ELSEVIER

European Journal of Pharmacology 335 (1997) 127–132

ejp

A dual effect of 5-HT_{1B} receptor stimulation on nociceptive dorsal horn neurones in rats

Johannes Gjerstad *, Arne Tjølsen, Kjell Hole

Department of Physiology, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway

Received 10 April 1997; revised 14 July 1997; accepted 18 July 1997

Abstract

In this study the modulatory effects of 5-HT_{1B} receptor activation on wide dynamic range neurones in the spinal cord were studied. Extracellular single unit recordings of dorsal horn neurones were performed in intact urethane-anaesthetized female Sprague–Dawley rats, and the receptive field distally on one hind paw was electrically stimulated with needle electrodes applied to the skin. The 5-HT_{1B} receptor agonist, CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one), the 5-$HT_{1A/B}$ receptor antagonist cyanopindolol, and the 5-HT_{1A} receptor antagonist WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride), were applied directly onto the spinal cord, and single unit responses were counted separately for A β-, A δ-, C-fibre responses and post-discharge according to the latencies. A dual effect of CP-93,129 was observed: 50 nmol CP-93,129 caused a clear inhibition of the A δ-fibre responses, whereas 50 and 150 nmol CP-93,129 produced a dose-dependent increase in post-discharge without affecting A β- and C-fibre responses. Application of 50 nmol cyanopindolol or 50 nmol WAY100635 alone did not affect neither the neuronal A-fibre nor the C-fibre responses, but when 50 nmol cyanopindolol was coadministered with 50 nmol CP-93,129 the effect of CP-93,129 alone was blocked: the A δ-fibre response was not inhibited and the post-discharge was not increased. In contrast, 50 nmol WAY100635 did not block the effect of 50 nmol CP-93,129 when the two drugs were coadministered. These results suggest that stimulation of the 5-HT_{1B} receptors may have both pro- and antinociceptive effects on wide dynamic range neurones in the dorsal horn after repeated electrical stimulation.

Keywords: Spinal cord; Nociception; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1B} receptor; CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one); Cyanopindolol; WAY100635

1. Introduction

Serotonergic pathways running from the rostroventral medulla in the brain stem to the dorsal horn of the spinal cord (Oliveras et al., 1979; Bowker et al., 1983) have long been considered to be essential in the mechanisms of descending inhibition (Yaksh and Wilson, 1979; Zemlan et al., 1980; Miletic et al., 1984), limiting the access of nociceptive information to higher centers. In the serotonergic neurones projecting to the spinal cord, 5-HT (5-hydroxytryptamine, serotonin) has been shown to coexist with substance P and thyrotropin releasing hormone (Johansson et al., 1981). The terminals of the descending serotonergic fibers are concentrated both in superficial and deeper laminae (Johansson et al., 1981; Miletic et al., 1984; Marlier et al., 1991) and appear to overlap in their anatomical distribution with terminals of the descending noradrenergic system in the spinal grey (Rajaofetra et al., 1992). Since only some of the serotonergic and noradrenergic vesicular profiles in the dorsal horn establish synapses, and the receptors appear to be located at some distance from the terminals, the spinal modulation of the sensory transmission by serotonin and noradrenaline probably is an example of monoaminergic 'volume transmission' in the central nervous system (Ridet et al., 1993).

In rats, the 5-HT_{1B} receptor subtype has been autoradiographically localized in all laminae in the spinal cord (Marlier et al., 1991; Thor et al., 1993). Within laminae III–VI, the 5-HT_{1B} receptors are distributed according to a dorsoventral gradient with the highest density in laminae III (Thor et al., 1993). The 5-HT_{1B} receptors represent approximately 18% of all 5-HT binding sites in the rat spinal cord (Huang and Peroutka, 1987). Since most 5-HT_1

* Corresponding author. Tel.: (47-55) 586-092; Fax: (47-55) 586-410; e-mail: johannes.gjerstad@pki.uib.no

receptors that are located on primary afferent neurones are of the 5-HT_{1A} receptor subtype (Daval et al., 1987), most 5-HT_{1B} receptors in the spinal cord probably are located on spinal neurones, or on the terminals of descending pathways from the raphe nuclei. Still, although the 5-HT_{1B} receptors have not been identified by specific labelling on primary afferent fibres, there is evidence for the presence of 5-HT_{1B} receptor messenger RNA in neurones of the rat trigeminal ganglia (Bruinvels et al., 1992).

