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RESEARCH ARTICLE

Changes in synaptic effectiveness of myelinated joint afferents during capsaicin-induced inflammation of the footpad in the anesthetized cat

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Abstract The present series of experiments was designed to examine, in the anesthetized cat, the extent to which the synaptic efficacy of knee joint afferents is modified during the state of central sensitization produced by the injection of capsaicin into the hindlimb plantar cushion. We found that the intradermic injection of capsaicin increased the N2 and N3 components of the focal potentials produced by stimulation of intermediate and high threshold myelinated fibers in the posterior articular nerve (PAN), respectively. This facilitation lasted several hours, had about the same time course as the paw inflammation and was more evident for the N2 and N3 potentials recorded within the intermediate zone in the L6 than in the L7 spinal segments. The capsaicin-induced facilitation of the N2 focal potentials, which are assumed to be generated by activation of fibers signaling joint position, suggests that nociception may affect the processing of proprioceptive and somato-sensory information and, probably also, movement. In addition, the increased effectiveness of these afferents could activate, besides neurons in the intermediate region, neurons located in the more superficial layers of the dorsal horn. As a consequence, normal joint movements could produce pain representing a secondary hyperalgesia. The capsaicin-induced increased efficacy of the PAN afferents producing the N3 focal potentials, together with the reduced post-activation depression that follows high frequency autogenetic stimulation of these afferents, could further contribute to the pain sensation from non-inflamed joints during skin inflammation in humans. The persistence, after capsaicin, of the inhibitory effects produced by stimulation of cutaneous nerves innervating non-inflamed skin regions may account for the reported reduction of the articular pain sensations produced by trans-cutaneous stimulation.

Keywords Joint afferents · Capsaicin · Post-activation depression · Central sensitization · Spinal cord

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Introduction

A previous study (Rudomin et al. 2007) has indicated that in the anesthetized cat, the pathways involved in the generation of the intraspinal focal potentials produced by stimulation of low threshold myelinated afferents in the posterior articular nerve (PAN) are subjected to a rather weak tonic inhibitory GABAA control and show very little post-activation depression after high frequency autogenetic stimulation. In contrast, the pathways activated by stimulation of intermediate and particularly of high threshold myelinated afferents are subjected to a stronger tonic GABAergic inhibition and show a clear post-activation depression that lasts several minutes and is not significantly affected by picrotoxin, a GABAA blocker.

Post-activation depression produced by stimulation of the PAN afferents appears not to be due to a GABAA-induced decrease of synaptic effectiveness, but rather to other mechanisms, among them, to transmitter depletion or to reduced release from the previously activated fibers (see Hultborn et al. 1996). The degree of post-activation depression may differ depending on the type of afferent and on the intraspinal location of the target neurons (Hammar et al. 2002), because of the intrinsic features of the intraspinal fiber terminals and/or because of extrinsic factors, for example a differential monoaminergic modulation of the

synaptic effectiveness of the afferent fibers (see below and Jankowska et al. 1997).

The injection of capsaicin or carrageenan into the knee joint capsule produces a state of central sensitization leading to increased neuronal responsiveness to afferents innervating adjacent unaffected regions. In addition, neurons originally transmitting nociceptive information from the joints respond then to light mechanical stimulation of the skin (Cervero et al. 1991; Schaible et al. 1991; Cervero and Laird, 1996a, b). This phenomenon, secondary hyperalgesia, persists for long periods of time (Neugebauer and Schaible 1990; Cervero et al. 1991; Schaible et al. 1991) and has been considered an expression of spinal cord plasticity, equivalent to the long term potentiation seen in other systems (Sandkuhler and Liu 1998; Rygh et al. 2006). It has a supraspinal component (Woolf 1995; Urban and Gebhart 1999; Gardell et al. 2003; Vanegas and Schaible 2004; Rygh et al. 2006), which most likely includes descending monoaminergic modulation (Bras et al. 1989; Jankowska et al. 1997; Garraway and Hochman 2001).

The present series of experiments was designed to examine, in the anesthetized cat, the extent to which the synaptic efficacy of intermediate threshold (assumed to convey information on joint position), and high threshold myelinated articular afferents (assumed to convey nociceptive information) is modified during the central sensitization produced by the injection of capsaicin into the hindlimb plantar cushion. The results obtained indicate that during this state of central sensitization there is a significant increase of the synaptic efficacy of these afferents lasting several hours, as well as a weaker post-activation depression of their intraspinal responses that follows high-frequency autogenetic stimulation. This may contribute to the development of pain sensations in uninflammed joints during skin swelling (Woolf 1995). Some of these observations have been published in abstract form (Hernández and Rudomin 2006).

Methods

General procedures

The experiments were carried out in 21 adult cats of either sex. Guidelines contained in Principles of Laboratory Animal Care (NIH publications 85-23, revised in 1985) were followed in all cases. These experiments were approved by the Institutional committee.

The animals were anesthetized with pentobarbitone sodium 40 mg/kg i.p. Additional doses were given intravenously to maintain a deep level of anesthesia, tested by assessing that withdrawal reflexes were absent, that the pupils were constricted and that arterial blood pressure was between 100 and 120 mm Hg. The carotid artery, radial vein, trachea and urinary bladder were cannulated. A solution of 100 mM of sodium bicarbonate with glucose 5% was given i.v. (0.03 ml/min) to prevent acidosis (Rudomin et al. 2007). When necessary, dextran 10% or ethylephrine (Effortil, Boering-Ingelheim) was administered to keep blood pressure above 100 mm Hg.

Peripheral nerves

The left sural (SU) and superficial peroneal (SP) nerves were dissected free, kept in continuity and mounted on bipolar hook electrodes for stimulation. Cuff electrodes were used to stimulate the left intact saphenous (Saph) and quadriceps (Qx) nerves. The left posterior biceps and semitendinosus (PBSt) and the posterior articular (PAN) nerves were also dissected free, but sectioned, and their central ends mounted on pairs of stimulating hook electrodes. Stimulus strengths are expressed as multiples of the minimum strength required to produce evoked cord dorsum potentials (CDPs) in each nerve (xT).

Cord dorsum and intraspinal recordings

Proper placement of the micropipettes used for intraspinal recording was aided by recording the CDPs elicited in different spinal segments in the left side (L5-S1) by stimulation of the ipsilateral muscle (PBSt and Qx), cutaneous (SU and SP) and PAN nerves by means of silver ball electrodes against an indifferent electrode placed on the paravertebral muscles (band pass filters 0.3 Hz to 10 kHz). In later series of experiments we also recorded the CDPs produced by stimulation of the plantar cushion in the left hindlimb through a pair of fine needle electrodes inserted into the skin, close to the site of capsaicin injection.

Intraspinal field potentials (IFPs) evoked by stimulation of sensory nerves were recorded with glass micropipettes (2–3 μm tip diameter) filled with a 2 M solution of NaCl (see Rudomin et al. 2007), inserted in those segments where the PAN N3 and plantar cushion CDPs were largest (rostral L7-caudal and middle L6; see "Results"). Evoked CDPs and IFPs were stored digitally for subsequent processing after the experiment.

Intradermic application of capsaicin

Capsaicin (30 μl of 1% solution, in a 10% Tween 80 and 90% saline) was injected in the plantar cushion of the left hindlimb (see Sorkin and McAdoo 1993). Cord dorsum and intraspinal field potentials produced by stimulation of sensory fibers were recorded at different times before and after the intradermic injection of capsaicin. To avoid desensitization, capsaicin was injected only once (Sakurada et al. 2005).

In eight experiments the degree of cutaneous inflammation due to CAP injection was assessed by measuring the paw

perimeter (p) with a silk thread placed around the shaved paw at the level indicated by the asterisk in the diagram of Fig. 4c. Measurements were made before and at different times after CAP injection (usually every 30 min). Each measurement was repeated three times and the mean used to calculate the paw diameter (d), using the relation $d = p/\pi$. Changes are presented as the difference in paw diameter before and after CAP injection to allow comparison with the changes in paw-thickness produced in the rat by the intradermic injection of capsaicin reported by Lin et al. (1999c).

