PAIN

NSAID-induced cyclooxygenase inhibition differentially depresses long-lasting versus brief synaptically-elicited responses of rat spinal dorsal horn neurons in vivo

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Abstract

This electrophysiological study examined the effects of NSAID administration on synaptically-elicited responses of rat single spinal dorsal horn neurons to natural stimulation of peripheral receptive fields. Nociceptive responses consisted of a fast initial discharge during the stimulus followed by a slowly-decaying afterdischarge. The cyclooxygenase inhibitor, indomethacin (2.0–8.0 mg/kg, i.v.), was without effect on the on-going rate of discharge but dose-dependently inhibited synaptically-elicited responses to noxious cutaneous mechanical stimulation (fast initial discharge: $n = 3/3$ with 2 mg/kg, 5/8 with 4 mg/kg, 5/6 with 8 mg/kg; slowly-decaying afterdischarge: $n = 3/3$ with 2 mg/kg, 6/8 with 4 mg/kg, 6/6 with 8 mg/kg) and thermal (fast initial discharge: $n = 7/9$ with 8 mg/kg; slowly-decaying afterdischarge: $n = 3/4$ with 4 mg/kg, $n = 7/9$ with 8 mg/kg). The inhibitory effect of indomethacin started within 2–4 min and lasted up to 120 min. To eliminate any effect of indomethacin via cutaneous sensory receptors it was tested on the responses of some neurons to high intensity electrical stimulation of the sciatic nerve; indomethacin depressed these evoked responses (fast initial discharge: $n = 5/6$ with 2 mg/kg, $n = 7/7$ with 4 mg/kg; slowly-decaying afterdischarge: $n = 6/6$ with 2 mg/kg, $n = 7/7$ with 4 mg/kg). The brief excitatory responses to innocuous pressure (fast initial discharge: $n = 2/3$ with 2 mg/kg, $n = 6/8$ with 4 mg/kg, $n = 4/6$ with 8 mg/kg) and hair ($n = 2/7$ with 2 and 4 mg/kg, respectively) stimulation in both non-nociceptive and wide dynamic range neurons were also depressed but to a lesser extent. However, the prolonged excitation of three wide dynamic range neurons to continuous hair stimulation was almost entirely inhibited by indomethacin. Overall, inhibition of the afterdischarge and the excitatory effect of long-lasting synaptic input were greater than inhibition of the fast synaptic input-evoked initial discharge. The evidence supports the suggestion that systemically-administered indomethacin has an effect in the spinal cord and demonstrates an action specifically in the dorsal horn. The data are interpreted to suggest that sensory inputs are more involved than input-independent excitation of dorsal horn neurons in leading to de novo synthesis of eicosanoids and that the time course of this synthesis brings the levels to a point where COX inhibition can have an observable effect during prolonged excitation. Although the data suggest that COX inhibition differentially inhibits nociceptive versus non-nociceptive mechanisms at the cellular level, irrespective of the modality of the stimulus, this is the first direct demonstration that prolonged activation of synaptic mechanisms are preferentially inhibited. According to this it would be predictable that NSAIDs would be more effective on nociceptive types of pain characterized by time or prolonged inputs of primary afferents. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used clinically for decreasing peripheral inflammation, as well as for depressing inflammation-induced pain. These effects occur by inhibition of cyclooxygenase (COX), which catalyzes arachidonic acid to prostaglandins. While an obvious site of action of NSAIDs is in the periphery, at the site of the inflammation, recent work has suggested that a site of the analgesic action of COX inhibitors may also be central (Jurna and Brune, 1990), specifically at the level of the spinal cord (Jurna et al., 1992).

This suggestion has been supported by a variety of studies. COX mRNA expression is observed in the dorsal horn (Beiche et al., 1996; 1998a; Hay and De Belleroche, 1997; Willingale et al., 1997). Prostaglandin synthase is

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expressed in neurons in laminae II and III (Vesin et al., 1995; Vesin, 1996). Intrathecal administration of NSAIDs inhibits the tail-flick reflex (Wang et al., 1994), the second phase of nociceptive scores in the formalin test (Malmberg and Yaksh, 1992b; 1994b), withdrawal of the hind paw to noxious thermal stimulation (Malmberg and Yaksh, 1992a) and responses in the writhing test and the colorectal distension test (Björkman, 1995). Thus, it is well established that antinociceptive effects of NSAIDs can be elicited at the level of the spinal cord.

While it is intuitively likely that these effects are expressed by an action in the dorsal horn, this has not yet been established experimentally. Although evidence has indicated that intrathecal administration of a COX inhibitor inhibits the tail-flick reflex without any motor effects (Wang et al., 1994), prostaglandin D synthase and COX immunoreactivity is found, not only in the dorsal horn but also in motoneurons in the ventral horn (Vesin et al., 1995; Vesin, 1996; Willingale et al., 1997). Thus, it is possible that at least some of the antinociceptive effects of NSAIDs, administered intrathecally, may occur via an action in the ventral horn.

It has also not been established whether the spinal effects of prostaglandins are expressed selectively on nociceptive versus non-nociceptive mechanisms. Functional studies on COX inhibitors at the spinal level have generally used only pain tests (Uda et al., 1990; Minami et al., 1992; Minami et al., 1994; Minami et al., 1995; Ferreira and Lorenzetti, 1996). One paper reported an inhibitory effect of intravenous administration of $R(-)$- or $S(+)$-flurbiprofen on dorsal horn neuronal responses to innocuous stimuli in rats with acute knee joint inflammation, although the specific components of the responses of dorsal horn neurons to nociceptive and non-nociceptive stimuli were not determined (Neugebauer et al., 1995). Otherwise, the effects of NSAIDs in functionally specific pathways are not clearly known.

Therefore, the purpose of the present study was to investigate the role of COX in the transmission of sensory information in normal animals, specifically in the responses of dorsal horn neurons to peripheral stimulation-induced synaptic input. At the cellular level, an important question that remains, is whether the action of COX mediates responses to only noxious stimulation or whether it also mediates responses to non-noxious stimuli. Furthermore, as the duration of responses of dorsal horn neurons to non-nociceptive or nociceptive input are different, we also investigated the effect of COX inhibition in long-lasting versus brief synaptically-elicited responses. Thus, the present study determined the effects of COX inhibition on synaptically-elicited responses of dorsal horn neurons to natural stimulation of the cutaneous receptive field in the rat and determined whether there is a preferential effect on nociceptive versus non-nociceptive inputs and prolonged versus brief responses. To eliminate any effect of indomethacin via cutaneous sensory receptors it was also tested on responses to high intensity electrical stimulation of the sciatic nerve.

Some of the results in this study have been reported previously in abstract form (Pitcher and Henry, 1996).

2. Materials and methods

2.1. Animal preparation

Experiments were done on adult, male Sprague–Dawley rats from Charles River (St. Constant, Quebec, Canada). Guidelines regarding 'The Care and Use of Experimental Animals' as outlined by the Canadian Council on Animal Care (Vols. I and II) were strictly followed. Rats (350–375 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Abbott Laboratories, Montreal, Quebec) followed by supplements of 10 mg/kg per h, i.v. The right common carotid artery and jugular vein were catheterized for continuous monitoring of arterial pressure and for injection of drugs, respectively. The temperature of the rat was maintained at 37.5°C using an infrared heating lamp when required.

Spinal cord segments L_1–L_3 were exposed for recording, as this is the location of synaptic input from the cutaneous receptive fields in the hind limbs. The spinal cord was transected at the T_9 vertebral level to eliminate supraspinal influences on the activity of lumbar dorsal horn neurons; to minimize spinal shock xylocaine (0.05 ml of 1%; Astra Pharma, Mississauga, Ontario) was injected into the cord at the level of transection just prior to transection. The spinal cord was covered with mineral oil (Marcol 72, Imperial Oil; Montreal, Quebec) at 37.5°C to prevent drying.

The left sciatic nerve was exposed via blunt dissection through the biceps femoris muscle and was isolated from surrounding connective tissue using glass probes. Bipolar stainless steel hook electrodes were then inserted under the isolated nerve.

Each rat normally breathed spontaneously during the experiment. However, in experiments where electrical stimulation was used, the anesthetized animal was also paralyzed with pancuronium bromide (1 mg/kg i.v. supplemented as necessary; Pavulon, Organon, Scarborough, ON) and ventilated mechanically according to standard parameters (Kleinman and Radford, 1964).

