Antinociceptive role of 5-HT$_{1A}$ receptors in rat spinal cord

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Background. Intrathecal administration of 5-hydroxytryptamine (5-HT) is antinociceptive to noxious heat and electrical stimuli. The contributions of different receptor subtypes to the antinociceptive effects of 5-HT are controversial. The main reasons for this are the poor receptor subtype selectivity of some agonist drugs and the difficulty of restricting drug action to the spinal cord in some experimental paradigms. This study investigated the roles of different 5-HT receptor subtypes involved in the spinal cord control of the nociception produced by these two nociceptive testing paradigms.

Methods. Tail-flick latency and electric current threshold for nociception were measured in an acute pain model that allowed the study of the antinociceptive effects of intrathecally administered drugs that were due to actions of these drugs at spinal cord receptors. Experiments were performed in male Wistar rats with chronically implanted lumbar subarachnoid catheters. Dose–response curves for spinally mediated antinociceptive effects of agonists selective for 5-HT receptor subtypes were constructed.

Results. The 5-HT$_{1}$ agonist 1-(3-chlorophenyl)-piperazine dihydrochloride caused a dose-dependent antinociceptive effect, measured by both nociceptive tests. However, 8-hydroxy-DPAT (selective 5-HT$_{1A}$ agonist) produced antinociception assessed by electric current but not tail flick. A 5-HT$_{1A}$-selective antagonist, 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine, reversed the antinociception in the electrical test produced by both of these agonists but the tail-flick latency effects after intrathecal 1-(3-chlorophenyl)-piperazine were not suppressed by this antagonist.

Conclusions. We conclude that 5-HT$_{1A}$ receptors in the spinal cord are involved in the nociceptive mechanisms assessed by noxious electrical stimuli. Other 5-HT$_{1}$ receptors (non 5-HT$_{1A}$ receptors) are involved in the spinally mediated antinociception assessed by thermal noxious stimuli.

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The antinociceptive effects of spinally administered 5-hydroxytryptamine (5-HT) are well established. Intrathecally administered 5-HT mimics the effect of electrical activity of tracts descending from neurones located in brainstem raphe nuclei, electrical stimulation of which also produces antinociceptive effects in single dorsal horn neurones and awake animals. The 5-HT antagonist methysergide has been shown to reverse the antinociception produced by spinal 5-HT and to produce hyperalgesia in rats when used alone intrathecally. These latter experiments suggest that 5-HT receptors are involved in spinal antinociception. More recent experiments have shown the involvement of subtypes of 5-HT receptors in the spinal cord control of nociception. In mice, a 5-HT$_{2}$ agonist given intrathecally produced biting and scratching behaviour, whereas a 5-HT$_{1A}$ agonist caused antinociception, assessed by tail-flick latency (TFL). Receptors for 5-HT$_{1A}$ have also been implicated in correlates of nociception in single
neurones in the spinal cord dorsal horn. Others have concluded, from experiments in which a selective 5-HT<sub>1A</sub> antagonist (WAY-100635) was given intrathecally, that 5-HT<sub>1A</sub> receptors in the spinal cord are responsible for the bulbospinally mediated antinociceptive effects of fentanyl administered intracerebroventricularly. Much experimental work has been published in this area, but the question of which subtypes of 5-HT receptor are involved, particularly in the spinal cord control of nociception, is still controversial. This controversy stems from the lack of selectivity of the drugs for the receptors used in some studies and from the inability of some experimental paradigms to limit intrathecal drug action to the spinal cord. Thus, incorrect conclusions about the subtype of 5-HT receptors involved in the spinal cord control of nociception could be made if the drug administered has activity at other 5-HT receptors at the same dose, or if the drug spreads away from the spinal cord site of injection to sites with 5-HT receptors in the brain that then mediate the antinociceptive effects. This has clear clinical implications for the development of a drug targeting spinal cord receptors for analgesia, perhaps by spinal injection.

