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Effect of intrathecal administration of serotonin in chronic pain models in rats

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Abstract

The present study examined the effects of intrathecal (i.t.) administration of 5-hydroxytryptamine (5-HT; 0.1–100 μg) on mechanical hyperalgesia associated with neuropathic pain (chronic constriction of the sciatic nerve model and diabetic model) and inflammatory pain (carrageenan and polyarthritic models) in rats. Results demonstrated that the hyperalgesia observed in the mononeuropathic and diabetic rats was attenuated by 5-HT; the active dose, however, was 100- to 1000-fold higher than that required in normal rats, and was moderately effective. In the two experimental models of inflammatory pain, 5-HT was not markedly or similarly active. In the carrageenan model, 5-HT at the highest dose was only weakly effective whereas in the polyarthritic model it was inactive.

Together, these results show that 5-HT has antinociceptive effects in several rat pain models, except in the model of diffuse pain (polyarthritic rats). Its antinociceptive effects in these models, however, are slight and differ from those observed in normal rats.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Chronic pain; Spinal cord; Paw pressure test; (Rat)

1. Introduction

While the antinociceptive effect produced by serotonin (5-HT, 5-hydroxytryptamine) has been well documented mainly after i.t. administration and in normal animals (Yaksh and Wilson, 1979; Crisp et al., 1991a,b; Bardin et al., 1997), very few studies have considered the effects of this monoamine in experimental models of pain. However, taking into account that tricyclic antidepressants (which inhibit the reuptake of both 5-HT and noradrenaline) are used in the treatment of chronic pain in humans (Onghena and Van Houdenhove, 1992; McQuay et al., 1996; Eschalier et al., 1999) and induce antinociceptive effects against neuropathic and inflammatory pain in rats (Ardid et al., 1991; Ardid and Guilbaud, 1992; Bianchi et al., 1995; Esser and Sawynok, 1999; Sawynok et al., 1999), a study of the effects of serotonin in conditions of chronic pain needs to be considered. We have, therefore, chosen to determine the spinal antinociceptive effect of 5-HT in several rat models of clinical pain to determine and compare its effect according to the aetiology of the pain.

The following models of neuropathic and inflammatory pain, which are widely used to examine anti-inflammatory and analgesic drugs, and which, based on behavioural, pharmacological and electrophysiological evidence, appear to represent appropriate models to evaluate "clinical" pain and analgesic activity, were used: (1) a model of persistent neuropathic pain produced by chronic constriction injury of the sciatic nerve (CCI model; Bennett and Xie, 1988); (2) a model of chronic pain with signs of hyperalgesia and allodynia in rats rendered diabetic by an intraperitoneal injection of streptozocin (Courteix et al., 1993); (3) a model of more localized and less persistent inflammatory pain in rats produced by an intraplantar injection of carrageenan (Winter et al., 1962) and (4) a model of diffuse, persistent inflammatory pain in rats rendered polyarthritic by an injection of Freund's adjuvant in the tail (Pearson and Wood, 1959).

Mechanical hyperalgesia was examined by measuring the vocalization threshold to pressure applied to the hind

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paw, which is considered to be a centrally integrated test (Kayser and Guilbaud, 1990).

2. Materials and methods

All the experiments were conducted in accordance with the guidelines on ethical standards for investigations of experimental pain in animals defined by the International Association for the Study of Pain (IASP; Zimmermann, 1983). In particular, the duration of the experiments was as short as possible and the number of animals was kept to a minimum.

2.1. Animals

Male Sprague–Dawley rats (Charles River, St-Aubin-Lès-Elbeuf, France) weighing 200–250 g were used. On arrival at the laboratory, rats were allowed to acclimate for 1 week, in groups of five rats per cage, with free access to food and water.

2.2. Neuropathic pain models

2.2.1. Chronic constriction injury: CCI model

Neuropathy was produced in the right hind paw, according to the method described in detail by Bennett and Xie (1988). Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and four chromic gut (5–0) ligatures were tied loosely (with about 1 mm spacing) around the common sciatic nerve. The nerve was constricted to a barely discernible degree, so that circulation through the epineurial vasculature was not interrupted. The same surgery was performed on the opposite site, without ligating the sciatic nerve (sham procedure). The rats were tested 2 weeks after the sciatic nerve ligature, when the pain-related disorders were at their maximum (Attal et al., 1990).