2.2. Electrical recording and data acquisition

Single unit spikes were recorded extracellularly using seven-barreled or single barreled micropipettes (overall tip diameter 2-5 μm). The multi-barreled electrodes were used because iontophoretic drug experiments were also run in some cases. A solution of 2.7 M NaCl was placed in the central recording barrel (impedance 2–4 MΩ measured at 1 kHz with the tip submerged in saline). Single unit recordings were made at depths ranging from 250 to 1300 μm in the dorsal horn of the spinal cord. The raw data were ampli-

fied using a unity-gain preamplifier built in-house, displayed on an oscilloscope (Tektronix 5111) and stored on video cassette tapes using a digital data recorder that incorporated a digital pulse code modulation technique (VR-100A, Instrutech, Great Neck, NY) and a conventional video cassette recorder. The signals were also relayed to a frequency counter/gating unit which counted single unit spikes per unit time (bin widths were 1 s). The output of the gating unit, recorded as the rate of discharge, was displayed continuously on a Grass 79D polygraph. Sampling and analysis were done using the data acquisition program, 'Spike 2' (Version 2.02; Cambridge Electronic Design, Cambridge, UK) and an IBM Pentium computer.

2.3. Functional classification of dorsal horn neurons

Functional classification of neurons was based on the responses to natural stimulation of their respective receptive fields in the ipsilateral hind limb using both noxious and innocuous stimuli. The following natural stimuli were used as search stimuli to elicit synaptic input while penetrating the dorsal horn and to characterize functionally a neuron once stable single unit recording was obtained: (i) an air stream passed over the receptive field sufficient to move only the hairs continuously or for 3 s periods (ii) light touch (iii) moderate pressure (0.2 N for 3 s) (iv) noxious mechanical stimulation using a calibrated clip (21 N for 3 s) and (v) noxious radiant heat (measured to reach 50°C at the skin surface). The thermal stimulation was applied for a duration of 8 s and was cycled at a fixed interval of 1 or 2 min. The mechanical stimulation was applied for 3 s every 3 or 4 min.

Classification of the identified neurons was in three categories (Henry, 1976): (i) non-nociceptive neurons that responded only to non-noxious stimuli such as hair, touch and/or pressure stimulation (some receptive fields on the rat hind limb did not have hair) (ii) wide dynamic range neurons that responded to both noxious and innocuous stimuli and (iii) nociceptive-specific neurons that responded only to noxious stimuli. In addition, all the units that responded to the noxious range of mechanical and/or thermal stimulation showed a characteristic slowly-decaying afterdischarge, as described previously (Henry, 1976; De Koninck and Henry, 1991).

For each neuron the receptive fields for hair movement, light touch, moderate pressure, noxious mechanical and noxious thermal stimulation were represented on a schematic diagram of the hind limb. Receptive field sizes generally remained unchanged throughout the experiments and were not investigated further in this study.

Some neurons were also tested for their response to a train of high intensity electrical stimuli applied directly to the exposed left sciatic nerve using a bipolar electrode. The stimulus consisted of 8 mA current pulses of 1 ms duration given at 20 Hz for 3 s. These parameters have been used in our laboratory to evoke excitation of high threshold afferent fibers. This was considered to include activation of nociceptive primary afferents as it produced a slowly-decaying afterdischarge similar to that appearing in response to noxious mechanical or noxious thermal stimuli. The response in wide dynamic range neurons, thus consisted of a fast initial discharge followed by a slowly-decaying afterdischarge.

2.4. Drug administration

Indomethacin (RBI, Natick, Massachusetts) was dissolved in 2% sodium bicarbonate and this solution was titrated to pH 7.4 using sodium monophosphate. Indomethacin was administered intravenously in a volume of 1 ml/kg body weight. As a control, the vehicle, 2% sodium bicarbonate (pH 7.4 using sodium monophosphate), was administered in a similar fashion.

Indomethacin is generally considered a non-selective COX-1/-2 inhibitor (Mitchell et al., 1994; Gierse et al., 1995; Yamamoto and Nozaki-Taguchi, 1996; Riendeau et al., 1997; Harada et al., 1998) and was used in this study to inhibit activation of both COX isoforms. Previous studies used doses ranging from 1 to 50 mg/kg (Jurna and Brune, 1990; Chapman and Dickenson, 1992; Hu et al., 1994; Buritova et al., 1995; Honoré et al., 1995; Bustamante et al., 1996). In the present study, pilot experiments indicated that 2 mg/kg of indomethacin were effective in depressing the effects of noxious, as well as innocuous stimulation-induced synaptic input to dorsal horn neurons. Furthermore, in a previous study, we found this dose to be effective in depressing the responses to iontophoretic application of the glutamate receptor agonists, *N*-methyl-D-aspartic acid (NMDA) and quisqualate and α-amino-3-hydroxy-5-methyl-isoxazolepropionic acid (AMPA), and of the NK-1 receptor agonist, substance P (Pitcher and Henry, 1996).

2.5. Data analysis

Quantization of the magnitude of a response to stimulus-induced synaptic input was done as follows. The total number of spikes was calculated over a period of 3 s, from the onset of the neuronal responses to hair and pressure. As the responses to pinch, noxious radiant heat and electrical stimulation consisted of not only a fast initial discharge but also a slowly-decaying afterdischarge, sampling for these responses included the two respective periods. Thus, the number of spikes in the fast initial discharge in response to pinch and electrical stimulation was determined over the 3 s period of the stimulus. The number of spikes in response to noxious thermal stimulation was determined over the 3 s period at the end of the stimulus. For the slowly-decaying afterdischarge the sample period was 60 s, beginning immediately after the fast initial discharge. The magnitude of the response was then calculated for each sample period by subtracting the number of spikes over the 3 or 60 s period immediately preceding the stimulus.
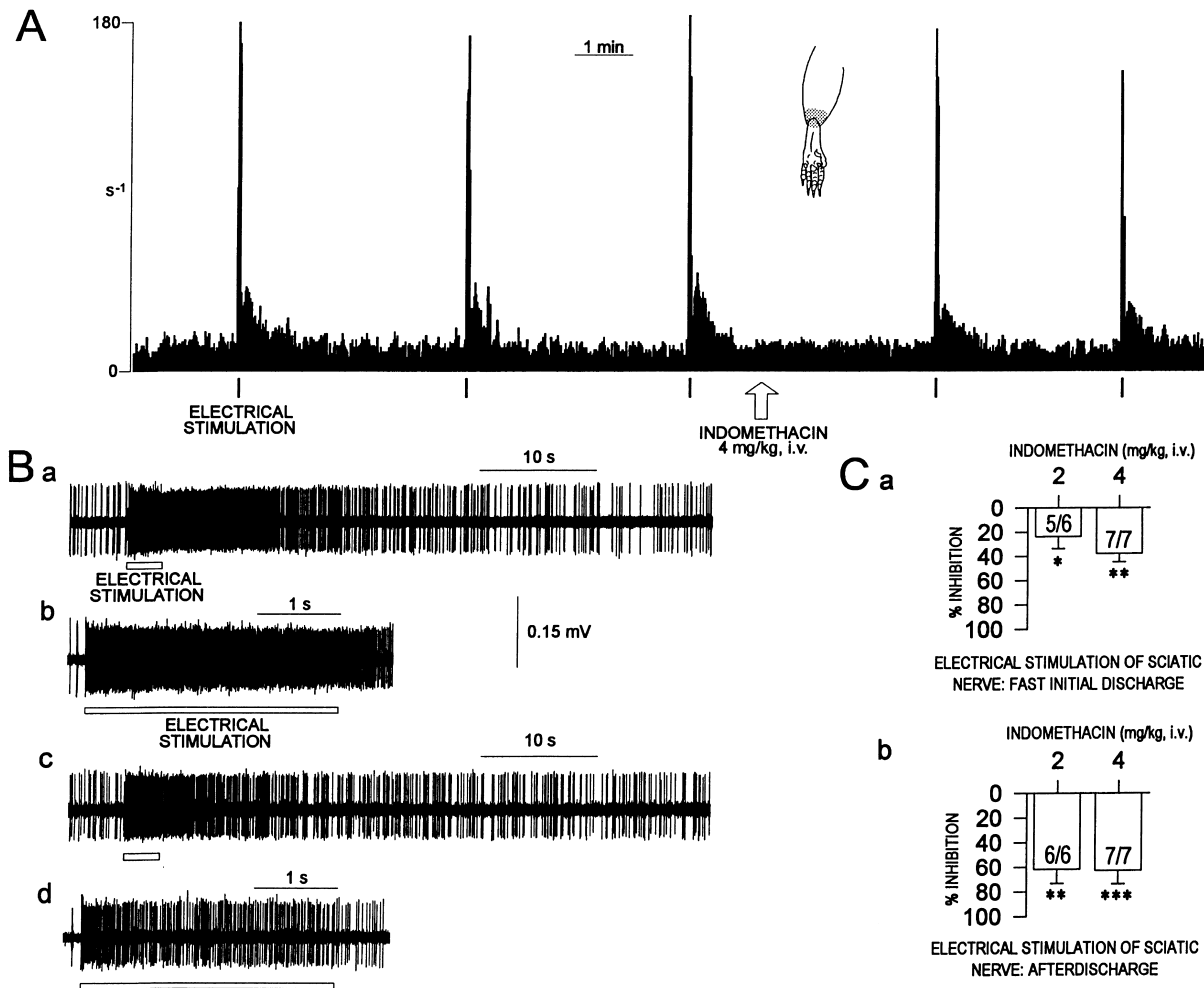
Fig. 1. Indomethacin depresses the response to high intensity electrical stimulation of the sciatic nerve. (A) High intensity electrical stimulation (1 ms rectangular pulses of 8 mA at 20 Hz for 3 s) to the exposed left sciatic nerve produced a fast initial discharge which lasted only for the duration of the stimulus and a slowly-decaying afterdischarge which persisted for up to approximately 1 min. The vertical axis represents the firing frequency in spikes/s. The horizontal axis is time. Indomethacin inhibited the fast initial discharge and the slowly-decaying afterdischarge, with a preferential depression of the latter. The time of administration of indomethacin is depicted by the open arrow. The time and duration of the electrical stimulus are shown by the narrow rectangles below the histogram. The neuron was found 1148 μm deep from the dorsal surface of the spinal cord. The inset shows the cutaneous receptive field on the left hind paw of the rat. (B) Extracellular recordings showing representative single unit excitatory responses to a high intensity electrical stimulus (shown by the clear rectangle below each trace) of another wide dynamic range neuron (700 μm). (a) The fast initial discharge is demonstrated by the high frequency firing rate during the stimulus. This is followed by a relatively slowly-decaying afterdischarge lasting approximately 30–40 s. (b) Magnified representation of the fast initial discharge shown in (a). Note the increase in firing rate during the electric train compared with the prestimulus baseline firing rate. (c) At 30 min after administration of indomethacin (4 mg/kg, i.v.) firing frequency of the fast initial discharge and the slowly decaying afterdischarge are noticeably decreased. Note that the duration of the afterdischarge is also attenuated compared with the duration of the afterdischarge shown in (a) before indomethacin was given. (d) Magnified representation of the fast initial discharge shown in (c) after administration of indomethacin. A considerable decrease in firing rate is demonstrated. Note that while the firing frequency was decreased by indomethacin, spike amplitude remained unaltered. (C) Dose-response histogram summarizing the effects of indomethacin on (a) the fast initial discharge and (b) the slowly-decaying afterdischarge. Each vertical axis represents the mean (± SEM) percent inhibition expressed as a percentage of the rate of discharge prior to the administration of indomethacin. Each ratio is the number of dorsal horn neurons inhibited over the number tested. *$P < 0.05$, ** $P < 0.01$ or ***$P < 0.001$ vs. vehicle (0% inhibition). The maximum percent inhibition of the fast initial discharge and the slowly-decaying afterdischarge at 2 mg/kg indomethacin are significantly different ($P < 0.05$).

