0022-3565/94/2693-1182$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 269, No. 3
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Printed in U.S.A.

Serotonin Receptor Subtypes in Spinal Antinociception in the Rat[1]

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Accepted for publication February 2, 1994

ABSTRACT

The aim of the present study was to clarify the subtypes of serotonin (5-HT) receptors involved in spinal antinociception in the rat. 1) Intrathecal (i.t.) injection of 5-HT (25–200 μg) produced a dose-dependent increase in tail-flick latency. 2) Intrathecal injection of fluoxetine, a 5-HT uptake blocker (25–40 μg), resulted in a bell-shaped dose-related antinociception with peak effects occurring at 10 μg. 3) A bell-shaped antinociceptive effect was obtained by i.t. injection of the 5-HT_{1A} agonist (+)-hydroxy-2-(di-N-propylamino)tetralin (0.25–2 μg), with the maximal effect occurring at 0.5 μg, which can be prevented by the 5-HT_{1A} antagonist spiperone (25 μg i.t.). 4) A similar dose-response curve was obtained following the i.t. injection of the 5-HT_{1B} agonist 1-[3-(trifluoromethyl)phenyl]-piperazine maleate (1–125 μg) with the maximal effect observed at 25 μg. 5) Neither the 5-HT_2 agonist (±)-α-methyl-5-HT-maleate nor the 5-HT_3 agonist (±)-2-methyl-5-HT-maleate produced significant antinociceptive effects at doses up to 50 μg. Spontaneous tail-flicks emerged at doses higher than 50 μg. 6) The antinociceptive effect induced by 5-HT (200 μg i.t.) could be attenuated dose-dependently either by the 5-HT_{1A} antagonist spiperone (5 and 25 μg i.t.) or by the 5-$HT_{1C/2}$ antagonist mianserin (0.5–50 μg i.t.), but not by the 5-HT_2 antagonist 1-(1-naphthyl)piperazine hydrochloride or the 5-HT_3 antagonist 3-tropanyl-indole-3-carboxylate. The results suggest that significant antinociceptive effects can be induced by spinal 5-HT *via* 5-HT_1 receptors and 5-$HT_{1C/2}$ receptors, whereas 5-HT_3 receptors do not seem to be involved.

In spite of the wealth of literature showing the importance of the bulbospinal serotonergic descending system in spinal pain modulation (Yaksh and Wilson, 1979; Zemlan *et al.*, 1980; Schmauss *et al.*, 1983), several key issues remain to be clarified. For example, is the 5-HT modulation of the spinal dorsal horn neuron facilitatory (Le Bars *et al.*, 1978; Duggan, Griersmith and North, 1980; Zemlan *et al.*, 1983) or inhibitory (Proudfit and Hammond, 1981; Berge *et al.*, 1983; Fasmer *et al.*, 1984)? Is the influence tonic or phasic (Larsen and Arnt, 1984; Rivot *et al.*, 1987; Proudift and Yaksh, 1980)? In addition, controversy remains as to which types of 5-HT receptor are involved in mediating the serotonergic antinociceptive effect (Hwang and Wilcox, 1987; Millan *et al.*, 1991; Alhaider *et al.*, 1991).

Recent studies using radioligand binding assays and molecular biology techniques have revealed at least three types of 5-HT receptors in the vertebrate CNS, *i.e.*, 5-HT_1, 5-HT_2 and 5-HT_3 receptors (Peroutka and Snyder, 1979; Kilpatrick *et al.*, 1987), whereas the 5-HT_1 receptor has been further divided into four subtypes, $_A$, $_B$, $_C$ and $_D$ (Pedigo *et al.*, 1981; Pazos *et al.*, 1985; Heuring and Peroutka, 1987). Several points need to be stressed in any attempt to differentiate of the roles played by various types of 5-HT receptors in pain modulation, among which are the specificity and the proper dosage of the pharmacological tool (agonist or antagonist) directed to the 5-HT receptor, as well as the reliability of the methodology being used for measuring nociception. Results of the tail-flick test, an extensively used nociceptive test, can be seriously misinterpreted if the TT is not monitored properly (Ren and Han, 1979). Thus, vasodilatation of the tail as a consequence of i.t. drug administration could result in an increase in the TT and a decrease in TFL, which could have been interpreted as "hyperalgesia." In the present study care has been taken to correct the TFL elicited by TT changes, the importance of which has been stressed recently by Eide *et al.* (1988), Eide and Rosland (1989) and Eide and Tjolsen (1988) in their studies in mice by using a formula which was almost identical with that developed in our laboratory in rats a decade ago (Ren and Han, 1979; Han and Ren, 1991).

Methods

Wistar rats of both sexes weighing 200 to 250 g were obtained from the Animal Center of the Beijing Medical University (Beijing, P.R.

Received for publication November 15, 1991.
[1] This work was supported by Grant DA 03983 from National Institute of Drug Abuse and a grant from the National Natural Science Foundation of China.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); CNS, central nervous system; TT, tail skin temperature; i.t., intrathecal; TFL, tail-flick latency; AUC, area under the curve; MAUC, mean area under the curve; 8-OH-DPAT, (+)-8-hydroxy-2-(di-N-propylamino)tetralin hydrobromide; TFMPP, 1-[3-(trifluoromethyl)phenyl]-piperazine hydrochloride; 1-NP, 1-(1-naphthyl)piperazine hydrochloride; ICS 205–930, 3-tropanyl-indole-3-carboxylate; DMSO, dimethylsulfoxide; NS, normal saline; ANOVA, analysis of variance; α-CH_3-5-HT, (±)-α-methyl-5-hydroxytryptamine maleate; 2-CH_3-5-HT, (±)-2-methyl-5-hydroxytryptamine maleate; STF, spontaneous tail flicks.

