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Prostaglandins Inhibit Endogenous Pain Control Mechanisms by Blocking Transmission at Spinal Noradrenergic Synapses

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Spinal intrathecal injections of the nonsteroidal antiinflammatory analgesics (NSAIAs) indomethacin and acetylsalicylic acid, which inhibit prostaglandin synthesis, cause dose-dependent hypoalgesia in the rat. Intrathecal injections of prostaglandin-E_2 (PGE_2) produce dose-dependent hyperalgesia. To determine whether this action of prostaglandins on the central nervous system is mediated through pain-generating or analgesia pathways, we studied the effect of intrathecal PGE_2 on endogenous opioid-induced analgesia. Intrathecal PGE_2 antagonized the analgesia produced by both brain stimulation and intracerebroventricular morphine. In contrast, the NSAIAs synergized with brain stimulation and morphine-induced analgesia. The alpha-adrenergic antagonist phentolamine and the catecholaminergic selective neurotoxin 6-hydroxydopamine, used to block tonic catecholamine activity in endogenous opioid-mediated analgesia systems, prevented the hyperalgesia induced by intrathecal PGE_2. Phentolamine did not, however, block the hyperalgesia caused by intradermal PGE_2. These findings suggest that prostaglandins can block endogenous opioid-mediated analgesia systems by inhibiting the bulbospinal noradrenergic component of this analgesia pathway.

Nonsteroidal anti-inflammatory agents (NSAIAs), including aspirin (i.e., acetylsalicylic acid) and indomethacin, are effective in a variety of pain syndromes, but are most often used to treat the hyperalgesia or tenderness associated with inflammatory lesions. This hyperalgesia is presumed to originate from the liberation of various inflammatory mediators, mainly prostaglandins, that can sensitize peripheral terminals of primary afferent nociceptors (Ferreira, 1972; Ferreira et al., 1973; Willis and Cornelsen, 1973). NSAIAs inhibit cyclooxygenation of arachidonic acid by inhibiting prostaglandin synthetase, thus preventing the production of hyperalgesia-inducing prostaglandins (Ferreira and Vane, 1974; Moncada et al., 1975).

Lim and colleagues used the crossed-perfused dog spleen preparation to demonstrate that NSAIAs produce analgesia peripherally, not centrally (Lim et al., 1964). Studies using local injection of small amounts of NSAIAs into inflammatory lesions confirmed that there was a peripheral site of action (Ferreira et al., 1978). Subsequent reports, however, indicate that prostaglandins may also contribute to nociception by an action in the central nervous system. For example, noxious stimuli elicit the release of prostaglandins from the spinal cord (Ramwell et al., 1966); low doses of prostaglandins applied via a spinal intrathecal (IT) cannula lower nociceptive thresholds (Ferreira et al., 1978; Yaksh, 1982; Ferreira, 1983); IT administration of low doses of NSAIAs antagonizes peripherally induced nociceptive effects (Ferreira et al., 1978; Ferreira, 1983); and simultaneous administration of NSAIAs into a peripheral inflammatory lesion and into the intracerebroventricular space produces analgesic effects greater than those produced at either site alone (Ferreira et al., 1978).

The site at which prostaglandins exert their central effects on nociception is unknown. It has generally been assumed that they act centrally, as they do peripherally, on neurons that transmit the nociceptive message (Yaksh, 1982). Prostaglandins could, however, act at several spinal sites of nociceptive control, including the recently discovered neural circuitry mediating opioid-induced analgesia. This circuitry includes local spinal enkephalin and dynorphin neurons and serotonin and noradrenaline terminals of descending brain-stem projection neurons (Basbaum and Fields, 1984). Since E-type prostaglandins inhibit noradrenergic neurotransmission (Bergstrom et al., 1973; Hedqvist, 1973), it is possible that the CNS system effects of E-type prostaglandins is produced by their effects on neurotransmitters at the spinal terminals of brain stem noradrenergic neurons.