To quantitate the effects of indomethacin, three responses before administration of indomethacin were averaged and three responses at the maximum inhibition after administration of indomethacin were averaged. The mean value 'during' was subtracted from the mean value 'before' and this was divided by the mean value before indomethacin. This was then multiplied by 100 to yield a percent inhibi-

tion. Thus, no effect of indomethacin would be 0% inhibition, while complete inhibition of a response by indomethacin would be 100% inhibition. Some neurons were tested more than once with indomethacin to determine the dose–response relationship. This was done only using a higher subsequent dose and only following full recovery from the lower dose tested to ensure that there was no
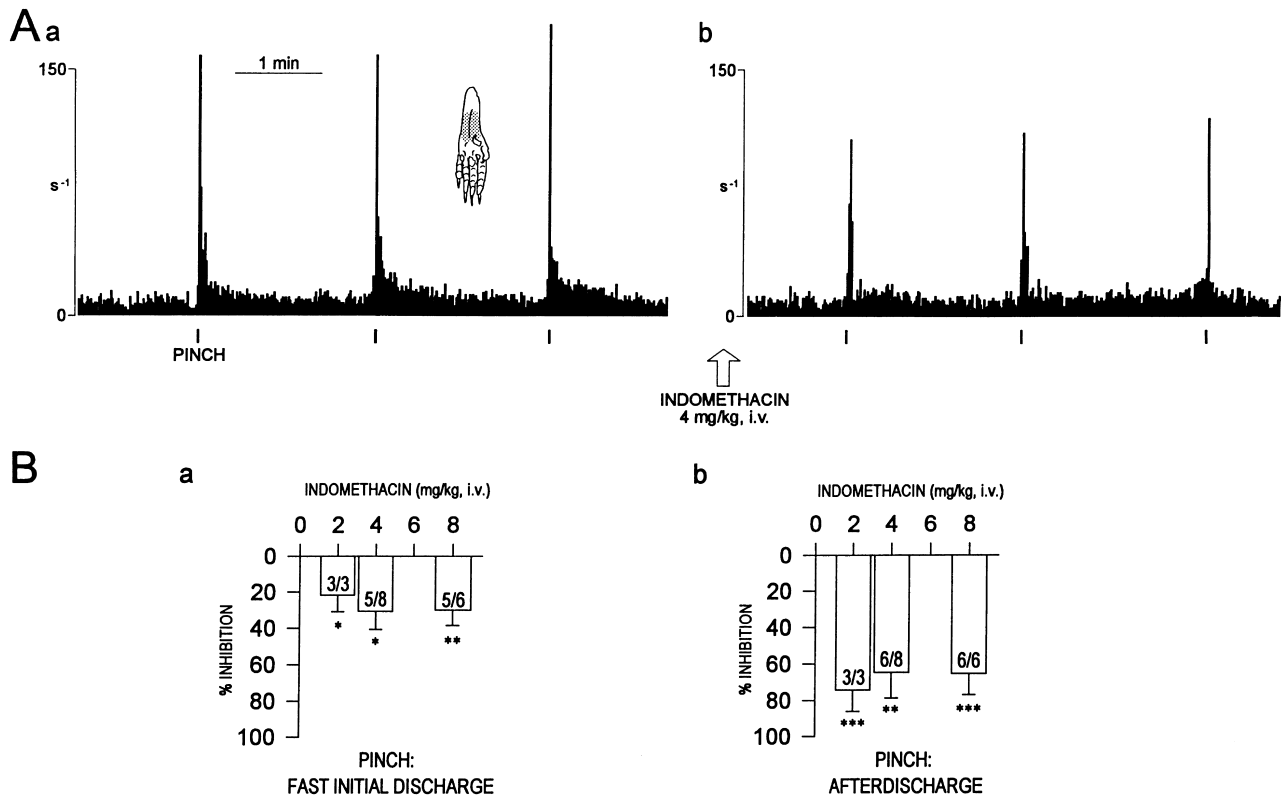
Fig. 2. Indomethacin depresses the response to noxious mechanical stimulation of the cutaneous receptive field. A(a) Noxious mechanical stimulation (pinch) produces a fast initial discharge which lasts only for the duration of the stimulus and a slowly-decaying afterdischarge which persists for up to 1 min in a wide dynamic range neuron (647 μm deep from the dorsal surface of the spinal cord). The time and duration of the pinch applications are shown by the narrow rectangles below the histogram. The inset shows the cutaneous receptive field on the left hind paw of the rat. (b) Inhibitory effect of indomethacin on the fast initial discharge and slowly-decaying afterdischarge responses of this neuron. This record was taken 20 min after the end of (a). (B) Dose-response histogram summarizing the effects of different doses of indomethacin on (a) the fast initial discharge and (b) the slowly-decaying afterdischarge. Each vertical axis represents the mean ($\pm$ SEM) percent inhibition expressed as a percent of the rate of discharge prior to the administration of indomethacin. To calculate significance, the mean percent inhibition was compared with the mean effect of vehicle administration. *$P < 0.05$, **$P < 0.01$ or ***$P < 0.001$ vs. vehicle. The maximum percentage inhibition of the fast initial discharge and the slowly-decaying afterdischarge at 2 and 4 mg/kg indomethacin, respectively, are significantly different ($P < 0.05$).

remaining effect of the drug. As a result, each dose group was derived from different neurons. Responses of neurons that were insensitive to indomethacin were included in the ratios of the dose-response histograms.