China). They were housed six in a cage with food and water *ad libitum*. The tail flick test was used for assessment of nociception (Ren and Han, 1979). The rats were restrained in plastic holders with the tail and hind legs protruding. The intensity of the radiant heat was adjusted to obtain a TFL within the range of 4 to 6 sec in resting conditions. Results from the first three measurements (5 min apart) were averaged as the basal TFL. Values from subsequent measurements were expressed as percentage of change from the base-line level, with +150% as the cutoff limit to avoid unnecessary damage to the tissue. The values of the three or six postdrug measurements (in a period of 30 or 60 min) in each rat were averaged as the AUC and the MAUCs, representing the mean antinociceptive effect within 30 or 60 min.

Room temperature was kept within the range of 21 ± 1°C during the experiment. Rat TT was measured before the TFL assessment by a thermistor of type 219, model MGA-III, Nihon Kohden (Tokyo, Japan). The base-line TT was usually 0.5°C above the room temperature. When the changes of TT were 2°C above the base-line TT, the TFL value was corrected by a factor of 0.25 sec/°C according to the regression coefficient introduced by Ren and Han (1979).

Intrathecal cannulation was performed according to Yaksh and Rudy (1976). A PE-10 polyethylene catheter was inserted caudally through the incised atlanto-occipital membrane into the subarachnoid space for 6.5 to 7.5 cm to reach the lumbar enlargement.

Throughout the study, dosages of drug refer to the weight of the salt. Fluoxetine HCl was kindly donated by Eli Lilly and Co. (Indianapolis, IN); 5-HT creatinine sulfate and mianserin HCl were purchased from Sigma Chemical Co. (St. Louis, MO); 8-OH-DPAT, TFMPP, spiperone, 1-NP and ICS 205-930 were from Research Biochemicals Inc. (Wayland, MA). ICS 205-930 and spiperone were first dissolved in 100% DMSO at a concentration of 10 mg/ml, and diluted with NS to the desired concentration (Glaum and Anderson, 1988). The other drugs were prepared fresh in NS before use. DMSO-containing vehicle and NS solutions were administered to control groups, respectively.

Data were expressed as means ± S.E.M.. Comparisons between groups were made by using ANOVA followed by Duncan's test. Significance was accepted at the $P < .05$ level.

Results

Figure 1 illustrates the antinociceptive effects induced by i.t. 5-HT at 4 doses (25, 50, 100 or 200 μg) in four groups of rats ($n = 8$). Compared with the control group which received 10 μl of NS, i.t. 5-HT produced a clear cut dose-related antinociception (ANOVA, $P < .01$). The 25-μg dose was ineffective on the tail-flick test.

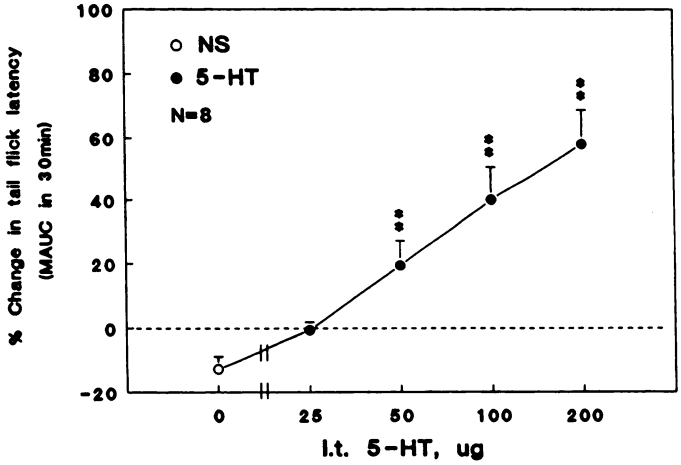

Fig. 1. Dose-dependent antinociceptive effect of intrathecal 5-HT. 0: NS, 10 μl. **P < .01, ANOVA followed by Duncan's test compared with the NS control group.

To explore whether spinal serotonergic nerve terminals exhibit a tonic release, we injected fluoxetine, the selective 5-HT reuptake inhibitor (Wong *et al.*, 1983; Stark *et al.*, 1985), i.t. to potentiate the effect of endogenously released 5-HT. Fluoxetine in the dose range of 5 to 20 μg produced a significant, although mild, antinociceptive effect. The mean increase of TFL in a period of 30 min reached 25.8 ± 7.2% at a dose of 10 μg. This effect of fluoxetine, however, became smaller at higher doses (fig. 2).

Fluoxetine also was used to potentiate the effect of exogenously injected 5-HT. Rats were injected i.t. with fluoxetine at the dose range of 2.5 to 40 μg, followed 10 min later by a subliminal dose of 5-HT (25 μg i.t.). Marked analgesia (71.8 ± 17.3%, $n = 13$) was obtained when the subliminal dose of 5-HT was preceded by an optimal (10 μg) dose of fluoxetine ($P < .05$ as compared to 25.8 ± 7.2% induced by fluoxetine alone). Taken together, the results shown in figure 2 suggest that a constant 5-HT release and reuptake process is going on in the spinal cord, albeit at a relatively low level.

In order to characterize the subtype specificity of the 5-HT_1 receptors in inducing spinal antinociception, the selective 5-HT_{1A} agonist 8-OH-DPAT (Schmidt and Peroutka, 1989) or the 5-HT_{1B} agonist TFMPP (Bobker and Williams, 1989) were injected i.t. The results are shown in figure 3. Both agonists produced a bell-shaped antinociceptive effect. However, the effective dose range was much broader in the 5-HT_{1B} agonist TFMPP (1-125 μg) as compared to the 5-HT_{1A} agonist 8-OH-DPAT (0.25-2 μg). Concerning the 5-HT_{1C} receptor, although this subtype has been characterized by a relatively specific antagonist mianserin, a selective agonist or antagonist is not yet available (Fozard, 1987; Cheetham *et al.*, 1989; Schmidt and Peroutka, 1989).