The role of the 5-HT_{1B} receptor in the spinal nociceptive modulation have so far been controversial, and only a few selective ligands have been available. Claims that RU 24969 (5-methoxy-3-[1,2,3,6-tetrahydro-pyridin-4-yl]-1H-indole succinate), TFMPP (1-[3-(trifluoromethyl)-phenyl] piperazine) and CGS 12066B (7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline 1:2 maleate salt) are 5-HT_{1B} receptor-selective have not been confirmed (Hoyer et al., 1994). Although the evidence is week and the data available inconsistent, most behavioral and electrophysiological studies suggest that dorsal horn-localized 5-HT_{1B} sites mediate an antinociceptive action in rodents (Murphy et al., 1992; Alhaider and Wilcox, 1993; El-Yassir et al., 1988; Ali et al., 1994). Nevertheless, action on the 5-HT_{1B} receptor subtype in the spinal cord also have been reported to have pro-nociceptive effects (Solomon and Gebhart, 1988; Murphy et al., 1992).

The aim of the present study was to observe the effect of 5-HT_{1B} receptor stimulation on dorsal horn-localized wide dynamic range neurones in the spinal cord. The role of the 5-HT_{1B} receptor subtype in the nociceptive transmission was investigated using the selective 5-HT_{1B} receptor agonist, CP-93,129 (Macor et al., 1990), the 5-$HT_{1A/B}$ receptor antagonist, cyanopindolol (Hoyer et al., 1994), and the selective 5-HT_{1A} receptor antagonist WAY100635 (Forster et al., 1995; Gjerstad et al., 1996). Single cell recordings were made of dorsal horn neurones in the lumbar region where the 5-HT_{1A} as well as the 5-HT_{1B} receptor subtype are abundantly present. The effects of the agonist and the antagonists on electrically evoked single unit activity were studied.

2. Materials and methods

2.1. Animals and surgery

Female Sprague–Dawley rats (Mol:SPD, Møllegaard) weighing 200–300 g were anaesthetized with urethane (1.4–1.8 g/kg body weight i.p.), placed on a heating pad and mounted in a rigid frame. The core temperature was kept constant at 36–37°C. According to the procedure previously described (Gjerstad et al., 1996), a laminectomy was made between vertebrae T13 and L2, and the dura mater and arachnoidea were removed so that a rostro–caudally orientated rectangular area (1×2 mm) of the spinal cord surface (segment L5–S1) was exposed. The vertebrae rostral and caudal to the laminectomy were supported by clamps to hold the cord rigid, and a tungsten electrode was lowered into the dorsal horn by means of an electronic stepper with depth control.

2.2. In vivo electrophysiology

The technique used have been described previously (Gjerstad et al., 1996). Briefly, the receptive fields of the dorsal horn neurones were stimulated electrically with a pair of steel needle electrodes applied to the skin. Every 5 min, a train consisting of 16 rectangular 2 ms wide pulses, at a strength of $1.5 \times$ threshold for the C-fibre-mediated response with a frequency of 0.5 Hz, was given.

Extracellular recordings were made from neurones that responded to both A-fibre and C-fibre input. The recorded signals were amplified and band-pass filtered with 1/2 amplitude cutoff values of approximately 1000 and 1250 Hz corresponding to the duration of the action potentials (0.8–1 ms). Action potentials were accumulated separately for Aβ-, Aδ-, C-fibre response and post-discharge according to latencies. In this study, Aβ-, Aδ- and C-fibre evoked responses were taken as action potentials recorded 0–20 ms, 20–90 ms and 90–400 ms after stimulation, respectively, whereas the action potentials recorded 400–800 ms after stimulation were considered a post-discharge. The data were captured and analyzed using a CED 1401 interface connected to an IBM compatible PC.

2.3. Drugs

The drugs used in these experiments were CP-93,129 ([3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one]; Macor et al., 1990), a gift from Pfizer (UK), cyanopindolol (Hoyer et al., 1994), a gift from Sandoz Switzerland, and WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; Forster et al., 1995), a gift from Wyeth Research UK). CP-93,129 was dissolved in 0.9% NaCl (pH 7.5), cyanopindolol was dissolved in 0.4 M acetic acid and pH adjusted with NaOH and 0.9% NaCl back to neutrality (pH 6–8), whereas WAY100635 was dissolved in NaOH and 0.9% NaCl (pH 6–8). All doses of the drugs were dissolved at room temperature and applied directly onto the exposed area of the spinal cord in a volume of 50 μl.