In the case of continuous hair stimulation the total number of spikes was calculated over a period of 3 min before the stimulation and during the continuous air stream. The magnitude of the response was the difference in the number of spikes. A similar difference was calculated at the maximum inhibition after the administration of indomethacin. To quantitate the effect of indomethacin, the value after indomethacin administration was subtracted from the value before and this was divided by the value before and multiplied by 100 to yield a percent inhibition.

To calculate significance, the mean ($\pm$SEM) percent inhibition following a particular dose of indomethacin was compared with the mean percent inhibition (0%) following vehicle administration. Statistical analysis of the data was done using one-way ANOVA and Student–Newman–Keuls test. A difference between test and control responses was considered significant with a P value <0.05.

3. Results

Indomethacin was tested on responses of dorsal horn neurons to stimulation of the cutaneous receptive field and to electrical stimulation of the sciatic nerve in 39 rats. Indomethacin could not be given iontophoretically due to its high resistance property in the ejection barrel of the electrode. Accordingly, the data to follow were all obtained from systemic administration of indomethacin. Data from a neuron were included only if full testing could be completed. Only neurons that showed full recovery from the effects of indomethacin were included in this study. Indomethacin was found to be consistently without effect on spike amplitude. It was also without effect on arterial pressure and respiration.

3.1. Tests of indomethacin on on-going activity

On-going baseline activity was unaltered by administration of indomethacin. The records in the figures illustrate this lack of effect.

3.2. Effect of indomethacin on response to high intensity electrical stimulation of the sciatic nerve

High intensity electrical stimulation of the exposed sciatic nerve produced a transient excitatory response (fast initial discharge) followed by a slowly-decaying afterdischarge which persisted for about 1 min after the end of the stimulus (Fig. 1A). This effect was observed in all 17 wide dynamic range neurons tested.

The effect of indomethacin on the response of a neuron to this stimulation is shown in Fig. 1A. The firing frequency of the fast initial discharge and the slowly-decaying afterdischarge were both attenuated following indomethacin administration (4 mg/kg, i.v.). The predominant effect of indomethacin was attenuation of the duration and amplitude of the slowly-decaying afterdischarge.

Spike activity of another single wide dynamic range neuron illustrating excitatory responses to electrical stimulation of the sciatic nerve is shown in Fig. 1B. The fast initial discharge and the slowly-decaying afterdischarge are clearly seen, the fast initial discharge being the high frequency firing during the electrical stimulus (Fig. 1Ba,Bb). The slowly-decaying afterdischarge persisted until approximately 30–40 s after the end of the stimulus of this particular neuron (Fig. 1Ba). Thirty minutes after administration of indomethacin (4 mg/kg, i.v.) both the initial discharge (Fig. 1Bc,Bd) and the afterdischarge (Fig. 1Bc) were attenuated.

The effect of indomethacin (2 or 4 mg/kg, i.v.) on the response to electrical stimulation was tested in a total of 13 wide dynamic range neurons. Twelve exhibited a depression of the fast initial discharge. All 13 showed inhibi-tion of the slowly-decaying afterdischarge. Fig. 1Ca shows that 2 and 4 mg/kg indomethacin depressed the fast initial discharge by $23.72 \pm 10.22\%$ ($n = 5$, $P < 0.05$ vs. control) and $37.83 \pm 7.05\%$ ($n = 7$, $P < 0.01$), respectively. The slowly-decaying afterdischarge was also depressed by 2 and 4 mg/kg indomethacin ($61.46 \pm 11.71\%$; $n = 6$, $P < 0.01$ and $62.51 \pm 10.94\%$; $n = 7$, $P < 0.001$), respectively (see Fig. 1Cb). The maximum percent inhibition of the fast initial discharge and the slowly-decaying afterdischarge at 2 mg/kg indomethacin are significantly different ($P < 0.05$). In all cases, full recovery of the response to peripheral electrical nerve stimulation was observed. Indomethacin-induced inhibition of the response of dorsal horn neurons to peripheral nerve stimulation typically began at about 2–4 min after administration and generally lasted for 90–120 min following injection.

Neuronal responses to electrical nerve stimulation were unaffected by 2% sodium bicarbonate ($n = 5$).

3.3. Effect of indomethacin on response to noxious mechanical stimulation

Noxious mechanical stimulation of the peripheral cutaneous receptive field produced the typical excitatory response in all 18 wide dynamic range neurons tested. This response consisted of a fast initial discharge occurring throughout the duration of the stimulus, and a slowly-decaying afterdischarge lasting beyond the end of the stimulus (Henry, 1976).

Fig. 2Aa shows the response of a wide dynamic range neuron to noxious pinch applied to the cutaneous receptive field on the left hind paw of the rat. The fast initial discharge lasted for the duration of the 3 s pinch and the slowly-decaying afterdischarge persisted approximately 1 min after the end of the pinch stimulus. Both the fast initial discharge and the slowly-decaying afterdischarge were attenuated by 4 mg/kg of indomethacin i.v. (Fig. 2Ab). While the fast initial discharge was decreased by about 30%, the record shows an almost complete block of the slowly-decaying afterdischarge.

Indomethacin depressed the pinch-induced fast initial discharge and the slowly-decaying afterdischarge in a dose-related manner. Doses of 2, 4 and 8 mg/kg were given to a total of 14 wide dynamic range neurons. Indomethacin at a dose of 2 mg/kg depressed the fast initial discharge of all three wide dynamic range neurons tested ($21.73 \pm 9.09\%$, $P < 0.05$ vs. control). Higher doses of 4 and 8 mg/kg indomethacin depressed the fast initial discharge of five out of eight neurons ($30.61 \pm 10.07\%$, $P < 0.05$) and five out of six neurons ($29.94 \pm 8.58\%$, $P < 0.01$), respectively. Indomethacin at doses of 2, 4 and 8 mg/kg depressed the slowly-decaying afterdischarge of three out of three ($74.39 \pm 11.67\%$, $P < 0.001$), six out of eight ($64.72 \pm 13.97\%$, $P < 0.01$) and six out of six ($65.44 \pm 11.53\%$, $P < 0.001$) wide dynamic range neurons, respectively. The maximum percent inhibition of the fast initial discharge and the slowly-decaying afterdischarge at 2 and 8 mg/kg indomethacin, respectively, are significantly different ($P < 0.05$). In cases where inhibition occurred, full recovery of the response to a noxious pinch was always observed.

Generally, the firing frequency of the fast initial discharge was attenuated as soon as 4 min following administration of indomethacin and the slowly-decaying afterdischarge as soon as 2 min. Indomethacin attenuated not only the amplitude but also the duration of the slowly-decaying afterdischarge (see Fig. 2Ab). Responses of wide dynamic range neurons to repeated noxious stimulation recovered from indomethacin-induced depression approximately 90–120 min after higher doses were given.