Similar experiments were performed by using the 5-HT_2 receptor agonist α-CH_3-5-HT and the 5-HT_3 receptor agonist 2-CH_3-5-HT (Richardson *et al.*, 1985; Kilpatrick *et al.*, 1987) for i.t. injection. Neither α-CH_3-5-HT nor 2-CH_3-5-HT produced significant antinociceptive effects at doses up to 50 μg (table 1). At doses higher than 50 μg, a series of abnormal behaviors emerged, including irregular STF, an elevation of the tail to a level higher than that of the body axis, which prevented accurate measurement of the TFL.

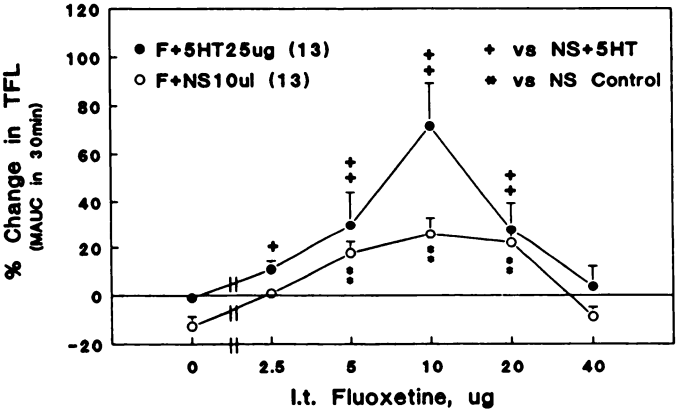

Fig. 2. The potentiating effect of fluoxetine (F) on 5-HT-induced analgesia. F was injected i.t. at the dose range of 2.5 to 40 μg, followed 10 min later by a subliminal dose of 5-HT (25 μg i.t.). A bell-shaped dose-response curve was obtained with maximal enhancement occurring at the 10-μg dose of F. *P < .05, **P < .01 and +P < .05, ++P < .01, ANOVA followed by Duncan's test compared with the NS control and the NS + 5-HT group, respectively.

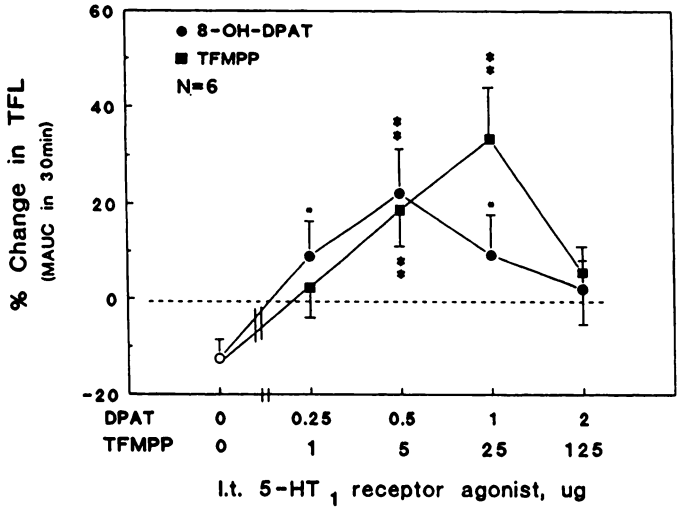

Fig. 3. The antinociceptive effect of i.t. 5-HT_{1A} receptor agonist 8-OH-DPAT and 5-HT_{1B} receptor agonist TFMPP. Bell-shaped dose-response curves were obtained with peak effect occurring at 0.5 μg of 8-OH-DPAT and 25 μg of TFMPP. 0: NS, 10 μl. *P < .05 or **P < .01, ANOVA followed by Duncan's test compared with the NS control group.

TABLE 1

Effects of the i.t. 5-HT_2 receptor agonist α-CH_3-5-HT and the 5-HT_3 receptor agonist 2-CH_3-5-HT on nociceptive threshold

Shown in the table are MAUCs in 60 min. 0: NS, 10 μl; n = 8–9.

	i.t. Dose (μg)			
	0	12.5	25	50
α-CH_3-5-HT	−13 ± 4	−2 ± 12	+6 ± 10	+12 ± 16
2-CH_3-5-HT		+4 ± 8	+9 ± 11	+11 ± 12

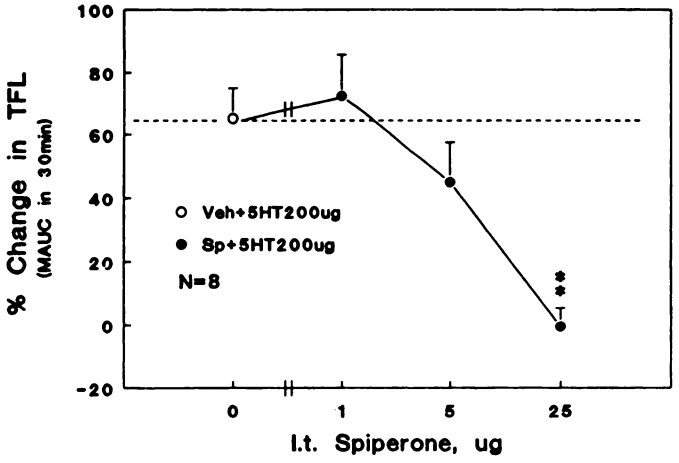

Fig. 4. Effect of i.t. 5-HT_{1A} receptor antagonist spiperone (Sp) on 5-HT-induced analgesia. The antinociceptive effect of i.t. 5-HT (200 μg) was antagonized significantly by i.t. Sp (25 μg). **P < .01, ANOVA followed by Duncan's test compared with the vehicle (Veh) control group.