In this report, we provide evidence to support the hypothesis that the central effects of prostaglandins on nociception are mediated by their interaction with an endogenous, opioid-mediated analgesia system, and that this action of prostaglandins is on the bulbospinal noradrenergic component of the system.

Materials and Methods

The experiments were performed on 250–350 gm male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA), which were implanted with a spinal cannula in the lumbar intrathecal (IT) space and stereotaxically either with a 22-gauge stainless steel guide in the third ventricle or with a bipolar stimulating electrode (Plastic Products, Roanoke, VA) placed in the nucleus reticularis paragigantocellularis (NRPG) (Satoh et al., 1980). The location of cannulae and electrodes was histologically confirmed.

The substances used were prostaglandin-E_2 (PGE_2), the prostaglandin-synthesis inhibitors indomethacin and acetylsalicyclic acid (Sigma, St. Louis, MO), morphine sulfate (Elkins Sinn, Cherry Hill, NJ), the alpha-adrenergic receptor antagonist phentolamine (Ciba-Geigy, Summit, NJ), and the noradrenergic-specific neurotoxin 6-hydroxydopamine (6-OHDA; Calbiochem-Behring, La Jolla, CA). PGE_2 was dissolved in a

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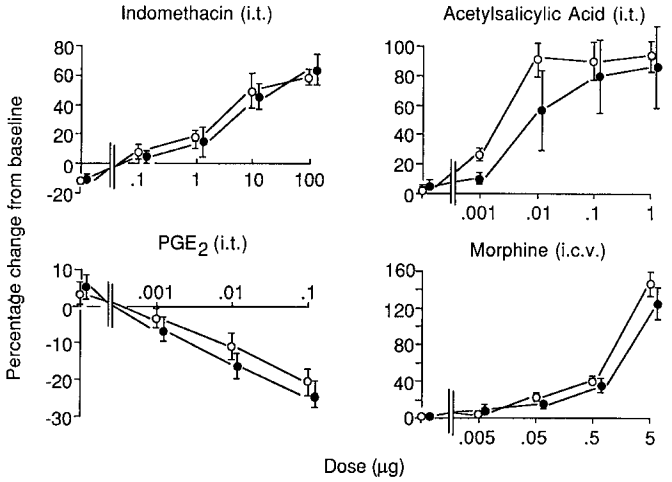

Figure 1. Dose-dependence relationships of the effects on paw-withdrawal (○) and tail-flick (●) thresholds of intrathecal (IT) NSAIAs (indomethacin, $n = 8$ for both; acetylsalicylic acid, $n = 18$ and $n = 9$, respectively), prostaglandin E_2 (PGE_2) (both, $n = 14$) and intracerebroventricular (ICV) morphine ($n = 32$ and $n = 16$, respectively). The response is calculated as a percentage change from baseline nociceptive threshold ($\bar{X} \pm 1$ SEM). The symbols for the responses in the tail-flick test (●) are displaced slightly to the right for clarity.

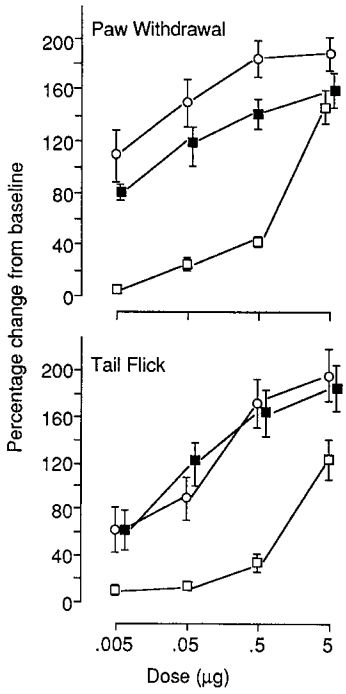

Figure 2. Dose-dependence relationships of the effects of ICV morphine (□) ($n = 32$ and $n = 16$, respectively) and the combinations of ICV morphine with IT indomethacin (10 μg) (○) ($n = 12$ and $n = 9$, respectively) or with acetylsalicylic acid (1 ng) (■) ($n = 10$ and $n = 9$, respectively) on nociceptive threshold.

