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European Journal of Pharmacology 318 (1996) 315–321

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

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Received 27 June 1996; revised 7 October 1996; accepted 11 October 1996

Abstract

Spinal 5-HT_{1A} receptor subtypes are involved in regulation of nociception. This study was performed to investigate the effect of stimulation of these receptors on wide dynamic range neurones in the spinal cord. Extracellular single unit recordings of dorsal horn neurones were performed in intact urethane-anaesthetized female Sprague-Dawley rats. The receptive field distally on one hind paw was electrically stimulated with needle electrodes applied to the skin. The 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), 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 Aβ-, Aδ-, C-fibre responses and post-discharge according to the latencies. Only 500 nmol 8-OH-DPAT caused a significant inhibition of all the neuronal responses. Cells with a pronounced wind-up, limited C-fibre response before drug application and relatively large receptive field for pinch in laminae III–IV were most powerfully inhibited by 500 nmol 8-OH-DPAT. 50 nmol WAY100635 alone did not affect the neuronal responses but blocked the effect of 500 nmol 8-OH-DPAT. These results suggest that stimulation of 5-HT_{1A} receptors inhibits the activity in spinal wide dynamic range neurones after repeated electrical stimulation.

Keywords: Spinal cord; Pain; Nociception; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1A} receptor; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); WAY100635

1. Introduction

Nociceptive transmission in the spinal dorsal horn has been considered to be under both segmental and supraspinal control. Descending 5-HT (5-hydroxytryptamine, serotonin) pathways from the raphe nuclei in the brainstem to the spinal cord are important in this system (Oliveras et al., 1979; Bowker et al., 1983).

The terminals of the descending serotonergic fibres are concentrated in both superficial and deeper laminae (Johansson et al., 1981; Miletic et al., 1984; Marlier et al., 1991a). An inhibitory effect of serotonin on spinal neuronal responses to noxious stimuli has been described (Yaksh and Wilson, 1979; Zemlan et al., 1980).

During the last decade many different binding sites for 5-HT have been identified, and selective ligands for some of these 5-HT receptor subtypes are now available (Hoyer et al., 1994). Recent studies have suggested that different 5-HT receptor subtypes have different roles in the modulation of nociception; even opposite effects have been described (Eide and Hole, 1993).

The 5-HT receptors can be classified according to pharmacological criteria into four receptor subtypes. They comprise the 5-HT_1, 5-HT_2 and 5-HT_3 receptors, whose genes have been cloned, as well as the uncloned 5-HT_4 receptor. Recently, genes for several additional receptors have been cloned (i.e., the 5-ht_5, 5-ht_6 and 5-ht_7), but these receptors have yet to be characterised in intact tissues (Hoyer et al., 1994). In mammals, the 5-HT_1 subtype is further divided into 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}. The 5-HT_{1B} receptor exists predominantly in rodents (rats and mice) while the 5-HT_{1D} receptor exists in many non-rodents (guinea pig, dog, pig, human). Nevertheless, although their concentration is very low, there is evidence that the 5-HT_{1D} receptor also exists in the rat (Hoyer et al., 1994).

In rats, 5-HT_{1A} and 5-HT_{1B} receptor subtypes represent approximately 27 and 18%, respectively, of all 5-HT_1 binding sites in the spinal cord (Huang and Peroutka, 1987). Within the spinal cord, both the 5-HT_{1A} and the

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PII S0014-2999(96)00819-9

5-HT_{1B} receptors are distributed along a rostro-caudal gradient with higher concentrations occurring caudally. 5-HT_{1A} receptors are mainly present in the dorsal horn, whereas 5-HT_{1B} receptors are present throughout all laminae (Marlier et al., 1991b).

Despite receiving a great deal of attention, the role of the 5-HT_{1A} receptor subtype in spinal nociceptive modulation is unclear. Some studies indicate that stimulation of the 5-HT_{1A} receptors reduces nociceptive responsiveness (Archer et al., 1987; Eide et al., 1988), while other studies show the opposite effect of 5-HT_{1A} receptor stimulation (Solomon and Gebhart, 1988; Ali et al., 1994).

In this study the modulatory role of the 5-HT_{1A} receptor subtype in nociceptive transmission in the spinal cord was investigated using the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; Middlemiss and Fozard, 1983; Van-Wijngaarden et al., 1990), and the selective 5-HT_{1A} receptor antagonist, WAY100635 (Fletcher et al., 1994; Forster et al., 1995). Single cell recordings were made from dorsal horn neurones in the lumbar region where this receptor subtype is abundantly present. The effects of the agonist and the antagonist on electrically evoked single unit activity were studied.