Should 5-HT induce an antinociceptive effect *via* 5-HT_1 receptors in the spinal cord, the effect of 5-HT should be prevented or reversed by i.t. injection of 5-HT_1 receptor antagonists. Three groups of rats were given an i.t. injection of spiperone, the 5-HT_{1A} receptor antagonist (Williams *et al.*, 1988; Bobker and Williams, 1989; Hoyer, 1989), at doses of either 1, 5 or 25 μg followed 10 min later by another i.t. injection of 200 μg of 5-HT. In the control group 10 μl of vehicle was used instead of spiperone. Figure 4 shows the dose-dependent antagonism of 5-HT-induced antinociception. A complete blockade was reached when the dose of spiperone was increased to 25 μg, which, by its own had no significant influence on basal TFL (data not shown). The effectiveness of spiperone in antagonizing the 5-HT_1 receptor was shown in another experiment by using the selective 5-HT_1 receptor agonist 8-OH-DPAT. Two groups of 10 rats were given i.t. injections of vehicle (10 μl) or spiperone (25 μg) followed 10 min later by 8-OH-DPAT (0.5 μg i.t.). The antinociceptive effect of 8-OH-DPAT shown in the vehicle control rats (+35 ± 10%) was abolished completely in the rats pretreated with spiperone (−10 ± 4.6%, P < .01).

Similar experiments were performed to assess whether the 5-HT-induced antinociception could be blocked by the 5-$HT_{1C/2}$ receptor antagonist mianserin (Fozard, 1987; Cheetham *et al.*, 1989). Intrathecal injection of mianserin (50 or 100 μg/animal) produced no significant effect on the basal TFL (data not shown). However, the antinociceptive effect of 5-HT (200 μg i.t.; MAUC = 53 ± 12%, n = 8) was almost blocked completely by prior injection of mianserin (50 μg i.t.; MAUC = 14 ± 12%, n = 8, P < .01) (fig. 5).

Attempts were made to assess whether the 5-HT-induced antinociception could be prevented by prior i.t. injection (10 min) of the 5-HT_2 receptor antagonist 1-NP (Glennon, 1987) or the 5-HT_3 receptor antagonist ICS 205-930 (Schmidt and Peroutka, 1989). The results are shown in tables 2 and 3. Intrathecal injection of 1-NP (10 and 20 μg) or ICS 205-930 (0.1, 10 and 100 μg) produced no significant changes on TFL (data not shown), nor did these antagonists affect the antinociception induced by 5-HT.

In order to clarify whether the TFL changes result from the influence of TT changes, the effect of temperature is taken into

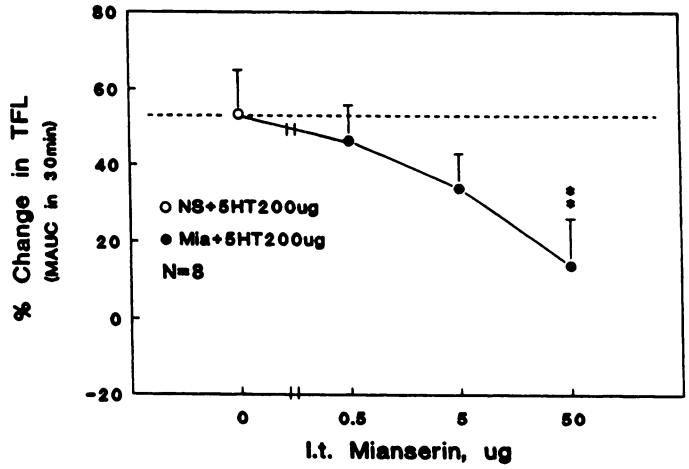

Fig. 5. Effect of i.t. 5-$HT_{1C/2}$ receptor antagonist mianserin (Mia) on 5-HT-induced analgesia. The antinociceptive effect of i.t. 5-HT (200 μg) was antagonized significantly by i.t. Mia (50 μg). **P < .01, ANOVA followed by Duncan's test compared with the NS control group.

TABLE 2

Effect of the 5-HT_2 receptor antagonist 1-NP on 5-HT (200 μg i.t.)-induced antinociception

Shown in the table are the MAUCs in 60 min after the i.t. injection of 5-HT (200 μg). No significant difference was found between the 1-NP groups and the NS control group. 1-NP was injected 10 min before 5-HT.

	NS	1-NP (μg)		
		5	10	20
MAUC*	38.1 ± 20.1	48.9 ± 20.3	45.9 ± 14.9	35.7 ± 17.0
n	10	7	10	10

TABLE 3

Effect of the 5-HT$_3$ receptor antagonist ICS 205-930 on 5-HT (200 μg i.t.)-induced antinociception

Shown in the table are MAUC in 60 min after the i.t. injection of 5-HT (200 μg). ICS 205-930 was injected i.t. 10 min before 5-HT. Number in parentheses, number of injections.

Dose	ICS 205-930	Vehicle	P
μg			
0.1	39.5 ± 21.8 (8)	39.2 ± 17.0 (9)	> .05
10	50.0 ± 18.0 (8)	45.5 ± 18.3 (9)	> .05
100	61.1 ± 21.4 (8)	48.6 ± 17.7 (9)	> .05

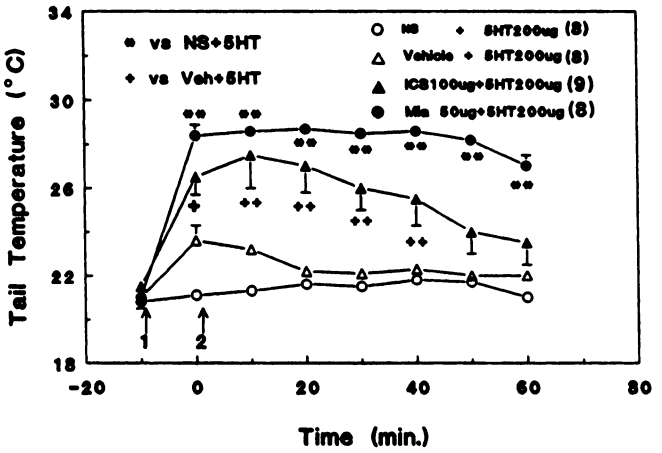

Fig. 6. Effect of i.t. 5-HT$_{1C/2}$ receptor antagonist mianserin (Mia) (50 μg) and 5-HT$_3$ receptor antagonist ICS 205–930 (100 μg) on TT. Arrow 1, i.t. injection of NS or vehicle (Veh) and ICS or Mia, respectively; arrow 2, i.t. injection of 5-HT 10 min later. Mia and ICS raised TT significantly. *P < .05, **P < .01, compared with the NS control, +P < .05, ++P < .01, compared with DMSO Veh group, ANOVA followed by Duncan's test. Number of animals in each group are shown in parentheses.