2.2.2. Induction of diabetes

The rats were rendered diabetic by an i.p. injection of 75 mg/kg streptozocin (Zanosar, Upjohn) dissolved in distilled water (Courteix et al., 1993). Four weeks later, the presence of diabetes was confirmed by measurement of tail vein blood glucose levels with Ames Dextrostix and a reflectance colorimeter (Ames Division, Miles Laboratories). Blood samples were obtained from the tail by pinprick, and only rats with a final blood glucose level > 14 mM were included in the study.

2.3. Inflammatory pain models

2.3.1. Carrageenan-evoked inflammation

Carrageenan (λ-carrageenan, Sigma, France) was used to induce unilateral paw inflammation. The rats received a s.c. injection of carrageenan (0.2 ml of a 2% solution of the polysaccharide in saline) in the plantar surface of the left hind paw. The rats were tested 2 h later, when both oedema and hyperalgesia had developed (Kayser and Guilbaud, 1987).

2.3.2. Adjuvant-induced arthritic pain model

Polyarthritis was induced by injecting 0.05 ml of heat-killed *Mycobacterium butyricum* (5 mg/ml suspended in mineral oil) intradermally into the tail base as described previously (Colpaert, 1987). The rats were tested 4 weeks later, when symptoms were at their maximum (Calvino et al., 1987; Colpaert, 1987).

2.4. The Randall–Selitto mechanical hyperalgesia test

The antinociceptive effect was determined by measuring the vocalization threshold elicited by pressure on the left hind paw (both hind paws in the CCI and carrageenan models), using the Ugo Basile analgesimeter. This instrument generates a linearly increasing mechanical force applied by a dome-shaped plastic tip (diameter: 1 mm) placed on the dorsal surface of the rat's hind paw. The force was applied until the rat squeaked. This centrally integrated response is especially sensitive to analgesic drug effects, particularly in the rat models used here (Ardid and Guilbaud, 1992; Attal et al., 1991; Kayser and Guilbaud, 1987, 1990).

2.5. Intrathecal injection and experimental design

Intrathecal injections of 5-HT or vehicle were performed as previously described by Mestre et al. (1994). Briefly, the rat was held in one hand by the pelvic girdle, and a 25-gauge $\times$ 1 in. needle connected to a 25-μl Hamilton syringe was inserted between the spinous processes of L5 and L6 into the subarachnoidal space, until a tail flick was elicited. The syringe was held in position for several seconds after the injection (injection volume: 10 μl/rat).

The experiments were performed blind in a quiet room by a single experimenter using the method of equal blocks with randomization of treatments. Vocalization thresholds were determined before the induction of persistent pain, except for the arthritic rats, and in all groups after the induction of persistent pain, 5, 15, 30, 45 and 60 min after an i.t. injection of either 5-HT (0.1, 1, 10 or 100 μg) or saline (NaCl 0.9%). 5-HT (Sigma, France) was dissolved in physiological saline (NaCl 0.9%). Solutions were prepared immediately before testing.

2.6. Data analysis and statistics

Vocalization thresholds to paw pressure are given in grams. Data are expressed as means $\pm$ S.E.M. The maximal percent effect (MPE) was calculated, at the peak of the time–response curve as follows: [(maximal postdrug value − predrug value)]/[(cut-off value − predrug value)] × 100.

The cut-off value corresponds to the maximal pressure that the apparatus applied (750 or 500 g for the neuropathic pain and inflammatory pain models, respectively). The time course of the effects of the various treatments was analyzed statistically by means of an analysis of variance (ANOVA) followed by Dunnett's test. The significance level was $P < 0.05$.

3. Results

No behavioural modification was induced by the four doses of 5-HT whatever the model used. The lowest two doses of 5-HT (i.e. 0.1 and 1 μg) did not produce antinociceptive effects in any of the four models (data not shown).

3.1. Effects of 5-HT in the CCI model

Before surgery, the vocalization threshold did not differ significantly between the hind paws: 275 ± 5 and 278 ± 6 g for the nerve-ligated and sham-operated hind paws, respectively. Two weeks after surgery, the vocalization threshold for the ligated side (i.e. 185 ± 5 g) was significantly ($P < 0.05$) and markedly ($-33 \pm 2\%$) decreased (Fig. 1A). The vocalization threshold for the non-ligated side was moderately decreased to 245 ± 3 g ($P < 0.05$). The injection of saline did not influence the vocalization threshold to paw pressure on either hind paw, but 5-HT, at the highest dose (100 μg), significantly increased the vocalization threshold for both hind paws (Fig. 1A). The MPE was $+39 \pm 8\%$ and $+44 \pm 12\%$ for the ligated and the sham-operated hind paws, respectively. The antinociceptive effects were apparent 5 min after the injection and lasted 45 min for the ligated paw and 30 min for the sham-operated paw.

