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RESEARCH ARTICLE

Descending inhibition selectively counteracts the capsaicin-induced facilitation of dorsal horn neurons activated by joint nociceptive afferents

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Abstract
Previous studies from our laboratory showed that in the anesthetized cat, the intradermal injection of capsaicin in the hindpaw facilitated the intraspinal field potentials (IFPs) evoked by stimulation of the intermediate and high-threshold myelinated fibers in the posterior articular nerve (PAN). The capsaicin-induced facilitation was significantly reduced 3–4 h after the injection, despite the persistence of hindpaw inflammation. Although this effect was attributed to an incremented descending inhibition acting on the spinal pathways, it was not clear if it was set in operation once the capsaicin-induced effects exceeded a certain threshold, or if it was continuously operating to keep the increased neuronal activation within manageable limits. To evaluate the changes in descending inhibition, we now examined the effects of successive reversible spinal blocks on the amplitude of the PAN IFPs evoked at different times after the intradermal injection of capsaicin. We found that after capsaicin the PAN IFPs recorded in laminae III–V by activation of high-threshold nociceptive Aδ myelinated fibers increased gradually during successive reversible spinal blocks, while the IFPs evoked by intermediate and low threshold proprioceptive Aβ afferents were only slightly affected. It is concluded that during the development of the central sensitization produced by capsaicin, there is a gradual increase of descending inhibition that tends to limit the nociceptive-induced facilitation, mainly by acting on the neuronal populations receiving inputs from the capsaicin-activated afferents without significantly affecting the information on joint angle transmitted by the low threshold afferents.

Keywords Articular afferents · Capsaicin · Nociception · Descending inhibition

Introduction

The effects of intradermal injection of capsaicin have been widely used as a model to investigate the development of the process of central sensitization induced by nociceptive stimulation (Russell and Burchiel 1984; Simone et al. 1989; LaMotte et al. 1991, 1992; Torebjörk et al. 1992; Kilo et al. 1994; Park et al. 1995; Sluka 1997; Szolcsányi 2004). Capsaicin activates transient receptor potential V1 channels (TRPV1; see Winter et al. 1995; Caterina et al. 1997), expressed in polymodal sensory nerve endings of unmyelinated fibers projecting mainly to Rexed's laminae I–II, and in myelinated Aδ fibers acting on nociceptive-specific neurons in lamina I and wide dynamic range neurons in the intermediate zone (mainly in lamina V; see Julius and Basbaum 2001; Basbaum et al. 2009; Todd 2010). The capsaicin-induced increment in the activity of afferent fibers promotes the release of neuropeptides such as substance P and calcitonin gene-related peptide leading to increased activity of second-order neurons and the development of a central state of sensitization associated with hyperalgesia and allodynia (Hayes and Tyers 1980; Lundberg et al. 1985; Franco-Cereceda et al. 1987; Oku et al. 1987; Schaible et al. 1990, 1992; Willis 2002; Latremoliere and Woolf 2009).

Previous studies in anesthetized cats and rats have shown that during acute articular inflammation produced by the injection of carrageenan into the joint capsule, there is a significant increase of the responses of single spinal neurons to extreme joint movements and that these responses are further incremented during spinal block. The

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effects of spinal block were attributed to the suppression of a compensatory descending inhibition that is mediated, at least in part, by raphe magnus and reticular descending pathways (Schaible et al. 1991; Quevedo et al. 1995; Ren and Dubner 1996, 2002). These observations were based on the analysis of changes induced in preparations with intact joint afferents, whose peripheral receptors were sensitized by the intra-articular injection of carrageenan (Schaible et al. 1991; Ren and Dubner 1996). Hence, the relative participation of afferent peripheral sensitization versus central mechanisms in the facilitation of neuronal responses produced by nociceptive stimulation was not elucidated.

In this context, we have shown in a previous study (Rudomin and Hernández 2008) that the intradermal injection of capsaicin in the footpad facilitated the N2 and N3 components of the cord dorsum potentials (CDPs) and of the intraspinal field potentials (IFPs) produced by electrical stimulation of the intermediate and high-threshold myelinated fibers of the posterior articular nerve (PAN). The capsaicin-induced facilitation of the N3 component of the PAN IFPs was reduced within 2–3 h after the injection, despite the persistence of foot pad inflammation, and it was suggested that this effect was due to a gradual increase of supraspinal inhibitory influences that mitigate the process of central sensitization initiated by the nociceptive stimulus (see also Contreras-Hernández et al. 2018).

Yet, it was not established if the descending inhibition generated in response to the nociceptive stimulus had a fixed threshold and was set in operation once the neuronal activity exceeded a certain limit, or whether it was operating continuously as part of a dynamic mechanism that provides the negative feedback required to keep neuronal activation within manageable limits. To this end, we examined the effects of successive spinal (T10) cold blocks on the CDPs and IFPs produced by stimulation of the intermediate and high-threshold PAN afferents tested before and after the intradermal injection of capsaicin.

We found that after the intradermal injection of capsaicin, there was a gradual increase of the N3 component of the PAN CDPs and IFPs evoked during each of the successive spinal blocks while the N2 PAN responses were only slightly increased, suggesting that there was a continuous buildup of a tonic descending compensatory inhibition preferentially acting on the pathways conveying nociceptive rather than proprioceptive information. These changes were more prominent in the L6 that in the L7 segment, a finding suggesting that the descending inhibition was preferentially exerted on those neuronal ensembles that were facilitated by the action of capsaicin. Some of these observations have been reported previously in abstract form (Ramírez-Morales et al. 2011, 2014).

Methods

Ethical approval

Cats were bred and housed under veterinarian supervision at the Institutional Animal Care unit (SAGARPA permission AUT-B-C-0114-007). They were kept in individual comfortable cages and had access to food and water ad libitum. All experiments were approved by the Institutional Ethics Committee for Animal Research (Protocol no. 126-03) and comply with the ethical policies and Mexican regulations (NOM-062-ZOO-1999; see also Grundy 2015). The Guide for Care and Use of Laboratory Animals (National Research Council, 2010) was followed in all cases.

General procedures

The experiments were carried out in 18 adult cats of either sex (2.4–4.6 kg) initially anesthetized with pentobarbitone sodium (40 mg kg^{-1} I.P.). The carotid artery, radial vein, trachea and urinary bladder were cannulated. The left posterior articular nerve (PAN) was dissected free and sectioned. The lumbo-sacral and low thoracic spinal cord segments were subsequently exposed by a laminectomy.

During the dissection, additional doses of pentobarbitone sodium (see below) 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 mmHg. When necessary, dextran 10% or ethylephrine (Effortil, Boering-Ingelheim) was administered to keep blood pressure above 100 mmHg. A solution of 100 mM of sodium bicarbonate with 5% glucose was given I.V. (0.03 ml min^{-1}) to prevent acidosis (Rudomin et al. 2007).

After the surgical procedures, the animals were transferred to a stereotaxic metal frame allowing fixation of the vertebral column. To prevent desiccation of the exposed tissues, pools were made with the skin flaps, filled with paraffin oil and maintained between 36 and 37 °C by means of radiant heat. Subsequently, the animals were paralyzed with pancuronium bromide (0.1 mg kg^{-1}) and artificially ventilated. The tidal volume was adjusted to maintain 4% of CO_2 concentration in the expired air.