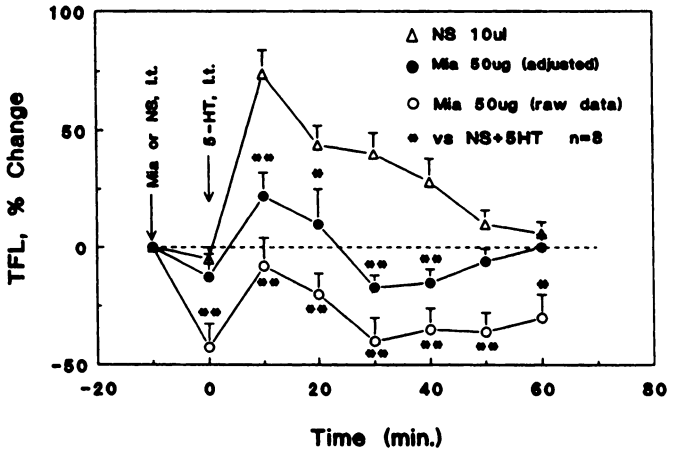

Fig. 7. The antinociceptive effect of 5-HT (200 μg i.t.) was antagonized by mianserin (Mia, 50 μg i.t.) by using TFL data adjusted with TT. A conclusion of hyperalgesia would have been drawn if the raw data of TFLs were used for computation. *P < .05, **P < .01, ANOVA followed by Duncan's test compared with the NS control group.

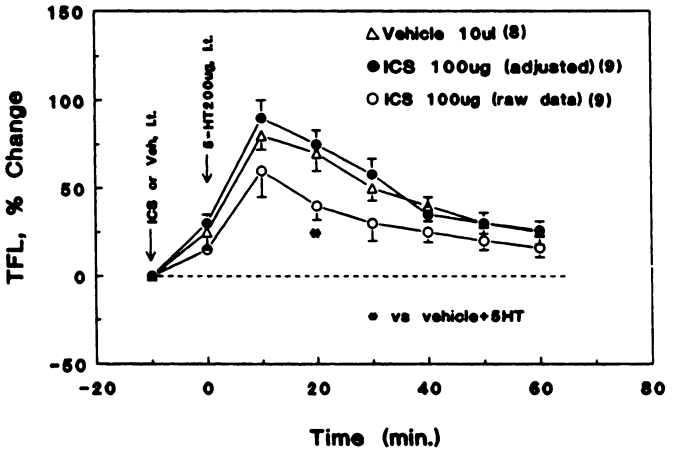

Fig. 8. The antinociceptive effect of 5-HT was antagonized (P < .05) by ICS 205–930 when the raw data of TFLs were used. This antagonism became statistically nonsignificant when the TFLs were adjusted with TT. Veh, vehicle.

account. We found that i.t. injection of the 5-HT and 5-HT receptor agonists used in the present study failed to change TT significantly, nor did i.t. injection of the 5-HT$_{1A}$ receptor antagonist spiperone and the 5-HT$_2$ receptor antagonist 1-NP; whereas the 5-HT$_{1C/2}$ receptor antagonist mianserin and the 5-HT$_3$ receptor antagonist ICS 205–930 increased the TT significantly (fig. 6). The increase of TFL induced by the i.t. injection of 5-HT (200 μg) was reduced significantly by i.t. injection of mianserin (50 μg) and ICS 205–930 (100 μg) if the TFL was not adjusted by TT (figs. 7 and 8). After the TFL was adjusted by TT (Ren and Han, 1979), mianserin still decreased the 5-HT-induced antinociceptive effects significantly (figs. 5 and 7); the attenuation induced by ICS 205–930 was no longer significant (fig. 8).

Discussion

One of the aims of the present study was to determine whether the nature of the serotonergic modulation of spinal nociceptive reflex is facilitatory (Le Bars *et al.*, 1978; Duggan *et al.*, 1980; Zemlan *et al.*, 1983) or inhibitory (Proudfit, 1980a,b; Fasmer *et al.*, 1984). The results shown in figure 1 indicate clearly a suppression rather than a facilitation of the tail-flick reflex, which is consistent with the findings of a behavioral pharmacology study reported by Yaksh (1979) and Eide *et al.* (1990, 1991), and disagrees with that of Zemlan *et al.* (1983) and Millan *et al.* 1991.