2.4. Experimental procedure

Single cell activity was recorded in the dorsal horn at depths of 400–1000 μm, corresponding to laminae III–VI. Only wide dynamic range neurones (one in each animal), i.e., cells responding to non-noxious touch and to noxious forceps pinch, were studied.

Every 5 min a train of stimuli was delivered and the responses were recorded. Following three stable train re-

sponses, i.e., the responses of each train of 16 stimuli varying by less than 10%, the drug was applied and the neuronal responses were studied for at least 60 min. The number of spikes per 16 stimuli after the last three stimuli trains before application of the drug served as the control response for subsequent drug experiments on the cell.

The effects of the 5-HT_{1B} receptor agonist, CP-93,129, and the 5-$HT_{1A/B}$ and 5-HT_{1A} receptor antagonists cyanopindolol and WAY100635, respectively, were calculated as the number of spikes per 16 stimuli 15–30 min after drug application as percent of the control response. Furthermore, 30 min after the application of each antagonist, a mixture of the 5-HT_{1B} receptor agonist and the antagonist, were applied, and the effect was calculated as the number of spikes per 16 stimuli 15–30 min after the application of the mixture, again as percent of the control response. The rat was killed immediately after the experiment.

2.5. Statistics

The results are shown as means ± S.E.M. Mean responses were compared with the controls using Student's *t*-test, a paired two-sample test for means. The interaction between the agonist and the antagonists was analysed using a two-way analysis of variance (ANOVA). Significance was accepted at the 5% level.

3. Results

3.1. Effects of the 5-HT_{1B} receptor agonist CP-93,129

The 5-HT_{1B} receptor agonist CP-93,129 was topically applied in doses of 15–500 nmol. 50 nmol CP-93,129 caused a clear inhibition of the A δ-fibre responses, whereas 50 and 150 nmol CP-93,129 produced a dose-dependent increase in post-discharge without affecting A β- and C-fibre responses. The largest dose of CP-93,129 (i.e., 500 nmol) caused a pronounced post-discharge and spontaneous neuronal firing (i.e., discharges in the absence of electrical stimulation) that completely masked the electrically evoked A β-, A δ- and C-fibre responses of the neurons (Fig. 1).

During the first 15 min after application of 50 nmol of CP-93,129, the A δ-fibre responses of all 9 neurones tested were reduced. At the same time a progressive increase in the post-discharge was observed. In the test period 15–30 min after application the A δ-fibre responses of the neurones became stabilized, whereas the post-discharge of some of the neurones, in contrast, were still increasing. In most neurones the effects on the A δ-fibre response and post-discharge were partly reversed when CP-93,129 was replaced with saline on the spinal cord (washout). An example of the neuronal responses of a single neurone after application of 50 nmol CP-93,129 and during washout is shown (Fig. 2).

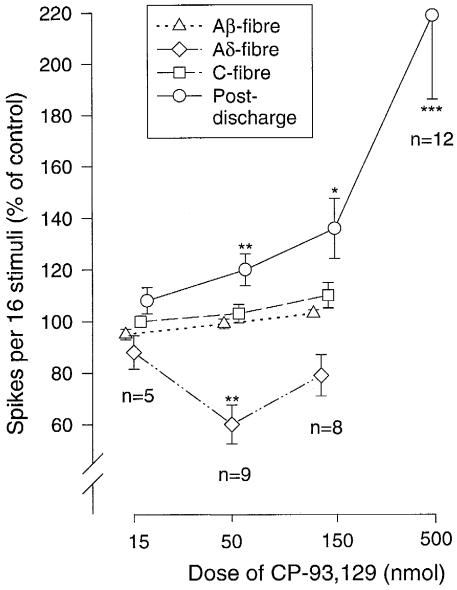

Fig. 1. The effect of CP-93,129 on evoked activity in dorsal horn neurones. Neuronal responses following different doses of CP-93,129 are shown as percent of control responses before drug application. (Means ± S.E.M. $n = 5–12$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, paired Student's *t*-test between the neuronal control responses and responses after application of CP-93,129).