It has been shown that intrathecal injection of 5-HT in such a way that its actions are confined to the spinal cord causes antinociception, as revealed by the response to noxious electrical stimuli [electric current threshold (ECT) test] and by TFL. Methysergide reverses these antinociceptive effects, indicating the involvement of spinal cord 5-HT receptors. The investigators went on to show, in selective antagonist and cross-tolerance experiments, that the antinociception revealed by the noxious electrical stimulation paradigm (but not TFL) involved spinal cord μ opioid and GABA<sub>A</sub> receptors. Because different nociceptive testing paradigms were used, it is uncertain what 5-HT receptor subtypes were involved in the μ opioid and GABA<sub>A</sub> receptor interaction.

The purpose of the present study was to investigate the involvement of 5-HT<sub>1</sub> receptor subtypes in the spinal cord control of nociception, assessed in the ECT and TFL paradigms. The response to noxious electrical stimulation and TFL were measured in a model that allowed the study of drug actions that were confined to the caudal segments of the spinal cord. Drugs selective for different 5-HT<sub>1</sub> receptor subtypes were injected intrathecally into conscious rats with chronically implanted lumbar subarachnoid catheters. Antinociceptive effects were assessed with noxious heat and electric current. Antagonism of these effects was investigated by intrathecal injection of a selective 5-HT<sub>1A</sub> antagonist.

Methods
Experiments were performed on male Wistar rats (weight 185±12 g (mean±SEM)). In all experiments, we followed international guidelines for the care of laboratory and experimental animals and guidelines for the investigation of pain in laboratory animals. This work was carried out with permission of the Monash University Standing Committee on Ethics in Animal Experimentation (SCEAE Project No. 93017).

Catheter implantation
Lumbar intrathecal catheters (dead space volume 3–5 μl) were implanted under halothane anaesthesia and aseptic surgical conditions as described previously. Portex catheters were introduced into the lumbar intrathecal space to lie next to the most caudal segments of the spinal cord, responsible for innervation of the tail and hind legs. The catheters were tunneled subcutaneously to an exit wound at the base of the neck. The exact volume of the catheter was measured before insertion, thus allowing accurate dosing of drugs injected through the catheter into the cerebrospinal fluid (CSF). Correct catheter placement was confirmed by injecting 2% lidocaine solution 10 μl down the catheter 10 min after recovery from general anaesthesia. The catheter was judged to be intrathecal if paralysis and dragging of the hind legs occurred within 30 s of this injection. All animals failing this test and those with neurological deficit after surgery were rejected from further study. The lidocaine test was also performed after each experiment to confirm that all drugs injected down the intrathecal catheters were injected into the lumbar intrathecal space. The rats were rested for 24 h before further experiments were performed. Only one experiment per day up to a maximum of five was performed on each animal.

Nociceptive tests
Two nociceptive tests were used in all experiments, the ECT test and the TFL test, as described previously. The electrical test allows demonstration of drug action at the level of the spinal cord after lumbar intrathecal injection, i.e. the measurement of an increase in nociceptive threshold in the tail with no change in the neck threshold measurements. Such a differential block proves that the effects of the drug are confined to the caudal segments of the spinal cord responsible for tail and hind limb innervation. A rise in TFL in the same experiment in which such a differential effect is demonstrated must also therefore be due to action of the drug on the caudal segments of the spinal cord. Both of these tests were applied in sequence to each rat every 5 min.

Electric current threshold test
Each rat was placed in a darkened restrainer and electrical stimulating electrodes were applied to the skin of the tail and neck. Brief bursts of electric current (0.5 s train, 2 ms pulse width, 50 Hz, 0–10 mA) were delivered in turn to each set of electrodes (tail first, 15 s before the neck) every 5 min. The minimum current necessary to evoke an obvious nociceptive response (a squeak or sharp withdrawal), defined by the ‘up–down’ method, was measured at each
skin site every 5 min until three stable consecutive readings had been obtained. The intrathecal drug was then given and measurements continued at each skin site every 5 min for a further 45–50 min. In order to standardize results obtained at each skin site, the response to intrathecal drug was calculated as a ratio with respect to the mean of the three stable readings taken before intrathecal drug injection, as described previously.18 These values for responses to each dose of drug were combined and expressed as the mean (SEM) on agonist dose–response curves.

