify spinal nocifensor reflex arcs. This issue has been raised because nocifensor withdrawal reflexes can sometimes be dissociated from more integrated nociceptive behaviors (King et al. 2003, 2007). It has also been suggested that the RVM output has a premotor function, shaping the withdrawal from noxious stimulation (Hellman and Mason 2012). Indeed, blocking the pronociceptive output from the RVM reduces the magnitude of the nocifensor withdrawal (Jinks et al. 2007). However, the argument for a premotor function is based primarily on correlations between cell activity and the dynamics of the motor response, and this correlation is less than robust (Devonshire et al. 2015). Moreover, lesion or reversible inactivation of the RVM does not eliminate motor responses, but rather alters the threshold for evoking the behavior (Heinricher and Kaplan 1991; Proudfit 1980; Young et al. 1984). More important, however, pharmacological manipulation presumed to activate the pain-facilitating output from the RVM supports conditioned place avoidance, in addition to hyperalgesia as measured by withdrawal threshold. Conversely, inactivation of the RVM in animals subjected to a nerve injury supports conditioned place preference (De Felice et al. 2011, 2013). These findings using place preference and avoidance as a measure of the affective state of the animal (King et al. 2009)

demonstrate that brainstem pain-modulating systems influence not just reflex arcs, but circuits contributing to pain as an aversive sensory experience.

8.3 Is Pain Modulation a Specific Function of the PAG-RVM System?

One underappreciated aspect of the function of this "pain-modulating" PAG-RVM circuit is that these same brain regions also regulate a range of physiological parameters, including heart rate, respiration, and body temperature, and contribute to a number of coordinated behaviors, including defense, reproduction, and maternal behaviors (Lovick 1997; McAllen et al. 2010; Bandler and Keay 1996; Bandler et al. 2000a; Behbehani 1995; Morrison 2011). Functional responses to stimulation in these regions can therefore include, in addition to antinociception, flight, jumping, gnawing, vocalization, apnea, and immobility (the latter incorrectly interpreted in early work as "pure analgesia" (Fardin et al. 1984, see Walker and Carrive 2003; Morgan and Carrive 2001 for discussion). For this reason, neither the periaqueductal gray nor the RVM should be considered as a pain-modulating "center," devoted exclusively to pain modulation. But does this mean that the idea of pain modulation as a specific brain function is invalid?

The long-established fact that pain modulation is invariably integrated with autonomic and other behavioral changes when engaged as part of organized responses to behavioral and physiologic challenges has led some to conclude that modulation of pain from the PAG-RVM system "…[cannot] be divorced from so-called side effects" (Mason 2011). In that view, the putative pain-modulating neurons in the RVM, the ON-cells and OFF-cells, are multifunctional and regulate physiological variables in addition to nociceptive processing. Consistent with this perspective, the firing of ON- and OFF-cells can sometimes be correlated with body temperature, respiratory parameters, or micturition (Baez et al. 2005; Hellman et al. 2007; Nason and Mason 2006). However, a neuron can fire in *association* with a physiological or behavioral parameter without *controlling* that parameter. Indeed, given that pain modulation is integrated with other behavioral and physiological parameters as part of a coordinated homeostatic response (Bandler and Shipley 1994; Bandler et al. 2000b; Lovick 1997; Fanselow 1991; Lovick 1993), one should expect that RVM outputs regulating different parameters, such as nociception, thermogenesis, or heart rate, would act in concert and show correlated activity due to shared inputs and/or local interactions. Simply demonstrating that the activity of putative pain-modulating neurons can be *correlated* with physiological parameters is not therefore an adequate strategy for determining whether these neurons play a role in *regulating* those parameters. Instead, *manipulation* of these populations is required to support a causal conclusion, and pain-modulating and autonomic functions of the RVM can be dissociated when recruited through endogenous mechanisms. For example, the activation of the dorsomedial hypothalamus, a model of

mild stress that can be employed in both awake and anesthetized preparations, gives rise to tachycardia, hyperthermia, and behavioral hyperalgesia (DiMicco et al. 2002, 2006; Martenson et al. 2009). Blocking excitatory amino acid transmission in the RVM with kynurenate selectively reduces ON-cell activity and prevents hyperalgesia, but not tachycardia or hyperthermia. However, blocking *all* RVM activity using the $GABA_A$ receptor agonist muscimol prevents both tachycardia and hyperthermia (Martenson et al. 2009). This implies that the hyperalgesic and autonomic effects of mild stress are mediated by different RVM cell populations. A similar dissociation has been seen in a model of conditioned fear in awake animals, where muscimol blocks cardiovascular responses, but kynurenate interferes with somatic signs of distress such as ultrasonic vocalizations or freezing (Vianna et al. 2008). Although it has been suggested that ON-cells could modulate respiratory function as well as pain (Phillips et al. 2012), it is likely that sympathoexcitatory pathways beyond the boundaries of the RVM were engaged in those studies (Dampney 2015).

Findings from studies using an experimental approach rather than relying on correlation therefore support a contrasting view that pain modulation is a specific function of ON- and OFF-cell classes, with other aspects of RVM function mediated by subsets of other neurons in the region. That is, although there is no question that pain modulation and other functions, including autonomic regulation, overlap at the level of the RVM *as a region*, it does not follow that these functions overlap at the level of *single neurons.* Instead, specificity of function is found at the level of individual neurons.

8.4 Inputs to the PAG-RVM Pain-Modulating System

Given a pronociceptive role for ON-cells and an antinociceptive role for OFF-cells, clues to the physiology and pathophysiology of nociceptive modulation may be derived from the behavioral and environmental conditions under which each class is active. ON- and OFF-cells respond to noxious inputs, producing a positive feedback signal that enhances behavioral sensitivity to subsequent stimuli delivered to any region of the body, including visceral structures (Foo and Mason 2003; Ramirez and Vanegas 1989; Sanoja et al. 2010). Excitability of ON- and OFF-cells also varies with arousal and behavioral context. In awake, unrestrained rats, RVM neurons that resemble ON-cells respond briskly to light touch and to sudden sound as well as to noxious inputs (Leung and Mason 1999; Oliveras et al. 1990). This finding suggests that such innocuous, but possibly behaviorally significant, environmental stimulation modulates nociceptive processing through the PAG-RVM system. Broadly speaking, correlations of ON- and OFF-cell discharge with behavioral state or physiological variables point to a potential role for these neurons in mediating the effects of a host of psychological and physiological variables on pain (Heinricher et al. 2009).

The organization of the RVM suggests that neurons of the ON- and OFF-cell classes function as a unit that exerts global, rather than topographically discrete,

control over pain transmission. Individual RVM neurons vary widely in responsiveness (Barbaro et al. 1989) but have large, often total body, receptive fields and likely project diffusely to the trigeminal dorsal horn and to multiple spinal levels (Huisman et al. 1981). Furthermore, many RVM neurons have axons that collateralize within the RVM itself, (Mason and Fields 1989) and, at least in anesthetized rats, cells of the same physiological class tend to fire at the same time (Barbaro et al. 1989). These fluctuations in ON- and OFF-cell population activity are correlated with modest but detectable and potentially behaviorally relevant shifts in nociceptive withdrawal threshold (Heinricher et al. 1989). Thus, RVM activity can have a broad influence over the general responsiveness of the organism to peripheral stimulation.

8.5 RVM Plasticity in Persistent and Chronic Pain

The RVM is increasingly recognized as a dynamic system, with altered physiology and function in chronic pain models (Terayama et al. 2000; Hurley and Hammond 2000, 2001; Schepers et al. 2008; Sykes et al. 2007; Guan et al. 2002, 2003, 2004; Ren and Dubner 2002). These changes develop over the course of many hours and days and likely reflect alterations in the intrinsic properties of RVM neurons and circuits, as well as altered afferent input.

There is evidence that the RVM can both contribute to behavioral hypersensitivity in chronic pain states and limit that hypersensitivity. *Acute* inflammation or injury is associated with a strong, sustained activation of ON-cells and suppression of OFF-cell firing (Kincaid et al. 2006; Xu et al. 2007). This shift in the balance between the ON- and OFF-cell outputs mediates secondary hyperalgesia, since blocking the activation of the ON-cells interferes with the lowering of behavioral threshold (Kincaid et al. 2006; Xu et al. 2007). At least in the short term, it appears that substance P contributes to the enhanced ON-cell output (Brink et al. 2012; Budai et al. 2007; Khasabov et al. 2012; Pacharinsak et al. 2008).

In *chronic* pain, however, the situation is significantly more complex. There is clear evidence that an active output from the RVM contributes to hypersensitivity in neuropathic pain models (Porreca et al. 2002). This output depends on cholecystokinin (Kovelowski et al. 2000), and taken together with the evidence that RVM ON-cells are activated by low concentrations of cholecystokinin to produce hyperalgesia (Heinricher and Neubert 2004), it is reasonable to think that ON-cells play some role in behavioral hypersensitivity in chronic inflammatory and neuropathic pain states. This idea receives further support from the evidence that ON- and OFF-cells are "sensitized" in both persistent inflammation and following nerve injury, responding to innocuous tactile stimuli (Carlson et al. 2007; Cleary and Heinricher 2013).