3.2. The effect of the 5-HT_1 receptor antagonists, cyanopindolol and WAY100635 on the action of CP-93,129

Application of 50 nmol cyanopindolol or 50 nmol WAY100635 alone did not affect neither the neuronal

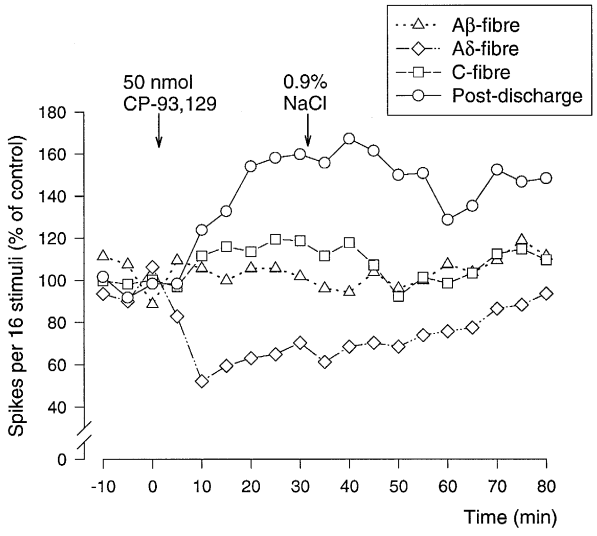

Fig. 2. The time course of the A β-, A δ-, C-fibre response and post-discharge evoked in a single dorsal horn neurone after 50 nmol CP-93,129 and 0.9% NaCl (washout). The A β-, A δ- and C-fibre response and the post-discharge (with 47, 59, 256 and 120 spikes per 16 stimuli, respectively, before application of the drug), are shown.

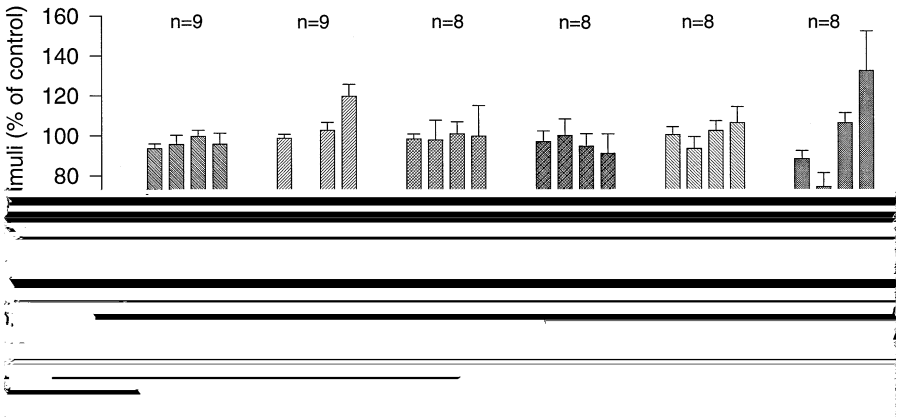

Fig. 3. The effect of application of 0.9% NaCl, 50 nmol CP-93,129, 50 nmol cyanopindolol, 50 nmol CP-93,129 coadministered with 50 nmol cyanopindolol, 50 nmol WAY100635, and 50 nmol CP-93,129 coadministered with 50 nmol WAY100635. The A β-fibre response (A β), the A δ-fibre response (A δ), the C-fibre response (C) and the post-discharge (PD) 15–30 min after drug application are shown. (Means ± S.E.M. $n = 8–9$.)

A-fibre nor the C-fibre responses in the present study (Fig. 3). When 50 nmol cyanopindolol were coadministered with 50 nmol CP-93,129 the effect of CP-93,129 alone was blocked: the A δ-fibre response was not inhibited and the post-discharge was not increased (Fig. 3). A significant interaction between CP-93,129 and cyanopindolol was discovered. (Two-way multivariate ANOVA, all responses A β, A δ, C, post-discharge: $P = 0.0038$, two-way univariate ANOVA, A δ: $P = 0.030$ and post-discharge $P = 0.14$, cyanopindolol and CP-93,129 interaction).