vehicle of 10% ethanol in saline. Indomethacin was dissolved in 2% sodium bicarbonate and then titrated to pH 7.2 with monosodium phosphate and 6-OHDA in saline containing 1 mg/ml ascorbic acid. The remaining drugs were dissolved in saline. Spinal IT injections were in a volume of 10 μl, followed by a 10 μl flush (i.e., the volume of the cannula) with vehicle, and intracerebroventricular (ICV) injections were in a volume of 1 μl. NRPG stimulation-produced analgesia was elicited with a variable-intensity 50 Hz train of 500 μsec monophasic pulses. The stimulating electrode consisted of a pair of 25 μm insulated stainless steel wires (Plastic Products). The stimulus was turned on 10 sec prior to testing nociceptive thresholds. The threshold for stimulation-produced analgesia was 134 ± 9 μA ($n = 39$) ($\bar{X} \pm 1$ SEM) in the control condition. After completion of the experiments, an electrolytic lesion was made to allow histological confirmation of the site of stimulation. The destruction of bulbospinal noradrenergic projection neurons by ICV 6-OHDA was confirmed by radioenzymatic assay of noradrenaline (DaPrada and Zurcher, 1976). ICV 6-OHDA produced a decrease of 77% in noradrenaline at the lumbosacral level of the spinal cord.

Two tests of nociception—the thermal tail-flick test (D'Amour and Smith, 1941) and the mechanical Randall-Selitto paw-withdrawal test (Randall and Selitto, 1957)—were employed in awake, restrained rats. To prevent thermally induced injury to the tail in analgesic rats, a cutoff of 20 sec, which has previously been shown to be noninjurious (Levine et al., 1980), was used. In the paw-withdrawal test, to prevent mechanically induced injury, a cutoff of 300 gm was used. The nociceptive thresholds were defined as the latency in seconds from the onset of the thermal stimulus to tail-flick and the weight in grams that elicited paw-withdrawal. The rats were trained in the nociceptive tests daily for the week prior to gathering data. On the day of the experiment, baseline nociceptive thresholds were measured before the various pharmacological and physiological manipulations were made. Responses to test agents were calculated as the percentage change from baseline threshold. Rats that went to cutoff (i.e., did not respond to the noxious stimulus) were assigned the cutoff value as their nociceptive threshold, for statistical comparisons. We used percentage change in threshold in calculating the effect of treatments in order to compensate for the differences in baseline threshold between groups. In addition, for all groups of rats whose responses to drugs were compared, there were no significant differences, by ANOVA, in baseline nociceptive threshold. Dose dependencies were tested by generating the best least-squares linear curve that fit the data and testing whether its slope differed significantly from zero. Other statistical comparisons were made by t tests corrected for multiple comparisons by Bonferroni's inequality.

Results

The independent effects of NSAIAs (i.e., indomethacin and acetylsalicylic acid), IT prostaglandins, and ICV morphine on nociceptive thresholds were first tested. IT administration of indomethacin and acetylsalicylic acid produced a significant dose-dependent analgesia (all p's < 0.01; see Fig. 1). Equivalent doses of indomethacin, given systemically (i.e., 0.5 mg/kg and 4.0 mg/kg, i.p.), did not significantly affect paw-withdrawal thresholds (7.7 ± 4.3%; $n = 6$ and 5.7 ± 3.5%; $n = 6$, respectively). IT PGE_2 produced a significant dose-dependent hyperalgesia in both tail-flick and paw-withdrawal tests (both p's < 0.01; see Fig. 1). Activation of pain control circuits by ICV injection of morphine (5 μg) produced a much greater analgesia than did the NSAIAs in both tail-flick and paw-withdrawal tests (Fig. 1). When IT PGE_2 (100 ng), but not its vehicle, was administered either 20 min before or 20 min after the ICV morphine, analgesia was prevented and blocked, respectively (p = n.s.; see Table 1). Submaximal doses of IT indomethacin (10 μg) and acetylsalicylic acid (1 ng) markedly synergized with ICV morphine, producing a shift to the left in the dose-dependence relationship for ICV morphine-induced analgesia. In fact, the analgesia produced by 0.05 μg of morphine, in combination with either acetylsalicylic acid or indomethacin, was significantly greater than the analgesia produced by 0.5 μg of morphine alone (all p's < 0.001) (see Fig. 2). Furthermore, injection of PGE_2 (100 ng, IT) 20 min before administration of a combination of ICV morphine and IT indomethacin prevented the development of analgesia (p = n.s.; see Table 1). These results are compatible with the hypothesis that release of prostaglandins in the spinal cord tonically modulates opiate-induced activation of pain control circuits.