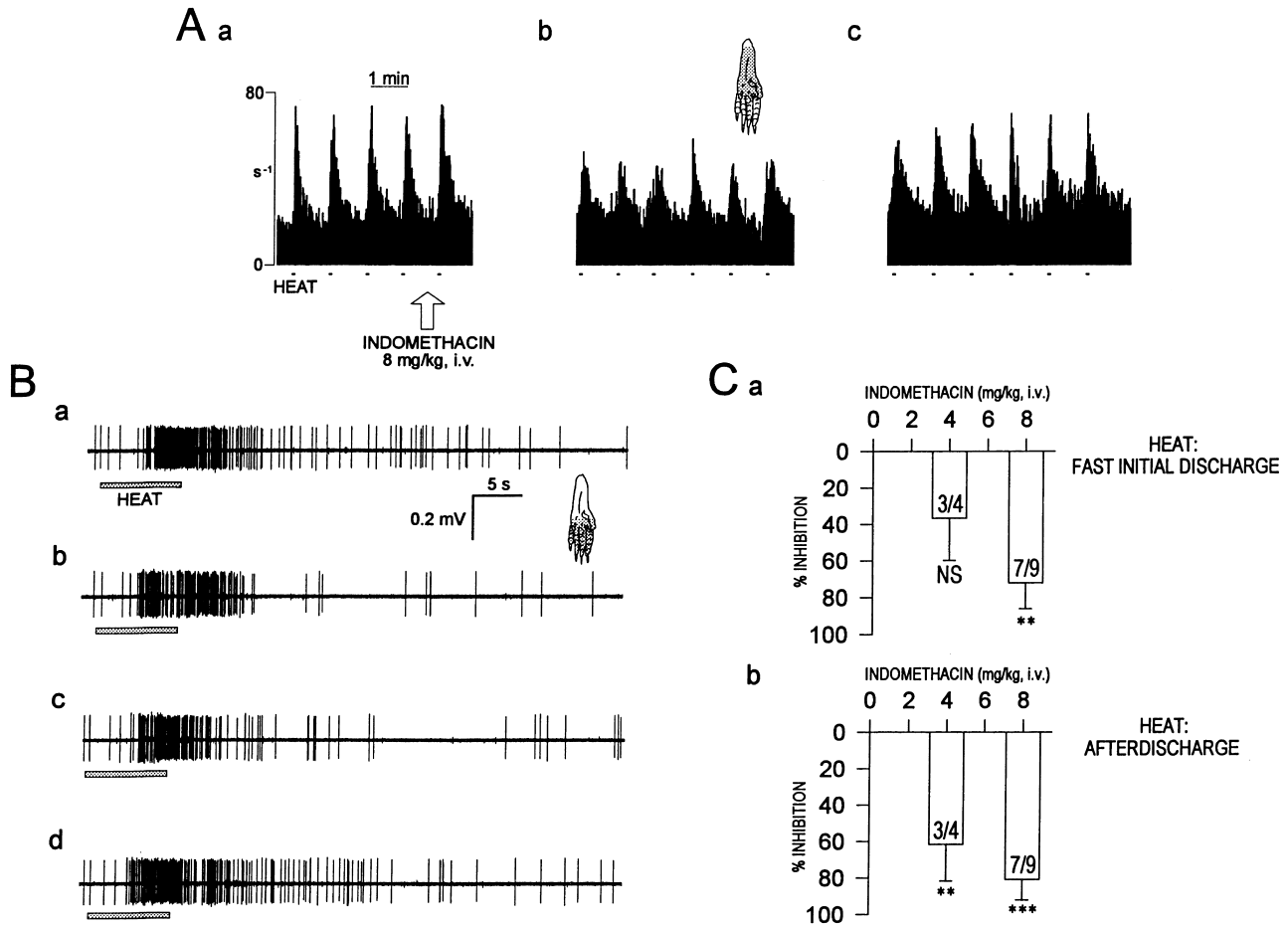As a control, 2% sodium bicarbonate was tested on the

Fig. 3. Indomethacin attenuates the response to noxious thermal stimulation (radiant heat) of the cutaneous receptive field. A(a) Noxious thermal stimulation (indicated by the narrow rectangles below the histogram) cycled at 1 min intervals elicited a fast initial discharge followed by the slowly-decaying afterdischarge in a wide dynamic range neuron (410 μm). (b) Inhibitory effect of indomethacin (8 mg/kg, i.v.) on the fast initial discharge and persistent responses. The record was taken 10 min after administration of indomethacin. The inset shows the cutaneous receptive field. (c) The response to noxious thermal stimulation had recovered by 90 min later. (B) Example of extracellular recordings showing representative single unit excitatory responses to noxious thermal stimulation (shown by the shaded rectangles below each trace) of another wide dynamic range neuron (938 μm). (a) The fast initial discharge is revealed by high frequency firing rate toward the end and slightly after application of the noxious thermal stimulus. This is followed by a relatively slowly-decaying afterdischarge. (b) At 5 min after administration of indomethacin (8 mg/kg, i.v.) firing frequency of the fast initial discharge is noticeably decreased and the slowly-decaying afterdischarge is almost entirely inhibited. Also the offset of the fast initial discharge after the end of the thermal stimulus is sooner. (c) At 30 min after indomethacin was given there remained a considerable inhibition of both the fast initial discharge and slowly-decaying afterdischarge. Note the increase in the latency of onset of the effect of noxious thermal stimulation in (c) compared with (a) and the decrease in the number of spikes. (d) At 100 min after indomethacin administration, there was a complete recovery of both the fast initial discharge and the slowly-decaying afterdischarge. Note that while the firing frequency was decreased by indomethacin, spike amplitude remained unaltered. (C) Dose–response histogram summarizing the effects of different doses of (a) indomethacin on the fast initial discharge and (b) the slowly-decaying afterdischarge neuronal responses to noxious thermal stimulation of the cutaneous receptive field. NS not significant, **$P < 0.01$ or ***$P < 0.001$ vs. vehicle.

pinch response in five neurons and was always without effect.

3.4. Effect of indomethacin on response to noxious thermal cutaneous stimulation

The effect of noxious thermal stimulation of the cutaneous receptive field on the hind paw of the rat was tested on 11 wide dynamic range neurons. This stimulus produced the usual response consisting of a fast initial discharge followed by a slowly-decaying afterdischarge which persisted after the end of the noxious thermal stimulus

(Fig. 3Aa). The fast initial discharge typically started 4 to 5 s after the bulb was turned on. The firing rate continued to increase throughout the application of the stimulus and reached a peak approximately 1 s after the stimulus ended. This typical response was seen with all 11 wide dynamic range neurons tested with noxious heat.

Fig. 3A shows the effect of 8 mg/kg of indomethacin on the response of one wide dynamic range neuron to noxious thermal stimulation of the cutaneous receptive field. Inhibition of the fast initial discharge and the slowly-decaying afterdischarge occurred within 10 min of administration of indomethacin (Fig. 3Ab). Fig. 3Ac shows
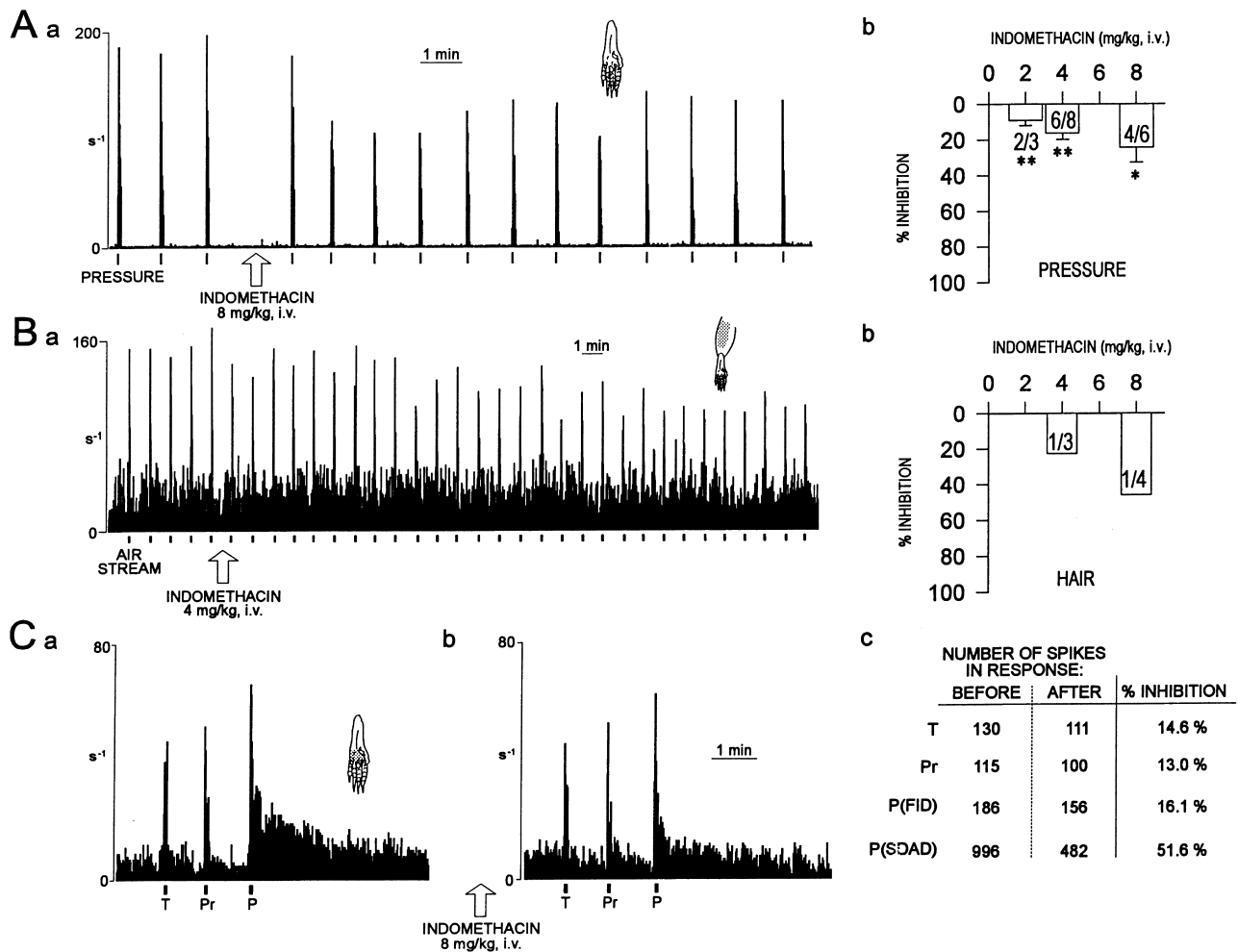
Fig. 4. Indomethacin induces relatively weak inhibition of responses to innocuous stimuli. A(a) Inhibitory effect of indomethacin on the response of a non-nociceptive neuron (935 μm) to moderate innocuous pressure (3 s duration) applied to the cutaneous receptive field (shown on inset of left hind paw of a rat) at 1 min intervals. (b) Dose–response histogram summarizing the effects of different doses of indomethacin on neuronal responses to pressure stimulation, *$P < 0.05$ or **$P < 0.01$ vs. vehicle. (B) Indomethacin induces relatively weak inhibition of the response to hair stimulation (air-stream for 3 s duration) of the receptive field in one neuron. (a) Effects of indomethacin on the responses of a non-nociceptive neuron (446 μm) to an air stream applied to the hairy skin of the left hind paw of a rat (receptive field depicted on inset of hind limb). (b) Dose–response histogram summarizing the effects of different doses of indomethacin on neuronal responses to hair stimulation, *$P < 0.05$ or **$P < 0.01$ vs. vehicle. C(a) Touch (T), pressure (Pr; 3 s) or noxious pinch (P; 3 s) stimuli on the cutaneous receptive field produced a fast initial discharge which lasted only for the duration of the stimulus in a wide dynamic range neuron (645 μm). A slowly-decaying afterdischarge following the fast initial discharge response is also is also produced by the pinch stimulus. (b) Indomethacin induced a relatively weak inhibitory effect on the fast initial response of this wide dynamic range neuron to touch, pressure and pinch applied to the cutaneous receptive field (shown on inset of left hind paw of a rat) 25 min after administration. However, indomethacin decreased noticeably the magnitude and duration of the slowly-decaying afterdischarge. (c) Number of spikes per response of this particular wide dynamic range neuron to touch (T), pressure (Pr) and pinch (P) before and after administration of indomethacin. Note that while there is only a slight decrease in the number of spikes per response to touch, pressure or the fast initial discharge (FID) to pinch stimulation after indomethacin administration, the number of spikes in the slowly-decaying afterdischarge (SDAD) is decreased to less than half of the number of spikes before indomethacin was given (51.6% inhibition).