Statistical analysis

All values are given as mean ± SEM. t Test, paired t test and Mann–Whithey Rank Sum test were used for statistical comparison. $P < 0.05$ values were considered as significant and are marked in the figures with two asterisks.

Histology

At the end of the experiments the glass micropipettes were broken and the tips left in the spinal cord. The animals were killed with pentobarbital overdose. The spinal cord was excised and fixed with 10% formalin. After dehydration, the segments containing the micropipette tips were made transparent with salicylate and cut transversely for identification of the recording sites.

Results

Features of the spinal CDPs and IFPs produced by stimulation of the PAN

Figure 1a illustrates the procedure followed to select the most adequate spinal segments to record the PAN focal potentials. CDPs evoked by stimulation of the left SP, SU and PAN nerves were recorded from the ipsilateral S1, L7, L6 and L5 segments. In agreement with previous studies (see Rudomin et al. 2007), we found that the SP-CDPs were largest in the L6 segment, the SU-CDPs in the rostral part of L7 and caudal part of the L6 segments and the PAN-CDPs in the rostral L7, L6 and caudal part of the L5 segments. The CDPs produced by stimulation of the left plantar cushion were largest in L6 and rostral L7, precisely in the same regions of the largest PAN-evoked CDPs.

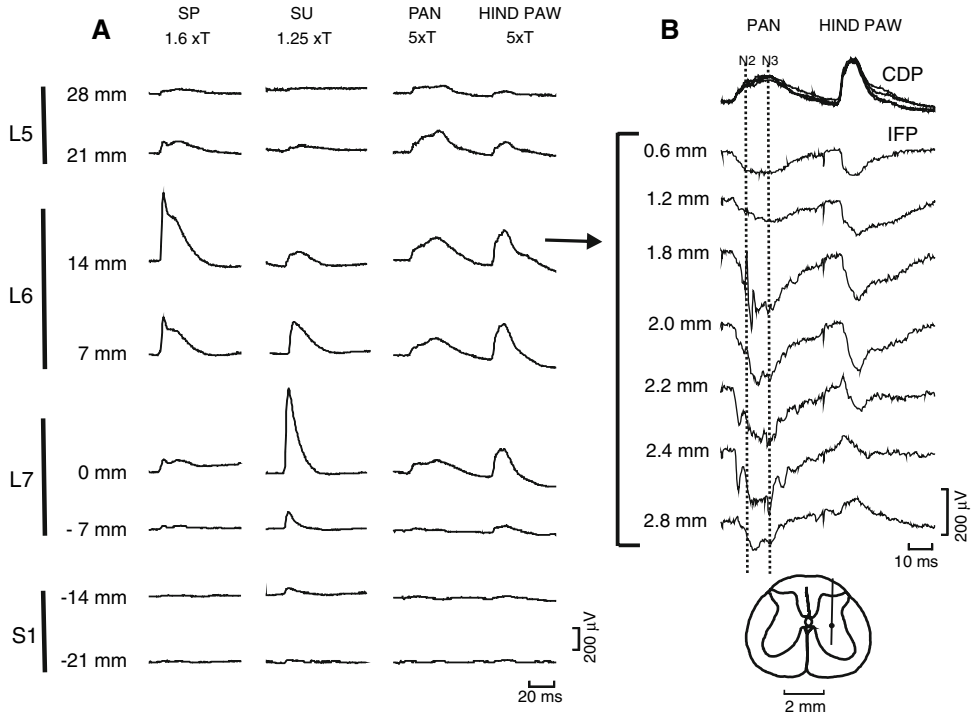

Fig. 1 Segmental and intraspinal projections of articular and plantar cushion afferents. **a** CDPs recorded from different segments in the left side of the spinal cord after stimulation of the ipsilateral SP, SU and PAN nerves and of the plantar cushion with single pulses at the indicated strengths. Distance in mm relative to the site of largest SU CDP is indicated. Note the substantial overlap of the PAN and plantar cushion CDPs in the L6 segment. **b** IFPs produced by stimulation of the PAN and of the plantar cushion recorded with a glass micropipette inserted in rostral L6. Same stimulus strengths as in A. Upper set of records show superposed CDPs. Lower set of records show IFPs recorded at different depths as indicated. *Vertical dotted lines* show N2 and N3 components of the PAN CDPs. The drawing shows the histological reconstruction of the electrode track. *Dot* shows location of recording site (1.8 mm depth) selected in this experiment to test the effects of intradermic capsaicin. In this and the other figures all traces are averages of 32 responses elicited at 1 Hz

After characterization of the segmental projections of the articular afferents, one or two recording micropipettes were inserted in the segments showing the largest PAN CDPs (rostral L6 in the example of Fig. 1b) and displaced down until finding the intraspinal layers showing the largest PAN N3 and plantar cushion focal potentials (usually between 1.8 and 2.0 mm depth. See histology in Fig. 1b; Rudomin et al. 2007). The recording micropipettes were left at these sites until the end of the experiment.

As reported by Rudomin et al. (2007) stimulation of the PAN produces cord dorsum and intraspinal focal potentials with at least three components (N1, N2 and N3). The onset latencies of the N1, N2 and N3 components varied from 0.49 to 2.06, 1.45 to 6.03 and 3.7 to 10.5 ms, with mean values of 1.12 ± 0.42, 2.6 ± 0.9 and 7.0 ± 1.6 ms, respectively. In general the N1 and N2 responses had a relatively low peripheral threshold (usually below 2 ×T). Nearly maximal responses were produced with pulses 2–5 ×T. In contrast, the N3 focal potentials had a higher threshold, usually above 2 ×T and acquired their maximal amplitudes with strengths above 5 ×T (see Fig. 5a, b; Schaible et al. 1986; Quevedo et al. 1993). In some experiments stimulation of the PAN with strengths 10–15 ×T produced a late N4 component (Fig. 5b), probably due to activation of unmyelinated afferents.

It has been suggested that the N1 responses are due to monosynaptic activation of neurons in the intermediate zone by a small population of group I muscle afferents that are usually present in the PAN (McIntyre et al. 1978). The N2 components appear to be due to oligosynaptic activation of neurons by afferents conveying information on joint position (Coleman et al. 2003b), while the N3 components would be due to polysynaptic activation by afferents most likely transmitting nociceptive information (Quevedo et al. 1993; Rudomin et al. 2007).

The intradermic injection of capsaicin increases the N2 and N3 focal potentials

Time course

We have investigated the effects of capsaicin injected in the plantar cushion of the left hindlimb on the intraspinal focal potentials produced by electrical stimulation of the PAN. The intradermic injection of capsaicin had either very small or no effect on the N1 components. In contrast, the N2 and the N3 components recorded from L6 were increased in 7/8 and 8/8 experiments, and those recorded from L7 in 3/5 and 8/8 experiments, respectively. It also increased the N4 components recorded in two experiments. The latency of the facilitation varied between 30 and 120 min and lasted up to 4 h, when the observations were terminated.

Figure 2a illustrates the time course of the effects produced in one experiment by the injection of capsaicin in the plantar cushion of the left hindlimb on the L6 CDPs and IFPs produced by stimulation of the PAN with single pulses 3 ×T. The CDPs and IFPs were recorded in the L6 segment, the latter within the intermediate zone (black square in histological diagram of Fig. 8e). In this particular experiment, 1 h after the injection of capsaicin, the PAN responses were still of the same magnitude as those recorded before the injection. By 2 h, the N2 as well as the N3 focal potentials were only slightly increased, but were clearly facilitated after 3 h (Fig. 2a, red traces). The facilitation of the N2 and N3 components increased steadily up to the end of the recording period (around 4 h after the injection of capsaicin). Figure 2b shows with more detail the time course of the effects of capsaicin on the amplitude of the PAN focal potentials partly illustrated in Fig. 2a, expressed as percentage change relative to control. The N1 components were only slightly facilitated, in contrast with the N2 and N3 components that were significantly increased, both with a similar time course.