2. Materials and methods

2.1. Animals and surgery

Female Sprague-Dawley rats (Mol:SPD, Møllegaard, Denmark) 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 by means of an electronic feedback control unit. 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. A tungsten electrode (2–5 MΩ, Frederick Haer, model 26-05-3) was inserted into the medial part of the dorsal horn by means of an electronic stepper with depth control (Significat Computer Controlled Stepper, model Scat-01, Digitimer).

2.2. In vivo electrophysiology

The receptive fields of the dorsal horn neurones were stimulated electrically with a pair of steel needle electrodes applied to the skin centrally in the receptive field. Because repetitive C-fibre stimulation with frequencies above 0.2–0.3 Hz is known to potentiate the neuronal C-fibre-mediated nociceptive response in the process called wind-up (Mendell, 1966), and the maximal potentiation occurs after stimulation at frequencies of 1–2 Hz (Schouenborg, 1984), a train consisting of 16 rectangular 2-ms-wide pulses, with a frequency of 0.5 Hz was given every 5 min. In order to study the neuronal responses, particularly the Aδ- and C-fibre-mediated responses, a stimulus strength sufficient to induce responses in these primary afferents was necessary. A stimulus strength of 1.5 × threshold for the C-fibre-mediated response (1.9–4.50 mA) that gives submaximal activation of the NMDA receptor (Chapman et al., 1994), was used.

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. As in previous studies (Chapman et al., 1994; Dickenson and Sullivan, 1987; Stanfa et al., 1992) the discharge 0–20 ms, 20–90 ms and 90–300 ms after stimulation was characterised as Aβ-, Aδ- and C-fibre response, respectively, and the discharge 300–800 ms after stimulation was considered a post-discharge.

The data were captured using a CED 1401 interface connected to an IBM compatible PC. Purpose-made software was used for control of stimulation, recording, accumulation of unit responses and data storage.

2.3. Drugs

The 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin; Middlemiss and Fozard, 1983; Van-Wijngaarden et al., 1990), was obtained from Research Biochemicals International, and the 5-HT_{1A} receptor antagonist, WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; Fletcher et al., 1994; Forster et al., 1995) was a gift from Wyeth Research (UK). The drugs were dissolved at room temperature in 0.9% NaCl. All doses of the drugs were dripped directly onto the exposed area of the spinal cord in a volume of 50 μl.

2.4. Experimental procedure

Using techniques that have been described previously (Schouenborg, 1984; Dickenson and Sullivan, 1987; Stanfa et al., 1992; Chapman et al., 1994), 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 a noxious forceps pinch painful when applied to human skin, were studied. Receptive fields for pinch were mapped, and their sizes were estimated.

Every 5 min a train of stimuli was delivered and the responses were recorded. Following three stable train responses, i.e., the responses of each train of 16 stimuli varying by less than 8%, the drug was applied and the

neuronal responses were studied for 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.

In each train of 16 stimuli the degree of wind-up was calculated according to the formula:

$$\text{Wind-up} = 100\% \times \left(\Sigma R_n - (16 \times R_1)\right)/(16 \times R_1)$$

where R_n = C-fibre response + post-discharge after stimulus n (Stanfa et al., 1992). The wind-up in the three control trains was used for calculating the average wind-up in the control for each cell.

The effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the 5-HT_{1A} receptor antagonist, WAY 100635, were calculated as the number of spikes per 16 stimuli 15–30 min after drug application as percentage of the control response. Furthermore, 30 min after the application of the antagonist, a mixture of the agonist and the antagonist was 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 percentage 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 effect of the highest dose of 8-OH-DPAT on C-fibre response as a function of other parameters was expressed in dot-plots, and regression analyses and multiple regression analyses were performed. The interaction between the agonist and the antagonist was analysed using an univariate two-way analysis of variance (ANOVA). Significance was accepted at the 5% level.

3. Results

3.1. Effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT

8-OH-DPAT was applied in doses of 5–500 nmol. Only 500 nmol 8-OH-DPAT caused a significant inhibition of all the neuronal responses. This dose of 8-OH-DPAT caused a pronounced inhibition of the Aδ-, C-fibre response and post-discharge, whereas the Aβ-fibre response was less inhibited (Fig. 1).