3.2. Effects of 5-HT in diabetic rats

Four weeks after the induction of diabetes, the vocalization threshold was significantly decreased by $-27 \pm 1\%$ compared to the preinduction value (206 ± 4 g vs. 281 ± 3 g) (Fig. 1B). Injection of saline did not influence the vocalization threshold. 5-HT, at 10 and 100 μg, significantly and dose-dependently increased the vocalization threshold (Fig. 1B). The MPE was $+23 \pm 8\%$ and $+37 \pm 8\%$ for 10 and 100 μg, respectively, 5 min after the injection. The effects lasted 60 min.

3.3. Effects of 5-HT on carrageenan-induced inflammatory pain

The intraplantar injection of carrageenan into the right hind paw significantly decreased ($-37 \pm 1\%$) the vocalization threshold for the injected paw (141 ± 2 g) compared with the pre-carrageenan control (223 ± 2 g) (Fig. 2A). Contralateral hyperalgesia was also observed ($-20 \pm$

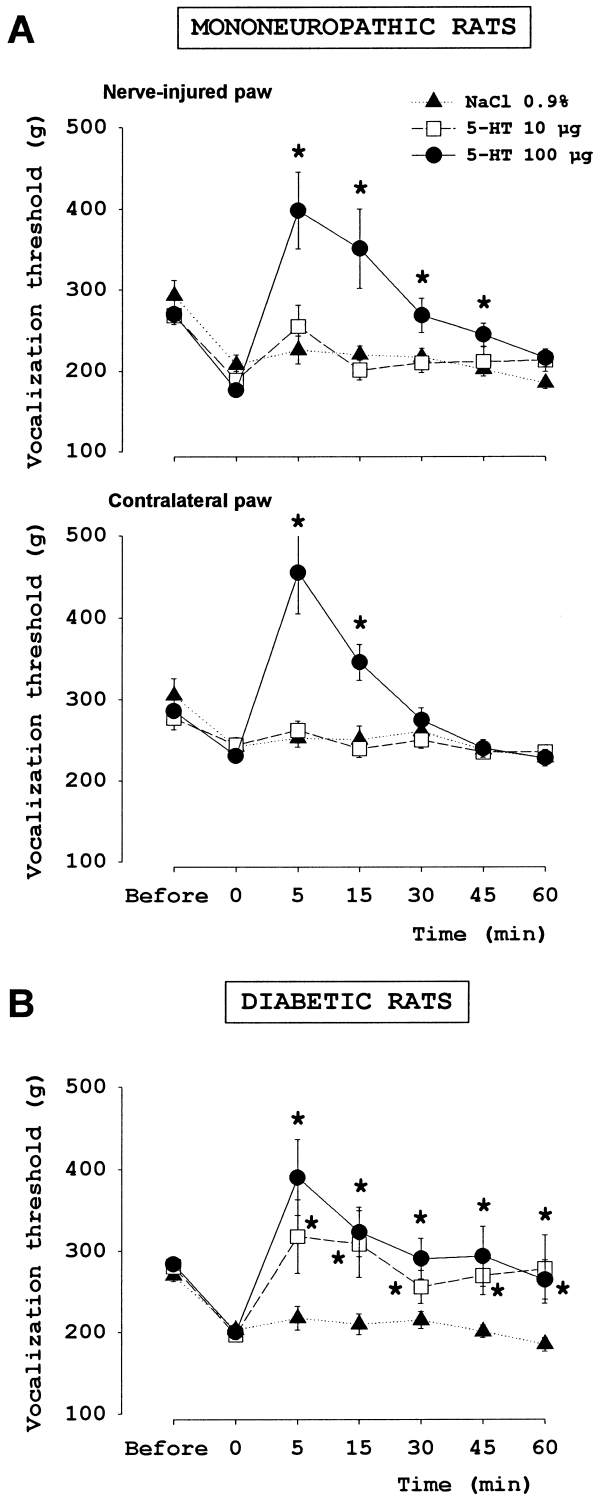

Fig. 1. Time course of the effect of i.t. injection of 5-HT on mechanical pain threshold in mononeuropathic (A) and diabetic (B) rats. Vocalization threshold determined before (0) and after drug injection is expressed as grams (g). Bars = mean ± S.E.M.; ($n = 10$ per group). * $P < 0.05$ versus saline-treated group.