During paralysis, adequacy of anesthesia was ensured with supplementary doses of anesthetic (2 mg kg^{-1} in an hour) and by assessing that the pupils remained constricted and that heart rate and blood pressure were not changed following a noxious stimulus (paw pinch).

Stimulation and recordings

Cord dorsum potentials (CDPs) evoked by stimulation of the central end of the left PAN and of the left plantar cushion were recorded by means of silver ball electrodes placed on the L4–S1 segments against an indifferent electrode inserted in nearby paravertebral muscles. In addition to the CDPs, we recorded the intraspinal field potentials (IFPs) with a pair of glass micropipettes (tip diameter 2–3 µm) filled with 2 M NaCl (1–2 MΩ) that were inserted in the left side of the L6 and L7 segments with a rostro-caudal separation of 7 mm and positioned at different depths within the dorsal horn. This allowed comparison of the effects of capsaicin and spinal block on the dorsal horn neuronal ensembles located in two different segments, both receiving the major inputs from both the PAN and from the hind paw (see Fig. 1). The CDPs and IFPs were recorded with separate preamplifiers (bandpass filters 0.3 Hz to 1 kHz), visualized online and digitally stored for further analysis.

The central end of the already sectioned PAN was stimulated by means of a pair of hook electrodes. Stimuli were single 0.1 ms pulses of different strengths that were referred to as multiples of the minimum strength (threshold) producing a detectable CDP (*xT*). The plantar cushion in the left hindlimb was stimulated through a pair of fine needle electrodes inserted into the skin, close to the site of capsaicin injection (see below).

Location of segmental and intraspinal recording sites

Figure 1 shows the general procedures followed to locate the best sites to record the CDPs and IFPs produced by PAN and hindpaw stimulation. To this end, we made simultaneous

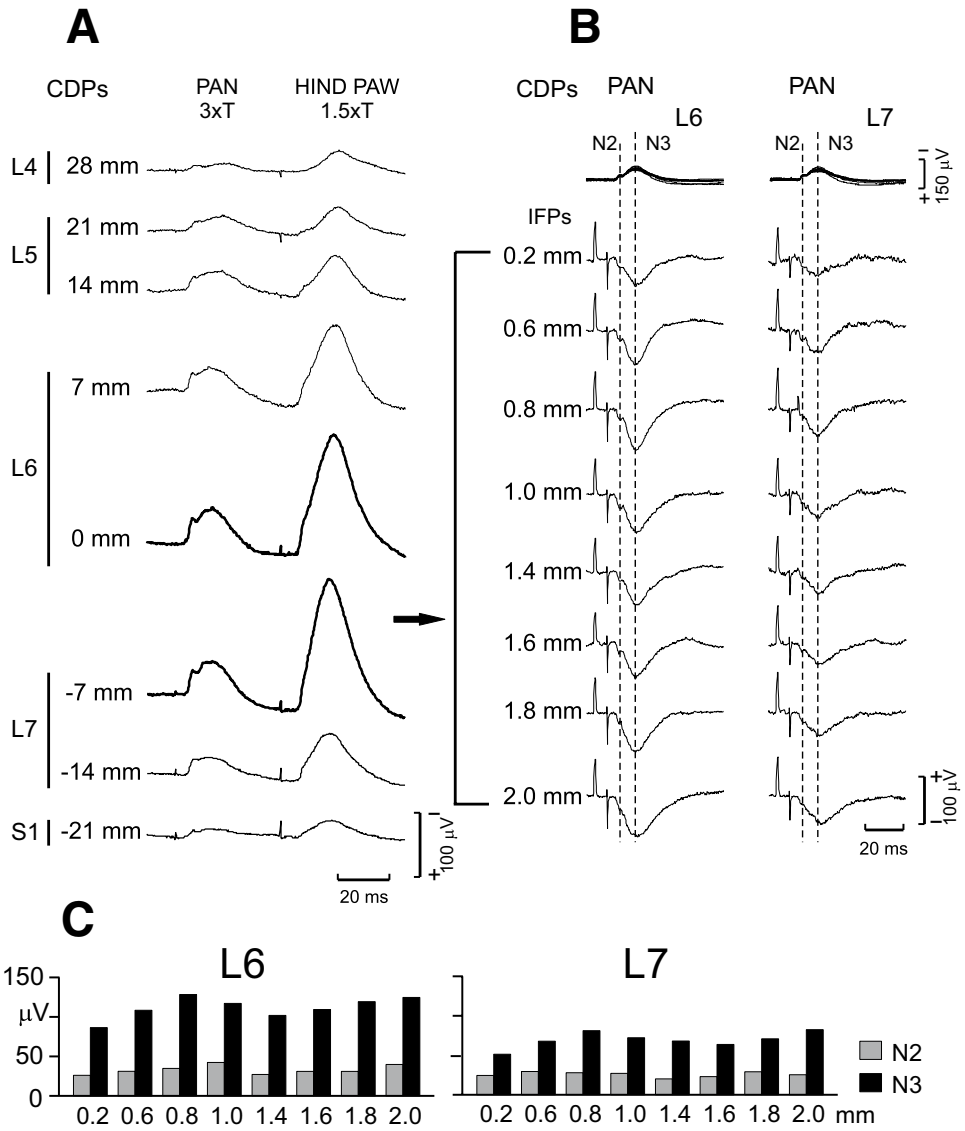

Fig. 1 Segmental and intraspinal projections of articular and plantar afferents. **a** CDPs evoked in the left L4–S1 segments by stimulation of the ipsilateral PAN and plantar cushion with single pulses 3 *xT* and 1.5 *xT* strength, respectively. Distance in mm relative to the site of largest PAN CDPs is indicated. **b** CDPs and IFPs produced by stimulation of the PAN with single pulses 3 *xT* recorded at different depths within the dorsal horn in the left L6 and L7 segments in the region where the evoked CDPs shown in **a** were largest (thick traces). Upper set of records in **b** shows superposed CDPs. **c** Amplitude of the N2 and N3 components of the PAN IFPs measured at the time shown by the vertical interrupted lines in **b**. Note that PAN N3 IFPs were largest in the superficial (0.8 mm) and deep layers (2.0 mm) in the dorsal horn. In this and the other figures, all traces are averages of 32 responses elicited at 1 Hz. Negativity upward for CDPs and downward for IFPs

recordings of the CDPs evoked in the left S1, L7, L6, L5 and L4 segments by stimulation of the ipsilateral PAN with single pulses 3 *xT* as well as by stimulation of the left plantar cushion through a pair of needle electrodes with pulses 1.5 *xT* strength. As in a previous study (Rudomin and Hernández 2008), we found that the CDPs produced by stimulation of the PAN and of the plantar cushion were largest in the rostral part of segment L7 and the caudal part of the L6 segment (thick traces in Fig. 1a).

Subsequently, two glass micropipettes were inserted in the left side of the L6 and L7 segments at the sites showing the largest CDPs evoked by PAN stimulation. Figure 1b shows the IFPs produced by electrical stimulation of the PAN with single pulses 3 *xT* at different depths in the L6 and L7 segments, as indicated. It may be seen that the largest N3 PAN responses were recorded in the superficial and deepest layers within the dorsal horn (0.8 and 2.0 mm depth) in the L6 as well as in the L7 segment (Fig. 1b, c). The responses produced by stimulation of the plantar cushion had a similar intraspinal distribution (not illustrated; see Rudomin and Hernández 2008) suggesting convergence of the spinal projections of the PAN and plantar afferents, possibly on the same set of second-order neurons (see Schaible et al. 1987a).