After application of 500 nmol 8-OH-DPAT the Aβ-, Aδ-, C-fibre responses and post-discharge in most wide dynamic range neurones (25/35) were decreased. In these cells, a progressive and pronounced decrease in the response was observed during the first 15 min. In the test period 15–30 min after application, the responses became stabilized. In neurones whose activity was inhibited, the effect was partly reversed when 8-OH-DPAT was replaced by saline (washout). An example of the C-fibre response of a single neurone after application of 8-OH-DPAT and washout is shown (Fig. 2).

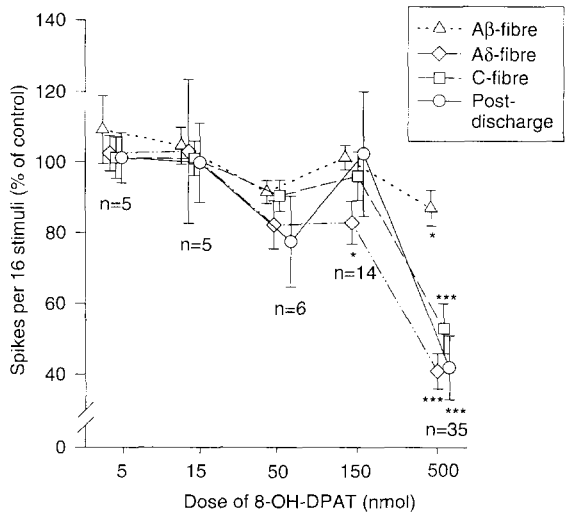

Fig. 1. The effect of 8-OH-DPAT on activity evoked in dorsal horn neurones. Neuronal responses following different doses of 8-OH-DPAT are shown as percentage of control responses before drug application. Mean ± S.E.M., $n = 5$–35. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, paired Student's t-test between the neuronal control responses and responses with 8-OH-DPAT.

The neurones whose activity was decreased after 500 nmol 8-OH-DPAT were studied further according to each single neurone's characteristics. A linear regression analysis was performed for the C-fibre response against each of the other parameters: wind-up in control, C-fibre response in control, depth in dorsal horn and size of the receptive field for pinch.

Most cells showed a positive wind-up before drug was applied. The Aδ-, C-fibre response and post-discharge were inhibited most strongly in cells with a pronounced wind-up in the control. The highly significant relationship between the C-fibre response and wind-up is shown (Fig. 3A).

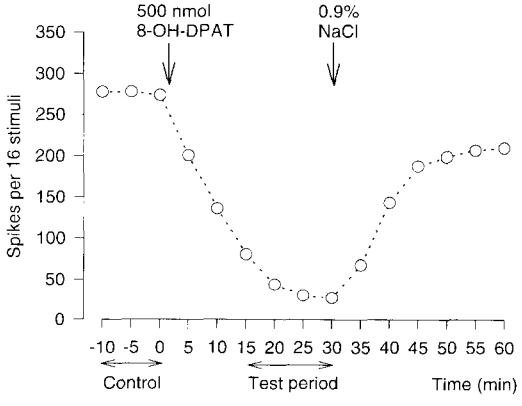

Fig. 2. Time-course of the C-fibre response evoked in a single dorsal horn neurone after 500 nmol 8-OH-DPAT and 0.9% NaCl (washout). Neuronal responses, i.e., spikes per 16 stimuli, are shown.