2%) (Fig. 2A). A slight decrease of the vocalization threshold for the inflamed paw was observed at the end of the experiment in the saline-treated group, which may be due to repeated testing. The i.t. administration of 5-HT

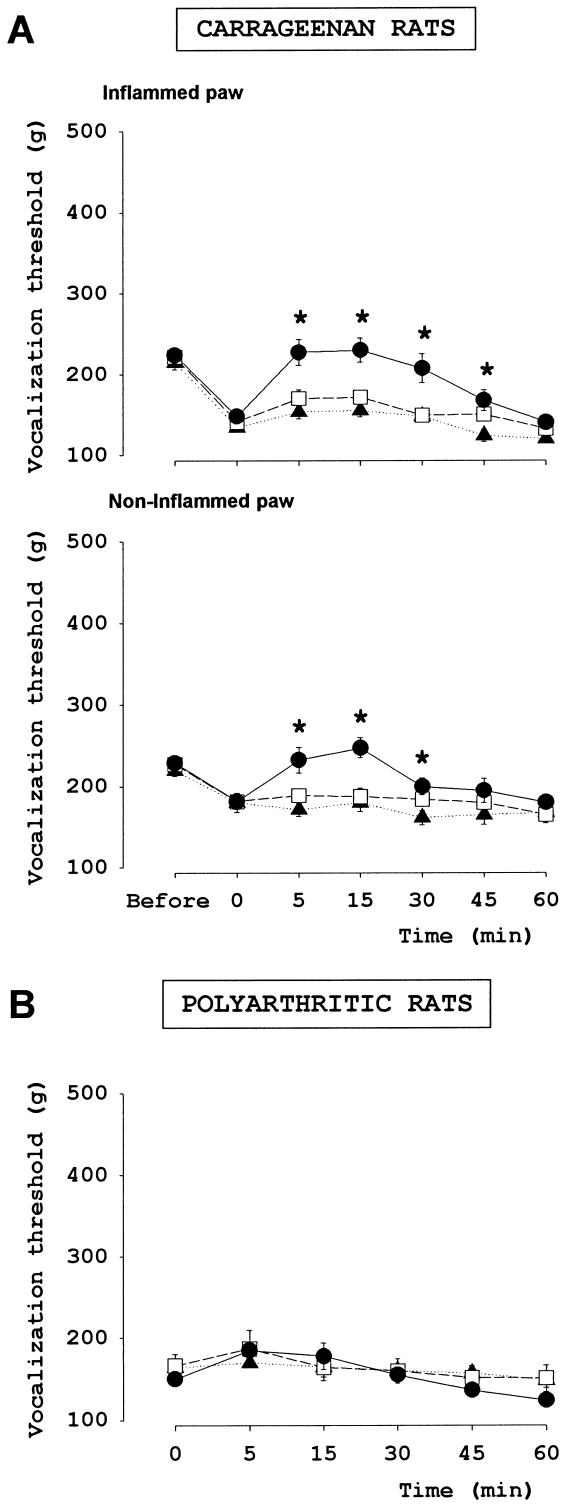

Fig. 2. Time course of the effect of i.t. injection of 5-HT on mechanical pain threshold in carrageenan (A) and polyarthritic (B) rats. Vocalization threshold determined before (0) and after drug injection is expressed as grams (g). Bars = mean ± S.E.M.; ($n = 8$ per group). $^{*}P < 0.05$ versus saline-treated group.

failed to produce a significant effect at the dose of 10 μg but induced a significant increase at the highest dose (100 μg) for both hind paws (Fig. 2A). The MPE was +24 ± 3% and +21 ± 3% for the inflamed and contralateral paws, respectively. The antinociceptive effects appeared 15 min after the injection and lasted 45 min for the inflamed paw and 30 min for the contralateral paw.

3.4. Effects of 5-HT in polyarthritic rats

Neither the injection of saline nor the injection of 5-HT at the doses used had any statistically significant effect (Fig. 2B).