that full recovery of both types of discharge in the response of this neuron to noxious thermal stimulation had occurred by approximately 100 min after administration of indomethacin.

Spike activity of another single wide dynamic range neuron demonstrating excitatory responses to noxious thermal stimulation of the cutaneous receptive field is shown in Fig. 3B. Five min after administration of indomethacin (8 mg/kg, i.v.; Fig. 3Bb) the initial discharge was noticeably decreased and the afterdischarge was almost entirely inhib-

ited. Thirty minutes after administration of indomethacin there remained considerable inhibition of both the initial discharge and the afterdischarge (Fig. 3Bc). Approximately 100 min after indomethacin there was complete recovery of both components (Fig. 3Bd).

Indomethacin was tested on each of the 11 wide dynamic range neurons. Full recovery was observed with each dose. Fig. 3C illustrates the dose-dependent inhibition by indomethacin of the initial discharge (Fig. 3Ca) and the afterdischarge (Fig. 3Cb) of wide dynamic range neurons tested
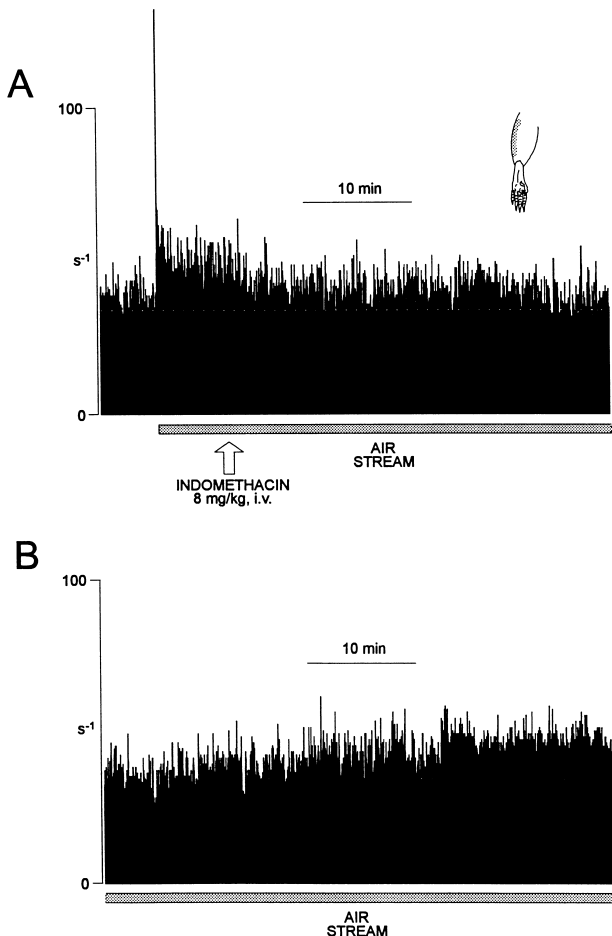
Fig. 5. Indomethacin depresses the on-going excitation induced by prolonged hair stimulation. (A) Continuous air stream (indicated by the hatched rectangle below the histogram) applied to the hairy receptive field increases firing frequency of a wide dynamic range neuron (844 μm). The pre-hair stimulation baseline firing frequency of 34 Hz is represented by the white dotted line spanning the record. Administration of indomethacin (8 mg/kg, i.v.) inhibits the hair stimulation-evoked increase in firing rate by 93% at 30 min after administration of indomethacin. (B) Gradual recovery of continuous hair stimulation-induced activity. The end of the trace is approximately 90–100 min after administration of indomethacin.

with noxious thermal stimulation. At 4 mg/kg of indomethacin, the slowly-decaying afterdischarge was depressed in three out of four neurons ($61.71 \pm 19.84\%$, $P < 0.01$ vs. control). Although the 4 mg/kg dose of indomethacin depressed the fast initial discharge ($36.62 \pm 23.18\%$), comparison of the mean percent inhibition with the mean percent effect of vehicle indicated that the response at this dose was not significant. However, 8 mg/kg of indomethacin significantly depressed the afterdischarge in seven out of nine neurons ($80.69 \pm 11.17\%$, $P < 0.001$) as well as the initial discharge in seven out of nine neurons ($72.13 \pm 13.98\%$, $P < 0.01$). Neuronal responses to noxious thermal stimulation were unaffected by administration of vehicle ($n = 5$).

3.5. Effect of indomethacin on response to innocuous mechanical stimulation

On neurons responding to innocuous peripheral mechanical stimulation, moderate pressure ($n = 14$) or touch ($n = 14$) evoked transient excitatory effects which were rapid in onset and termination (Fig. 4Aa,C).

The response to pressure was depressed by indomethacin. This effect is illustrated in Fig. 4Aa, where 8 mg/kg of indomethacin decreased the response of a non-nociceptive neuron to pressure stimulation of the cutaneous receptive field. The onset of the inhibitory effect occurred within 2–4 min following administration.

Indomethacin was tested on the responses to pressure of nine non-nociceptive and five wide dynamic range neurons. Responses of two out of three non-nociceptive neurons were depressed by 2 mg/kg of indomethacin ($8.87 \pm 3.10\%$, $P < 0.01$ vs. control), four out of five non-nociceptive and two out of three wide dynamic range neurons by 4 mg/kg indomethacin ($16.04 \pm 3.61\%$, $n = 6$, $P < 0.01$) and two out of two non-nociceptive and two out of four wide dynamic range neurons ($24.17 \pm 8.28\%$, $n = 4$, $P < 0.05$) by 8 mg/kg of indomethacin. Some dorsal horn neurons were insensitive to indomethacin (see Fig. 4Ab). It is also important to note that the inhibitory effect of indomethacin on pressure responses of wide dynamic range neurons was not greater than the inhibitory effect of indomethacin on the responses of non-nociceptive neurons to this stimulus. Generally, the response to pressure recovered 60–100 min after administration of indomethacin.