Spinal cord cold block

A silver-plated thermode covered with insulating lacquer was placed over the surface of the exposed spinal cord at low thoracic level (T10). The thermode had an attached thermocouple that allowed measurement of the temperature at the surface of the cord, below the cooling chamber. The temperature of thermode was changed from a warm (37 °C) to a cold (− 22 °C) circulating fluid that lowered the spinal cord temperature to between 0 and − 2.5 °C. The effectiveness of this procedure to block impulse conduction was tested previously (see Quevedo et al. 1993). Switching to the warm liquid allowed restoration of impulse conduction in the spinal cord within a couple of minutes. Spinal blocks lasting 5–9 min proved to be reversible and were employed several times in the same experiment (see also Laird and Cervero 1990; Cervero et al. 1991; Schaible et al. 1991; Quevedo et al. 1993).

Intradermal administration of capsaicin

Cord dorsum and intraspinal potentials produced by electric stimulation of posterior articular nerve (PAN) were recorded at different times during reversible spinal cord block before and after the intradermal injection of capsaicin in the plantar cushion of the left hindlimb (30 µl of 1% solution, in a 10% Tween® 80 solution and 90% saline). Capsaicin was injected only once to avoid desensitization (see Sorkin and McAdoo 1993; Sakurada et al. 2005).

Histology

At the end of the experiment, the animal was euthanized with a pentobarbital overdose and perfused with 10% formalin; the spinal cord was removed, leaving the recording micropipettes in place. After fixation and dehydration, the spinal cord segment containing the recording micropipettes was placed in a solution of methyl salicylate for clearing. Subsequently, the spinal cord was cut to obtain sections containing the recording micropipettes. The tracks left by the micropipettes were drawn with a camera lucida (Wall and Werman 1976).

Data analysis

During the experiment, the CDPs and IFPs evoked by PAN stimulation with different strengths (1.5–10 *xT*) were averaged online (32 samples at 1 Hz) and the results digitally stored for subsequent processing. To assess the overall effects of capsaicin and spinal block on the amplitude of the N2 and N3 IFPs, we pooled the data obtained in all experiments with several stimulus strengths (usually 3–5). The numbers inside each column in Figs. 6 and 7 indicate sample size. All values are given as mean ± SEM. *t* Test, paired *t* test and Mann–Whitney rank sum test were used for statistical comparison. $p < 0.05$ values were considered as significant and are marked in the figures with asterisks, as follows: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

Results

Effects of intradermal injection of capsaicin and spinal block

As shown in previous studies (Quevedo et al. 1993; Rudomin et al. 2007), stimulation of the PAN with increasing strengths produces a series of CDPs and IFPs with different components. Stimulation of the low and intermediate threshold afferents (1.1–2 *xT*) evoked responses with short latency components (N1 and N2) that have been attributed to the activation of articular afferents conveying information on joint position (McIntyre et al. 1978), while, as shown by Rudomin and Hernández (2008), stronger stimulation (usually above 2 *xT*) activated Aδ afferents conveying nociceptive information and produced the N3 component (see also Schaible et al. 1986; Quevedo et al. 1993).

The recordings displayed in Fig. 2 show the effects of the intradermal injection of capsaicin on the L6 CDPs as well as on the IFPs recorded at different depths in the L6 segment evoked by electrical stimulation of the PAN with single pulses 5 *xT*. Before the injection of capsaicin, the N2 and N3 components of the PAN IFPs were larger within the