4. Discussion

This study examined the antinociceptive potency and efficacy of spinally administered 5-HT in several rat models of persistent pain. The results show that 5-HT had antinociceptive effects in all models, but not in a model of diffuse pain (polyarthritic rats) (Table 1). Its effects, however, were less marked and occurred only at doses that were 100- to 1000-fold higher than those producing antinociception in normal rats (Table 1; Bardin et al., 1997).

4.1. Effects of 5-HT on neuropathic pain

In rats with persistent neuropathic pain-related disorders, the i.t. administration of 5-HT had antinociceptive effects, as evidenced by its ability to attenuate mechanical hyperalgesia in mononeuropathic and diabetic rats. These results confirm and extend previous findings that 5-HT is active against pain in normal rats (Yaksh and Wilson, 1979; Solomon and Gebhart, 1988; Crisp et al., 1991a,b; Alloui et al., 1996) and in the CCI model in rats (Eaton et al., 1997). The present results, however, suggest that 5-HT is less potent and less efficacious in this chronic pain

Table 1
Maximal percent of effect (MPE) of i.t. 5-HT on mechanical pain threshold in different experimental models of pain

	Doses (μg)	MPE (%)[a]
Normal rats[b]	0.1	+23 ± 3
	1	+42 ± 7
	10	+72 ± 7
Mononeuropathic	10	ns
(ligatured hindpaw)	100	+39 ± 8
Diabetic	10	+23 ± 8
	100	+37 ± 8
Carrageenan-induced	10	ns
hyperalgesia (inflammed paw)	100	+24 ± 3
Polyarthritic	10	ns
	100	ns

[a] The MPE was calculated, at the peak of the time course curves, as follows: [(maximal postdrug value − predrug value)]/[(cut-off value − predrug value)] × 100. ns = no significant.
[b] From Bardin et al., 1997.

model. Its active dose was up to 1000-fold higher in mononeuropathic and diabetic rats than in normal rats. Moreover, its active dose was less effective, because the maximal analgesia obtained with 100 μg was $+39 \pm 8\%$ and $+37 \pm 8\%$ in mononeuropathic and diabetic rats, respectively, and $+72 \pm 7\%$ after 10 μg in normal rats. The low potency and efficacy of 5-HT to produce antinociceptive effects in models of persistent pain suggest that serotonergic systems may not be strongly involved in chronic pain. Another reason to question the importance of 5-HT was the inability of low doses, which were active in normal rats, to induce antinociceptive effects in the sham-operated paw in mononeuropathic rats. This finding is similar to that reported by Anderson and Goodchild (1996), who found that 5-HT, which potently produced antinociceptive effects (measured by means of an electrical test) in normal rats, failed to produce antinociceptive activity in mononeuropathic rats. Also, several studies have shown a poor sensitivity and efficacy of serotonergic antidepressants against experimental (Courteix et al., 1994) and clinical (Max et al., 1992) neuropathic pain, as confirmed by the meta-analysis of the clinical efficacy of antidepressants (Onghena and Van Houdenhove, 1992; McQuay et al., 1996). Moreover, Kishore-Kumar et al. (1989) found buspirone to be ineffective in the treatment of human neuropathic pain. The mechanisms underlying the limited antinociceptive efficacy of 5-HT are certainly complex, and few explanations have been proposed to date. An alteration of the serotonergic system might be related to this reduced sensitivity. Recently, Padayatti and Paulose (1999) reported that diabetic rats showed a decreased affinity for [^{3}H]5-HT binding to 5-HT$_1$ receptors in the brain. They also reported changes in the synthesis and metabolism of 5-HT serotonergic nerves. In the same model, the supraspinal level of 5-HT was found to be decreased, whereas the number of 5-HT$_{1A}$ and 5-HT$_2$ receptors had increased (Sandrini et al., 1997). Also, a decreased release of 5-HT from the bulbospinal pathways (Suh et al., 1996), which are known to be activated by morphine (Hammond, 1990), has been reported in diabetic rats. Other mechanisms may also account for the reduced efficacy of 5-HT. The development of neuropathic pain is thought to involve the reorganization of primary afferents fibres, central sensitization and loss of inhibitory interneurons (Coderre et al., 1993; Ochoa, 1994; Goff et al., 1998). Recent studies confirmed that different 5-HT receptors are present on the terminals of primary afferents fibres (see Hamon and Bourgoin, 1999). A reduction in the number of presynaptic 5-HT receptors, or a reorganization of serotonergic fibres, could also explain the lower efficacy of 5-HT in neuropathic pain. It is worth noting that morphine is also less effective in models of neuropathic pain, and this loss of effectiveness has been attributed to a reduction in presynaptic opioid receptors resulting from degeneration of primary afferent fibres subsequent to nerve damage (Yaksh et al., 1995). In addition, reduced activity of γ-amino-butyric acid (GABA)-ergic interneurons, which may play a key role in mediating the effects of 5-HT in the dorsal horn (Alhaider et al., 1991; Peng et al., 1996; Millan, 1997; Yang et al., 1998), has also been shown in neuropathic pain (Dray et al., 1994; Ibuki et al., 1997). These observations taken together can therefore be advanced to explain the weak analgesic effect of 5-HT against neuropathic pain.