However, persistent pain is not a simple continuation of the state seen immediately after the injury or early in inflammation. Despite the fact that ON- and OFF-cells are sensitized to innocuous stimuli, the *ongoing* firing of ON- and OFF-cells is normalized (Cleary and Heinricher 2013; Carlson et al. 2007). Tonic descending

inhibition is restored and can limit abnormal nociceptive processing. Persistent pain likely represents, at least in part, a failure of this compensatory rebalancing of the RVM output (Porreca et al. 2002; De Felice et al. 2011; Leong et al. 2011; Cleary and Heinricher 2013). Top-down mechanisms, for example from medial prefrontal cortex (Millecamps et al. 2007), could play a role in the extent to which the system can rebalance. At the same time, the ability of innocuous stimuli to cause a state-change in RVM, evoking a burst of activity in the ON-cells and causing the OFF-cells to cease firing, implies that the system is less stable than in uninjured animals.

8.6 Conclusions

There is now increasing evidence that pathological pain states are at least in part driven by changes in the brain itself. Descending modulatory pathways are known to mediate top-down regulation of nociceptive processing, transmitting cortical and limbic influences to the dorsal horn. However, these modulatory pathways are also intimately intertwined with ascending transmission pathways through positive and negative feedback loops. Models of persistent pain that fail to include descending modulatory pathways are thus incomplete. Although teasing out individual links in a recurrent network is never straightforward, it is imperative that understanding of pain modulation be fully integrated into how we think about pain.

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Chapter 9
Advances in the Treatment of Neuropathic Pain

Li Xu*, Yuguan Zhang*, and Yuguang Huang

Abstract Neuropathic pain is pain that arises as a direct consequence of a lesion or diseases affecting the somatosensory system. Treatments for neuropathic pain include pharmacological, nonpharmacological, and interventional therapies. Currently recommended first-line pharmacological treatments include antidepressants and anticonvulsants (gabapentin and pregabalin). However, in some cases, pharmacological therapy alone fails to give adequate control of the chronic pain. New techniques have been invented and have been proved effective on neuropathic pain, such as behavioral, cognitive, integrative, and physical therapies. In this review, we focused on the advances in the treatment of central neuropathic pain, diabetic peripheral neuropathy, postherpetic neuralgia, and cancer pain.

Keywords Neuropathic pain • Antidepressant • Anticonvulsant • Radio frequency • Neural stimulation

9.1 Introduction

Clinical evaluation of neuropathic pain (NP) requires a thorough history and physical examination to identify characteristic signs and symptoms. In many cases, other laboratory investigations and clinical neurophysiological testing may help identify the underlying etiology and guide treatment selection. Mechanisms for NP include aberrant ectopic activity in nociceptive nerves, peripheral and central sensitization, impaired inhibitory modulation, and pathological activation of microglia. Available treatments essentially provide only symptomatic relief and may include nonpharmacological, pharmacological, and interventional therapies.

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Most extensive evidence is available for pharmacological treatment, and currently recommended first-line treatments include antidepressants (tricyclic agents and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin). Individualized multidisciplinary patient care is facilitated by careful consideration of pain-related disability as well as patient education, repeat follow-up and strategic referral to appropriate medical/surgical subspecialties, and physical and psychological therapies. Despite the availability of many effective drugs and guidelines for the treatment of NP, evidence from the United States and Europe suggests that they are not widely used, and many cases remain under- or untreated (Gilron et al. 2015). This chapter focuses on the advances in the treatment of NP.

Neuropathic pain mechanisms relevant to diagnosis and treatment include ectopic activity, peripheral sensitization, central sensitization, impaired inhibitory modulation, and activation of microglia (Hehn et al. 2012). Although the signs and symptoms characteristic of neuropathic pain varies a lot, the sensory quality descriptors "tingling" (or "pins and needles" or "prickling"), "burning" (or "hot"), and "shooting" (or "electrical shocks") are included in nearly all these various tools, and these three descriptors are perhaps the most characteristic of neuropathic pain. Much of this characteristic has emerged from the development and publication of several screening tools. Such as the Michigan Neuropathy Screening Instrument (MNSI), Neuropathic Pain Scale (NPS), Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), Neuropathic Pain Questionnaire (NPQ), "Douleur Neuropathique en 4 questions," Pain Quality Assessment Scale, and the Short-Form McGill Pain Questionnaire-2.

Through the signs, symptoms, and tests, neuropathic pain can be diagnosed. However, epidemiological surveys have indicated that many patients with NP do not receive appropriate treatment for their pain. A number of pharmacological agents have been found to be effective in NP on the basis of randomized controlled trials including, in particular, tricyclic antidepressants, serotonin and norepinephrine reuptake inhibitor antidepressants, pregabalin, gabapentin, opioids, lidocaine patches, and capsaicin high-concentration patches. Evidence-based recommendations for the pharmacotherapy of NP have recently been updated. The treatment protocol is described in Fig. 9.1. Improving the current way of conducting clinical trials in NP could contribute to reduce therapeutic failures and may have an impact on future therapeutic algorithms (Attal and Bouhassira 2015; Helfert et al. 2015).

The treatment strategies for neuropathic pain involve a variety of methods such as physical therapy, psychotherapy, teamwork medical, traditional Chinese medicine, transcutaneous electrical nerve stimulation, and interventional therapy.

9.2 Pharmacological Treatment

The first line drugs include tricyclic antidepressants (TCAs), selective serotonin norepinephrine reuptake inhibitors (SSNRI), anticonvulsants, and topical lidocaine. TCAs are efficacious for several types of neuropathic pain including DPN, nerve

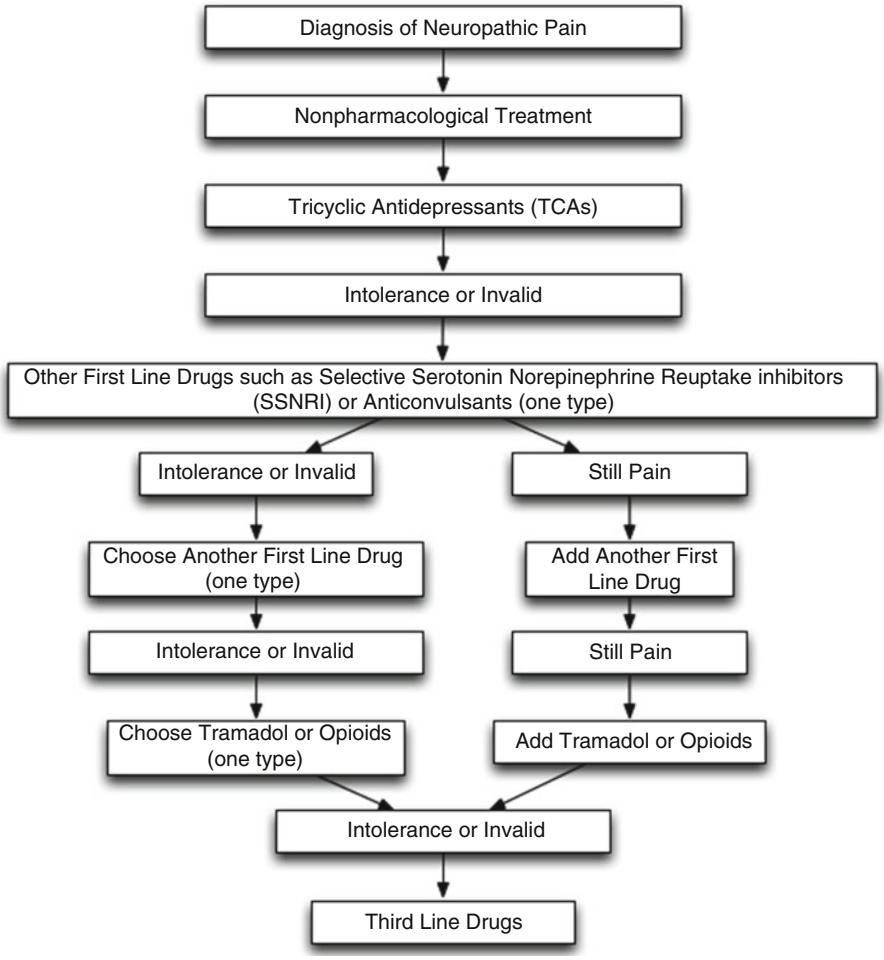

Fig. 9.1 Treatment protocol

injury pain, PHN, and central post-stroke pain (Xu et al. 2012). The analgesia effects of TCAs are attributed to inhibiting reuptake of serotonin and noradrenaline from presynaptic terminals. And they show analgesia efficacy as well as antidepressant effect, the pain-relieving effect is independent of their mood-elevating properties SSNRIs such as duloxetine and venlafaxine have shown consistent efficacy in DPN. Anticonvulsants, such as gabapentin and pregabalin, are quite used as the first choice of neuropathic pain. The efficacy of gabapentin for PHN and DNP has been repeatedly demonstrated. The use of gabapentin in a variety of neuropathic pain conditions was recently reviewed. Overall, the efficacy of gabapentin (50 % pain relief compared to baseline) in PHN, DNP, complex regional pain syndrome type I (CRPS-1), nerve injury pain, small fiber sensory neuropathy, phantom pain, and