Vehicle had no effect on responses of neurons to pressure stimulation-induced synaptic input ($n = 5$).

3.6. Effect of indomethacin on response to hair stimulation

Hair stimulation, using an air stream, also produced a transient excitatory response occurring only for the duration of the stimulus. The response to brief pulses of air was tested with indomethacin on three non-nociceptive and three wide dynamic range neurons. Fig. 4Ba shows the response of a non-nociceptive neuron to brief periods of hair movement. Only this neuron was depressed by 4 mg/kg of indomethacin and this occurred in a dose-dependent manner using 4 and 8 mg/kg (22.59 and 45.98 %, respectively; see Fig. 4Bb). The response of this neuron to hair stimulation recovered, approximately, 70 min after indomethacin administration. Vehicle had no effect on the response to hair-induced synaptic input to dorsal horn neurons ($n = 5$).

In three wide dynamic range neurons tested, continuous hair stimulation elicited an on-going increase in the firing frequency. Fig. 5A shows that this excitation was decreased by 93% at 30 min after administration of indomethacin (8 mg/kg, i.v). Fig. 5B shows the recovery of the excitatory effects of continuous application of hair stimulation approximately 90–100 min after the administration of indomethacin.
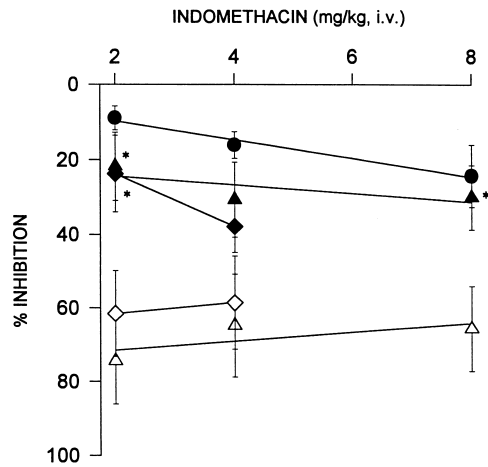
Fig. 6. Dose-dependent reduction of the responses of dorsal horn neurons to innocuous pressure (●, fast initial discharge; $r = 0.50$), noxious pinch (▲, fast initial discharge; $r = 0.30$; △, slowly-decaying afterdischarge; $r = 0.10$) and electrical stimulation of the sciatic nerve (◆, fast initial discharge; ◇, slowly-decaying afterdischarge) by indomethacin. Each symbol (mean ± SEM) represents the maximum percent inhibition (vertical axis) of peripheral stimulation-evoked responses of dorsal horn neurons following intravenous administration of 2, 4 or 8 mg/kg indomethacin (horizontal axis), respectively. *$P < 0.05$ vs. △ or ◇, respectively.

Indomethacin at a dose of 8 mg/kg decreased the response to constant hair stimulation of three wide dynamic range neurons. The excitation was decreased maximally by $91.95 \pm 0.72\%$ at approximately 25 min after administration of indomethacin. Vehicle had no effect on the response to hair-induced synaptic input to dorsal horn neurons ($n = 3$).

3.7. Comparison of effects of indomethacin on noxious and innocuous sensory inputs

Fig. 4Ca shows the effects of touch, pressure and noxious pinch stimuli on the activity of a wide dynamic range neuron. A slowly-decaying afterdischarge remained after the end of application of the noxious pinch stimulus. Indomethacin (8 mg/kg) decreased predominantly the slowly-decaying afterdischarge response of this neuron to the pinch while having only a slight inhibitory effect on the fast initial discharge (Fig. 4Cb). Responses of this neuron to touch and pressure stimuli were also less affected by indomethacin. Fig. 4Cc illustrates the number of spikes per response to touch (T), pressure (Pr) or pinch (P) before and after the administration of indomethacin (8 mg/kg, i.v.). The slowly-decaying afterdischarge of this wide dynamic range neuron was more sensitive to the effect of indomethacin (51.6 % inhibition) than the response to touch (14.6% inhibition), pressure (13.0% inhibition) or the fast initial discharge to the pinch stimulus (16.1% inhibition). Fig. 6 shows that indomethacin selectively inhibits the pinch-induced slowly-decaying afterdischarge.

4. Discussion

This study demonstrates that systemic administration of the cyclooxygenase inhibitor, indomethacin, selectively and dose-dependently depresses nociception-induced responses of spinal dorsal horn neurons, particularly the slowly-decaying afterdischarge of the response to noxious cutaneous stimuli or to high intensity electrical stimulation of the sciatic nerve. The fast initial discharge responses of wide dynamic range neurons to brief noxious or innocuous stimulation, and of non-nociceptive neurons to innocuous stimulation, were depressed considerably less by indomethacin. Importantly, in three wide dynamic range neuron tested, the prolonged excitatory effect of continuous hair stimulation of the receptive was inhibited by indomethacin.

The inhibitory effects of indomethacin on synaptically-induced activation of dorsal horn neurons cannot be accounted for by changes in arterial pressure. Such changes would cause movement of the cord vis-à-vis the electrode tip, resulting in a change in extracellular spike amplitude. However, the extracellular traces had constant spike amplitude throughout experiments, indicating stable recording. In addition, in the present study, blood pressure and respiratory rate remained unchanged following administration of indomethacin.

4.1. Spinal versus peripheral site of action of indomethacin

Indomethacin does not lend itself well to iontophoretic application, and administration was necessarily via a systemic route. This seemed reasonable in view of the report that indomethacin crosses the blood–brain barrier (Bannwarth et al., 1990) and the short time for effects can be seen centrally after systemic administration (Bustamante et al., 1996). However, as application was systemic in the present study, this raises the possibility that at least some of the effects reported here may have been due to a peripheral site of action, particularly in the cutaneous receptive field. COX products of arachidonic acid metabolism can alter the threshold of cutaneous nociceptors in the rat (Taiwo and Levine, 1990). In fact, it has been suggested that prostaglandins are directly involved in sensitizing cutaneous nociceptors (Gold et al., 1994; 1996). While we shall not argue against this possibility, it is reasonable to expect that at least some of the effects seen here were due to an action in the spinal cord. This is substantiated by the fact that we have completed experiments (Pitcher and Henry, 1996) in which indomethacin given systemically depressed the excitatory effects of iontophoretic application of excitatory neurotransmitters onto dorsal horn neurons.

A second line of evidence supporting a role for COX in sensory mechanisms in the spinal cord is that responses to electrical stimulation of the sciatic nerve were inhibited by indomethacin. As this electrical stimulation bypassed cutaneous receptors, the data indicate that at least some of the

inhibitory effects of indomethacin had to be at the level of the spinal dorsal horn.

A third line of support for a central site of action in the present study is the evidence of COX expression in the spinal cord (Beiche et al., 1996; 1998b; Hay and De Belleroche, 1997; Goppelt-Struebe and Beiche, 1997) and of prostaglandin synthase in superficial laminae (Vesin et al., 1995; Vesin, 1996). This evidence provides support for the existence of a mechanism at the spinal level by which the effects of indomethacin can be expressed.

Finally, another reason that the effects may have been due to an action in the spinal cord is physiological evidence that intrathecal administration of NSAIDs blocks nociceptive responses in the formalin test (Malmberg and Yaksh, 1992b; 1993; Morgan et al., 1992) and depresses hyperalgesia in response to intrathecal administration of glutamate and substance P (Malmberg and Yaksh, 1992a).

Thus, while we must entertain the possibility of a peripheral contribution to the present results, this study also strongly leans toward a central involvement of eicosanoids, specifically in mediating the effects of sensory inputs in the spinal dorsal horn in the normal rat.

4.2. COX involvement in on-going versus evoked activity of dorsal horn neurons

The lack of effect of indomethacin, at least with the doses used in this study, on the on-going discharge of dorsal horn neurons may be interpreted to indicate that eicosanoids are normally not involved in mediating or regulating the basal activity or excitability of these neurons. It is not implied that arachidonic acid or prostaglandins are not present during on-going activity, but it is suggested that eicosanoids are not normally synthesized at levels which are functionally significant at least in terms of contribution to or regulating on-going spiking activity.