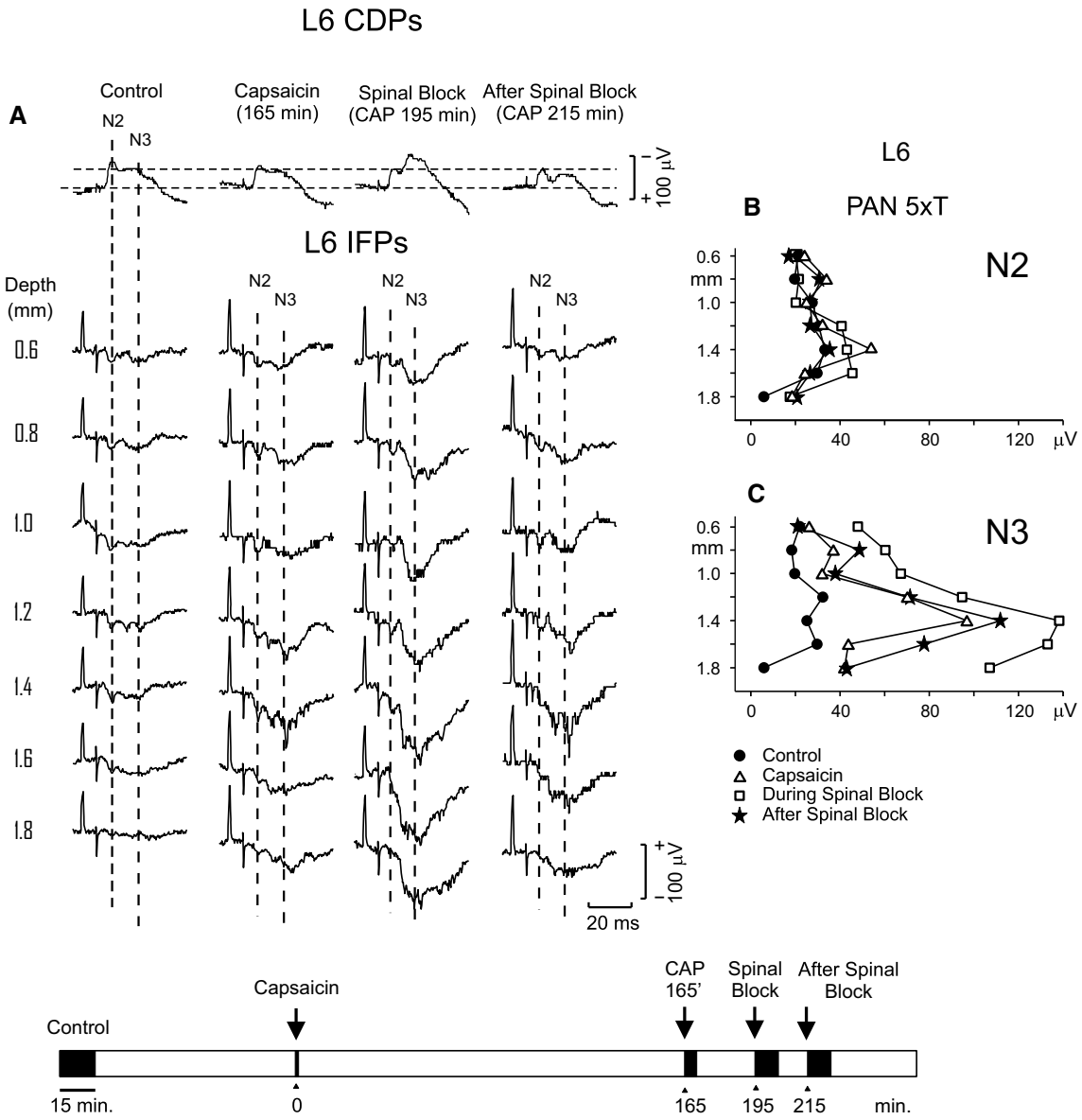

Fig. 2 Spinal block facilitates the N3 PAN CDPs and IFPs elicited after the intradermal injection of capsaicin. **a** CDPs and IFPs produced by PAN stimulation with pulses 5 *xT*. IFPs were recorded from the left rostral L6 segment at different depths within the dorsal horn, as indicated. First column shows control responses. Second column, responses obtained 165 min after the intradermal injection of capsaicin. Third column, responses recorded during a 9 min spinal block applied 195 min after the capsaicin injection. Fourth column, responses recorded 10 min after removal of the spinal cold block (215 min after the injection of capsaicin). **b**, **c** Amplitude changes of the N2 and N3 IFPs recorded at different depths measured at the time indicated by the vertical interrupted lines in **a**. Note additional increase during spinal block of the capsaicin-facilitated N3 IFPs, in contrast with the small changes of the N2 IFPs. After the removal of the spinal block, the N3 IFPs recovered most of their previous values. Ordinates indicate IFP recording depth measured relative to cord surface. Abscissa shows amplitude of the responses in µV. Bar at the bottom shows timing of the different procedures relative to the time of capsaicin injection. Further explanations are in text

intermediate zone (1.2–1.6 mm, see first column in Fig. 2a and black circles in Fig. 2b, c). By 165 min after the intradermal injection of capsaicin, the amplitude of the N3 CDPs and IFPs recorded between 1.2 and 1.6 mm was clearly increased, while the N2 responses were barely affected (second column in Fig. 2a and open triangles in Fig. 2b, c; see also Rudomin and Hernández 2008).

A cold spinal cord block applied 195 min after the injection of capsaicin produced an additional increase of the already facilitated PAN CDPs and IFPs. It was rather small for the N2 CDPs and IFPs, and quite prominent for the N3 IFPs recorded in deeper regions (between 1.2 and 1.8 mm; third column in Fig. 2a and open squares in Fig. 2b, c). It should be noted that the N3 IFPs recorded in the most

superficial layers (0.6–1.0 mm) were also facilitated during spinal block.

After removing the spinal block, the amplitude of the N3 PAN CDPs and IFPs recorded at all depths was decreased almost to its previous values (fourth column in Fig. 2a and stars in Fig. 2b, c), suggesting that the spinal block was reversible and the descending inhibitory influences reached again the spinal segments.

Effects of successive spinal blocks made before and after the intradermal injection of capsaicin

Figure 3a, b shows the CDPs and IFPs produced by PAN stimulation with single pulses of 2 and 3 *xT* strength during three successive spinal blocks, each lasting 4 min to ensure reversibility of the cold block (see Quevedo et al. 1993). It may be seen that during each spinal block, the amplitude of the N3 CDPs and of the negative component of the N3 IFPs produced by stimulation of the PAN nerve with the 2 and 3 *xT* stimuli was increased in a consistent manner, and that the responses recorded after the spinal block nearly recovered the amplitude attained before spinalization. In other words, under control conditions, the successive spinal blocks produced rather stable and reversible increases of the negative component of the N3 PAN IFPs (n-wave) as shown in Fig. 3c.

An additional and rather interesting finding was that the control CDPs as well as the IFPs showed only a negative component (n-wave). During spinal block, this component was increased and was in addition followed by a clear positive component (p-wave; see Fig. 3a–c). The p-wave is generated by the current flows associated with primary afferent depolarization (PAD; see Eccles et al. 1962), suggesting that in control conditions (that is, before the intradermal injection of capsaicin) there was a tonic descending inhibition exerted on the spinal pathways mediating PAD and presynaptic inhibition that was suppressed during reversible spinalization (see Quevedo et al. 1993).

Figure 4 shows that the descending inhibition exerted on the N3 CDPs and IFPs increased gradually after the intradermal injection of capsaicin. In this experiment, the PAN IFPs and CDPs produced with single pulses 3 *xT* were simultaneously recorded in segments L6 and L7. During each procedure (before, during and after the successive spinal blocks), both micropipettes were displaced to record the IFPs evoked at two different depths within the dorsal horn, one of them in the superficial layers (0.6 mm) and the other in deeper layers within the intermediate zone (2.0 mm), as indicated.

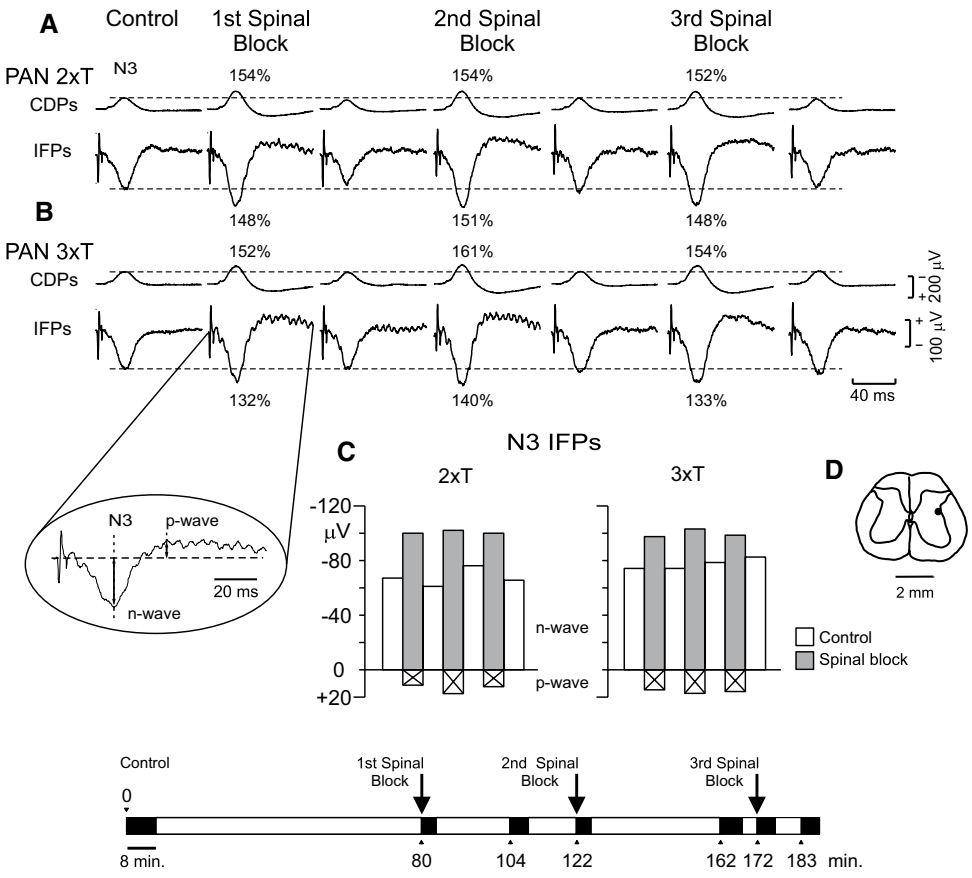

Fig. 3 Effects of successive spinal blocks on the CDPs and IFPs produced by stimulation of high-threshold PAN afferents. **a** Effects of successive spinal blocks on the CDPs and IFPs produced by stimulation of the PAN with single pulses 2 *xT*. **b** The same, but with PAN stimulation 3 *xT*. Note that the N3 components of the CDPs and IFPs were facilitated to about the same extent during successive spinalizations and that the control amplitudes were recovered after removing the spinal block. Percentage changes of CDPs and IFPs peak amplitudes relative to control are indicated. **c** Amplitude changes of the negative (n-wave, solid bars) and positive (p-wave, crossed bars) components of the N3 PAN IFPs recorded before, during and after spinal block, as indicated. Bar at the bottom shows timing of the different procedures. Spinal site of IFPs recording is shown in **d**. Further explanations are in text