mixed neuropathic pain was reported to be superior to placebo (Risk ratio: RR 1.7, 95 % CI: 1.46–1.99; NNT: 6.8, 95 % CI: 5.4–9.2) at the expense of relatively frequent, but most often tolerable, adverse effects (Moore et al. 2011). Fatigue is one of these adverse effects. There are an increasing number of clinical studies on pregabalin that provides supportive evidence for the treatment of DNP, PHN, and other neuropathic pain conditions. The suggestion that the overall cost of care may be reduced in gabapentin-refractory neuropathic pain by switching to pregabalin has been made. A retrospective analysis of data from nine controlled trials of pregabalin for painful diabetic peripheral neuropathy and postherpetic neuralgia suggests that the advantages of pregabalin are the twice-daily administration schedule, a narrower dosage range (between 150 and 600 mg/day), fewer potential adverse effects, and a more rapid therapeutic effect (Sharma et al. 2010). The combination of pregabalin with oxycodone did not clearly show benefit compared with pregabalin alone in a randomized controlled trial (RCT) for PHN or DNP (Jongen et al. 2014; Wettermark et al. 2014). The most widely studied relevant clinical presentations of localized neuropathic pain (LNP) are postherpetic neuralgia, diabetic neuropathy, and neuropathic postoperative pain. They successfully respond to treatment with 5 % lidocaine-medicated plaster with equal if not better pain control but with fewer side effects versus conventional systemic treatments (Casale and Mattia 2014; Likar et al. 2012; Zis et al. 2014). The choice of first line drugs on neuropathic pain is concluded in Table 9.1.

Table 9.1 The first line drugs on neuropathic pain

Type of drug	Level	Side effect	Relative contraindication	Main indication
Tricyclic antidepressants (TCAs)	+	Drowsiness, dry mouth, blurred vision, weight gain, urinary retention	Heart disease, glaucoma, suicide risk, epilepsy, combined with tramadol	DPN, PHN, central post-stroke pain
Selective serotonin norepinephrine reuptake inhibitors (SSNRI)	+	Nausea	Liver function damage, renal inadequacy, alcohol abuse, combined with tramadol	DPN
Anticonvulsants	++	Drowsiness, dizziness, peripheral edema	Renal inadequacy	PHN, DNP, complex regional pain syndrome type I (CRPS-1), small fiber sensory neuropathy, phantom pain, mixed neuropathic pain
Topical Lidocaine	++	Skin rash	None	PHN, allodynia

DPN diabetic peripheral neuropathy, *PHN* postherpetic neuralgia

Table 9.2 The second line drugs on neuropathic pain (TCAs, tricyclic antidepressants; SSNRIs, selective serotonin norepinephrine reuptake inhibitors)

Type of drug	Level	Side effect	Relative contraindication	Other benefits
Opioids	+	Nausea, vomiting, constipation, drowsiness and dizziness	Drug abuse, suicide risk	Fast
Tramadol	+	Nausea, vomiting, constipation, drowsiness and dizziness, epilepsy	Drug abuse, suicide risk, epilepsy, combined with TCA, SSNRI	Fast

The second line drugs include opioids, tramadol. Due to the side effects of opioids, it limits the usage on the treatment of neuropathic pain. However, opioids are being prescribed in stronger potencies and larger doses for musculoskeletal injuries (Mai et al. 2015). A comparative review of available extended release tramadol formulations shows that these medications are not equivalent in their pharmacokinetic profile and this may have implications for selecting the optimal therapy for patients with pain syndromes where tramadol is an appropriate analgesic agent (Kizilbash and Ngo-Minh 2014). The choice of second line drugs on neuropathic pain is concluded in Table 9.2.

The third line drugs include antiepileptic drugs such as citalopram, paroxetine, mexiletine, and others. New evidence which genotyped 34 participants from a placebo-controlled trial of escitalopram in peripheral neuropathic pain for polymorphisms in five genes: the serotonin receptor 2A (HTR2A) gene, the serotonin receptor 2C (HTR2C) gene, the ABCB1 gene encoding for the P-glycoprotein, the CYP2C19 gene, and the serotonin transporter gene (SLC6A4), shows that the serotonin receptor 2C is involved in pain relief in patients with neuropathic pain during treatment with escitalopram, which is the pharmacologically active S-enantiomer of citalopram (Brasch-Andersen et al. 2011).

9.3 Nonpharmacological Treatment

A number of new techniques have been invented and have been proved effective on chronic pain. Nonpharmacologic approaches can be classified as behavioral, cognitive, integrative, and physical therapies. Core principles in developing a treatment plan are explaining the nature of the chronic pain condition, setting appropriate goals, and developing a comprehensive treatment approach and plan for adherence. Clinicians should become familiar with these interventions so that they can offer patients flexibility in the pain management approach. Effective noninvasive treatment modalities for chronic pain include behavioral therapy for short-term pain relief; cognitive behavioral therapy for reducing long-term pain and disability; hypnosis as adjunctive therapy; guided imagery, diaphragmatic breathing, and muscle

relaxation, especially for cancer-related pain; mindfulness-based stress reduction for patients with chronic low back pain; acupuncture for multiple pain conditions; combination manipulation, manual therapy, endurance exercise, stretching, and strengthening for chronic neck pain; animal-assisted therapy; and S-adenosyl-L-methionine for joint pain (Chang et al. 2015).

Neural blockade therapy is a classic method on pain management, the role of it for chronic pain syndromes is still to be discovered. There are some evidences that neural blockade is a valid method on chronic pain. A case of an 18-year-old girl who underwent an uneventful laparoscopic cholecystectomy complained of chronic pain at the site of the surgery postoperatively. Multiple interventions had failed to relieve the pain. However, a successful transversus abdominis plane (TAP) block confirmed the peripheral (somatic) source of the abdominal pain and provided temporary analgesia, after which an indwelling catheter was inserted, which provided prolonged pain relief (Guirguis et al. 2013). Neural blockade of the scalp may be used as an adjunct to general anesthesia or serve as the principal anesthetic for both intracranial and extracranial procedures. Effective scalp blockade typically requires anesthetizing multiple peripheral nerves, blockade of one or more of these is often used to diagnose and treat conditions such as chronic headache (Papangelou et al. 2013). There is a growing body of evidence suggesting that regional anesthesia, even if it requires supplement with sedation or general anesthesia, may be superior to opioids for improved pain control along with increased patient satisfaction and decreased perioperative morbidity and mortality comparing to general anesthesia in patients with significant medical disease(s). Despite successful implementation of neural blockade, and to avoid opioid withdrawal, at least half the chronic pain patient's daily pre-admission opioid dose should be continued daily throughout the perioperative period (Souzdalnitski et al. 2010).

Radio frequency (RF) treatment is a minimally invasive technique with multiple therapeutic applications. The basic researches supposed that RF could regulate some channel expression in the DRG (Liu et al. 2015). Pulsed radiofrequency (PRF) has been proved to reduce neuropathic pain after nerve injury, even though the underlying mechanism remains unclear. The components of PRF are described in Fig. 9.2 (Chua et al. 2011). A case report describes the use of ultrasound-guided PRF to reduce neuropathic pain in a double-level upper extremity nerve injury. And it showed that PRF is a useful tool when pharmacological therapy is inadequate for pain control in posttraumatic neuropathic pain (Magistroni et al. 2014).

Implantation of drug delivery (IDD) system replaced the administered routes such as oral, intravenous, subcutaneous, transdermal, and transmucosal. The system consists of an implantable pump that stores and delivers medication through a catheter to the IT space. Programmability is achieved by positioning an external devise over the implanted pump to change the mode of drug delivery. The innovations in programmable IT drug delivery systems are expanding more rapidly than ever before (wilkes 2014). Unfortunately, the clinical pain field suffers from a lack of randomized controlled trials (RCTs).