**Tail-flick latency test**
This was performed every 5 min immediately before the electrical measurement in the tail. The heat from a 150 watt projector bulb was directed onto the blackened tip of the tail and the power adjusted so that the rat flicked its tail away from the noxious stimulus in approximately 3 s. At this intensity of stimulation, three stable control (pre-drug injection) readings were obtained and then the test drug was injected. TFL measurement continued every 5 min thereafter for 30–45 min. The response to each dose of drug was calculated as a percentage of maximum possible effect (MPE):

\[
\text{%MPE} = \left( \frac{\text{TFL}_{\text{post-drug}} - \text{TFL}_{\text{pre-drug}}}{\text{cut-off time} - \text{TFL}_{\text{pre-drug}}} \right) \times 100,
\]

A cut-off time of 10 s was used. Values were combined for each drug dose, expressed as mean (SEM) and used to produce dose–response curves.

**Agonist dose–response relationships**
A range of doses (0.005–5.0 μmol) of 1-(3-chlorophenyl)piperazine dihydrochloride (non-selective agonist of 5-HT1)19 20 was given intrathecally to six rats with chronically implanted subarachnoid catheters. Sixteen experiments were performed (n=4 per dose of agonist). Similarly, a range of doses (0.01–0.5 μmol) of 8-hydroxy-2-dipropylaminotetralin hydrobromide (a 5-HT1A-selective agonist)21 22 was given intrathecally to 15 rats with chronically implanted subarachnoid catheters. Twenty-three experiments were performed (n=4–6 per dose of agonist). The drugs were dissolved in 6% dextrose solution to make the injectate hyperbaric compared with CSF. Doses were delivered into the lumbosacral CSF in 5 μl volumes while the rat was held at a 15° inclined plane, head up. This was done to restrict the spread of drug to the caudal segments of the spinal cord, as described previously.23 The dextrose solution has been shown not to affect nociceptive thresholds when given alone.18 Once-daily injections of agonist drugs were given on up to five occasions to individual rats. This has been shown in previous work on intrathecal 5-HT not to cause tolerance or tachyphylaxis.15 16

**Antagonist dose–response relationships**
Doses of agonist drugs were chosen from their dose–response curves for the experiments with antagonists. These were the roughly equieffective doses that produced a near-maximal response for tail ECT. This dose was 0.1 μmol for 8-hydroxy-DPAT hydrobromide and 0.5 μmol for 1-(3-chlorophenyl)-piperazine dihydrochloride. The agonist was given intrathecally alone at the beginning and at the end of a series of once-daily experiments in each rat. In the intervening days the same dose of the same agonist was given intrathecally combined with a range of doses (0.001–0.05 μmol) of a 5-HT1A-selective antagonist [4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine] dissolved in 6% dextrose. Twenty experiments were performed in nine rats using 8-hydroxy-DPAT hydrobromide (n=4–5 observations per dose of antagonist). Eleven rats were used in 27 experiments with 1-(3-chlorophenyl)-piperazine dihydrochloride (n=4–5 observations per dose of antagonist). Antinociceptive effects were measured using ECT in the tail and neck and TFL. The pre-series and post-series measurements of the antinociceptive effect for agonist alone in each rat were then averaged (R). The percentage suppression of agonist effect by each dose of antagonist was calculated using the following equation, described previously:18

\[
\text{% suppression} = \frac{R - r}{R - 1} \times 10
\]

where \( r \) is the individual response to agonist mixed with a particular dose of antagonist and \( R \) the mean response to agonist given intrathecally alone. The values of percentage suppression were calculated for both ECT and TFL tests. Values were combined to produce means and SEM, which were then plotted as dose–response relationships for suppression of the antinociceptive effects of each agonist.