Table 1.

Treatment(s) (Route of administration)	% Change in nociceptive thresholds (20 min posttreatment, $\bar{X} \pm$ SEM)	
	Tail-flick	Paw-withdrawal
Morphine (ICV)		
0 μg (saline)	+1.8 ± 2.1 (n = 12)	+2.1 ± 1.6 (n = 12)
5 μg	+124.3 ± 17.4 (n = 16)	+146.8 ± 12.9 (n = 32)
+ PGE_2, 100 ng (IT)		
Premorphine	−4.6 ± 2.7 (n = 12)	−4.7 ± 2.4 (n = 12)
Postmorphine	−2.1 ± 4.1 (n = 6)	−1.2 ± 3.5 (n = 6)
0.5 μg	+33.8 ± 8.2 (n = 16)	+41.7 ± 4.8 (n = 32)
+ Indomethacin, 10 μg (IT)	+156.3 ± 14.5 (n = 9)	+183.6 ± 14.3 (n = 12)
+ PGE_2, 100 ng (IT)	−3.5 ± 1.8 (n = 12)	−8.2 ± 2.1 (n = 12)
Morphine, 5 μg (ICV)		
+ Phentolamine, 1 ng (IT)	+13.5 ± 10.8 (n = 5)	+9.4 ± 11.2 (n = 10)
Phentolamine, 1 ng (IT)		
+ PGE_2, 100 μg (IT)	−2.5 ± 2.5 (n = 6)	+3.1 ± 1.9 (n = 12)
+ PGE_2 100 μg (ID)	n.a.[a]	−23.0 ± 2.7 (n = 12)
+ Indomethacin, 100 μg (IT)	+0.5 ± 1.8 (n = 6)	−4.2 ± 1.6 (n = 6)
6-Hydroxydopamine 250 μg (IT)		
+ PGE_2, 100 ng (IT)	−2.2 ± 2.4 (n = 6)	−2.1 ± 2.7 (n = 6)
+ Indomethacin, 10 μg (IT)	−3.2 ± 3.2 (n = 6)	−2.1 ± 3.7 (n = 6)
6-Hydroxydopamine, 250 μg (ICV)		
+ PGE_2, 100 ng (IT)	+2.1 ± 2.6 (n = 12)	+11.7 ± 2.4 (n = 12)
+ Indomethacin, 10 μg (IT)	+3.1 ± 4.9 (n = 12)	−2.1 ± 6.4 (n = 12)

[a] Not applicable.

Activation of pain control circuits by electrical brain stimulation in the NRPG also produced potent analgesia in both paw-withdrawal and tail-flick tests. In a group of rats that had a baseline paw-withdrawal threshold of 124.0 ± 6.0 gm (n = 36) and a tail-flick latency of 5.5 ± 0.2 sec (n = 25), brain stimulation (134 ± 9 μA; n = 39) produced analgesia with thresholds greater than cutoff in all rats. IT PGE_2 (100 ng) returned paw-withdrawal threshold and tail-flick latency to baseline (122.0 ± 4.0 gm and 4.8 ± 1.2 sec, respectively) when tested 20 min after administration. On the other hand, indomethacin (10 μg), injected IT, significantly decreased the current required to produce this level of analgesia by 43 ± 3.5% (from 179.7 ± 14.3 to 97.5 ± 11.5 μA; n = 16, $p < 0.001$). The first of these 2 experiments demonstrates that brain stimulation-produced analgesia can be blocked by PGE_2, and the second provides further evidence that there is a tonic release of cyclooxygenase products that influences descending inhibitory controls.