Neural stimulation has been widely used in Europe for many years. It involves spinal cord stimulation (SCS), transcutaneous electrical nerve stimulation (TENS),

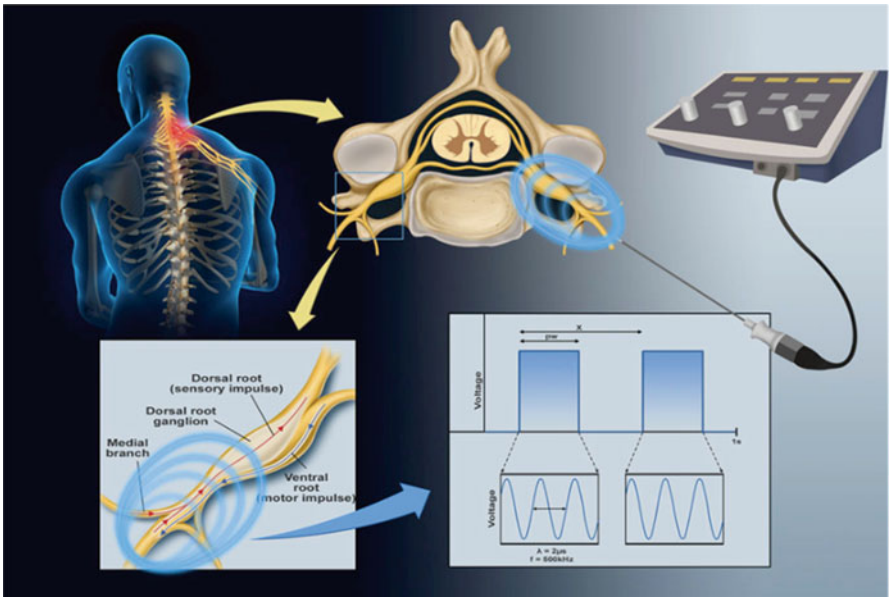

Fig. 9.2 The components of PRF(*23*) λ wavelength of intrinsic RF current; f frequency of intrinsic RF current at usually 500 kHz; pw pulse width; *x* duration of each pulse cycle and therefore pulse frequency = 1/*x* (Adapted from Chua et al. 2011)

peripheral nerve stimulation (PNS), and motor cortex stimulation (MCS). Spinal cord stimulation consists of implantation of peri-epidural electrode in the posterior columns of the spinal cord at the spinal level of the dermatomes on which we want to produce the analgesic effect. The mechanism of function of SCS is that the stimulation is applied directly to the posterior horns of the spinal cord does not allow us to conclude on the specific neurophysiological mechanisms of this analgesia. The stimulation may recruit afferents from the periphery, afferents from the spinal cord to the higher centers, local neuron circuits, and even fibers of the anterior horns of the spinal cord. Some studies seem to suggest that the efficacy of SCS in reducing neuropathic pain is probably related to a direct effect on central sensitization measured by temporal summation (TS) (Marchand 2015; Zhang et al. 2014). Transcutaneous electrical nerve stimulation (TENS) relieves pain by inhibiting pain-related potentials on the spinal and supraspinal level, known as "gate control." It is alternating current or modulated DC, comprising rectangular impulses. The analgesic effects of TENS are seen in both the ipsilateral and contralateral spinal segmental regions (Samuel and Maiya 2015). Peripheral nerve stimulation (PNS) is a neuromodulation technique in which electrical current is applied to the peripheral nerves to ameliorate chronic pain through preferential activation of myelinated fibers, inducing long-term depression of synaptic efficacy (Johnson et al. 2015). When damage to the peripheral nerves causes severe pain that does not respond to targets in the spinal cord, such as postherpetic neuralgia, occipital or C2 neuritis,

intercostal nerve pain from trauma or disease, and ilioinguinal nerve entrapment. When SCS alone fails to give adequate control of the pain, peripheral nerve stimulation alone, or in combination with SCS, will often salvage a good outcome (Deer 2011). Motor cortex stimulation (MCS) was first used for the treatment of central post stroke pain and now has been proved more effective in the treatment of chronic neuropathic pain of central post stroke pain and peripheral neuropathic pain types than in the treatment of SCI pain in the long-term follow-up (Im et al. 2015). There is a concern that infectious complications related to IDD system and SCS at a comprehensive cancer pain center. Researchers reported that 142 devices were implanted in 131 patients during the examined period. Eighty-three of the devices were IDD systems and 59 were SCS systems. Four infectious complications were noted with an overall infectious risk of 2.8 %. The infection rate was 2.4 % for IDD systems versus 3.4 % for SCS systems ($P = 1$) (Engle et al. 2013).

9.4 The Treatment of Common Neuropathic Pain

9.4.1 Central Pain

Central pain, also is named as central neuropathic pain, is the pain raised from the brain and the spinal disease such as central post stroke pain (CPSP), multiple sclerosis (MS), spinal cord injury (SCI), Parkinson's disease (PD), and central nervous infection. For pharmacological treatment, amitriptyline, an adrenergic antidepressant, is currently the first-line drug for CPSP. GABAergic drugs with potential calcium channel-blocking effects, such as gabapentin or pregabalin, have recently emerged as a potentially useful therapy. Pregabalin may improve pain-related anxiety and sleep disturbances. Given the safety, efficacy, well tolerability, and lack of interaction with other drugs, a recent study suggests gabapentin to be more considered as a first line therapy or as add-on therapy for reducing the pain severity in patients with thalamic syndrome (Hesami et al. 2015). It is important to note that large randomized, controlled trials on gabapentin and pregabalin have shown an improved safety profile over the older antiepileptic agents. Indeed, studies have shown gabapentin's superiority over placebo for chronic SCI pain, and other randomized controlled trial have verified the efficacy of oral pregabalin for patients with SCI with central neuropathic pain, at least for 3-month increments (Lee et al. 2013). Lamotrigine, an antiepileptic, was also found to be effective in a controlled trial and can be used as an alternative or additive therapy (Kim 2014).

Nonpharmcological treatment on central pain has been studied in recent years. A study recruited 14 consecutive patients with thalamic pain, atypical facial pain, post-brachial plexus avulsion injury pain, phantom pain, and pain in syringomyelia were treated with motor cortex stimulation. It suggests that MCS significantly reduces the intensity of neurogenic pain. The best long-term results in the present study were achieved in patients with thalamic syndrome (Sokal 2015). Cury et al. observed the effects of deep brain stimulation on pain and other nonmotor symptoms

in Parkinson disease and found that subthalamic nucleus deep brain stimulation (STN-DBS) decreased pain after surgery, but had different effects in different types of PD-related pain. Motor and nonmotor symptom improvements after STN-DBS did not correlate with pain relief (Cury et al. 2014).

Practitioners should carefully consider factors including concomitant disease states, renal function, and side effect of the drugs when prescribing the oral agents for spinal cord injury patients. Patients with post-spinal cord injury may suffer depression for numerous reasons, having a tricyclic antidepressant or serotonin / norepinephrine reuptake inhibitor as first-line or part of combination therapy would be recommended. When choosing combination therapies, using agents with different mechanisms of action. For example, using gabapentin with tricyclic antidepressants or serotonin/norepinephrine reuptake inhibitors would provide multiple targets at the nerves, while using combinations such as gabapentin with pregabalin will only augment side effects (DeFrates and Cook 2011). A number of studies have begun to use non-invasive neuromodulatory techniques therapeutically to relieve neuropathic pain and phantom phenomena in patients with SCI. The utility of these protocols in combination with pharmacological approaches should also be explored (Nardone 2014).

9.4.2 Peripheral Pain

Diabetic peripheral neuropathy (DPN) is the most common peripheral neuropathy and has been studied for many years. The management of diabetic neuropathic pain consists basically in excluding other causes of painful peripheral neuropathy, improving glycemic control as a prophylactic therapy, and using medications to alleviate pain. First line drugs for pain relief include anticonvulsants, such as pregabalin and gabapentin, and antidepressants, especially those that act to inhibit the reuptake of serotonin and noradrenaline. There is difference with respect to the maximum approved dose of pregabalin for the treatment of DPN in the United States (300 mg/day) and European Union (600 mg/day), though clinical data demonstrate that pregabalin doses >300 mg/day may be beneficial in some patients. Pregabalin has shown efficacy (and is approved) as a monotherapy for DPN. There are data demonstrating the efficacy of pregabalin in some patients with DPN who have not responded to other pharmacological treatments, including those unresponsive to treatment with gabapentin (Juhn et al. 2015). In addition, there is experimental and clinical evidence that opioids can be helpful in pain control, mainly if associated with first line drugs. A study which examines the proportion of DPN patients receiving pharmacologic DPN treatments and specifically identifies the rates and factors associated with opioid use and first-line opioid use proves that 53.47 % had DPN-related opioid use and 33.33 % received opioid as first-line treatment (Patil et al. 2015). Other agents, including for topical application, such as capsaicin cream and lidocaine patches, have also been proposed to be useful as adjuvants in the control of diabetic neuropathic pain, but the clinical evidence is insufficient to support their use (Schreiber et al. 2015).

Table 9.3 The therapy protocol on PHN

Patient	Age	Treatment		
		Antivirus	Anti-inflammatory	Analgesia
Normal immune function	<50	+ (within 70 h)	+	Sympathetic blockade
	>50	–	+	Epidural, body/sympathetic nerve block and infiltration
Insufficient immune function	<50	+ (within 70 h)	–	Oral analgesics
	>50	+ (within 70 h)	+	Nerve block and adjuvant use of oral analgesics

– no need
+ useful
++ necessary

Another common peripheral neuropathy is postherpetic neuralgia (PHN). The treatment of PHN contains two stages, the acute stage and the postherpetic neuralgia stage. The therapy protocol on PHN is described in Table 9.3.