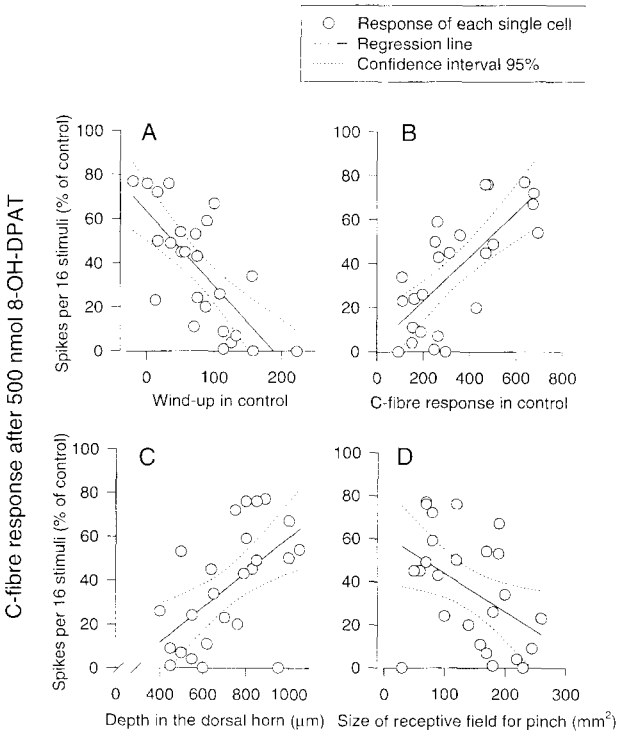

Fig. 3. The effect of 500 nmol of 8-OH-DPAT related to wind-up in control, C-fibre response in control, depth in the dorsal horn and receptive field for pinch. Single cell responses following 8-OH-DPAT are shown as percentages of the control responses. (A) Wind-up in control. Linear regression $P < 0.0001$. (B) C-fibre response in control. Linear regression $P < 0.0001$. (C) Depth in the dorsal horn. Linear regression $P = 0.0028$. (D) Receptive field for pinch. Linear regression $P = 0.022$.

Only for the C-fibre mediated response was there a significant relationship between the control value and the effect of 8-OH-DPAT. The C-fibre responses in cells with a limited C-fibre response before application of the drug were most strongly inhibited (Fig. 3B). No significant relationship was found between wind-up and the control value.

The cells studied were located in deeper laminae (III–VI) in the dorsal horn. The cells were evenly distributed within this depth interval. A significant relationship between the effect of 8-OH-DPAT on the C-fibre response and depth was discovered. The C-fibre responses of cells in laminae III–IV seemed to be more strongly inhibited than the same responses of cells in deeper laminae (Fig. 3C). Interestingly, no such relationships were found for the A-fibre-mediated responses and the post-discharge.

The size of the receptive field for pinch varied from the plantar side of just one toe to the entire plantar aspect of the paw. The C-fibre response was inhibited most strongly in cells with a relatively large receptive field for pinch (Fig. 3D). No relationship was found between depth and the size of the receptive field.

A multiple regression analysis was performed with C-fibre response as the dependent parameter and the wind-up in the control, C-fibre response in the control, depth and size of the receptive field as independent parameters. The first two parameters significantly affected the C-fibre response inhibition (wind-up in control: $P = 0.0019$, C-fibre response in control: $P = 0.036$) in this analysis also, whereas the other two parameters did not (depth: $P = 0.43$, size of the receptive field: $P = 0.40$). Since depth and size of receptive field did not significantly add to the equation of the predicted C-fibre response they were excluded from the final multiple regression analysis (wind-up in control: $P = 0.0014$, C-fibre response in control: $P = 0.0011$).

3.2. The effect of the 5-HT_{1A} receptor antagonist, WAY100635, and 8-OH-DPAT

According to the results of the present study, 5-HT_{1A} receptor stimulation has a powerful inhibitory effect on cells with a wind-up in the control > 50% and C-fibre response in the control < 400 spikes/16 stimuli (Fig. 3A and B). WAY100635, 50 nmol, and a mixture of 50 nmol WAY100635 + 500 nmol 8-OH-DPAT were tested on 8 cells with these characteristics, and the responses were compared to the responses of a new group of 5 cells exposed to 0.9% NaCl with the same characteristics and to those cells with wind-up in the control > 50% and C-fibre response in the control < 400 spikes/16 stimuli already exposed to 8-OH-DPAT alone, i.e., 15 of the 35 cells exposed to 500 nmol 8-OH-DPAT in the first place (Fig. 4).

A univariate two-way analysis of variance (ANOVA) was performed. The effect of 50 nmol WAY100635 and 500 nmol 8-OH-DPAT on Aδ-, C-fibre response and post-discharge compared to the effect on the same responses after coadministration showed a clear interaction between the two drugs (Aδ: $P = 0.0004$, C: $P = 0.0065$, post-discharge $P = 0.031$, WAY100635 and 8-OH-DPAT interaction, ANOVA). Whereas 50 nmol WAY100635 alone affected neither the A- nor the C-fibre responses, the same dose significantly blocked the inhibition of the Aδ-,