In five experiments we recorded the PAN focal potentials simultaneously from two different spinal segments. In the example of Fig. 3 these potentials were recorded from one rostral (middle L6) and one caudal (middle L7) location, both within the intermediate zone, as shown by the histology (black circles in Fig. 8e). In this experiment the N1 components recorded rostrally following PAN stimulation with pulses 3 ×T strength appeared on the rising phase of the N2 components. Nevertheless, it was clear that the N2 as well as the N3 components were already facilitated 90 min after the intradermic injection of capsaicin. Maximal facilitation was attained 160 min after the injection and declined slightly thereafter, but was still appreciable after 4½ h (Fig. 3a red traces; Fig. 3c).

The intraspinal focal potentials recorded with the caudal micropipette (middle L7) showed less differentiated N2 and N3 components. Both were also facilitated after capsaicin, but to a smaller extent than those recorded in the more rostral segment, as shown in Fig. 3b (red traces) and d. Similar differences were found in four other experiments. At the end of each experiment, we verified that crushing the PAN centrally to the stimulating electrodes abolished the intraspinal responses (see lower traces in Fig. 3a, b), as it would be expected if these responses were due to activation of PAN afferents and not to current spread activating nearby nerves.

Figure 4a and b shows the time course of the effects of capsaicin on the amplitude of the PAN N2 and N3 L6 and L7 focal potentials obtained by pooling data from all experiments. The changes in amplitude (expressed as percentage relative to control) were grouped in four time categories

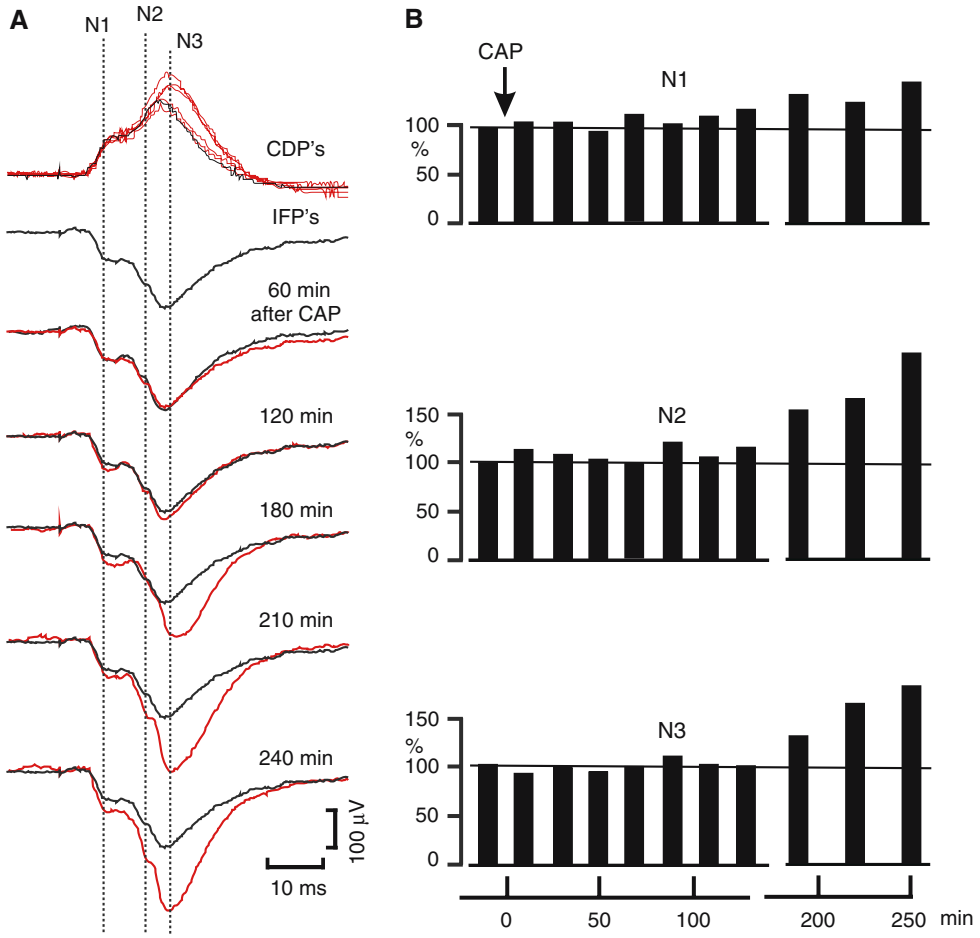

Fig. 2 The intradermic injection of capsaicin increases the PAN focal potentials produced by stimulation of intermediate and high threshold myelinated afferents. **a** stimulation of the PAN with single pulses 3 ×T produces CDPs and IFPs with distinct N1, N2 and N3 components, as indicated by the vertical dotted lines. IFPs were recoded from rostral L6 segment within the intermediate zone at the location indicated by the filled square in the histological diagram of Fig. 8e. *Black traces* control responses. *Red traces* responses obtained at different times after the injection of capsaicin in the plantar cushion, as indicated. Note that 180 min after the injection of capsaicin, the N2 and N3 components were already facilitated, while the N1 components were only slightly increased. The facilitation of the N2 and N3 focal potentials persisted for at least 285 min. **b** time course of the effects of CAP injection on the amplitude of the N1, N2 and N3 focal potentials produced by stimulation of the PAN with single pulses 3 ×T. Data taken from the same series as in **a**. Ordinates show amplitude of the responses expressed as percentage of control. Abscissa, time after capsaicin injection (CAP; see *arrow*)

(10–30, 40–90, 100–180 and 190–330 min after capsaicin). The N2 and N3 PAN focal potentials were already increased 30 min after the injection of capsaicin both in the L6 and L7 segments. This increase was largest between 100 and 180 min after the injection of capsaicin and declined thereafter.

It is to be noted that the N3 focal potentials were more facilitated than the N2 potentials, both in the L6 and L7 segments and that the N3 focal potentials recorded in the L6 segment were more facilitated than the potentials recorded in the L7 segment (as in Fig. 3). It thus seems that the magnitude of the capsaicin-induced facilitation of the N2 and N3 PAN focal potentials is largest in those segments receiving the strongest projections from both the plantar cushion and the articular afferents.

Graded stimulation

Recording the focal potentials produced by graded stimulation of the PAN provided further evidence that the N1 components were only marginally or not at all affected after the intradermic injection of capsaicin, while the N2 and N3 potentials were largely facilitated. In the experiment illustrated in Fig. 5a, PAN stimuli of 1.1 and 1.2 ×T produced only N1 focal potentials. Slightly stronger stimuli (1.5–2 ×T) produced N2 potentials, and stimuli above 2.5 ×T produced clear N3 potentials that were already maximal with strengths around 5 ×T (Fig. 5a, black traces). Two hundred and ten minutes after the injection of capsaicin into the left plantar cushion, the N1 components produced by the weakest stimuli were about the same as before the injection, in