9.4.3 Cancer Pain

Pain and neuropathic symptoms impact quality of life in patients with cancer. A study shows that over 40 % of the patients with moderate to severe pain also have neuropathic symptoms, causing increased interference with daily activities (Oosterling et al. 2015). The most efficacious adjuvant analgesics used as first-line treatment for NP includes tricyclic antidepressants, calcium channel α2-d ligand anticonvulsants, and serotonin-norepinephrine reuptake inhibiters. Adjuvant analgesics are often combined with opioids when NP is refractory or severe (Smith et al. 2014). A number of studies suggest that traditional herbal medicine (THM) combined with conventional therapy is efficacious as an adjunctive therapy for patients with cancer pain (Lee et al. 2015). Interventional pain management techniques are an indispensable arsenal in pain physician's armamentarium for severe, intractable pain and can be broadly classified into neuroablative and neuromodulation techniques. An array of neurolytic techniques (chemical, thermal, or surgical) can be employed for ablation of individual nerve fibers, plexuses, or intrathecalneurolysis in patients with resistant pain and short life expectancy. Neuraxial administration of drugs and spinal cord stimulation to modulate or alter the pain perception constitutes the most frequently employed neuromodulation techniques. Laying standardized guidelines based on existing and emerging evidence will act as a foundation step towards strengthening, credentialing, and dissemination of the specialty of interventional cancer pain management (Bhatnagar and Gupta 2015).

9.5 Conclusion

The mechanism of neuropathic pain is complicated. We haven't found a single way to effectively relief the neuropathic pain up to present. Gabapentin and pregabalin are the first line drugs which are widely used in PHN, DNP, and SCI patients. Besides pharmacological therapy, physical therapy, psychotherapy, teamwork medical, traditional Chinese medicine, electrical nerve stimulation and interventional therapy are effective in many cases.

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Chapter 10
Integrated, Team-Based Chronic Pain Management: Bridges from Theory and Research to High Quality Patient Care

Mary A. Driscoll and Robert D. Kerns

Abstract Chronic pain is a significant public health concern. For many, chronic pain is associated with declines in physical functioning and increases in emotional distress. Additionally, the socioeconomic burden associated with costs of care, lost wages and declines in productivity are significant. A large and growing body of research continues to support the biopsychosocial model as the predominant framework for conceptualizing the experience of chronic pain and its multiple negative impacts. The model also informs a widely accepted and empirically supported approach for the optimal management of chronic pain. This chapter briefly articulates the historical foundations of the biopsychosocial model of chronic pain followed by a relatively detailed discussion of an empirically informed, integrated, multimodal and interdisciplinary treatment approach. The role of mental health professionals, especially psychologists, in the management of chronic pain is particularly highlighted.

Keywords Chronic pain • Biopsychosocial model • Multidisciplinary treatment • Cognitive-behavioral therapy • Complementary and integrative care

10.1 Pain Prevalence

Chronic pain is a significant and costly public health problem. For example, in the United States, it is estimated that over 100 million individuals, roughly one third of the US population, suffer from pain. According to the U.S. Institute of Medicine Committee on Advancing Pain Research, Care, and Education (Institute of Medicine 2011), the total direct and indirect costs associated with pain are believed to be

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between $560 and $635 billion, annually. This is more than twice the amount spent on heart disease and cancer, each year, combined (Roger et al. 2011). The epidemic of chronic pain is not unique to the USA. Recent epidemiological investigations in China estimate that 26–35 % of individuals report pain that is chronic in nature (Wong and Fielding 2011; Jackson et al. 2011). Global investigations put the prevalence of chronic pain at 20 % (Goldberg and McGee 2011).

10.2 Defining Chronic Pain

The International Association for the Study of Pain (IASP) is globally acknowledged to be the world leader in the study of pain. According to IASP, pain is defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Volinn et al. 1991). The definition of chronic pain is one that is similarly accepted. Though the duration of chronic pain may vary, it is characterized by the presence of persistent pain and by interference in multiple domains of life.

Unlike acute pain, chronic pain is not reliably linked to structural tissue damage. A large and developing literature suggests an absence of relationship between reports of chronic pain and sensitive markers of biological disease (Boden et al. 1990; Jarvik et al. 2001). For example, findings reveal that 100 % of adults over the age of 65 evidence structural changes on magnetic resonance imaging (MRI) that are consistent with degenerative disc disease, yet the majority of these individuals remain asymptomatic (Boden et al. 1990). Similarly, MRI tests reveal structural abnormalities consistent with herniated and bulging discs in over a third of asymptomatic adults. Existing research using sensitive biological markers also fails to identify specific structural tissue damage in a large percentage of individuals complaining of pain. Support for the efficacy of powerful biological agents (e.g. opioids) in the management of back pain, the most commonly endorsed site of pain, is similarly underwhelming (Martell et al. 2007). Thus, a singular focus on structural etiologies and pharmacological treatments for chronic pain is overly simplistic.

Indeed, patient experiences speak to the physical, functional, social, psychological, and socioeconomic complexities of chronic pain. Unlike those with common chronic problems that are largely asymptomatic (e.g. hypertension), those with pain are keenly and constantly aware of their diagnosis. The noxious physical sensations associated with pain beget difficulties in other critical domains. People with chronic pain complain of sleep disruptions, difficulty concentrating, and emotional distress, along with social repercussions including isolation and interpersonal conflict. Interference in functional activities and financial difficulties stemming from lost wages, medical bills, and unemployment are also common, as are frustrations with medical systems that are often unsympathetic to or unable to adequately address their complaints. Thus, the experience of chronic pain is one marked by significant burden in multiple domains (Banks and Kerns 1996).

Patient narratives underscore these burdens. One military veteran described his experience with chronic pain as follows, "When I think about the day I was injured, I can feel the pain in my back flare up right where I was hurt." Another can be quoted as saying, "My whole day seems to be spent waiting for the time to take my next pain pill. I know they don't help that much, but it's all I have." Still another has said, "Whenever I'm laying in bed at night and my shoulder starts hurting, I find myself wondering when this will all end, and I start having thoughts of taking things into my own hands."

Narratives like these serve as reminders of the complex interplay between the experience of chronic pain and mental health. In their substantive work in this domain, Gatchel and colleagues report that approximately 60 % of those with chronic pain report symptoms consistent with at least one psychiatric diagnosis (Gatchel 2004). Notably, the presence of co-morbid psychiatric problems has been found to portend greater pain intensity and disability (Holzberg et al. 1996; Katon and Ciechanowski 2002). Aside from the heightened suffering experienced by those with co-morbid pain and psychiatric disorders, it is acknowledged that co-occurring mental health problems may interfere with the effectiveness of pain interventions (Gatchel 1996; Burns et al. 1998).

Collective consideration of these extenuating factors in the chronic pain experience renders strict biomedical conceptualizations of chronic pain to be insufficient. The transition from an overly simplistic and purely reductionistic biomedical model to a more comprehensive biopsychosocial model has been gradual, but substantive. In 1965, Melzack and Wall introduced the Gate Control Theory of Pain which described pain as a centrally mediated experience in which peripheral sensory inputs are regulated by a complex gate mechanism within the spinal cord that determines the amount of pain signals that actually reach the brain from distant parts of the body (Melzack and Wall 1965). If the spinal gate is wide open, all available pain signals reach the brain, whereas, if the gate is completely closed, no pain messages reach the brain. In reality, the gate is never completely opened or closed. Instead, the gate works like a dimmer switch to determine the intensity of pain signals. The gate mechanism is controlled by a variety of factors including physical factors (e.g. nerve impulses, medications, tension, inflammation), cognitive factors (e.g. distraction, helplessness, worry, optimism), and behavioral factors (e.g. sedentary behaviour, exercise, activity pacing, relaxation), among others. Building on his earlier Gate Control Theory, Melzak introduced the Pain Neuromatrix Model in 2001 which conceptualized pain as a multidimensional experience produced by patterns of nerve impulses generated by a variety of inputs including psychological, cognitive, motivational, and affective sources in addition to sensory ones (Melzack 2001). Thus, somatic or sensory input is only part of the larger matrix of impulses acknowledged to modulate pain.