The second aim was to verify the tonic release of 5-HT in the spinal cord of the conscious rat, making use of fluoxetine, which blocks the reuptake of 5-HT by the nerve terminals. Hwang and Wilcox (1987) reported that i.t. injection of fluoxetine did not prolong the TFL of mice. Yaksh and Wilson (1979) reported that parenteral administration of fluoxetine had no significant effect on the basal TFL of the rat, yet it potentiated the analgesia induced by exogenously administered 5-HT. In the present study, i.t. administered fluoxetine not only potentiated the effects of exogenous 5-HT, but it also produced dose-dependent analgesia by itself, suggesting a potentiation of endogenously released 5-HT, which is supported by the evidence that fluoxetine increased the content of 5-HT in microdialysate of the cat spinal cord (Sorkin *et al.*, 1991). The difference in these studies may result from differences in animal species. Besides, the effect of 5-HT in the peripheral terminals of the primary afferent C fibers has been shown to facilitate noxious input (Fozard, 1984; Richardson *et al.*, 1985), as opposed to the central effects of 5-HT which suppress the transmission of noxious input at the dorsal horn neurons (Belcher *et al.*, 1978; El-Yassir *et al.*, 1988; Griersmith and Duggan, 1980; Nakagawa *et al.*, 1990). Parenterally adminis-

tered fluoxetine may facilitate both central and peripheral actions of 5-HT, resulting in no net effect.

The tonicity of the descending serotonergic inhibitory pathway could be explored by another approach, *i.e.*, a potential lowering of base-line TFL after i.t. injection of the 5-HT receptor antagonists (Proudfit and Hammond, 1981; Berge *et al.*, 1983). The experimental results obtained in the present study revealed no significant change in TFL when the 5-HT_{1A} receptor antagonist spiperone or the 5-$HT_{1C/2}$ receptor antagonist mianserin were administered i.t. at doses sufficient to block the analgesic effect induced by i.t. 5-HT (1, 5 and 25 μg for spiperone and 50 and 100 μg for mianserin). More study is needed to resolve the conflict between the results of fluoxetine which is in favor of, and that of the 5-HT blockers which is against, the hypothesis of tonic release of 5-HT at the spinal level. A combined administration of antagonists for 5-HT receptor subtypes $_{1A}$, $_{1B}$ and $_{1C}$ may be worthwhile when selective 5-HT_{1B} and 5-HT_{1C} antagonists are available.

Substantial evidence in the literature indicates that 5-HT_1 receptors predominate in the rat spinal cord (Blackshear *et al.*, 1981; Monroe and Smith, 1982; Marlier *et al.*, 1991), and that both 5-HT_1 and 5-HT_{1A} receptor subtypes are present mainly in the superficial laminae of the dorsal horn (*i.e.*, laminae I-II). The 5-HT_{1B} receptor is present throughout the spinal cord, exhibiting high densities in the caudal-most part of the dorsal horn in lamina X (Pazos and Palacios, 1985; Molineaux *et al.*, 1989; Marlier *et al.*, 1991). The 5-HT_{1C} receptor also has been reported in the superficial (I-II), lamina V of the dorsal horn (Pazos and Palacios, 1985; Molineaux *et al.*, 1989). Controversy exists, however, as to the possible role played by the three receptor subtypes in the spinal cord for pain modulation. Thus, the function of the 5-HT_{1A} receptor has been reported to suppress non-nociceptive responses of dorsal horn neurons (El-Yassir *et al.*, 1988), to facilitate nociceptive responses of the dorsal horn neurons (Murphy and Zemlan, 1990) or to be inactive in spinal nociception (Fasmer *et al.*, 1986). It is interesting that Millan *et al.* (1991) reported that the 5-HT_{1A} agonist 8-OH-DPAT (s.c.) elicited STF and attenuated *mu*- but not *kappa*-opioid antinociception in mice and rats (Millan *et al.*, 1991; Millan and Colpaert, 1991a,b). The function of 5-HT_{1B} receptors appears to be the suppression of nociceptive responses of dorsal horn neurons (El-Yassir *et al.*, 1988), especially responses of spinal wide-dynamic range neurons (Murphy and Zemlan, 1990). Although *in situ* hybridization studies revealed a wide distribution of the mRNA encoding the 5-HT_{1C} receptor in CNS sites relevant to pain conduction and modulation (relay nuclei of the thalamus, PAG, raphe nuclei, spinal lamina V and VII, etc.), no clear functional evidence has yet been presented which substantiates this prediction (Molineaux *et al.*, 1989). El-Yassir *et al.* (1988) found that the descending serotonergic inhibitory control on dorsal horn neurons is mediated by the 5-HT_{1A} receptor for non-noxious signals and by the 5-HT_{1B} receptor for noxious signals. On the contrary, reports from Zemlan *et al.* (1983) and Murphy and Zemlan (1990) indicated that activation of the spinal 5-HT_{1A} receptor facilitated noxious reaction, whereas activation of the 5-HT_{1B} receptor inhibited discharges of wide-dynamic range neurons induced by noxious stimuli. Based on the behavioral studies in rats, Fasmer *et al.* (1986) concluded that the 5-HT_{1A} receptor in spinal cord may not be involved in spinal nociceptive reflexes. This conclusion is in sharp contrast with the results obtained in mice by several groups (Archer *et al.*, 1987; Eide *et al.*, 1990, 1991; Eide and Hole, 1991) who showed that the 5-HT_{1A} agonist 8-OH-DPAT (s.c. and i.t.) produced significant antinociceptive effects.

In the present study coherent results have been obtained which indicate that 5-HT_1 receptors ($_{1A}$ and $_{1B}$) in the rat spinal cord are involved in pain modulation. Thus, i.t. injection of the 5-HT_{1A} or the 5-HT_{1B} agonists produced dose-related antinociception, whereas the 5-HT_{1A} and the 5-$HT_{1C/2}$ antagonists produced dose-related blockade of 5-HT-induced antinociception. However, it should be stressed that the reliability of the results of pharmacological studies depends on the availability and selectivity of the pharmacological tools. Currently, we have a selective agonist but no good antagonist for the 5-HT_{1B} receptor; spiperone has been used as an antagonist for 5-HT_{1A} receptor, but it also exhibits a high affinity for the 5-HT_2 receptor (Williams *et al.*, 1988; Bobker and Williams, 1989; Hoyer, 1989). Concerning the 5-HT_{1C} receptor, neither agonist nor selective antagonist is available. Whereas mianserin has been used as an antagonist for the 5-HT_{1C} receptor, it also binds to the 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptor with lower or equal affinity (Fozard, 1987; Cheetham *et al.*, 1989; Schmidt and Peroutka, 1989). It is thus obvious that the availability of the more selective 5-HT agonists and antagonists are crucial for progress in this field.