In contrast, 50 nmol WAY100635 did not block the effect of 50 nmol CP-93,129 when the two drugs were coadministered (Fig. 3). Statistical analysis showed no significant interaction. (Two-way multivariate ANOVA, all responses A β, A δ, C, post-discharge: $P = 0.17$, two-way univariate ANOVA, A δ: $P = 0.35$ and post-discharge: $P = 0.96$, WAY100635 and CP-93,129 interaction).

4. Discussion

The present study is the first to demonstrate the effect of the selective 5-HT_{1B} receptor agonist CP-93,129 on single wide dynamic range neurones in the dorsal horn. A dual effect was observed: the A δ-fibre response was inhibited, whereas the post-discharge was increased after topical application of CP-93,129. In contrast, no significant changes in the A β- and C-fibre responses were detected.

These findings suggest that the nociception mediated by the fast conducting A δ-fibres is inhibited, whereas the nociception mediated by fibres with low conduction velocity, i.e., the C-fibre mediated post-discharge, is enhanced by 5-HT_{1B} receptor stimulation. The 5-HT_{1B} receptor agonist CP-93,129 may therefore, theoretically, have both pro- and antinociceptive effects depending upon the type of spinal input, i.e., the A δ- versus the C-fibre input and/or the synchronous characteristics of this input, to the spinal cord.

The heat-activated rat tail flick reflex is mediated by fibres with low conduction velocity (Danneman et al., 1994). In accordance with the results of the present study where the 5-HT_{1B} receptor agonist CP-93,129 caused no change in the C-fibre response and an increased post-discharge, Solomon and Gebhart (1988) reported a reduced tail flick latency in rats after intrathecal application of the 5-HT_{1B} receptor agonist RU 24969. However, the rat tail flick latency also has been reported to be prolonged (Murphy et al., 1992) or unaffected (Crisp et al., 1991; Mjellem et al., 1992; Ali et al., 1994) after intrathecal application of RU 24969 and/or TFMPP. The reason for the conflicting results in the these tests is not clear, but methodological problems related to spontaneous tail flicks (i.e., tail flicks without any noxious stimulation), that is shown to be induced in rats by the 5-HT_{1B} receptor agonist RU 24969 (Bervoets et al., 1993), may be of importance. In addition, differences in tail skin temperatures in the tail-flick tests may confound the results if not corrected for (Tjølsen et al., 1989).

Our results suggest that the 5-HT_{1B} receptor agonist CP-93,129 causes a selective inhibition of the A δ-fibre mediated responses of dorsal horn-localized wide dynamic range neurones. This is consistent with the findings in earlier electrophysiological studies that the effect of the 5-HT_{1B} receptor agonists RU 24969 (El-Yassir et al., 1988) and TFMPP (Ali et al., 1994) is predominantly an inhibition of mechanical and heat evoked nociceptive responses of dorsal horn neurones. On the other hand, these results contrast with the findings in the present study, where 5-HT_{1B} receptor agonist CP-93,129 significantly increases the electrically evoked post-discharge and probably enhance the excitability of these neurones in the dorsal horn. However, the 5-HT_{1B} receptors could have separate and dissociable functions depending upon the type of

spinal input, and the effects of the 5-HT_{1B} agonists may be due to the use of different stimuli.

At least some of the spinal 5-HT_{1B} sites appear to be autoreceptors, i.e., 5-HT_{1B} receptors located on the terminals of descending serotonergic projections, inhibiting the spinal release of serotonin (Monroe and Smith, 1985; Matsumoto et al., 1992; Yang et al., 1994). Interestingly, clonidine, a selective α_2 adrenoceptor agonist, inhibits the release of 5-HT, whereas the 5-HT_{1B} receptor agonist RU 24969 inhibits the release of noradrenaline in spinal cord synaptosomes (Matsumoto et al., 1990). Thus, noradrenergic fibres could potentially regulate the 5-HT neurone activity through release of noradrenaline and subsequent action on the α_2 heteroreceptor apparently located on descending 5-HT terminals (Matsumoto et al., 1990). Likewise, regulation of noradrenergic neurones by endogenous release of 5-HT, and thereby stimulation of 5-HT_{1B} heteroreceptors possibly located on descending noradrenergic terminals from the raphe nuclei, cannot be excluded. In any case, topical application of the 5-$HT_{1A/B}$ receptor antagonist cyanopindolol, blocked the effect of CP-93,129, whereas the same type of application of the selective 5-HT_{1A} receptor antagonist WAY100635 (that appear to block the effect of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT on neurones in laminae III–VI; Gjerstad et al., 1996), did not. The data of the present study is therefore consistent with the previous finding that CP-93,129 is highly selective for the 5-HT_{1B} receptors versus the 5-HT_{1A} receptors in rats (Macor et al., 1990).