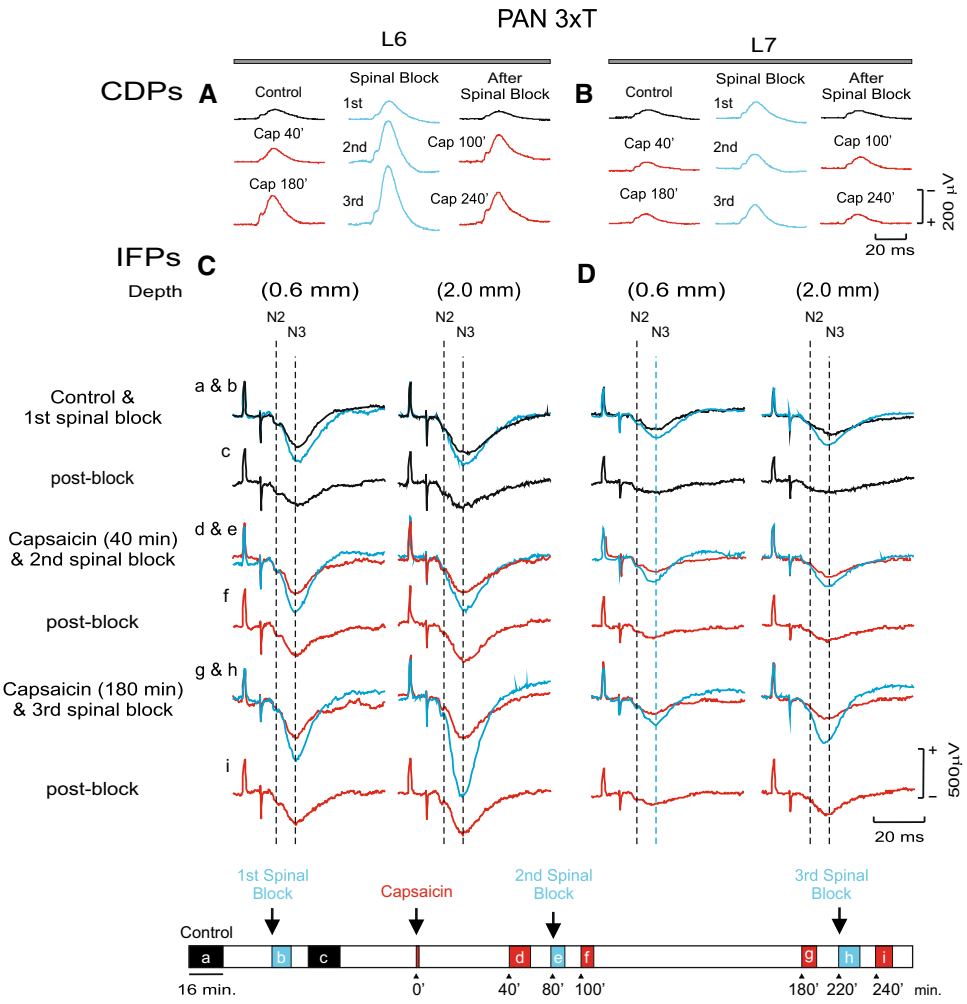

Fig. 4 Differential effects of capsaicin and of successive spinal blocks on the PAN CDPs and IFPs recorded in the L6 and L7 segments. **a**, **b** CDPs produced by stimulation of the PAN with single pulses 3 *xT* simultaneously recorded from the L6 and L7 segments, respectively. **c**, **d** IFPs produced with the same PAN stimulation recorded in the superficial (0.6 mm) and deep (2.0 mm) layers of the dorsal horn in the L6 and L7 segments, as indicated. **c**, **d** Superposed traces of the IFPs recorded before (black traces) and during the spinal block (blue traces) applied at different times after the intradermal injection of capsaicin (red traces), as indicated. Vertical interrupted lines show time of N2 and N3 measurements. Note that the facilitation of the N3 component during the successive spinal blocks was stronger in the deeper than in the superficial layers in both segments and also stronger in the L6 than in the L7 segment. Bar at the bottom shows timing of the different procedures relative to the time of capsaicin injection. Further explanations are in text

It may be seen that between 40 and 180 min after the injection of capsaicin the amplitude of the N3 CDPs as well as of the N3 IFPs recorded in the L6 segment was clearly increased, in contrast with the responses recorded in the L7 segment that displayed smaller increments. This figure also shows that N3 IFPs recorded in both segments increased gradually after successive spinal blocks, being largest by 180 min after the injection. It is to be noted that the spinal block produced a stronger and reversible facilitation of the negative components of the N3 CDPs and IFPs (n-waves) as well as of the positive component (p-waves) mainly in the L6 segment and that these changes were more evident for the IFPs recorded in the deeper dorsal horn (2.0 mm).

Figure 5a–d provides a quantitative assessment of the amplitude changes of the N2 and N3 IFPs illustrated in Fig. 4. In both the L6 and L7 segments, the N2 responses recorded after the intradermal injection of capsaicin were barely affected during the spinal block (Fig. 5a, b; see also Fig. 6). In contrast, the amplitude of the N3 superficial and deep IFPs was gradually increased during the successive spinal blocks (gray bars) and was larger for the IFPs recorded in the L6 than in the L7 segment. Note also that the positive components of the L6 and L7 IFPs (p-waves) recorded at 0.6 mm depth were increased during the successive spinal blocks, in contrast with the p-waves recorded at 2.0 mm that were generated only during the third spinal block (crossed bars; Fig. 5c, d).

It should be noted that the facilitation (measured in µV) was larger for the negative component of the N3 IFPs recorded in the L6 than in the L7 segment (Fig. 5c, d). Nevertheless, as shown in the figure, the percentage changes relative to control appeared to be larger in the L7 deeper dorsal horn.

Altogether we examined in ten experiments the effects of successive spinal blocks applied at different times before and after the intradermal injection of capsaicin. Figure 6 summarizes the percentage changes relative to control of the negative components of the PAN N2 and N3 IFPs recorded in the L6 segment at two different depths, one superficial (0.2–0.6 mm, laminae I–II) and one deeper (1.4–3.0 mm, laminae III–V; see histology in Fig. 6e, f). It should be noted that two out of the three spinal blocks applied before the