Millan *et al.* (1991) and Millan and Colpaert (1991a,b) reported recently that s.c. injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT dose-dependently elicited STF and antagonized *mu*-, but not *kappa*-opioid antinociception in mice and rats . In our hands, no STF were observed after i.t. injections of 8-OH-DPAT at doses of 0.25 to 2 μg. The difference in results may have been accounted for mainly by the difference in the route of administration. There has been evidence suggesting that s.c. administration of 8-OH-DPAT attenuated the release of 5-HT in the CNS (hypothalamus, nucleus accumbens, frontal cortex, etc.) by acting on the 5-HT_{1A} autoreceptors in raphe neurons (Hjorth and Sharp, 1991; Gartside *et al.*, 1992). It is possible that the STF and the attenuation of morphine-induced analgesia elicited by s.c. administration of 8-OH-DPAT resulted from the decrease of central serotonergic function. This is supported by the recent findings in this laboratory (W. Xu and H. S. Han, unpublished observations) that i.t injections of the 5-HT_{1A} antagonist spiperone and the 5-$HT_{1C/2}$ antagonist mianserin can antagonize the analgesic effect induced by i.c.v administration of ohmefentanyl, a highly selective *mu* receptor agonist (Xu *et al.*, 1987; Zhu *et al.*, 1987; Goldstein and Naidu, 1989), which is in agreement with the well documented findings that spinal 5-HT descending system mediates morphine-induced analgesia (Yaksh, 1979; Suh *et al.*, 1989; Suh and Tseng, 1990). Moreover, drugs administered s.c. can have peripheral action, and the peripheral action of 5-HT has been known to facilitate nociception (Fozard, 1984; Richardson *et al.*, 1985). It is thus obvious that the i.t. route of administration should be more relevant to the spinal action of the drug under consideration, which is supported by recent behavioral studies showing that i.t. injections of 8-OH-DPAT (0.1–100 μg) failed to evoke STF or any other marked behavioral effects of rats, suggesting that the spinal 5-HT_{1A} receptor subtype is not involved in this behavior (Fone *et al.*, 1991).

It was demonstrated recently that i.t. injection of higher doses of 8-OH-DPAT (300 nmol) and TFMPP (100–600 nmol) produced significant antinociceptive effects on the hot-plate test, and lower doses of 8-OH-DPAT (30–100 nmol) produced no significant changes in the hot-plate latency. In the tail-flick

test, however, the 300 nmol dose of 8-OH-DPAT produced a hyperalgesic response, and the 100 to 600 nmol of TFMPP did not alter TFL (Crisp *et al.*, 1991). In comparison, much smaller doses were used in the present study. Thus, i.t. administration of lower doses of 0.25 to 1 μg (0.8–3.0 nmol) of 8-OH-DPAT and 1 to 25 μg (3.8–93.7 nmol) of TFMPP induced a dose-dependent increase in TFL. No significant changes in TFL were observed when the doses of 8-OH-DPAT and TFMPP were increased up to 2 (6.1 nmol) and 125 μg (468.7 nmol), respectively. It might be possible that a hyperalgesic effect will be observed in rats if the 8-OH-DPAT dose was increased from 6.1 to 300 nmol. It is worthwhile to mention the findings of Dedeoglu and Fisher (1991) that i.c.v. administration of low doses of 8-OH-DPAT (0.3–3 nmol) elevated arterial pressure and heart rate, whereas higher doses of 8-OH-DPAT (10–100 nmol) decreased both arterial pressure and heart rate.

The 5-HT_2 receptor has an uneven distribution in the CNS, with cerebral cortex having higher levels than the cord as revealed by radioligand experiments and dot blot hybridization studies of the 5-HT_2 receptor mRNA (Hoyer *et al.*, 1986; Molineaux *et al.*, 1989). In the rat spinal cord, the 5-HT_2 receptor is present mostly in the sympathetic area and in the ventral horn; the dorsal horn exhibits few 5-HT_2 receptors (Marlier *et al.*, 1991). Concerning the possible role played by the 5-HT_2 receptor in spinal pain modulation, literature data present a picture of great divergence. Activation of the 5-HT_2 receptor has been reported to facilitate (Wilcox and Alhaider, 1990; Eide *et al.*, 1991) or to inhibit (Soloman and Gebhart, 1988) the nociceptive reaction in dorsal horn neurons, or not to be involved in serotonergic descending inhibition (El-Yassir *et al.*, 1988). Results obtained in our study seem to support the work of El-Yassir *et al.* (1988), because i.t. injection of the 5-HT_2 receptor agonist produced no antinociceptive effect, and the antinociception produced by i.t. 5-HT was not affected by the 5-HT_2 receptor antagonist 1-NP. At doses higher than 50 μg, the 5-HT_2 agonist α-CH_3-5-HT elicited STF, which were considered as a supersensitivity to non-nociceptive stimuli (Millan *et al.*, 1991). Recently, it was reported that i.t injection of the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) produced a dose-dependent behavioral syndrome consisting of biting or licking directed toward the caudal part of the body and reciprocal hindlimb scratching (Eide *et al.*, 1991). Inasmuch as the 5-HT_2 receptor is present mostly in the ventral horn and sympathetic area of the rat spinal cord (Marlier *et al.*, 1991), it is not possible to exclude motor mechanisms acting on the ventral horn, which has been supported by recent behavior studies showing the involvement of the 5-HT_2 receptor of the rat spinal cord in the motor behaviors (Fone *et al.*, 1991).