In conclusion, the 5-HT_{1B} receptor agonist CP-93,129 inhibited the Aδ-fibre response and increased the post-discharge in wide dynamic range neurones in the dorsal horn. In contrast, the Aβ- and C-fibre responses of the same neurones were not significantly altered. Action on both 5-HT_{1B} auto- and heteroreceptors in the dorsal horn as well as action on 5-HT_{1B} receptors on primary afferent terminals, cannot be excluded. These results suggest that the 5-HT_{1B} receptor subtype may have both pro- and antinociceptive effects on wide dynamic range neurones in the dorsal horn after repeated electrical stimulation.

References

Alhaider, A.A., Wilcox, G.L., 1993. Differential roles of 5-hydroxytryptamine$_{1A}$ and 5-hydroxytryptamine$_{1B}$ receptor subtypes in modulating spinal nociceptive transmission in mice. J. Pharmacol. Exp. Ther. 265, 378–385.

Ali, Z., Wu, G., Kozlov, A., Barasi, S., 1994. The actions of 5-HT_1 agonists and antagonists on nociceptive processing in the rat spinal cord: Results from behavioral and electrophysiological studies. Brain Res. 661, 83–90.

Bervoets, K., Rivet, J.M., Millan, M.J., 1993. 5-HT_{1A} receptors and the tail flick response. IV. Spinally localized 5-HT_{1A} receptors postsynaptic to serotoninergic neurones mediate spontaneous tail flicks in the rat. J. Pharmacol. Exp. Ther. 264, 95–104.

Bowker, R.M., Westlund, K.N., Sullivan, M.C., Wilber, J.F., Coulter, J.D., 1983. Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei: A multiple transmitter complex. Brain Res. 288, 33–48.

Bruinvels, A.T., Landwehrmeyer, B., Moskowitz, M.A., Hoyer, D., 1992. Evidence for the presence of 5-HT_{1B} receptor messenger RNA in neurones of the rat trigeminal ganglia. Eur. J. Pharmacol. 227, 357–359.

Crisp, T., Stafinsky, J.L., Spanos, L.J., Uram, M., Perni, V.C., Donepudi, H.B., 1991. Analgesic effects of serotonin and receptor-selective serotonin agonists in the rat spinal cord. Gen. Pharmacol. 22, 247–251.

Danneman, P.J., Kiritsy-Roy, J.A., Morrow, T.J., Casey, K.L., 1994. Central delay of the laser-activated rat tail-flick reflex. Pain 58, 39–44.

Daval, G., Verge, D., Basbaum, A.I., Bourgoin, S., Hamon, M., 1987. Autoradiographic evidence of serotonin1 binding sites on primary afferent fibres in the dorsal horn of the rat spinal cord. Neurosci. Lett. 83, 71–76.

El-Yassir, N., Fleetwood-Walker, S.M., Mitchell, R., 1988. Heterogeneous effects of serotonin in the dorsal horn of rat: The involvement of 5-HT_1 receptor subtypes. Brain Res. 456, 147–158.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88.

Gjerstad, J., Tjølsen, A., Hole, K., 1996. The effect of 5-HT_{1A} receptor stimulation on nociceptive dorsal horn neurones in rats. Eur. J. Pharmacol. 318, 315–321.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203.

Huang, J.C., Peroutka, S.J., 1987. Identification of 5-hydroxytryptamine binding site subtypes in rat spinal cord. Brain Res. 436, 173–176.

Johansson, O., Hökfelt, T., Pernow, B., Jeffcoate, S.L.W.N., Steinbusch, W.M., Verhofstad, A.A.J., Emson, P.C., Spindel, E., 1981. Immunohistochemical support for three putative transmitters in one neurone: Coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone like immunoreactivity in medullary neurones projecting to the spinal cord. Neuroscience 6, 1857–1881.

Macor, J.E., Burkhart, C.A., Heym, J.H., Ives, J.L., Lebel, L.A., Newman, M.E., Nielsen, J.A., Ryan, K., Schulz, D.W., Torgersen, L.K., Koe, B.K., 1990. 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one: A potent and selective serotonin (5-HT_{1B}) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. J. Med. Chem. 33, 2087–2093.