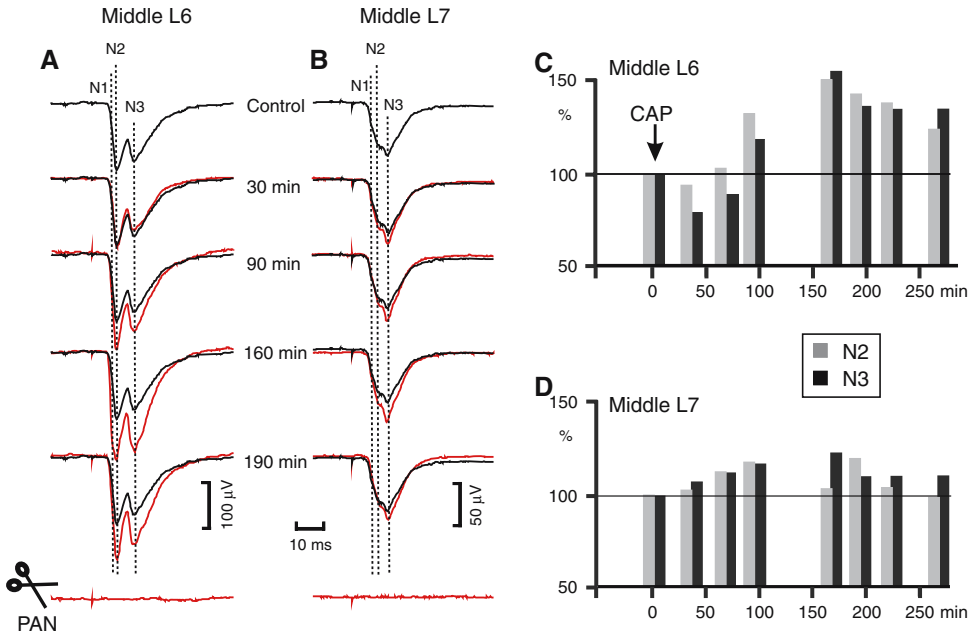

Fig. 3 Differential effects of the intradermic capsaicin on the PAN focal potentials. Focal potentials produced by stimulation of the PAN with single pulses 3 ×T were simultaneously recorded from the middle L6 and middle L7 segments, as indicated. Intraspinal location of recording sites is indicated by the filled circles in Fig. 8e. **a**, **b** Averaged IFPs recorded at different times after the intradermic injection of capsaicin, as indicated. *Upper traces in black* show control responses recorded just before the injection of capsaicin (control). These traces are reproduced below to facilitate comparison with the responses obtained after capsaicin (*red traces*). The *lowest traces* in **a**, **b** show the responses obtained after crushing the PAN (marked with scissors). **c**, **d** Time course of the effects of capsaicin on the amplitude of the N2 (*gray bars*) and N3 focal potentials (*black bars*) simultaneously recorded from both spinal segments. Amplitude changes are expressed as percentage relative to control. Note that capsaicin produced a stronger facilitation of the N2 and N3 components recorded in the rostral than in the caudal segments

contrast with the N3 components that were almost doubled in size. Although the N2 components appeared as an inflection in the rising phase of the N3 components, it was clear that they were also increased after the injection of capsaicin (Fig. 5a, red traces).

In the experiment of Fig. 5b the focal potentials were recorded from the middle L6 segment within the intermediate nucleus (black circle in the L6 diagram of Fig. 8e). The N1 focal potentials produced by the weakest PAN stimuli (1.1 ×T) 90 min after the injection of capsaicin were unaffected. Stimuli above 2 ×T produced distinct N2 as well as N3 components, both of which were facilitated after intradermic capsaicin. Stimuli 10 and 15 ×T produced in addition a delayed potential (N4), probably due to activation of unmyelinated afferents, that was also facilitated.

Time course of paw inflammation

In several experiments (as in Figs. 2, 3) the latency of the facilitation of the N2 and N3 focal potentials appeared to be much longer than the latency of capsaicin-induced subjective pain and hyperalgesia felt by humans (Serra et al. 2004), the sensitization of peripheral C-fibers (Lin et al. 1999a, b), the facilitation of spinal neuronal on-going activity and responses to peripheral stimulation (Sun et al. 2004), and the increased blood flow in the skin close to the injection site (Lin et al. 1999c). It thus seemed that the time course of the facilitation was more related to paw inflammation than to a direct action of capsaicin on the afferent fibers.

To estimate the time course of the paw inflammation produced by capsaicin injected into the plantar cushion, we measured in eight experiments the changes in paw thickness using a silk tread placed around the paw at the site shown in the diagram of Fig. 4c. During cutaneous inflammation there was plasma extravasation and edema with the consequent increase in paw diameter. Measurements made in five cats 30 min after the injection of capsaicin already showed a small swelling that was almost maximal between 100 and 180 min after the injection and remained so for 4–5 h (Fig. 4c, d). Before capsaicin, the calculated paw diameter varied in different animals from 1.9 to 2.5 cm (mean 2.0 ± 0.08 cm). By 190 and 330 min after the intradermic injection of capsaicin, when the inflammation was strongest, paw diameter was incremented between 3 and 5 mm (mean 3.9 ± 0.3 mm).

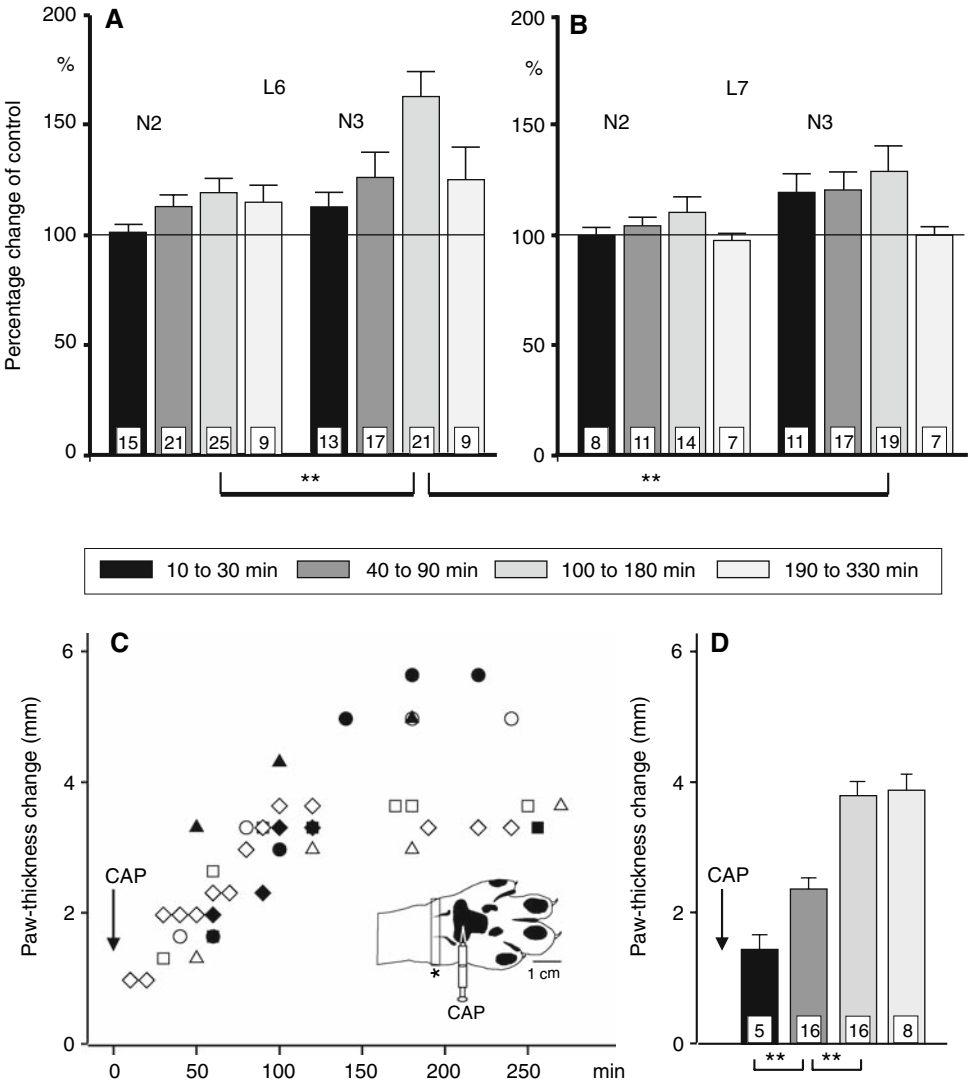

Fig. 4 Changes of PAN N2 and N3 focal potentials and of paw thickness at different times after intradermic injection of capsaicin. **a**, **b** Mean time course of the effects of capsaicin injected in the left plantar cushion on the amplitude of the ipsilateral PAN N2 and N3 focal potentials recorded from the L6 and L7 segments, as indicated. In five experiments responses were simultaneously recorded from L6 and L7, and in three experiments from L6 only. Mean and SEM are expressed as percentage change relative to control and are grouped in four time categories (10–30, 40–90, 100–180 and 190–330 min after the injection of capsaicin). Numbers in boxes indicate sample size. **c** time course of capsaicin-induced increments in paw thickness measured at the site indicated by the *asterisk* in the diagram (see "Methods"). Data obtained from eight different experiments, as indicated. **d** Mean and SEM of the data displayed in **c**, grouped in four different categories as indicated. Brackets with two *asterisks* indicate significant differences between the mean ($P < 0.05$).