The predominant, contemporary model of pain, however, is the biopsychosocial model (Fig. 10.1). Introduced by George Engel in 1978, it emphasizes the interrelatedness of biological, psychological and social factors in the context of disease and health (Engel 1978). In the context of pain, it encourages consideration of emotional, cognitive and behavioral aspects of pain over and above salient neural and biochemical

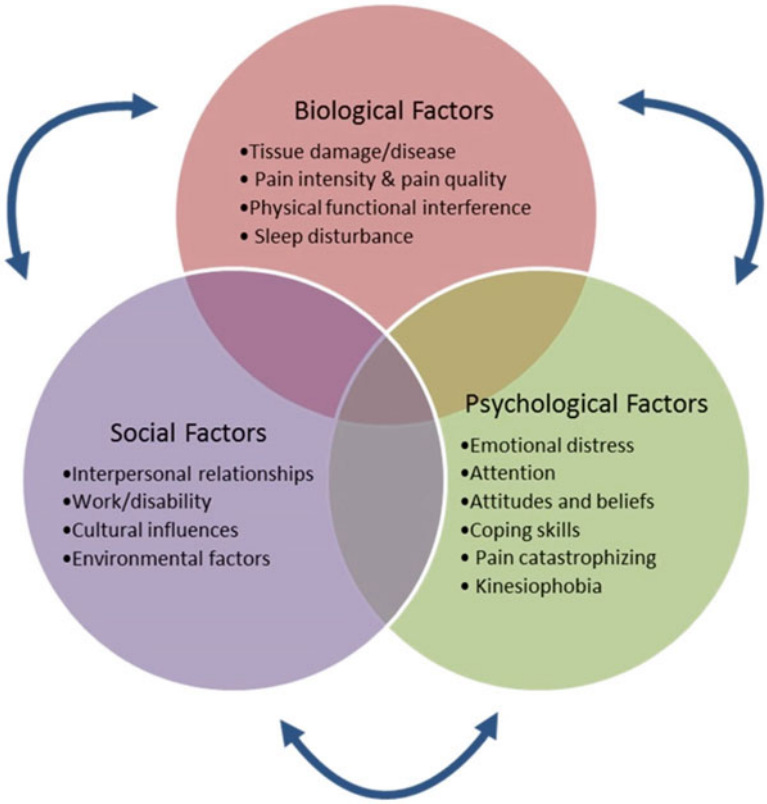

Fig. 10.1 Biopsychosocial model of chronic pain

processes (Turk and Monarch 1996). As such, this model allows for simultaneous consideration of disease related precipitants and a range of psychosocial factors (e.g. mood, attention, appraisal, social context) that may predispose an individual to experience pain, or alternatively that may function to exacerbate it. This model largely explains the wide variation in pain intensity, functional status and emotional distress observed among those with similar injuries. For example, two elderly persons with similar degrees of moderate spine disease may report quite dissimilar pain experiences. An otherwise healthy 85-year-old man with strong family support and social interests may report only mild pain and limited pain interference. On the other hand, another 85-year-old woman with a few friends and the recent loss of a spouse may report severe pain, substantial functional limitations and depression. One might also think of the biopsychosocial model as a systems model whereby changes in one domain necessarily precipitate changes in other domains (Keefe et al. 2002). Increases in stress may trigger inflammation and muscle tension which, in turn, may increase pain and emotional distress (depression, anxiety), while simultaneously reducing productivity.

Even before the advent of the biopsychosocial model, pioneers in the field of pain research observed that psychological and social factors might play a salient role in the pain experience. Some 40 years ago, William Fordyce described an operant behavioral model of chronic pain wherein "pain behaviors" characterized by complaints, guarding, posturing and demonstration of physical limitations could be reinforced by social contingencies such as positive attention, financial gain or avoidance of responsibility (Fordyce et al. 1968). He hypothesized that traditional biomedical treatments might be augmented with a focus on behaviour modification, to address these contingencies by minimizing dependence on others and encouraging engagement in productive activity (Fordyce et al. 1968). Several investigations lend support for the credibility of this treatment approach; substantial improvement was observed among chronic pain patients previously refractory to treatment (Fordyce et al. 1968; Turner and Clancy 1998).

In 1983, Dennis Turk published his seminal book describing a cognitive-behavioral perspective, launching a further revolution in the clinical application of social learning models of pain (Turk et al. 1983). The model postulated that individual differences in patient beliefs, attitudes, appraisals and coping abilities are critical determinants in the lived experience of pain. Put more simply, maladaptive perceptions about pain coupled with poor self-confidence in patient ability to address its inherent challenges interact with social contingencies and nociceptive inputs to predict greater disability and affective distress. This disability and distress then serve to confirm negative beliefs, thus exacerbating the pain experience. The past thirty years have seen the continued elaboration of the conceptual model. For example, Kerns and Jacob (1995) described a diathesis-stress model of chronic pain as an elaboration of the biopsychosocial model highlighting the context of social learning and interactions between predisposing person factors such as strengths and weaknesses in coping, instrumental behaviors, and emotional regulation and the stress of pain as determinants of the chronic pain experience, including the experience of pain, per se, as well as the impact of pain on physical and emotional functioning (Kerns and Jacob 1995). Refinement of the biopsychosocial model and the cognitive-behavioral perspective on chronic pain, as well as a large and continually expanding body of research, has led to further refinements in cognitive-behavioral therapy (CBT). By the beginning of the twenty-first century, CBT had emerged as the predominant psychological approach to chronic pain management (Hoffman et al. 2007; Kerns et al. 2011).

10.3 Goals of Chronic Pain Treatment

With the acceptance of biopsychosocial models of pain has also come an acknowledgement that treatments targeting pain intensity at the exclusion of other salient domains are insufficient (Kerns and Jacob 1995). Indeed, treatments that target the noxious experience of pain but ignore functional, social and emotional outcomes are sub-optimal and often ineffective. Understanding pain as a biopsychosocial

phenomenon rather than a purely medical one requires a major paradigm shift in the way providers assess, conceptualize and treat pain. Specifically, it is important that providers begin to recognize that chronic pain is a condition to be managed rather than a problem to be cured (IOM 2011). Moreover, it is important to identify and address goals related to the many dimensions of the chronic pain experience (Kerns et al. 2011). At the biological level, identification and treatment of underlying disease, pathology and/or tissue damage is warranted with simultaneous efforts to reduce both the intensity and severity of pain. Efforts to optimize functioning and productivity and to reduce suffering and emotional distress are equally important and are less likely to be achieved by interventions solely targeting underlying biology. Thus, the focus should be on managing the condition via selection of multimodal interventions tailored to the salient biopsychosocial dimensions identified by the individual patient as being most bothersome. Indeed, engaging patients as active participants in the selection of these interventions encourages self-efficacy and promotes adherence. Taking all of this into consideration, it stands to reason that such an approach might result in very different treatment plans for individuals presenting with similar underlying pathology.

10.4 Core Principles for Effective Pain Management

Effective pain management involves seven core principles. These include: (a) genuine empathic respect for the patient and their situation; (b) proactive efforts by providers to thoroughly assess patient adaptation in biological, psychological and social domains; (c) tailored and direct communication to manage expectations and to set realistic functional goals; (d) efforts to partner with patients to make shared medical decisions; (e) utilization of targeted, rational polypharmacy, where appropriate (f) consideration of multidisciplinary treatments as a means to address the specific biopsychosocial concerns of each patient; and (g) regular interactions to assess progress, to troubleshoot problems and to promote patient efforts to self-manage pain.

At the heart of these seven core principles is a commitment to patient-centered pain management which emphasizes (a) empowerment via reassurance, encouragement, education and collaboration along with (b) judicious use of analgesics/adjuvant medications, and perhaps, most importantly, (c) a commitment to help the patient develop adaptive strategies for self-managing their pain while securing appropriate specialty care to address co-morbidities that may interfere with patient efforts to do so.

10.4.1 Empathy

The Institute of Medicine (IOM) highlights that patients presenting with pain encounter significant challenges when dealing with the medical system. This is especially true for treatment refractory patients with uncertain pain etiologies. These individuals may be the unwelcome target of both conscious and unconscious

stigma by providers and caregivers (IOM 2011; Tait et al. 2009). In fact, findings from vignette studies lend support for this assertion (Chibnall et al. 1997, 2000). Interactions may be marked by suspicion regarding the validity of and/or motivation behind patient pain complaints. These interactions are frustrating for all parties and may lead to doctor shopping, dissatisfaction with care, distrust of the medical establishment, the use of multiple, costly and invasive diagnostic tests along with suggestions that the pain is in the patient's head or that the patient is malingering. This progression often begets an escalation in symptom reporting, help seeking behavior and increased emotional distress. This can serve to undermine self-efficacy and to exacerbate helplessness in the person with pain.

Recently, there has been a call for a renewed emphasis on empathy as a critical therapeutic instrument in the provision of pain care (Gallagher 2006). Practically, empathy saves time and avoids frustration by fostering an alliance between patient and provider. Such an alliance allows for the emergence of a mutual respect and encourages collaboration in the identification of treatment targets that are consistent with patient preferences. Failure to establish an alliance may lead to treatment recommendations that are at odds with patient desires. For instance, a provider assumption that a patient desires medical intervention may fail to recognize that the patient fears the pain of an injection, paralysis from surgery or the possibility of an addiction secondary to opioid use (Gallagher 2006). Failure to appreciate the clash between provider assumption and patient preference renders successful treatment outcomes less likely. An empathic relationship would encourage frank discussion of these factors to identify common ground.