Responses to synaptic input, on the other hand, were sensitive to indomethacin. The effects of eicosanoids appear to be stimulus-dependent and thus synaptic input may cause eicosanoid synthesis to levels which have physiological effects. Therefore, the effects of COX inhibition would be observed only during the period that eicosanoids are above the basal levels. This possibility is consistent with the data of Malmberg et al. who reported increased prostaglandin E_2-like immunoreactivity from the superfused spinal cord slice upon addition of capsaicin to the perfusate (Malmberg and Yaksh, 1994a).

Thus, from these combined data we propose that eicosanoids may not participate as a major component in mediating or regulating on-going activity, but that indomethacin is expressing its effects mainly upon altered levels of excitability coincident with synaptic input to dorsal horn neurons. It follows that synaptic activation of neurons in the spinal dorsal horn provokes de novo synthesis of eicosanoids presumably via activation of phospholipase A_2 and COX.

4.3. Involvement of COX in nociceptive versus non-nociceptive processing

We report here that indomethacin depresses the responses to both noxious and innocuous stimulation in wide dynamic range neurons, as well as the effects of innocuous stimulation in non-nociceptive neurons. The involvement of eicosanoids in central nociceptive processing is not a novel idea, as indicated above. Different systemically administered COX inhibitors including indomethacin have been shown to dose-dependently decrease responses of thalamic neurons to the effects of high intensity electrical stimulation of C fibers in the hind limb sural nerve (Jurna and Brune, 1990). Various systemically- (Bustamante et al., 1996) and intrathecally- (Bustamante et al., 1997) administered NSAIDs, including indomethacin, also depress the electrical stimulation-induced C fiber reflex in the rat. Therefore, our findings support the idea that eicosanoids are involved in nociceptive mechanisms in the spinal cord.

To the best of our knowledge, a role of eicosanoids in the transmission of nociceptive versus non-nociceptive information has not been investigated directly. One study has demonstrated that while the COX inhibitor, salicylic acid, depressed the activation of single neurons in the thalamus induced by electrical stimulation of C-fibers in the sural nerve (Jurna et al., 1992), no effect of this NSAID was observed on the response to electrically-evoked activation of low-threshold sensory afferents. Although low intensity stimulation was used, presumably to mimic the effect of non-nociceptive input, it is not clear whether or not non-nociceptive mechanisms were actually activated in this earlier study since the thalamic neurons that responded to electrical stimulation of the sural nerve did not respond to touch, gentle stroking or air puffs applied to the skin. Ours, thus, appears to be the first directed specifically to whether COX is associated only with nociceptive mechanisms in the central nervous system.

4.4. Selective involvement of eicosanoids in synaptic input-induced prolonged discharge

While our results show that COX inhibition is not associated exclusively with nociceptive mechanisms at the spinal level it is shown in this study that COX inhibition depresses the long-lasting discharge more than the fast initial discharge of nociceptive dorsal horn neurons in response to noxious stimuli. The transient responses to innocuous stimuli, of both nociceptive and non-nociceptive neurons, were also depressed by COX inhibition, but this depression was of similar magnitude as the depression of the fast initial discharge in response to noxious stimulation.

An explanation for this preferential effect does not come readily to mind. If eicosanoids are synthesized de novo when synaptic input occurs, as we suggest above, then in its simplest form, both types of input should be depressed equally. In the case of the transient responses, however, if

the time of synthesis of eicosanoids is at all delayed, it could quite well be that the synaptically-induced excitation is largely over by the time that eicosanoids reach their maximum level. On the other hand, if the synaptically-induced excitation is more prolonged, then eicosanoids are being synthesized continuously and one would, therefore, expect a greater effect of COX inhibition on the more prolonged types of excitation. However, given the relatively rapid turnover of eicosanoids in neurons (Wolfe and Horrocks, 1994), the data may be interpreted to suggest that, during the transient responses, eicosanoid synthesis is short-lasting, thus yielding presumably a less than maximal level of eicosanoids. In other words, brief excitation in the spinal dorsal horn, evokes limited eicosanoid synthesis. It follows that prolonged synaptically-induced excitation would result in increased eicosanoid synthesis as there is long-lasting activation of the eicosanoid signal transduction pathway. This could apply to any type of prolonged excitation, whether the action of slowly-acting chemical mediators of synaptic transmission or sustained input of fast-acting chemical mediators. This, in turn, would account for the preferential effect on the afterdischarge of the response of nociceptive neurons to noxious stimuli and to high intensity electrical stimulation of sensory fibers. It would also account for the rather strong effect of COX inhibition to the prolonged excitation induced by continuous activation of hair receptors.

The concept of an association between the tonic effects of peripheral stimulation-induced synaptic input and activation of the eicosanoid signal transduction pathway is not without support. For example, it is demonstrated that formalin injection increases the prostaglandin E_2 and excitatory amino acid diasylate levels in the lumbar spinal cord (Malmberg and Yaksh, 1995a,b). Furthermore, the increases in the levels of both of these chemicals correlate temporally with the behavioral nociceptive responses in the formalin test (Malmberg and Yaksh, 1995a). As the long-lasting behavioral effects of noxious formalin injection are attributed to continuous C-fiber afferent activity (Dallel et al., 1995; McCall et al., 1996; Puig and Sorkin, 1996), the finding that the increased diasylate levels of prostaglandin E_2 and excitatory amino acids concurs with the two phases of the formalin test suggests further that eicosanoids may be synthesized de novo and persist only as long as there is on-going primary afferent activity.

In the present study, in the case of the afterdischarge, substance P is a likely candidate mediating this prolonged excitation as administration of the substance P (NK-1) receptor antagonists including CP-96,345 and CP-99,994 have been shown to attenuate this type of afterdischarge (De Koninck and Henry, 1991; Radhakrishnan and Henry, 1991; 1995). Whether the tonic activation of NK-1 receptors is due to tonic release of substance P from primary afferents, presumably from C-fibers, throughout the afterdischarge, to persistence of the ligand in the synaptic cleft, to a slow removal or breakdown of substance P or to any other

mechanism is not specifically revealed in this study. Whichever the case, the tonic effects of substance P and maybe other slow-acting neurochemical mediators may be at least one mechanism which participates in increased activation of the eicosanoid pathway during the noxious stimulation-induced afterdischarge.

In the case of hair stimulation, the response to brief stimulation was affected to a minor degree, while that to sustained stimulation was depressed to a major degree. Both NMDA and non-NMDA receptor activation have been shown to mediate the effects of non-nociceptive inputs to neurons in the spinal dorsal horn (Radhakrishnan and Henry, 1993). Furthermore, we have found that COX participates in the excitatory responses to NMDA, AMPA and quisqualate receptor activation in dorsal horn neurons (Pitcher and Henry, 1996). Thus, the preferential effects of COX inhibition do not seem linked, so much to whether the type of chemical mediating the excitation is fast or slow acting; rather, the effect of COX inhibition is more closely tied to the time course of this excitation; we propose that the time course of de novo synthesis of the eicosanoids is such that they reach physiologically significant levels during the effect of prolonged excitatory input.

5. Conclusions

This electrophysiological study demonstrates that eicosanoids are involved in processing of sensory input in the spinal dorsal horn in the normal rat. It is revealed that indomethacin depresses the excitatory effect of electrical stimulation of the sciatic nerve on wide dynamic range neuronal activity, demonstrating a central mechanism of eicosanoids, specifically in the spinal dorsal horn. As indomethacin has no effect on on-going discharge of single dorsal horn neurons but inhibits the excitatory effects not only of nociceptive but also non-nociceptive peripheral cutaneous stimulation on both wide dynamic range and non-nociceptive neurons, it is suggested that the role of eicosanoids in synaptically-elicited excitation of these neurons involves de novo synthesis of the eicosanoid signal transduction pathway. Although the data suggest that eicosanoids may be involved specifically in the effects of nociceptive rather than non-nociceptive sensory input, the data also suggest that irrespective of the modality of the stimulus, long-lasting synaptically-elicited responses may be selectively mediated by eicosanoids. The finding that expression of COX-2 mRNA and protein in rat spinal dorsal horn neurons is increased in a model of chronic peripheral inflammation (Goppelt-Struebe and Beiche, 1997; Beiche et al., 1998a) suggests that the eicosanoid pathway can become upregulated during the effects of prolonged sensory input. Therefore, we propose that the eicosanoid signal transduction pathway is involved in normal sensory processing but is predominantly involved in the effects of long-lasting synaptic transmission with the

potential to become upregulated. These specific properties of the eicosanoid pathway in the spinal dorsal horn may be the mechanisms underlying the chronic pain conditions observed clinically which are sensitive to the effects of NSAIDs.

Acknowledgements

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