4.2. Effects of 5-HT on inflammatory pain

The results obtained with the two experimental models of inflammatory pain provide evidence that 5-HT, injected i.t., is not markedly or similarly active. In the model of sub-acute inflammatory pain, 5-HT was only weakly active and it was inactive against persistent inflammatory pain. The disparity between the results obtained in the two inflammatory models may be due to a difference in the intensity of inflammatory injury. In addition, it has been shown recently that the mechanical stimulus–response function of C fibres is different in inflammatory hyperalgesia and in neuropathic mechanical hyperalgesia (Ahlgren et al., 1997). Nevertheless, the inhibitory effect of 5-HT observed here in the rat carrageenan model is consistent with reports of antinociceptive activity of the selective serotonin reuptake inhibitors clomipramine (Ardid et al., 1991) and fluoxetine (Bianchi et al., 1995) in the same model. However, as in the neuropathic pain models, the active dose of 5-HT to reduce carrageenan-induced hyperalgesia was higher than the active dose in normal rats (Table 1) and was less effective: the maximal analgesia observed with 100 μg of 5-HT was $+24 \pm 3\%$ in carrageenan-induced hyperalgesia, but was $+72 \pm 7\%$ after 10 μg in normal rats. Further, only the highest dose was effective in the non-injected paw. To date, there is a lack of detailed information about the influence of 5-HT in nociceptive processing in the spinal cord in inflammatory pain models. It is conceivable, however, that the present results obtained with 5-HT in inflammatory pain models might also be related to changes in serotonergic systems. In polyarthritic rats, an increased synthesis of 5-HT in the spinal cord has been reported (Godefroy et al., 1987; Li et al., 1999). Enhanced serotonergic activity may modulate spinal 5-HT receptors and/or their signal transduction pathways in a manner that could lead to a reduced effectiveness of i.t. injected 5-HT in these models. However, some studies have suggested that chronic inflammatory pain directly activates the serotonergic descending pathways or indirectly evokes the release of endogenous opioid substances (enkephalin or endorphin) to alleviate pain (Kayser and Guilbaud, 1983; Ossipov et al., 1997; Li et al., 1999). In this and in other inflammatory pain models, the tissue levels of substance P (Lembeck et al., 1981; Colpaert et al., 1983; Schoenen et al., 1985) in the spinal cord are significantly increased. Interestingly, increased release of substance P at the spinal level induces long-term

inhibition of the neuronal responses to 5-HT (Eide and Hole, 1991), and in vitro experiments have shown that substance P decreases the affinity of [^{3}H]5-HT binding sites (mainly 5-HT$_{1A}$ receptors) in membrane preparations from the rat spinal cord (Agnati et al., 1983). Changes in inhibitory control have been observed also at supraspinal and spinal levels (Calvino et al., 1987; Guilbaud, 1988). Based on these considerations, as in neuropathic pain, changes in serotonergic systems might underlie the weak activity, or lack of activity, of i.t. injected 5-HT against inflammatory pain.

In summary, the present results show that 5-HT has only weak analgesic activity in models of persistent, neuropathic and inflammatory pain, suggesting that, in these models, serotonergic systems may be less involved than other systems, such as those involving noradrenaline (Kayser et al., 1992). This suggestion is in agreement with the superior analgesic efficacy of reuptake inhibitors of both 5-HT and noradrenaline as compared with selective serotonin reuptake inhibitors (Jett et al., 1997; Eschalier et al., 1999). The differential analgesic effects of 5-HT in normal rats and in rat models of pain are conceivably related to changes in serotonergic analgesic systems. A more detailed assessment of the involvement of serotonergic systems in persistent pain awaits further studies.

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