Indeed, findings suggest that patients often want to have their pain understood and validated, while providers are more focused on diagnosing and treating (Frantsve and Kerns 2007). Thus, patients are reticent to accept physician recommendations because they feel the provider doesn't really understand the problem or how it interferes with daily life. By taking an empathic approach early on, the patient feels heard and legitimized. This then allows the patient to be open to a mutual discussion about treatment targets. Establishing empathy can be difficult particularly if the patient has suffered for years with pain, has a personality disorder, demonstrates little insight, has unrealistic expectations of the provider or fails to assume at least partial responsibility for self-managing pain. In these instances, providers are challenged to acknowledge their personal frustration – and move past it. Responding empathically simply requires an acceptance of where the patient is. Statements that validate struggles or encourage a partnership are particularly effective, especially if they are communicated with genuine sentiment. Examples include: "you've been through a lot," or "together, we can work on ways to help you manage this pain better."

10.4.2 Biopsychosocial Assessment

Consistent with IOM recommendations, providers must conduct a thorough biopsychosocial assessment to (a) understand the salient factors contributing to pain and disability and (b) identify potential avenues of intervention that might be acceptable

to and appropriate for the patient. First and foremost, efforts to diagnose and understand the underlying physical pain process (e.g. deconditioning, inflammation, joint damage, etc.) are critical. Inherent in this, is the need for providers to simultaneously consider the patient's medical comorbidities and how these might influence pain itself. In addition, a careful psychosocial assessment will identify co-occurring mental health comorbidities (e.g. depression, anxiety, PTSD), as well as an understanding of the ways in which pain interferes with activities, and quality of life. Equally as important is an emphasis on the patient's coping and social resources, along with appraisals about pain and the patient's ability to manage it. Keeping in mind that the biopsychosocial model specifies that changes made in one domain can impact functioning in other domains, a thorough assessment offers information that may help the provider identify and discuss potential treatment options with the patient later on.

10.4.3 Manage Expectations/Set Functional Goals

Patient and provider agreement has been demonstrated to be an important predictor of outcomes in pain (Staiger et al. 2005). When disagreement occurs, treatment compliance, functional improvements, patient satisfaction and/or disruptions in the patient/provider relationship may occur. Without efforts to agree on treatment goals or clarify expectations patients may incorrectly believe the prescribed treatment will alleviate the pain entirely. In other cases, a patient may have expectations that far exceed the limits of modern pain management interventions. For example, an avid runner may have their heart set on resuming marathon training following a spine injury. They may incorrectly assume that provider recommendations will successfully help them attain this goal. Alternatively, provider assumptions about patient expectations, values or preferences may prompt selection of interventions that are not palatable for the patient, and thus not adhered to. For example, research suggests that patients prefer interventions that maximize impact while simultaneously minimizing interruptions in their daily life (Hornberger et al. 1995). By contrast, providers are far more likely to select interventions that require more deliberate effort, especially in circumstances when such interventions afford greater health benefits (Hornberger et al. 1995). Direct, empathic and honest communication can mitigate the future dissatisfaction and frustration that set in when treatment outcomes are inconsistent with expectations.

One suggested approach is to clarify the limits of what can be done, and educate the patient about their condition while simultaneously offering hope that the patient's situation can improve. For example, when speaking to an individual diagnosed with degenerative disc disease of the low back, a provider might say, "The bad news is that when pain has gone on for this long, it's not likely to go away. You have wear and tear arthritis of the bones in your low back. This triggers inflammation, muscle spasms, and pain. Though we can't make the pain go away, there are things we can do to improve the quality of your life and make the pain more toler-

able." Following this honest discourse with a conversation about the patient's goals is paramount to treating pain effectively. Asking the patient to identify specific ways in which pain interferes with their daily life sets the stage for establishing mutually agreed upon, specific *functional* goals.

The SMART framework is helpful for establishing such goals (Bovend'Eerdt et al. 2009). The SMART acronym stands for **S**pecific, **M**easureable, **A**ction-Oriented, **R**ealistic and **T**imely goals. It involves specification of a measureable target activity or behaviour that has become difficult or seemingly impossible. For example, a father confined to a wheelchair secondary to physical deconditioning stemming from chronic pain may wish to walk his daughter down the aisle, unassisted, when she gets married in 6 months. Such a goal is likely to be realistic if there is no underlying pathology other than deconditioning that is limiting him and if the timeline is sufficient. Together, he and his provider might then collaborate to evaluate the feasibility, to identify the treatment strategies best suited to address this goal and to determine the support necessary to achieve it (e.g., hands on, practical assistance, emotional support).

10.4.4 Partner with Patients to Make Shared Medical Decisions

Unlike acute and emergent medical problems, the treatment options for chronic illnesses, like pain, tend to be many and varied – what may be desired by one patient may be unacceptable for another. As highlighted above, empathic efforts to understand the lived experience of each patient with attention to their personal challenges, disabilities, capabilities and expectations fosters an environment wherein mutually agreed upon goals can be identified. And so begins the process of making shared medical decisions. Patient values and preferences play a major role in treatment planning under this model. Indeed, it is an interactive, collaborative, bidirectional process that emphasizes effective communication, education and selection of treatment options that best match individual patient factors. This model specifies the development of a partnership between patient and provider in which the preferences of both parties are incorporated into treatment planning. Thus, both are more engaged in decision-making and are invested in treatment outcomes which lead to greater satisfaction with the process and greater satisfaction with results (Charles et al. 1997). Indeed, studies suggest that patient/provider agreement is linked to greater treatment satisfaction and improved outcomes among those with pain (Staiger et al. 2005).

Throughout the decision-making process, it is incumbent upon providers to maintain the direct, honest, non-judgmental and empathic patient-centered communication. Where possible, efforts to offer a variety of treatment options and to help patients weigh the pros and cons of each are emphasized over singular treatment recommendations decided solely by the provider. Inherent in this is a genuine inter-

est in hearing and understanding the patient's perspective. Summarizing and reflecting the patient's concerns and preferences minimizes disagreement and allows for clarification with regard to mutually agreed upon goals.

10.4.5 Utilization of Targeted, Rational Polypharmacy

Effective chronic pain management frequently involves targeted, rational polypharmacy. Pain can be classified into two broad categories: (a) nociceptive, which is thought to originate from the noxious stimulation of peripheral nociceptors; and (b) neuropathic, which stems from damage to the nerves themselves. Clinically, it is important to distinguish nociceptive pain from neuropathic pain, as the pharmacologic agents appropriate for each do diverge. Nociceptive pain is best addressed with anti-nociceptive agents including non-steroidal anti-inflammatory medications, acetaminophen and opioids. By contrast, anti-neuropathic agents include some anti-convulsants, tricyclic antidepressants and selective norepinephrine reuptake inhibitors. The co-occurrence of nociceptive and neuropathic pain would certainly warrant combination treatment.

10.4.6 Employ Multidisciplinary Treatment Plan

As highlighted earlier, the association between reports of chronic pain and sensitive markers of biological disease is unreliable, at best (Boden et al. 1990; Jarvik et al. 2001). This coupled with the acknowledgement that chronic pain is a multifaceted problem marked by functional disability, affective distress and interpersonal difficulty suggests it would be naïve to believe that a single biologically based intervention (like pharmacotherapy) will be optimally effective. Consequently, a movement toward multidisciplinary treatment has gained traction. Multidisciplinary treatment generally involves some combination of two or more of the following: behavioral, pharmacology and physical or exercise based interventions (e.g. physical therapy, aquatherapy, yoga) to promote adaptive strategies that the patient can use to self-manage pain.

Flor and colleagues examined 65 controlled and non-controlled investigations to evaluate the efficacy of the multidisciplinary approach in the treatment of chronic low back pain (Flor et al. 1992). Findings revealed that this model was superior to no treatment, and to single discipline interventions like pharmacotherapy or physical therapy. Notably, at 2 years follow-up, people treated in a multidisciplinary setting functioned 75 % better than those who had received no treatment or uni-modal interventions. Additionally, they were almost twice as likely to return to work. Citing the limited availability of randomized interventions in this initial investigation, a more recent meta-analysis reviewed 12 randomized multidisciplinary chronic low back pain studies (Guzmán et al. 2001). Findings were largely consistent with

those highlighted by Flor et al. (1992). Specifically, there was moderate evidence suggesting that intensive multidisciplinary programs (>100 h; daily) improve function and reduce pain. However, in this investigation, it's important to note that a distinction was made between intensive and less intensive multidisciplinary programs (<30 h, once or twice weekly). The latter were not superior to usual care. In an effort to determine whether the costs associated with multidisciplinary interventions were justified in the long term, several other investigations have examined outcomes ranging from 18 months to 10 years (Cassisi et al. 1989; Bendix et al. 1998; Guck et al. 1985; Meilman et al. 1985). These studies demonstrate sustained improvements in a variety of domains, including pain intensity, and disability as well as activity level, and participation in social interactions.