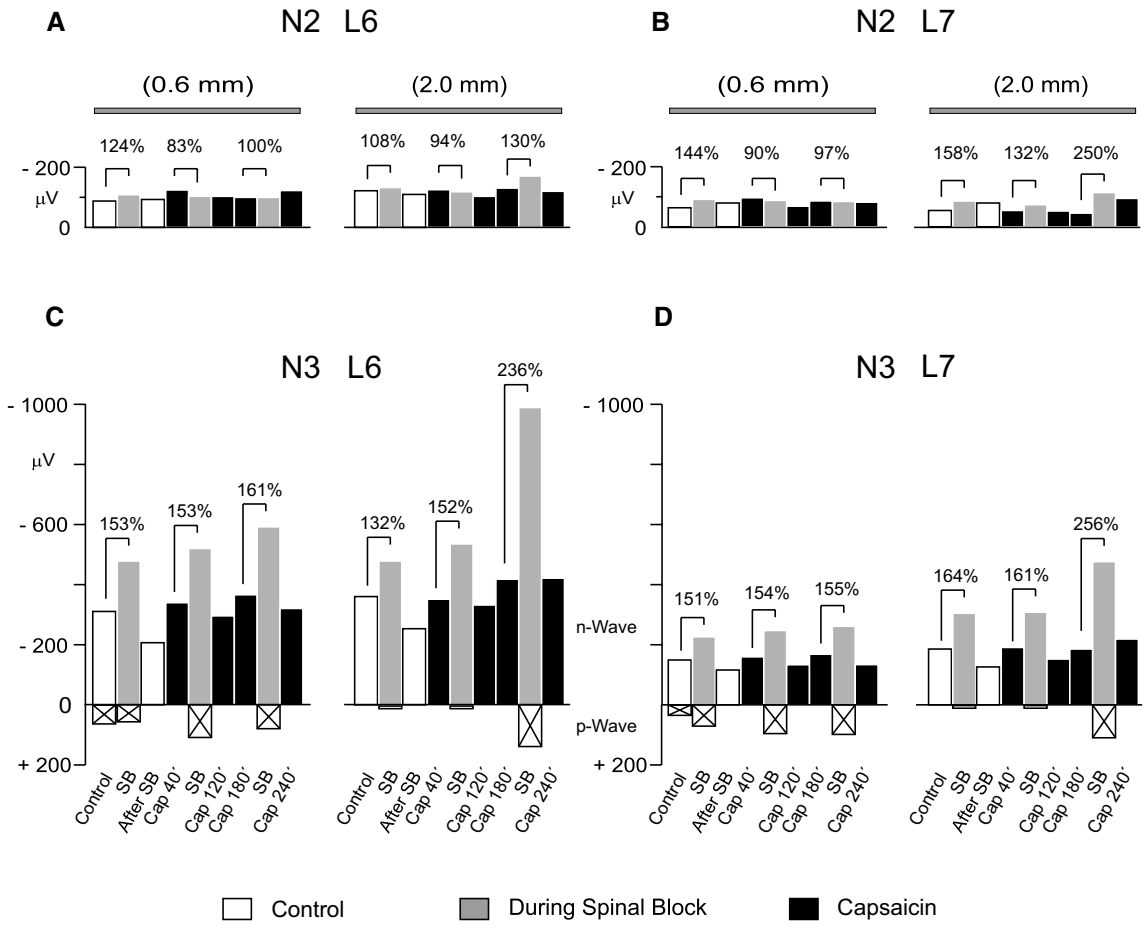

Fig. 5 Amplitude changes of the N2 and N3 IFPs produced during successive spinal blocks applied before and after the intradermal injection of capsaicin. Data obtained from the same experiment as that of Fig. 4. **a**, **b** Effects of capsaicin and reversible spinalization of the N2 IFPs recorded in the superficial (0.6 mm) and deep (2.0 mm) dorsal horn in the L6 and L7 segments, as indicated. Values above bars show percentage changes relative to the amplitude of N2 component recorded before each spinal block. Open bars, control responses obtained before and after the spinal block. Gray bars, during reversible spinal block (SB). Black bars, responses obtained after a single intradermal injection of capsaicin, at the indicated times in minutes. **c**, **d** Same for the N3 IFPs (n-waves and p-waves). Note the gradual increase in the amplitude of the N3 negative component during the successive spinal blocks applied after the intradermal injection of capsaicin. See text for further details

injection of capsaicin produced similar and statistically significant increases in the amplitude of the N3 responses (below $p < 0.05$) recorded in the superficial and deep dorsal horn and that the N2 responses were also increased in two of the three spinal blocks (Fig. 6a, c), but to a smaller extent ($p < 0.05$). After capsaicin, the N3 responses recorded both in the superficial and deep dorsal horn showed a clear and statistically significant facilitation that was gradually incremented during successive spinal blocks, particularly by 2–3 h after the injection (Fig. 6b, d). Yet, 4–5 h after the injection of capsaicin, there was a clear reduction of the facilitation of the N3 responses recorded during the spinal block.

It should be noted that the capsaicin-induced facilitation of the PAN N3 responses observed before the spinal block was not as large as that reported by Rudomin and Hernández (2008). Since the present analysis was made pooling data from several experiments, it is possible that in some cases (see Figs. 4, 5) the increase in tonic inhibition, as revealed by spinal cold block, compensated the capsaicin-induced increase of the N3 PAN responses. Nevertheless, as shown in Fig. 4a, the L6 unlike the L7 N3 CDPs showed a considerable facilitation after capsaicin. It thus seems that the descending compensation of the facilitatory action of capsaicin can be rather selective and may affect some but not all the dorsal horn neurons located in the L6 segment.

The observations described above show rather clearly that the descending control that is activated by the intradermal injection of capsaicin increases gradually with time and appears to be stronger on the neuronal networks located in deep dorsal horn. This is in agreement with the findings of Schaible et al. (1991) who showed that spinal block had