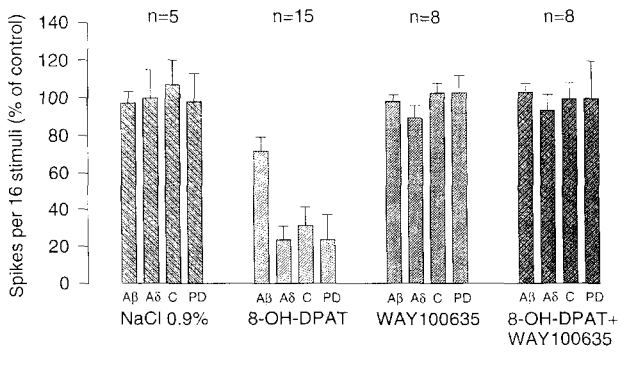

Fig. 4. The effect of application of 0.9% NaCl, 500 nmol 8-OH-DPAT, 50 nmol WAY100635, and 500 nmol 8-OH-DPAT coadministered with 50 nmol WAY100635. The Aβ-fibre response (Aβ), the Aδ-fibre response (Aδ), the C-fibre responses (C) and the post-discharge (PD) are shown. Mean ± S.E.M., $n = 5–15$.

C-fibre response and post-discharge caused by 500 nmol 8-OH-DPAT alone (Fig. 4).

4. Discussion

This study demonstrated that the 5-HT_{1A} receptor agonist, 8-OH-DPAT, decreased the evoked response in most wide dynamic range neurones in the dorsal horn. The strong inhibition of the Aδ- and C-fibre responses could be mediated through presynaptic 5-HT_{1A} receptors located on primary afferent Aδ- and C-fibres (Dunlap and Fischbach, 1978) or by a postsynaptic mode of action on spinal neurones. Some anatomical studies (Hoffert et al., 1983; Miletic et al., 1984) indicate that, in the superficial dorsal horn of the cat, descending serotonergic projections make contact predominantly on dendritic shafts and somata rather than on axons, providing evidence for postsynaptic 5-HT sites. However, in the rat, only some of the serotonergic vesicular profiles establish synapses (Maxwell et al., 1983; Marlier et al., 1991a), and the receptors are likely to be located at some distance from the serotonergic terminals. Other reports have shown that approximately 20–30% of the 5-HT_1 and 5-HT_{1A} receptors in rats are located presynaptically on primary afferent fibres (Daval et al., 1987).

Our results confirm the earlier findings from electrophysiological studies that the effect of 5-HT_1 and 5-HT_{1A} receptor agonists is predominantly inhibition of wide dynamic range neurones (El-Yassir et al., 1988; Zemlan et al., 1994). The antinociceptive effect of serotonin is also consistent with the results of studies of isolated rat capsaicin-sensitive dorsal root ganglion neurones with long duration action potentials (C-type primary sensory neurones). The effect of 5-HT and 5-HT_{1A} receptor stimulation is inhibition of a high-threshold Ca^{2+} channel current in these cells (Del Mar et al., 1994). This suggests that 5-HT, released in the spinal cord from descending fibres, may have an antinociceptive effect by inhibiting Ca^{2+} entry into primary afferent terminals of nociceptors via activation of 5-HT_{1A} receptors.

Behavioural studies have yielded far more conflicting results. Some authors have reported a reduced nociceptive response (Archer et al., 1987; Eide et al., 1988), while others have described the opposite effect for the same 5-HT_1 and 5-HT_{1A} receptor agonists (Solomon and Gebhart, 1988; Ali et al., 1994). The conflicting results may be related to different sources of error in experimental models, differences in skin temperatures in hot plate and tail-flick tests (Tjølsen et al., 1989), differences in the subjective behavioural scoring of nociceptive responses, different administration routes of drug and species differences.

Although the 5-HT_{1A} receptor agonist, 8-OH-DPAT, decreased the activity evoked in most wide dynamic range neurones, the responses were difficult to explain without considering the characteristics of each single neurone. This is the first report demonstrating the importance of wind-up properties, control responses, depth, and size of the receptive field of the neurones for the effect of 5-HT_{1A} receptor stimulation. Multiple regression analysis showed that only cells with a pronounced wind-up and limited control response were powerfully inhibited.