Inhibition of PAN responses by cutaneous afferents

Rudomin et al. (2007) showed that conditioning stimulation of cutaneous nerves inhibits the N2 and N3 focal potentials produced by PAN stimulation. We performed a series of experiments aimed to determine whether these inhibitory actions were affected during the central sensitization induced by the intradermic injection of capsaicin. In the experiment of Fig. 6a, the Saph nerve was stimulated with single pulses of increasing strengths applied 40 ms before a single PAN test stimulus 3 ×T that produced clear N2 and N3 focal potentials (Fig. 6a, black traces). Before the intradermic injection of capsaicin, conditioning stimulation of the Saph nerve with pulses up to 2 ×T had very little effect on the test PAN focal potentials, while stimuli of 3 ×T and above strongly inhibited the N2 and N3 potentials (Fig. 6a, red traces). After the intradermic injection of capsaicin, the PAN N2 and N3 focal potentials were strongly facilitated (Fig. 6b, black traces). Yet, conditioning stimulation of the Saph nerve with single pulses 3–10 ×T still inhibited the PAN responses (Fig. 6b, red traces). It should be noted that in this experiment the Saph-conditioned N3 potentials recorded after capsaicin were larger that those recorded before capsaicin. It is not clear however if this was merely a consequence of the increased effectiveness of the excitatory pathways involved in the generation of the N3 potentials or to reduced effectiveness of the inhibitory pathways activated by the cutaneous afferents.

Figure 7 summarizes the effects produced in eight different experiments by the intradermic injection of capsaicin on the inhibition of the L6 PAN intraspinal focal potentials produced by conditioning stimulation of muscle and cutaneous nerves. Since in most cases increasing the intensity of the cutaneous stimuli above 3 ×T did not significantly increase the magnitude of the inhibition (as in Fig. 6), the data have been grouped in two categories according to the strength of the conditioning stimuli (1.5–2 ×T and 3–10 ×T). The intensity of the PAN stimuli was set to produce

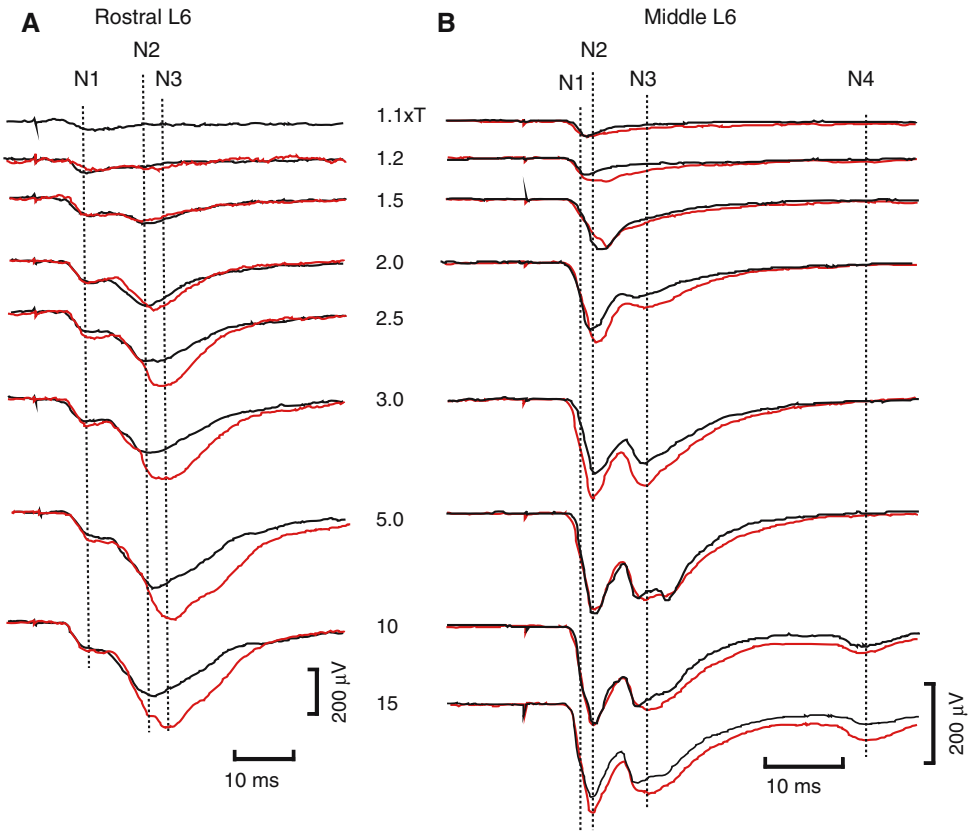

Fig. 5 Effects of intradermic capsaicin on the focal potentials produced by graded stimulation of the PAN. **a**, **b** Averaged IFPs produced by stimulation of the PAN with single pulses at the indicated strengths. Data obtained from two different experiments (same as those illustrated in Figs. 2 and 3, respectively). *Black traces* control responses. *Red traces* responses produced with the same stimulus strength as the control responses recorded 210 and 200 min after the intradermic injection of capsaicin, respectively. *Vertical lines* show the different components of the PAN focal potentials. Further explanations in text

distinct N2 and N3 potentials and varied between 2.5 and 5 ×T in these experiments. Before capsaicin, conditioning stimulation of cutaneous nerves inhibited the N2 as well as the N3 focal potentials. The magnitude of the inhibition depended on the strength and source of the conditioning stimuli. SP conditioning with single pulses 1.5–2 ×T inhib-