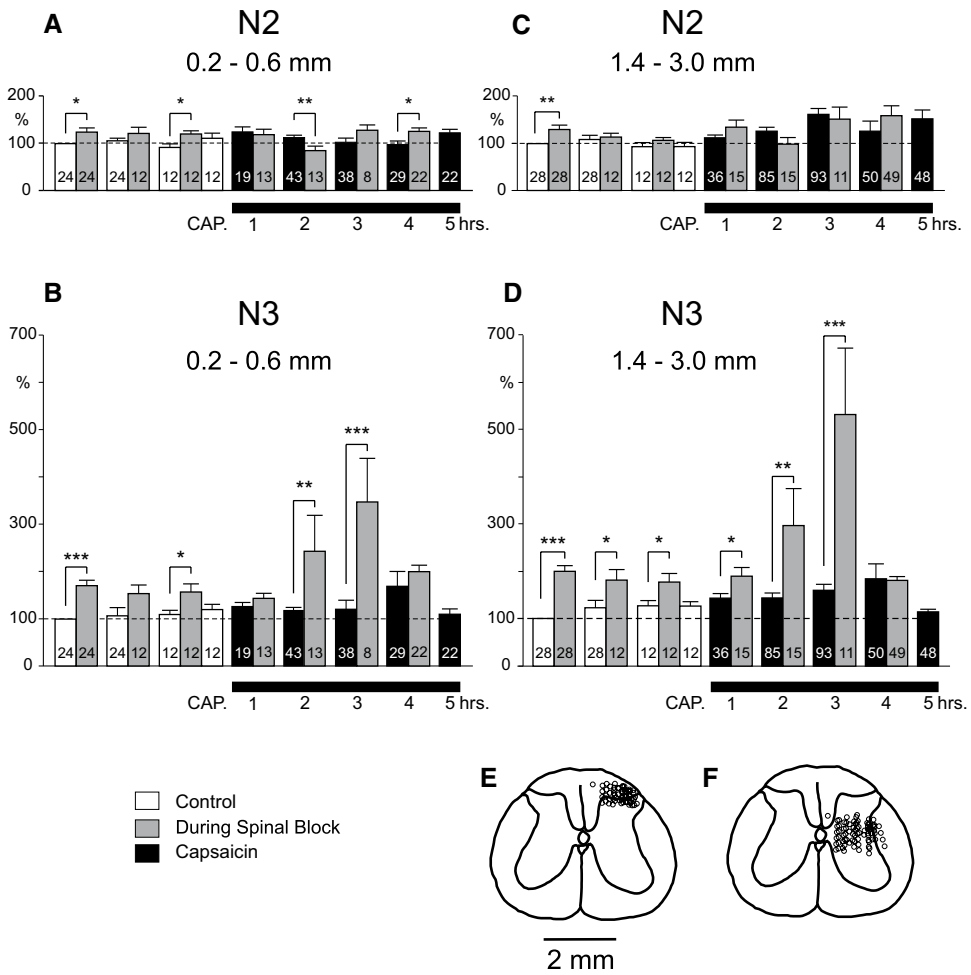

Fig. 6 Summary of the effects produced on the N2 and N3 PAN IFPs by successive spinal blocks applied before and after capsaicin injection. **a**, **b** Percentage changes relative to control of the N2 and N3 IFPs produced with single pulses between 1.5 and 10 *xT* recorded in the most superficial layers of the dorsal horn (0.2–0.6 mm) in the L6 segment. **c**, **d** Same, but for IFPs recorded in deeper layers (1.4–3.0 mm). Open bars, control responses before and after spinal block. Gray bars, during reversible spinal block. Black bars, responses produced after the intradermal injection of capsaicin, at the indicated times in hours. Asterisks indicate significant differences (see "Methods"). Mean, SEM and sample size are indicated in each bar. **e**, **f** Histological location of superficial and deep recording sites. Further explanations are in text

stronger effects on the deep neurons whose activity was already increased during inflammation than on the neurons located in more superficial layers of the dorsal horn.

The decline in the descending inhibition observed 4–5 h after the injection of capsaicin could be the expression of a homeostatic adjustment in response to a fading state of central sensitization (Schaible et al. 1991; Ren and Dubner 1996), to a decreased effectiveness of the neuromodulators released by the descending inputs because of desensitization (Docherty et al. 1991; Cholewinski et al. 1993; Koplas et al. 1997), or because of increased inhibitory interactions at spinal level (Murphy and Zemlan 1987).

Effects of capsaicin on the intraspinal distribution of the PAN N3 IFPs

As discussed above, the capsaicin-induced inflammation increases the descending inhibition exerted on the populations of spinal neurons activated by intermediate and high-threshold articular afferents. The observations depicted in Fig. 6 were obtained from responses recorded at two different depths. It thus seemed desirable to make a more detailed analysis of the changes produced by capsaicin and spinal block on the PAN N3 IFPs recorded at different depths within the L6 segment.

Figure 7a shows the changes in the intraspinal distribution of the PAN N3 IFPs produced by stimuli varying from 1.5 to 10 *xT* recorded at different depths during the first 2 h after the intradermal injection of capsaicin (range 10–120 min). It may be seen that the control responses (open bars) were largest at 0.6 mm and also between 1.8 and 2.0 mm depth, the latter well within layers III–V (see Rudomin and Hernández 2008). The capsaicin-induced facilitation was statistically significant for the responses recorded at 0.6 mm and between 1.2 and 2.0 mm depth, while the effects produced by spinal block were significantly largest between 0.8 and 1.2 mm.

By 120–240 min after capsaicin injection (Fig. 7b), the N3 IFPs were facilitated at all depths, mainly between 1.4 and 2.0 mm depth. Spinal block also produced a significant increment of the already capsaicin-facilitated responses practically at all depths (between 0.6 and 2.0 mm).

Altogether these observations indicate that spinal block induced by cooling increased the capsaicin-facilitated N3 PAN responses. In most experiments, this effect was seen

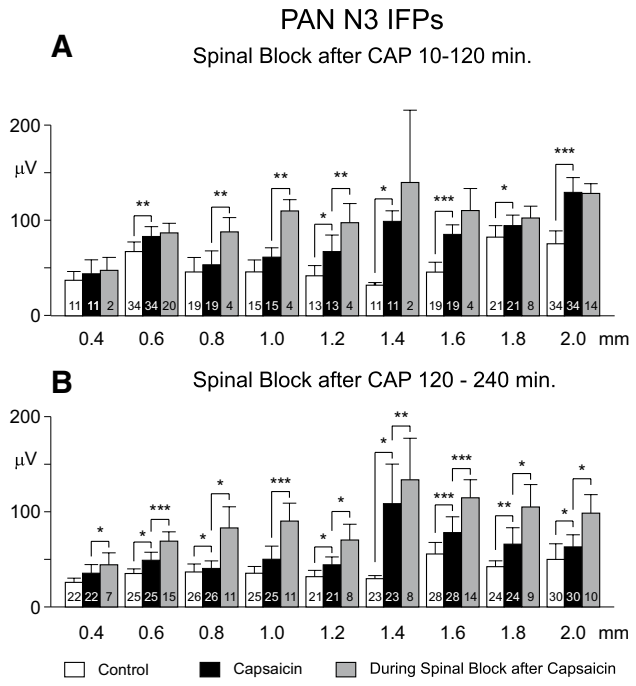

Fig. 7 Effects of spinal block on the N3 PAN IFPs recorded at different depths and at different times after the injection of capsaicin. **a**, **b** PAN N3 IFPs evoked with single pulses between 1.5 and 10 *xT* recorded in the dorsal horn at different depths in the left side of the L6 segment before and after a single intradermal injection of capsaicin, as indicated. Data were grouped into two categories according to the time elapsed after capsaicin injection (10–120 and 120–240 min). Ordinates, mean and SEM of the negative component of the N3 PAN field potentials in μV. Open bars, control. Black bars, after intradermal injection of capsaicin. Gray bars, during reversible spinal block. Asterisks indicate statistically significant differences (see "Methods"). A number of observations are indicated in each bar. Further explanations are in text

even 240 min after the intradermal injection of capsaicin and was mostly exerted on neurons located in the deepest layers in the dorsal horn.

Discussion

The present observations indicate that during the development of the central sensitization produced by capsaicin, there is a gradual and selective increase of descending inhibition that tends to limit the nociceptive-induced facilitation of the dorsal horn neuronal responses produced by the activation of Aδ afferents in the posterior articular nerve, without significantly affecting the proprioceptive information on joint angle transmitted by the low threshold articular afferents, a situation of possible relevance for limb movement during cutaneous inflammation.

Gradual increase of descending inhibition in response to nociceptive stimulation

Capsaicin has been used in several experimental models to investigate the involvement of different peripheral and central structures in the transmission of nociceptive information and pain perception during acute and chronic inflammation processes (Russell and Burchiel 1984; Lynn 1990; LaMotte et al. 1992; Szolcsányi 2004). Pain and primary hyperalgesia are produced by direct activation of peripheral nociceptors and their sensitization, while secondary hyperalgesia and allodynia result from increasing the activity of second-order neurons that receive synaptic inputs from nociceptors in the spinal cord (Simone et al. 1991; Dougherty and Willis 1992). Intradermal injection of capsaicin causes primary hyperalgesia at the injection site and secondary mechanical hyperalgesia and allodynia in the surrounding regions of the skin (LaMotte et al. 1991; see also Contreras-Hernández et al. 2018).