Marlier, L., Sandillon, F., Poulat, P., Rajaofetra, N., Geffard, M., Privat, A., 1991. Serotonergic innervation of the dorsal horn of rat spinal cord: Light and electron microscopic immunocytochemical study. J. Neurocytol. 20, 310–322.

Matsumoto, I., Combs, M., Brannan, S., Jones, D.J., 1990. Autoreceptor- and heteroreceptor-mediated regulation of monoamine release in spinal cord synaptosomes. N.Y. Acad. Sci. 604, 609–611.

Matsumoto, I., Combs, M.R., Jones, D.J., 1992. Characterization of 5-hydroxytryptamine$_{1B}$ receptors in rat spinal cord via [125I]iodocyanopindolol binding and inhibition of [3H]-5-hydroxytryptamine release. J. Pharmacol. Exp. Ther. 260, 614–626.

Miletic, V., Hoffert, M.J., Ruda, M.A., Dubner, R., Shigenaga, Y., 1984. Serotoninergic axonal contacts on identified cat spinal dorsal horn neurons and their correlation with nucleus raphe magnus stimulation. J. Comp. Neurol. 228, 129–141.

Mjellem, N., Lund, A., Eide, P.K., Størkson, R., Tjølsen, A., 1992. The role of the 5-HT_{1A} and 5-HT_{1B} receptors in spinal nociceptive transmission and in the modulation of NMDA induced behaviour. Neuroreport 3, 1061–1064.

Monroe, P.J., Smith, D.J., 1985. Demonstration of an autoreceptor modulating the release of [3H]5-hydroxytryptamine from a synaptosomal-rich spinal cord tissue preparation. J. Neurochem. 45, 1886–1894.

Murphy, A.Z., Murphy, R.M., Zemlan, F.P., 1992. Role of spinal serotonin1 receptor subtypes in thermally and mechanically elicited nociceptive reflexes. Psychopharmacology 108, 123–130.

Oliveras, J.L., Guilbaud, G., Besson, J.M., 1979. A map of serotoninergic structures involved in stimulation produced analgesia in unrestrained freely moving cats. Brain Res. 164, 317–322.

Rajaofetra, N., Ridet, J.L., Poulat, P., Marlier, L., Sandillon, F., Geffard, M., Privat, A., 1992. Immunocytochemical mapping of noradrenergic projections to the rat spinal cord with an antiserum against noradrenaline. J. Neurocytol. 21, 481–494.

Ridet, J.L., Rajaofetra, N., Teilhac, J.R., Geffard, M., Privat, A., 1993. Evidence for nonsynaptic serotonergic and noradrenergic innervation of the rat dorsal horn and possible involvement of neuron-glia interaction. Neuroscience 52, 143–157.

Solomon, R.E., Gebhart, G.F., 1988. Mechanisms of effects of intrathecal serotonin on nociception and blood pressure in rats. J. Pharmacol. Exp. Ther. 245, 905–912.

Thor, K.B., Nickolaus, S., Helke, C.J., 1993. Autoradiographic localization of 5-hydroxytryptamine$_{1A}$, 5-hydroxytryptamine$_{1B}$ and 5-hydroxytryptamine$_{1C/2}$ binding sites in the rat spinal cord. Neuroscience 55, 235–252.

Tjølsen, A., Lund, A., Eide, P.K., Berge, O.G., Hole, K., 1989. The apparent hyperalgesic effect of a serotonin antagonist in the tail flick test is mainly due to increased tail skin temperature. Pharmacol. Biochem. Behav. 32, 601–605.

Yaksh, T.L., Wilson, P.R., 1979. Spinal serotonin terminal system mediates antinociception. J. Pharmacol. Exp. Ther. 208, 443–453.

Yang, L., Jacocks III, H.M., Helke, C.J., 1994. Release of [3H]5-hydroxytryptamine from the intermediate area of rat thoracic spinal cord is modulated by presynaptic autoreceptors. Synapse 18, 198–204.

Zemlan, F.P., Corrigan, S.A., Pfaff, D.W., 1980. Norandrenergic and serotonergic mediation of spinal analgesia mechanisms. Eur. J. Pharmacol. 61, 111–124.