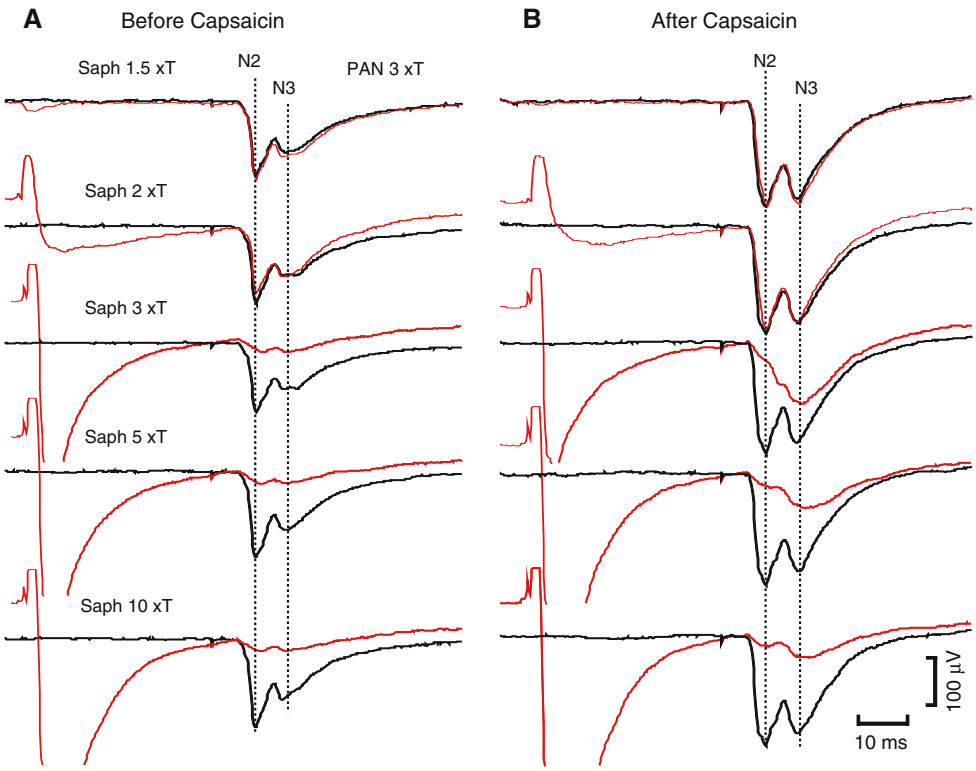

Fig. 6 Effects of the intradermic injection of capsaicin on the Saph-induced inhibition of the PAN focal potentials. **a** Unconditioned PAN focal potentials produced by single pulses 3 ×T (*black traces*) superposed with PAN potentials preceded by conditioning stimulation of the Saph nerve with increasing strengths (*red traces*), as indicated. **b** Same as **a**. Note the facilitation of the PAN N2 and N3 focal potentials and the reduction of the Saph-induced inhibition after the injection of capsaicin

ited both the N2 and the N3 focal potentials while stronger conditioning stimuli (3–10 ×T) increased the inhibition of the N3 responses almost without incrementing the inhibition of the N2 responses (Fig. 7a, b). Saph conditioning with pulses 1.5–2 ×T produced almost no inhibition of the N2 and N3 potentials, in contrast with stimuli 3–10 ×T that were clearly more effective (Fig. 7c, d; see also Fig. 6). On the other hand, conditioning stimulation of the SU nerve with pulses 1.5–2 ×T produced a weak inhibition of both the N2 and N3 potentials that was not incremented with higher intensities (Fig. 7e, f). Conditioning stimulation of muscle nerves (trains of four pulses at 400 Hz applied 35–40 ms before the PAN testing pulse) produced a rather small inhibition, even with trains of stimuli 10 ×T (Fig. 7g, h).

After capsaicin the mean amplitude of the PAN N3 focal potentials that were inhibited by SP and Saph conditioning with strengths above 3 ×T was larger than the mean amplitude of the unconditioned potentials (Fig. 7b, d). However, since these differences were not significant, it may be concluded that the intradermic injection of capsaicin had no major effects on the inhibition of the PAN N2 and N3 potentials produced by conditioning stimulation of sensory nerves.

Post-activation depression

We showed previously (Rudomin et al. 2007) that during autogenetic stimulation of the PAN with high frequency trains there was a strong depression of the N2 and N3 focal potentials produced by test stimuli applied to the same nerve. The depression of the N3 focal potentials persisted for several minutes after discontinuing the stimulation and was only marginally affected after systemic injections of picrotoxin.

As shown in the preceding paragraphs, the intradermic injection of capsaicin produced a prolonged state of central sensitization leading to incremented N2 and N3 PAN focal potentials that might have interfered with the post-activation depression produced by autogenetic conditioning. This was tested in seven experiments, and the results of one of these are illustrated in Fig. 8. In this particular case, the amplitude

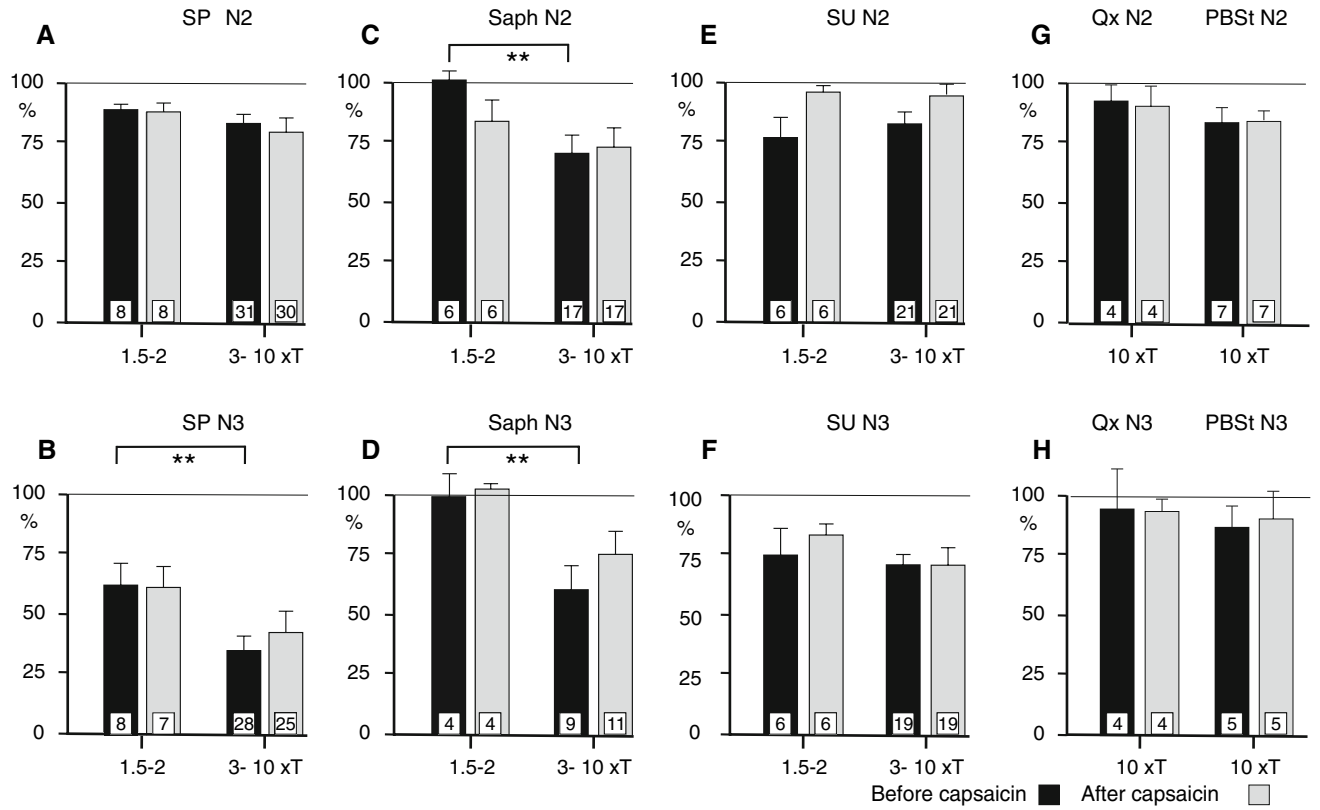

Fig. 7 Effects of intradermic injection of capsaicin on the inhibition of PAN focal potentials produced by cutaneous and muscle nerve conditioning. Data derived from eight different experiments. PAN focal potentials were produced with single pulses 2.5–5 ×T and recorded from the L6 segment. Conditioning stimuli to cutaneous nerves were single pulses applied 35 ms before the PAN test responses, as indicated. Muscle nerves were stimulated with trains of four pulses at 300 Hz, 10 ×T, also applied 35 ms before the PAN test responses. Data have been grouped in two categories according to the strength of the cutaneous conditioning stimuli (1.5–2 ×T and 3–10 ×T), as indicated. Ordinates, percentage change in amplitude relative to control. *Gray bars* before, *black bars* 2–3 h after capsaicin was injected in the plantar cushion. Numbers in boxes indicate sample size. Brackets with two *asterisks* indicate significant differences between the mean ($P < 0.05$). Further explanations in text