To evaluate whether the central effects of PGE_2 and indomethacin on descending controls are partly mediated by an action on the terminals of bulbospinal noradrenergic neurons, we used phentolamine (IT) and 6-OHDA (IT and ICV) to block or eliminate the terminals of the noradrenergic projection neurons. By themselves, the IT administration of phentolamine and 6-OHDA and the ICV administration of 6-OHDA decreased nociceptive thresholds in the tail-flick and paw-withdrawal tests (phentolamine: −9.1 ± 1.7% and −15.2 ± 2.4%, both n = 6; $p < 0.6$ and $p < 0.0009$, respectively; IT 6-OHDA: −8.2 ± 3.8% and −20.0 ± 4.3%, both n = 6; $p < 0.025$ and $p < 0.003$, respectively; ICV 6-OHDA: −31.2 ± 3.5% and −36.2 ± 4.4%, both n = 12; both p's < 0.025). The analgesia produced by ICV morphine (5 μg) was significantly antagonized by prior IT phentolamine (1 ng; ($p < 0.025$; see Table 1). In addition, this IT dose of phentolamine administered prior to PGE_2 (100 μg) or indomethacin (10 μg) prevented their effects on nociceptive thresholds. On the other hand, intradermal (ID) injection of PGE_2 still produced hyperalgesia in phentolamine-treated rats ($p < 0.01$). Thus, the ability of IT phentolamine to block the effects of PGE_2 and indomethacin on nociception does not appear to be due to ceiling effects of phentolamine on pain transmission neurons, but rather to an effect on spinal noradrenergic synapses. 6-OHDA-induced destruction of spinal noradrenergic terminals, either by IT or ICV injection (250 μg, 2 d prior to experiments; Bosland et al., 1981), also blocked the effects of PGE_2 and indomethacin on nociceptive thresholds, thus providing further evidence that PGE_2 inhibits spinal noradrenergic synapses in pain control circuits (see Table 1).

Discussion

These studies confirm a central effect of PGE_2 and prostaglandin-synthesis inhibitors (Ferreira et al., 1978; Yaksh, 1982) on nociception. In this study, indomethacin vehicle itself produced a significant hyperalgesia. This may underestimate the potency of indomethacin in these experiments and may thus explain why acetylsalicylic acid appears to be more potent. Our finding that IT PGE_2 blocks both stimulation-produced analgesia and the analgesia produced by supraspinally administered opiates strongly suggests that prostaglandins block bulbospinal projection neurons that operate in pain control circuits. Prostaglandin-synthesis inhibitors injected IT act synergistically with ICV morphine and electrical brain stimulation-produced analgesia, suggesting that there is tonic production of prostaglandins in the spinal cord that can affect activity in these circuits. Furthermore, blockade of noradrenergic synaptic transmission in the spinal cord eliminates the effects of IT PGE_2, acetylsalicylic acid, and

indomethacin on nociception, suggesting that these spinal effects of prostaglandins are mediated by inhibition of noradrenergic synaptic transmission in the spinal terminals of brain-stem projection neurons. This observation is also consistent with the known inhibition of the synaptic release of norepinephrine by prostaglandins (Bergstrom et al., 1973). We conclude that PGE_2 inhibits pain control circuits in the central nervous system by blocking the release of noradrenaline from the spinal terminals of brain-stem projection neurons. That these central effects of prostaglandins are, in fact, clinically important is suggested by a report that IT administration of low doses of salicylates in patients with chronic pain produced analgesia without concomitant sensory, motor, or autonomic side effects (Devoghel, 1983). Experiments are currently in progress to determine the source of the endogenous prostaglandins in the central nervous system and the mechanisms that control their production and release.

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