The powerful inhibition of both Aδ-, C-fibre response and post-discharge of cells with a pronounced wind-up may have at least two explanations: first, the 5-HT_{1A} receptors are located postsynaptically on neurones which also have receptors that are involved in the mechanism of the wind-up process, e.g., the NMDA receptors (Davies and Lodge, 1987; Dickenson and Sullivan, 1987) and the NK-1 receptors (Budai and Larson, 1996), or second, the 5-HT_{1A} receptors might be located presynaptically on primary afferent fibres that terminate on these neurones. In any case, it is interesting that 5-HT seems to modulate the activity preferentially in cells with a pronounced wind-up.

Furthermore the results of these studies showed that only wide dynamic range neurones with a relatively small response to electrical stimulation were powerfully inhibited by 5-HT_{1A} receptor stimulation. Probably, in cells with a pronounced C-fibre response, an intense C-fibre input causes a pronounced temporal summation of excitatory postsynaptic potentials and a postsynaptic depolarisation that considerably exceeds the spike threshold. Therefore, a large C-fibre response may be relatively resistant to inhibition. In contrast, a small C-fibre input will generate a postsynaptic depolarisation that only just exceeds the spike threshold, making cells with a small C-fibre input and thus a small C-fibre response more susceptible to inhibition. For this reason the C-fibre responses in the control also should be taken into account when one compares the response change of one group of wide dynamic range neurones with another.

The 5-HT_{1A} receptors within the spinal cord are distributed according to a dorsoventral gradient, with the highest density of these receptors in the superficial laminae of the dorsal horn. In the lumbar region the gradient throughout the spinal cord is conspicuous; the density in laminae I–II is 8 times the density in laminae III–VI (Marlier et al., 1991b). In the present study, the C-fibre-mediated responses of neurones in laminae III and IV seemed to be more inhibited than the same responses in more deeply located neurones. These results may be explained by the distribution of the 5-HT_{1A} receptors in the dorsal horn described in earlier studies, or by the ability of penetration of 8-OH-DPAT from the surface of the spinal cord to the receptor sites in the tissue. The fact that no relationship was found between the inhibition of the Aδ-fibre response and depth may indicate that the effect of 8-OH-DPAT on the C-fibre response is caused by a relatively higher density of these receptors in laminae III–IV than in deeper laminae on primary afferent C-fibres or dendrites that receive these fibres, rather than by a concentration gradient of the drug in the spinal cord.

All neurones that were studied had a specific receptive field for pinch. The size of these fields is dependent on the size of the area where the receptors of the primary afferent fibres are located and on the convergence of these fibres on the spinal neurone. A great number of primary afferent fibres may therefore terminate on dorsal horn neurones with large receptive fields. The modulation of transmitter release from all these terminals by 5-HT acting on the presynaptically located 5-HT_{1A} sites is probably an important supplement to the postsynaptic mode of action (Daval et al., 1987).

Because the 5-HT_{1A} receptors in rats are present in abundance in the spinal cord (Huang and Peroutka, 1987), and because stimulation of these receptors mainly seems to decrease the neuronal evoked activity, this activity would increase after blocking of these receptors if there were a tonic inhibitory serotonergic activity on the 5-HT_{1A} receptors. The fact that the 5-HT_{1A} receptor antagonist, WAY100635, did not alter neuronal activity argues against any tonic activity on 5-HT_{1A} receptors in these experiments. In any event, WAY100635 completely blocked the effect of 8-OH-DPAT on a subpopulation of neurones whose activity was powerfully inhibited by 8-OH-DPAT alone, indicating that the effect of 8-OH-DPAT is mediated by activation of specific 5-HT_{1A} receptor sites.

In conclusion, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, seems to inhibit the Aδ-fibre response, C-fibre response and post-discharge of many wide dynamic range neurones in the dorsal horn. Powerful inhibition was observed in cells with a pronounced wind-up, limited C-fibre response in the control, limited depth in the dorsal horn and a relatively large receptive field. No tonic inhibitory serotonergic activity on the 5-HT_{1A} receptors was detected in these experiments.

These results suggest that stimulation of the 5-HT_{1A} receptors inhibits the activity in highly convergent wide dynamic range cells, preferentially in laminae III–IV, involved in the enhancement of nociceptive responses after repeated electrical stimulation. Stimulation of 5-HT_{1A} receptors may therefore inhibit the first step of the central nervous system changes after peripheral stimulation and activity in primary afferent fibres that could be relevant for the development of pathological pain states.

References

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

Archer, T., E. Arweström, B.G. Minor, M.L. Persson, C. Post, E. Sundström and G. Jonsson, 1987, (+)-8-OH-DPAT and 5-MeODMT induced analgesia is antagonised by noradrenaline depletion, Physiol. Behav. 39, 95.