One model that has emerged in the United States is considered an exceptional framework for conceptualizing and measuring high quality pain care. The Stepped Pain Care Model, developed by the Department of Veterans Affairs, is graduated. See Fig. 10.2. At the base, self-care habits are emphasized; this may be sufficient to address the needs of a large percentage of patients with pain. It is self-implemented and involves self-directed attention to nutrition, exercise/conditioning, sufficient sleep, participation in relaxation/mindfulness, engagement in meaningful activities and social involvement with family/friends as a means to manage pain and maintain functioning. Individuals unable to independently address these domains, or those whose efforts to implement/maintain these self-directed efforts are unsuccessful are candidates for Step 2. Usually, more complex patients are eligible for this level of care which is typically administered within the patient's medical home (primary

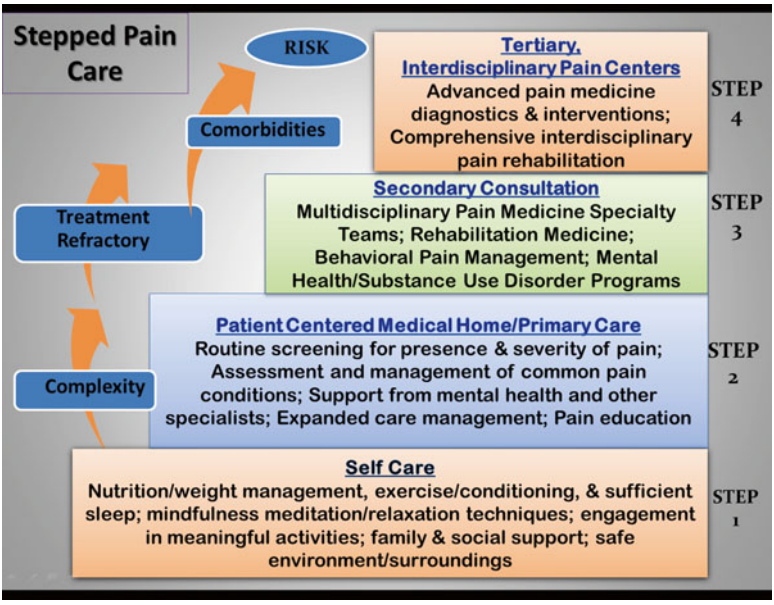

Fig. 10.2 U.S. Department of Veterans Affairs Model of stepped pain care

care). Here providers assess and manage common pain conditions, provide education around pain and, if appropriate, solicit support for the patient from mental health providers housed within primary care. Patients who fail to improve with this level of intervention then move on to more intensive, multidisciplinary interventions wherein they are evaluated by a team of specialists with expertise in the following disciplines, as appropriate: rehabilitation, behavioral pain management, neurology, rheumatology, substance use and mental health. Treatment plans are then developed and tailored using the shared-decision making model. Finally, patients who continue to be refractory or who demonstrate elevated risk (e.g. addiction) may be referred for the highest level of care which involves advanced pain medicine diagnostics/interventions and comprehensive intensive interdisciplinary pain rehabilitation.

Dobscha and colleagues demonstrated that an intervention to educate primary care providers and patients about the importance of a collaborative, multidisciplinary approach to pain management could improve pain outcomes (e.g. disability, intensity, depression) significantly more for those in the intervention arm relative to the treatment as usual arm (Dobscha et al. 2008, 2009). The intervention itself was developed to help providers deliver assessment results, formulate treatment recommendations and identify local resources in ways that would maximize compliance with treatment guidelines and optimize patient adherence. Primary care staff received skills training regarding safe/effective care for chronic pain that included basic principles of pain management, along with information regarding biopsychosocial treatment approaches, and the importance of patient self-management (Dobscha et al. 2008).

10.4.7 Reassess Progress

Pain, like other chronic illnesses is dynamic which calls for frequent reassessment of symptomatology, functioning, and interference in daily life. Indeed, reassessment is the only way to determine whether treatment is working and whether mutually agreed upon goals are being achieved.

10.5 The Role of Psychology in Managing Chronic Pain

Psychologists have played an important role in advancing the credibility of pain self-management and in the development of interventions that specifically support development of pain self-management skills. The use of cognitive behavioral principles to address pain began to gain traction in the 1980s. Designed to promote a shift from a sense of helplessness and hopelessness to a greater degree of personal control and pain self-efficacy in the management of pain, Kerns and colleagues demonstrated that a brief, goal-oriented, structured intervention could effectively reduce the impact of pain on functioning and emotional distress (Kerns et al. 1986).

The core components of cognitive-behavioral therapy for pain management include: relaxation, cognitive restructuring, activity pacing, pleasant activity scheduling, anger management and sleep hygiene. Together, these skills help the patient to reconceptualise the pain experience as less threatening and more controllable, to develop effective coping strategies and to build self-efficacy to manage pain.

One meta-analyses examining 25 randomized controlled trails demonstrated that cognitive behavioral interventions are effective for chronic pain in adults (Morley et al. 1999). When compared with control conditions, the average effect size across all domains was .50. Other meta-analyses have examined psychosocial interventions more broadly and included randomized controlled studies of various psychological interventions (e.g. behavioral, cognitive-behavioral, self-regulatory or supportive counseling). Indeed, one such meta-analysis examined 21 trials investigating the effectiveness of psychosocial interventions in the treatment of back pain, specifically. Results revealed moderate positive effects for pain intensity and health-related quality of life, along with small effects for pain interference (Hoffman et al. 2007). CBT specifically demonstrated a strong effect for pain intensity while self-regulatory interventions like biofeedback and relaxation training demonstrated a strong effect for both pain intensity and depression.[25] Additionally, a meta-analysis comparing psychosocial interventions (of which 70 % were CBT) to controls among persons with arthritis found large effect sizes for coping, moderate effect sizes for anxiety and joint swelling, and small but significant effect sizes for physical and psychological disability along with self-efficacy to manage pain (Dixon et al. 2007).

10.6 Complementary and Integrative Care

Beyond psychological interventions, like CBT and self-regulatory interventions, there are a large and growing number of behavioral, rehabilitative and complementary and integrative therapies that have demonstrated efficacy in the treatment of *chronic* pain. These include, but are not limited to: low impact aerobic exercise (Chou and Huffman 2007), stretching/strengthening (Chou and Huffman 2007), yoga (Chou and Huffman 2007; Cramer et al. 2013; Büssing et al. 2012), aquatherapy (Baena-Beato et al. 2014; Evcik et al. 2008), mindfulness and acceptance based therapies (Veehof et al. 2011), relaxation training (Linton 1985), biofeedback (Flor and Birbaumer 1993), massage (Chou and Huffman 2007; Cherkin et al. 2003), chiropractic (Chou and Huffman 2007; Cherkin et al. 2003), acupuncture (Chou and Huffman 2007) and trigger point injections (Scott et al. 2009). Though the extant literature suggests these interventions demonstrate superiority over non-active control conditions, it is difficult to claim, with any certainty, that any one of these interventions is any better than the other. Providers are, however, encouraged to review the extant evidence for each modality in light of the presenting pathology, as some interventions may demonstrate superiority over others within diagnostic categories, in the presence of complicating comorbidities or in combination with other treatments. Moreover, a thorough biopsychosocial assessment coupled with

consideration of patient goals and preferences will help providers to identify the most palatable and beneficial adjunctive treatments, thus, ensuring patients are both willing to engage with these modalities and optimistic about the outcomes.

10.7 Summary and Conclusions

Acknowledging the high burden of pain in the USA, the IOM's 2011 recommendations called for a cultural transformation in the way the epidemic of chronic pain is regarded (IOM 2011). Building on the IOM's recommendations, the National Pain Strategy (NPS) has advocated for a "population based, biopsychosocial approach to pain care that is grounded in scientific evidence, integrated, multimodal, and interdisciplinary while, at the patient level is tailored to individual needs" (The Interagency Pain Research Coordinating Committee 2015). At the heart of such a shift, the NPS places great importance on improving the competencies of the healthcare professionals who are challenged to address the burden of pain. Among its specific initiatives, the NPS recommends improvements in the following pain domains: basic knowledge, assessment, team based care, empathy and cultural competency. They further suggest that accreditation bodies and licensure boards are well placed to require that undergraduate and graduate institutions prioritize pain care, broadly, and knowledge in these domains, specifically, as critical components of their curriculum. NPS further recommends reimbursement reform that will incentivize providers to conduct comprehensive, integrated, multidisciplinary assessments early in the course of pain, before patients have failed multiple interventions. This will ensure timely, effective and safe care that may be optimally positioned to alleviate suffering and improve outcomes. Until such targeted efforts to improve the approach to pain care are addressed, the burden of pain will remain prominent for those who suffer with it and the healthcare providers who treat it.

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ERRATUM

Chapter 3
Modulation of C-nociceptive Activities by Inputs from Myelinated Fibers

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The original version of this chapter unfortunately contained a mistake. In Chap. 3 titled "Modulation of C-nociceptive Activities by Inputs from Myelinated Fibers", the family name and given name of the authors were reversed as Duan Wan-Ru and Xie Yi-Kuan. Hence the correct name should read as below.

Wan-Ru Duan and Yi-Kuan Xie.

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