The results described above are particularly interesting in relation to the recent suggestion that 5-HT_2 and 5-HT_{1C} receptors might be homologous in their genomic expression (Hartig, 1989). This is supported by the findings of 51% homology and a similarity in binding characteristics and postreceptor events (Hoyer, 1988; Hartig, 1989; Schmidt and Peroutka, 1989). It is for these reasons that the 5-HT_{1C} receptor has been defined as a subtype of the 5-HT_2 receptor family (Hartig, 1989). However, contradictory evidence exists, *e.g.*, the 5-HT_{1C} receptor and the 5-HT_2 receptor show characteristic CNS distribution of their own (Hoyer *et al.*, 1986; Molineaux *et al.*, 1989), and the 5-HT_2 receptor has been further divided into 5-HT_{2A} and 5-HT_{2B} subtypes (Pierce and Peroutka, 1989). If these two receptors are identical in nature, one would expect a functional similarity in pharmacological profiles. This seemed not to be supported by the results of the present study, because the 5-$HT_{1C/2}$ receptor antagonist mianserin blocked the 5-HT-induced antinociception, whereas the 5-HT_2 receptor antagonist 1-NP did not. Moreover, the 5-HT_2 agonist α-CH_3-5-HT produced STF instead of antinociception. Further identification of the functions between 5-HT_{1C} and 5-HT_2 receptors will be possible only when more selective 5-HT_{1C} agonists and antagonists are available.

The 5-HT_3 receptor has been shown to exist in peripheral tissue as well as in the CNS (Kilpatrick *et al.*, 1987), including the dorsal horn of the rat spinal cord (Glaum and Anderson, 1988). A similar degree of controversy exists concerning the possible role played by the 5-HT_3 receptor. Glaum and Anderson (1988), Glaum *et al.* (1990) and Alhaider *et al.* (1991) showed an antinociceptive effect of 5-HT_3 receptor activation. Rodgers *et al.* (1990) assigned the 5-HT_3 receptor a role in defeat-induced analgesia, although the location of 5-HT_3 receptor, in this particular case, was not identified. In the present study both agonist and antagonist were used and were found to exert no significant effect on nociception at the spinal level (tables 1 and 3).

The marked inconsistency between the results reported by different laboratories may reflect the complexity of the problem as well as the technical problems associated with the techniques being used; TFL has been used frequently and widely as an index of nociception. Being a spinal reflex, tail-flick reflex is constantly under the modulation of suprasegmental control. From a technical point of view, because the TFL is the time period needed for the TT to raise from the base-line level to 42.6°C (Ness and Gebhart, 1986), the accuracy of the measurement relies on the constancy of TT. Any increase or decrease of the TT would result in the decrease or increase of the TFL, producing a false impression of hyper- or hypoalgesia. Looking into the detail, the TT of the rat under normal condition is usually only 0.2–0.5°C higher than room temperature, it remains in the same level of room temperature and the tail stays at room temperature even in the postmortem animal. Therefore, the possibility of a decrease in TT by vasoconstriction is almost nonexistent. On the contrary, a dramatic increase in TT of more than 10°C can occur as a result of vasodilatation. In order to correct the changes of TFL induced by change in TT, Ren and Han (1979) developed a formula: TFL (seconds) = $-0.254 \times$ TT (°C) + 10.038, under the condition that room temperature was fixed in the range of 20 ± 1°C and the intensity of radiant heat was adjusted to induce a basal TFL of 5 sec. This observation was confirmed recently by Eide *et al.* (1988) in mice with a formula: TFL = -0.26 TT + 10.7, which was quite similar to our own formula for rats (Ren and Han, 1979). They also mentioned that the apparent "hyperalgesic" effect induced by i.t. 5,6-dihydroxytryptamine (a 5-HT chemical denervator) or some of the 5-HT receptor antagonists were in fact a result of their vasodilator effect (Berge *et al.*, 1988; Eide *et al.*, 1988; Eide and Tjolsen, 1988; Eide and Rosland, 1989). In the present study we also found a marked increase of TT in rats receiving i.t. injections of the 5-HT receptor antagonists mianserin and ICS 205–930 (fig. 6), quite similar with the observations of Eide and Tjolsen (1988). Failure to correct for changes in TT would have led us to a conclusion similar with that of Glaum *et al.* (1990). Our raw data showed that the antinociceptive effect of 5-HT could be decreased by both the 5-$HT_{1C/2}$ receptor antagonist mianserin and the 5-HT_3 receptor

antagonist ICS 205–930 (figs. 7 and 8). The decrease induced by mianserin was still significant after the raw data were corrected by changes in TT (fig. 7); the effect induced by ICS 205–930, however, was no longer significant (fig. 8), suggesting that spinal 5-HT_3 receptor may not play an important role in the 5-HT-induced antinociceptive effect when temperature change was taken into account.

On the other hand, it is worthwhile to mention the findings of Fone *et al.* (1991) that the i.t. injection of 2-CH_3-5-HT (50 and 100 μg) produced sideward tail flicks, quite similar with the STF observed in our present study, inconsistent with that of Alhaider *et al.* (1991) showing that the i.t. administration of 2-CH_3-5-HT produced dose-dependent antinociception in the tail-flick test and inhibited behaviors elicited by i.t. administration of agonists for excitatory amino acid and neurokinin receptors. Further work is needed by using other selective 5-HT_3 receptor agonists and antagonists in other nociceptive assays such as hot-plate analgesiometric test.

In summary, our results indicate that 1) spinal 5-HT, either endogenously released or exogenously administered, has an inhibitory effect on spinal nociceptive reflex; 2) the spinal 5-HT_1 receptor (at least the 5-HT_{1A} and the 5-HT_{1B} subtypes) and the 5-$HT_{1C/2}$ receptor are involved in modulating nociception; and 3) the spinal 5-HT_3 receptor may not be involved in mediating spinal serotonergic antinociception.

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