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

Budai, D. and A.A. Larson, 1996, Role of substance P in the modulation of C-fiber-evoked responses of spinal dorsal horn neurones, Brain Res. 197.

Chapman, V., A.H. Dickenson and A. Tjølsen, 1994, Bi-directional effects of intrathecal NMDA and substance P on rat dorsal horn neuronal responses, Neurosci. Lett. 178, 90.

Daval, G., D. Verge, A.I. Basbaum, S. Bourgoin and M. Hamon, 1987, Autoradiographic evidence of serotonin 1 binding sites on primary afferent fibres in the dorsal horn of the rat spinal cord, Neurosci. Lett. 83, 71.

Davies, S.N. and D. Lodge, 1987, Evidence for involvement of *N*-methyl aspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat, Brain Res. 424, 402.

Del Mar, L.P., C.G. Cardenas and R.S. Scroggs, 1994, Serotonin inhibits high-threshold Ca^{2+} channel currents in capsaicin-sensitive acutely isolated adult rat DRG neurones, J. Neurophysiol. 72, 2551.

Dickenson, A.H. and A.F. Sullivan, 1987, Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation, Neuropharmacology 26, 1235.

Dunlap, K. and G.D. Fischbach, 1978, Neurotransmitters decrease the calcium component of sensory neurone action potentials, Nature 276, 837.

Eide, P.K. and K. Hole, 1993, The role of 5-hydroxytryptamine (5-HT) receptor subtypes and plasticity in the 5-HT systems in the regulation of nociceptive sensitivity, Cephalalgia 13, 75.

Eide, P.K., K. Hole, O.G. Berge and O.J. Broch, 1988, 5-HT depletion with 5,7-DHT, PCA and PCPA in mice: differential effects on the sensitivity to 5-MeODMT, 8-OH-DPAT and 5-HTP as measured by two nociceptive tests, Brain Res. 440, 42.

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

Fletcher, A., D.J. Bill, I.A. Cliffe, E.A. Forster, D. Jones and Y. Reilly, 1994, A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist, Br. J. Pharmacol. 112, 91p.

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

Hoffert, M.J., V. Miletic, M.A. Ruda and R. Dubner, 1983, Immunocytochemical identification of serotonin axonal contacts on characterized neurons in laminae I and II of the cat dorsal horn, Brain Res. 267, 361.

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

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

Johansson, O., T. Hökfelt, B. Pernow, S.L.W.N. Jeffcoate, W.M. Steinbusch, A.A.J. Verhofstad, P.C. Emson and E. Spindel, 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.

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

Marlier, L., J.R. Teilhac, C. Cerruti and A. Privat, 1991b, Autoradiographic mapping of 5-HT_1, 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors in the rat spinal cord, Brain Res. 550, 15.

Maxwell, D.J., C. Leranth and A.A.J. Verhofstad, 1983, Fine structure of

serotonin-containing axons in the marginal zone of the spinal cord, Brain Res. 266, 253.

Mendell, L.M., 1966, Physiological properties of unmyelinated fiber projections to the spinal cord, Exp. Neurol. 16, 316.

Middlemiss, D.N. and J.R. Fozard, 1983, 8-Hydroxy-2-(di-*n*-propylamino)-tetralin discriminates between subtypes of the 5-HT_1 recognition site, Eur. J. Pharmacol. 90, 151.

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

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

Schouenborg, J., 1984, Functional and topographical properties of field potentials evoked in rat dorsal horn by cutaneous C-fibre stimulation, J. Physiol. 356, 169.

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

Stanfa, L.C., A.F. Sullivan and A.H. Dickenson, 1992, Alterations in neuronal exitability and potency of spinal mu, delta and kappa opioids after carrageenan-induced inflammation, Pain 50, 345.

Tjølsen, A., A. Lund, P.K. Eide, O.G. Berge and K. Hole, 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.

Van-Wijngaarden, I., M.T. Tulp and W. Soudijn, 1990, The concept of selectivity in 5-HT receptor research, Eur. J. Pharmacol. 188, 301.

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

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

Zemlan, F.P., A.Z. Murphy and M.M. Behbehani, 1994, 5-HT_{1A} receptors mediate the effect of the bulbospinal serotonin system on spinal dorsal horn nociceptive neurons, Pharmacology 48, 1.