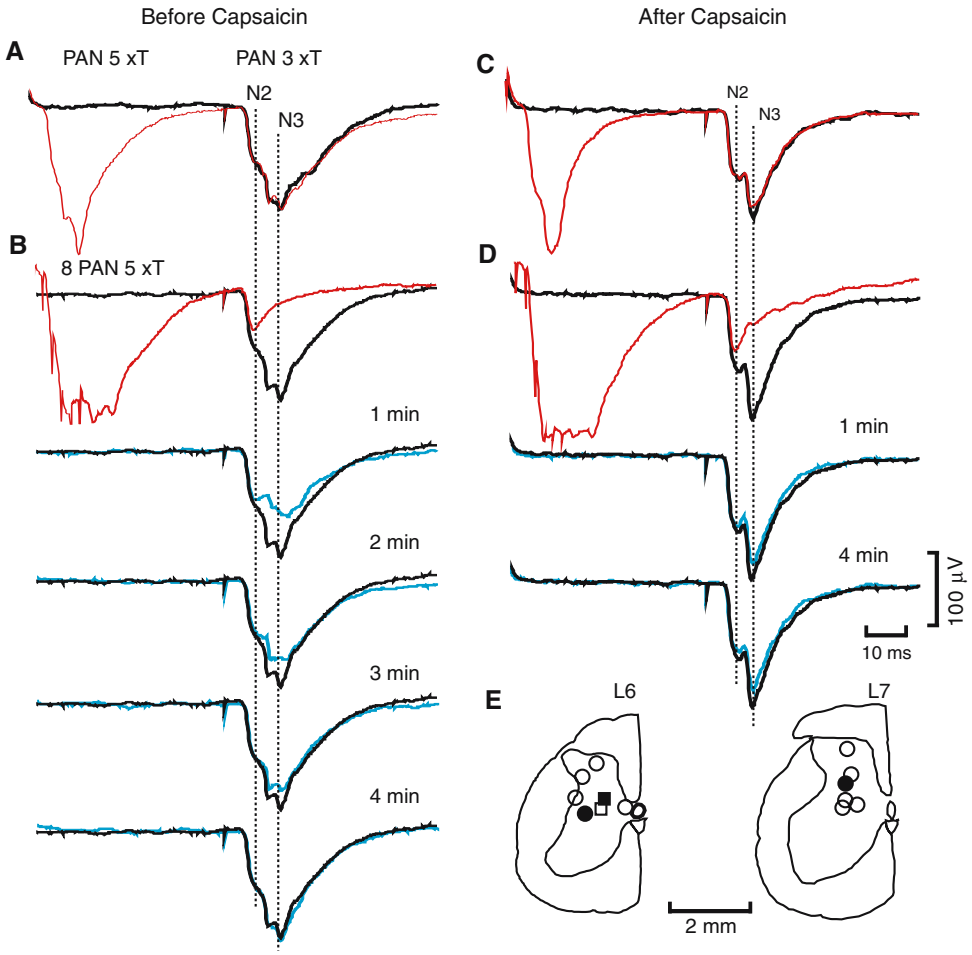

Fig. 8 Intradermic capsaicin reduces post-activation depression of N2 and N3 PAN focal potentials produced by autogenetic stimulation. **a** Conditioning autogenetic stimulation of the PAN with single pulses 5 ×T (*red trace*) barely depressed the test PAN focal potentials produced with single pulses 3 ×T strength (*black trace*). **b** Conditioning autogenetic stimulation of the PAN with trains of eight pulses at 600 Hz 5 ×T, strongly depressed the N2 as well as the N3 focal potentials (*red trace*), which remained depressed 3–4 min after discontinuing the high frequency conditioning autogenetic stimulus, as shown by the *blue traces*. For comparison, these responses appear together with the same unconditioned test response (*black trace*). **c**, **d** The same as **a** and **b**, but 330 min after the injection of capsaicin into the left foot pad. Although the test responses produced by PAN stimulation showed a rather small facilitation after capsaicin, there was practically no post-activation depression 1 min after discontinuing the high frequency conditioning autogenetic stimulation. **e** Location of recording sites in experiments aimed to test the effects of capsaicin on post-activation depression. *Open square* in L6 shows location of recording site of responses illustrated in **a**–**d**. *Filled square* shows location of recording site of potentials depicted in Figs. 2 and 5a, and filled circles of potentials illustrated in Fig. 3 and 5b

of both the N2 and N3 PAN focal potentials recorded within the intermediate nucleus in segment L6 (open square in Fig. 8e) was only slightly increased after the intradermic injection of capsaicin. PAN stimulation with single pulses 5 ×T preceding by 45 ms the test responses produced no inhibition before (Fig. 8a, red traces), or 330 min after the intradermic injection of capsaicin (Fig. 8c, red traces). In contrast, before capsaicin, conditioning stimulation of the PAN with high frequency trains with the same strength, strongly depressed the test N2 components and practically abolished the test N3 components (Fig. 8b, red traces). The N2 and particularly the N3 components remained depressed for up to 3 min (Fig. 8b, lower set of blue traces). After capsaicin there was a clear reduction of the post-activation depression of both the N2 and N3 components that recovered most of their control amplitude within the first minute after discontinuing the stimulation (Fig. 8d, blue traces).

Figure 9 summarizes the effects of intradermic capsaicin on the post-activation depression of the PAN focal potentials that follows autogenetic stimulation with high frequency trains. These potentials were recorded from a rostral (L6) location in seven experiments and a caudal (L7) location in six experiments. In most cases the recording electrodes were placed within the intermediate zone (see Fig. 8e). Before capsaicin, the N3 components were more depressed during PAN autogenetic conditioning stimulation

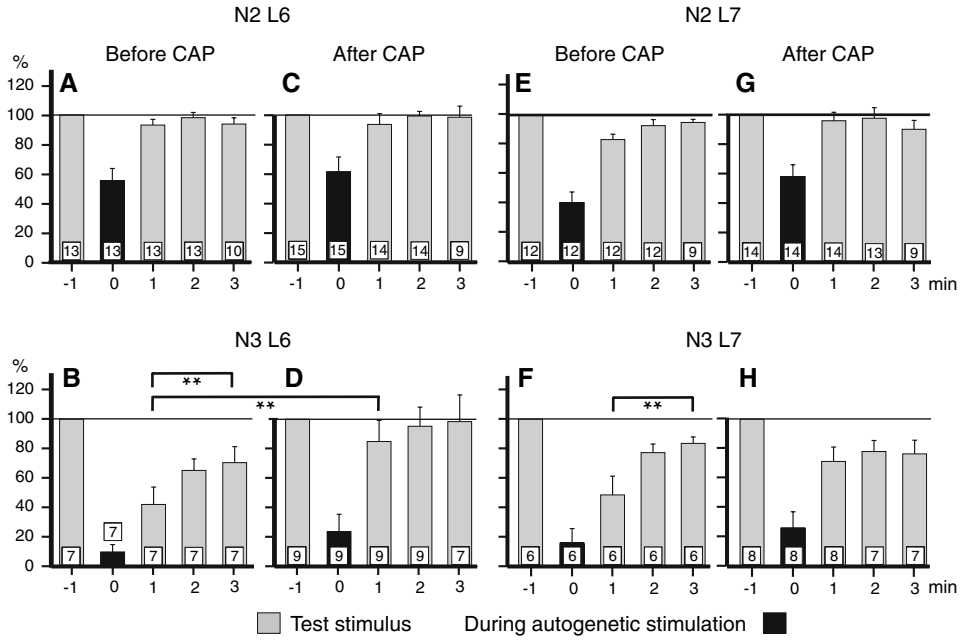

Fig. 9 Summary of the effects of capsaicin on autogenetic inhibition and post-activation depression of PAN focal potentials. PAN focal potentials were recorded from the L6 segments in seven experiments and from the L7 segment in six experiments (see Fig. 8E for location of recording sites). **a**, **b**, **e**, **f** Before, and **c**, **b**, **g**, **h** 90–300 min after the intradermic injection of capsaicin (CAP). *Black bars* show the percentage change in amplitude of the N2 and N3 components produced by PAN stimuli 3–5 ×T during autogenetic conditioning stimulation with a train of eights pulses at 600 Hz 5–10 ×T for 1 min. *Gray bars* show amplitude of PAN N2 and of N3 components recorded before (labeled −1) and 1–3 min after discontinuing the autogenetic conditioning train. *Bars* show mean and standard error. *Numbers in boxes* indicate sample size. After intradermic capsaicin, there was a clear reduction of the post-activation depression of the N3 components recorded in L6, particularly during the first minute after discontinuing the conditioning train. Brackets with two *asterisks* indicate significant differences between the mean ($P < 0.05$). Further explanations in text

than the N2 components (black columns in Fig. 9a, b, e, f). After discontinuing the conditioning autogenetic stimulation, the N2 components recovered faster than the N3 components, both in the L6 and L7 segments (Fig. 9a, b, e, f).