The gradual increase in the amplitude of the N3 PAN evoked responses observed during successive spinal blocks induced after the injection of capsaicin has been interpreted as evidence of an increased counteracting descending inhibitory action that tends to compensate the state of central sensitization produced by the nociceptive stimulus. In this sense, our observations agree with those of Schaible et al. (1991) who have shown that during the inflammation produced by the local injection of carrageenan into the joint, successive spinal blocks produce an additional increase of the neuronal activity evoked by activation of proprioceptive and nociceptive articular stimulation induced by limb flexion and extension. Yet, it should be mentioned that in the observations made by Schaible et al. (1991) the increment in the nociceptive-induced activity of the spinal neurons was due, at least in part, to the sensitization of the articular receptors produced by the pro-inflammatory agents (Schaible et al. 1991; Ren and Dubner 1996), while in our observations the PAN nerve was sectioned, so that the observed changes in the amplitude of the N3 PAN potentials produced by capsaicin before and during the spinal blocks should be solely attributed to changes in the functional connectivity between the dorsal horn neuronal networks in response to the capsaicin-induced activation of cutaneous nerves.

As shown in a recent study (Contreras-Hernández et al. 2018), the intradermal injection of capsaicin gradually increased the correlation between the ongoing CDPs recorded in different lumbar segments as well as the responses produced by mechanical stimulation of the skin within the region of primary and secondary hyperalgesia. This effect has been ascribed to an increased synchronization between dorsal horn neurons that induces non-random changes in their patterns of functional connectivity. This study has also indicated that the capsaicin-induced increase

in correlation occurs mostly in the L6 and L7 segments in the deep dorsal horn (laminae III–VI) where second-order neurons receive projections of the afferents activated by the nociceptive stimulus (Rudomin and Hernández 2008; Contreras-Hernández et al. 2018). The effects of capsaicin on the patterns of correlation between the spontaneous CDPs were notorious in animals with intact neuroaxis and were greatly attenuated when capsaicin was injected in previously spinalized preparations, suggesting that the capsaicin-induced reconfiguration of the patterns of neuronal connectivity depends on supraspinal influences.

The present set of observations complements and expands these findings by showing that the dorsal horn neuronal responses produced by stimulation of high-threshold articular afferents increased gradually during each of the successive spinal blocks applied after the intradermal injection of capsaicin. They support the proposal that there is a gradual building up of the descending inhibition exerted over spinal neurons activated by nociceptive stimulation that counteracts and regulates the increased neuronal activation produced by nociceptive stimulation.

Our observations also show that the descending inhibition, in this case of the N3 PAN responses, is mostly exerted on neurons in the deeper dorsal horn than in the more superficial layers and is mainly addressed to those spinal segments receiving the nociceptive input, in this case the L6 rather than the L7 segment (Julius and Basbaum 2001; Basbaum et al. 2009; Todd 2010). Most likely, the descending inhibition of the PAN IFPs results from changes in the synaptic activity of wide dynamic range neurons, which receive synaptic contacts from both low- and high-threshold primary afferents (Cervero and Plenderleith 1985; Schaible et al. 1991; Ren and Dubner 1996). However, it should be pointed out that the descending inhibition produced by intradermal injection of capsaicin also affects the neurons located in more superficial layers. These changes, although they were smaller in amplitude, may indicate that the nociceptive neurons of the superficial part of the dorsal horn are also under significant descending control (Fields et al. 1991; Omote et al. 1998; Vanegas and Schaible 2004). In fact, as shown by Light and Kavookjian (1988), cells of lamina II project to the deeper laminae, e.g., lamina V. Hence, the activation of these lamina II neurons during nociceptive stimulation could also contribute to the increased correlation between the activity in superficial and deep laminae during the action of capsaicin recently demonstrated by Contreras-Hernández et al. (2018).

Descending control of presynaptic inhibition

As reported previously (Quevedo et al. 1993), both the negative and positive components of the CDPs and IFPs as well as the DRPs produced by stimulation of high-threshold articular afferents were increased during reversible spinal block. This finding has been considered as evidence of a tonic descending inhibition exerted on the dorsal horn neurons as well as on the pathways mediating PAD and presynaptic inhibition.

The present series of experiments shows that in addition to the capsaicin-induced facilitation of the negative component of the CDPs and IFPs observed during the successive spinal blocks, there is also an increased facilitation of the p-wave, suggesting that during the state of central sensitization there is an increased descending inhibition exerted on the pathways presumably mediating PAD of the Aδ PAN afferents that is removed during the spinal block (see Ramírez-Morales et al. 2011, 2014). As it has been shown by several investigators, increases in PAD during nociceptive stimulation may also lead to antidromic activation of Aδ and C fibers that is transmitted to the periphery and contributes to the development of neurogenic inflammation (Lin et al. 1999, 2000; Wang et al. 2004; Ren et al. 2005). Therefore, the increased descending inhibition exerted during the action of capsaicin on the pathways mediating PAD could be interpreted as part of a control mechanism that prevents an excessive depolarization of the Aδ and C afferent fibers and the generation of the antidromic responses that contribute to further development of neurogenic inflammation (Lin et al. 1999, 2000).

In addition, the descending inhibition could also affect transmission along the pathways leading to autogenetic presynaptic inhibition of joint afferents. In the anesthetized cat, the PAN afferents show a weak autogenetic inhibition (Jankowska et al. 1993; Rudomin and Lomelí 2007), in contrast with tendon organ afferents, where this feature has been shown to effectively contribute to the self-regulation of their synaptic efficacy (Lafleur et al. 1992). Quite interestingly, preliminary observations (Ramírez-Morales et al. 2011, 2014) now indicate that the pathways leading to autogenetic PAD of single Aδ PAN afferents are facilitated after the intradermal injection of capsaicin. The increased autogenetic presynaptic inhibition developed during the capsaicin-induced state of central sensitization could act as an additional mechanism tending to limit the effectiveness of these nociceptive afferents, thus preventing the development of a self potentiating process that would lead to increased pain and possible development of allodynia and hyperalgesia (Schaible et al. 1991; Ren and Dubner 1996).

Further functional implications

It is interesting to note that although the N2 PAN responses recorded in the deep dorsal horn were slightly increased after the intradermal injection of capsaicin, they were barely affected during spinal block. The N2 PAN responses are produced by the activation of low threshold articular

afferents that transmit information regarding joint position that is utilized for the control of limb movement (McIntyre et al. 1978; Schaible et al. 1986; Quevedo et al. 1993). It is, therefore, possible that under the present experimental conditions (e.g., anesthetized and paralyzed preparations) the information transmitted by the proprioceptive joint afferents was marginally altered during cutaneous inflammation. However, it should be pointed out that stimulation of cutaneous and muscle afferents and of the brainstem reticular formation and the raphe magnus produces PAD of low threshold joint afferents (Jankowska et al. 1993; Rudomin et al. 2007; Rudomin and Lomelí 2007). Therefore, it is possible that during movement in behaving subjects, the information transmitted by the low threshold joint afferents would be also modulated by presynaptic mechanisms as has been shown in primates for cutaneous afferents during voluntary movements (Seki et al. 2003).

A final point, clinical observations indicate that during the state of central sensitization produced by inflammation of the skin (Ikoma et al. 2003, 2004), there is a perception of joint pain (Helliwell and Taylor 2005; Globe et al. 2009). This could be explained in view of the increased activity of the second-order wide dynamic range neurons that receive converging inputs from both the skin and articular afferents, i.e., of referred pain (Schaible et al. 1987a, b; Woolf 1995, 2011; Willis 2002).

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