The effects of intradermic injection of capsaicin on the post-activation depression of the N2 and N3 components were different in the L6 and L7 segments. In the L6 segment, one minute after discontinuing the conditioning autogenetic stimulation, the N3 components had practically recovered their control size (Fig. 9d, for data indicated by brackets $P < 0.05$, t test), while the N3 components recorded in the L7 segment were still depressed (Fig. 9h). In contrast, the N2 components recorded in segment L6 had a relatively small post-activation depression that was barely affected after the intradermic injection of capsaicin (Fig. 9a, c), while the N2 components recorded in segment L7 had a larger post-activation depression that was not significantly changed after capsaicin (Fig. 9g).

Discussion

The present observations show that the injection of capsaicin into the plantar cushion leads to the development of central sensitivity that includes increased synaptic effectiveness of articular afferents. The spinal projections of these two sets of afferents show a considerable overlap, particularly in segment L6, even though they innervate relatively distant hindlimb territories. These findings are in line with the finding in normal human subjects that focal nociceptive input in a single nerve territory can result in allodynia and hyperalgesia in a nerve territory adjacent to the input (Tal and Bennett 1994; Kingery et al. 1993; see also Sang et al. 1996).

The mechanisms involved in the development of the central sensitization triggered by the intradermic injection of capsaicin are rather complex. It is known that capsaicin directly activates vainilloid receptors in unmyelinated cutaneous afferents and increases their permeability to cations, particularly sodium and calcium with the consequent increase of their synaptic effectiveness (Mironov and Churiukanov 2006). This may in turn activate a number of biochemical systems around their intraspinal terminations that contribute to the development of central sensitization (for review see Winter et al. 1995; see also Sluka et al. 1997) and to peripheral neurogenic inflammation produced by the release of inflammatory peptides from the sensitized afferent terminals due, at least in part, to the antidromic activity generated by excessive primary afferent depolarization (see Willis 1999; Lin et al. 2000). The parallel time course of

paw inflammation and the facilitation of the N2 and N3 PAN focal potentials observed during the first 3 h after the intradermic injection of capsaicin (see Fig. 4) suggests that inflammation per se plays a relevant role in the facilitation of the PAN focal potentials. However, the finding that the N2 and N3 PAN focal potentials generated in the L6 segment are more facilitated than the potentials elicited in the L7 segment, suggests that these interactions have a local character that depends, to a great extent, on the degree of spatial overlap between the spinal projections of the PAN and plantar cushion afferents (see Fig. 1). Investigation of the actions of other inflammatory procedures such as the intradermic injection of carrageenan and/or mustard oil on the PAN focal potentials could provide further information on the role played by the inflammation itself in this process.

We have shown before that the spinal pathways leading to the generation of the N2 and N3 PAN responses are subjected to a tonic descending inhibition that is suppressed by reversible spinalization (Quevedo et al. 1993). It thus seems possible that, in addition to the increased activity in spinobulbar-spinal pathways (Gardell et al. 2003) that contributes to the development of the secondary hyperalgesia produced by intradermic injection of carrageenan (Neugebauer and Schaible 1990; Cervero et al. 1991; Schaible et al. 1991; Urban and Gebhart 1999; Vazquez et al. 2007), the descending inhibition is also modified during the development of the capsaicin-induced facilitation.

Capsaicin-induced changes of N2 focal potentials

The conduction velocities of the PAN afferents producing the N2 components (Rudomin and Lomeli 2007) overlap with those of the articular afferents shown to convey information on joint position (Coleman et al. 2003a, b; see also Dorn et al. 1991). Under normal conditions, the pathways involved in the generation of the N2 components are subjected to a relatively small tonic GABAergic inhibition, show a relatively minor post-activation depression and a rather small autogenetic PAD (Jankowska et al. 1993; Rudomin et al. 2007; Rudomin and Lomeli 2007). This could contribute to the preservation of the original information conveyed by the position-signaling articular afferents, which may be essential for the control of limb position (Bosco and Poppele 2003; Poppele and Bosco 2003; Bosco et al. 2005).

The finding that capsaicin injected in the plantar cushion sensitizes spinal responses to stimulation of myelinated joint afferents does not necessarily imply that normal joint movements would now produce pain. In principle, it could only imply the usual function of these afferents would now be enhanced. This is in itself very interesting, because it deals with the effect of nociception upon the processing of proprioceptive and somatosensory information and, probably, movement, an issue that to our knowledge has not been sufficiently explored. Alternatively, it is possible that the increased effectiveness of the fibers producing the N2 components, activates, in addition of neurons in the intermediate region, neurons located in the more superficial layers of the dorsal horn, including laminae I and II, that transmit nociceptive information. As a consequence, normal joint movements would now produce pain representing a secondary hyperalgesia. This could function as a protective response preventing excessive joint displacements (Woolf 1995). A similar mechanism could prevail in the development of the allodynia after chronic damage of nerves or chronic inflammation (see Baba et al. 1999; Okamoto et al. 2001; Kohno et al. 2003), although in this case afferent sprouting could be also involved (Woolf et al. 1992; Koerber et al. 1994).

Capsaicin reduces post-activation autogenetic depression of N3 focal potentials

As discussed previously (Rudomin et al. 2007), the N3 focal potentials produced by PAN stimulation reflect activation of polysynaptic pathways, which are subjected to a tonic GABAA inhibition. The capsaicin-induced facilitation of the N3 components could be due, at least in part, to a reduction of the tonic GABAA inhibition, as suggested by the findings of Moore et al. (2002) and Zhou et al. (2007). Other possible mechanisms could be local events in the dorsal horn that follow the action of capsaicin in the peripheral cutaneous afferents and increase spinal neuronal activity (Lin et al. 1999a; Tominaga et al. 2004; Pilyavskii et al. 2005), or change descending modulation (Lin et al. 1999b; Gardell et al. 2003).

In addition to the increased amplitude of the N3 components, after capsaicin there is a clear reduction of the post-activation depression that follows autogenetic stimulation. The post-activation depression may involve presynaptic mechanisms related to transmitter availability and release in the afferent fibers (Capek and Esplin 1977; Lev-Tov and Pinco 1992), or may reflect reduced efficacy of transmission along the involved interneuronal pathways, which are unable to follow high stimulating frequencies (Rudomin et al. 2007). Since the post-activation depression of the N3 PAN components is marginally affected by GABAA blockers (Rudomin et al. 2007), its reduction after capsaicin may involve other mechanisms. For example, activation of descending serotonergic or other monoaminergic pathways (Cardona and Rudomin 1983) or activation of GABAB receptors in the afferent fibers (Castro-Lopes et al. 1995; Peshori et al. 1998; Castro et al. 2006).

The capsaicin-induced facilitation of the N3 PAN focal potentials and the reduction of their post-activation depression after autogenetic stimulation would further contribute

to increased central sensitization and play some role in the development of pain sensations in non-inflamed joints during skin inflammation (Woolf 1995). The finding that during the capsaicin-induced central sensitization the PAN responses are still inhibited by conditioning stimulation of cutaneous afferents could in addition account for the effectiveness of trans-cutaneous stimulation to reduce articular pain sensations (Mannheimer et al. 1978; Ma and Sluka 2001; Oh et al. 2006).

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