**Results**
Positive lidocaine tests were obtained in each rat and after each experiment that contributed values to the dose–response relationships reported in this paper. Thus, all agonist and antagonist drugs injected into intrathecal catheters were delivered to the lumbosacral intrathecal space. Both agonist drugs caused dose-related increases in tail ECT with no change in neck ECT. Thus, both drugs caused antinociception by an action in the caudal segments of the spinal cord responsible for tail innervation (Figs 1 and 2). The non-selective 5-HT1 agonist 1-(3-chlorophenyl)piperazine dihydrochloride caused simultaneous dose-related antinociceptive effects, as assessed with the TFL test (Fig. 1). By contrast, the selective 5-HT1A agonist 8-hydroxy-DPAT hydrobromide caused no such TFL effects, the antinociception being confined to ECT (Fig. 2).
Figure 3 shows dose–response curves for the suppression of tail ECT and TFL antinociceptive effects of 1-(3-chlorophenyl)-piperazine dihydrochloride in the ECT and TFL tests. Intrathecal 1-(3-chlorophenyl)-piperazine dihydrochloride produced dose-related antinociception in both tests. ECT (neck) values did not increase after intrathecal injections of 1-(3-chlorophenyl)-piperazine dihydrochloride, indicating that the drug was confined in its actions to the caudal segments of the spinal cord responsible for tail innervation. Data points are means and bars are SEM (n=4).

Figure 3 shows dose–response curves for the suppression of tail ECT and TFL antinociceptive effects of 1-(3-chlorophenyl)-piperazine dihydrochloride (Fig. 3A) and 8-hydroxy-DPAT hydrobromide (Fig. 3B) by the antagonist 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)-piperazine. This selective 5-HT<sub>1A</sub> antagonist caused dose-related antagonism of the antinociception assessed by the ECT test for both agonist drugs. However, the TFL effects of the non-selective 5-HT<sub>1</sub> receptor agonist 1-(3-chlorophenyl)-piperazine dihydrochloride were unaffected by intrathecal injection of the selective 5-HT<sub>1A</sub> antagonist (Fig. 3A).

Discussion
Many studies have reported antinociception after intrathecal administration of serotonergic agonists. Many of these involved injections of drugs via catheters reported to be intrathecal but no proof of catheter position and site of drug action at the level of the spinal cord was given. Although conclusions about spinal cord 5-HT receptors were drawn, the antinociceptive effects observed could have been due to actions of the drugs on the brain after spread via CSF.

The study reported in this paper concentrated on the restriction of drug actions to the spinal cord and the provision of support for such confinement. The experiments were performed in rats with positive lidocaine tests, and antinociceptive effects occurred only in the tail, not the neck. It has been shown previously in this model that intrathecally administered 5-HT caused its ECT antinociceptive effects by involvement of spinal cord GABA and µ opioid receptors but no such interactions occurred for the TFL effects. The results of the experiments reported in this paper extend these findings. Again, there was a difference in the mechanisms of antinociception revealed by the TFL and ECT tests. The ECT test reveals antinociception involving 5-HT<sub>1A</sub> receptors, whereas the TFL test involves other 5-HT<sub>1</sub> (but not 5-HT<sub>1A</sub>) receptors.

One study in mice used the same 5-HT<sub>1A</sub>-selective agonist as that used in the present study (8-hydroxy-DPAT). The investigators concluded that 5-HT<sub>1A</sub> receptors in the spinal cord were responsible for TFL antinociception. This result is contrary to the findings of the experiments reported in this paper. This may represent a supraspinal effect of the drug. Many of these drugs are very soluble in water and thus spread easily throughout the CSF. The investigators made no measurements, such as a comparison of rostral and caudal dermatome nociceptive thresholds, that would indicate confinement of drug action to the caudal segments of the spinal cord.

The importance of distinguishing spinal and supraspinal effects was highlighted recently in a study of the involvement of 5-HT<sub>1A</sub> receptors in opioid-induced bulbospinal inhibition. It was shown that fentanyl injected into CSF in the brain inhibited sural–gastrocnemius reflex responses. Intrathecal administration of the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 significantly reduced this inhibition. In a separate group of experiments, i.v. fentanyl
depressed the sural–gastrocnemius reflex. This inhibition was not affected by intrathecal WAY-100635 (100 μg), but combined administration of the 5-HT₁A antagonist with an α₂ adrenoceptor antagonist (RX 821002) significantly reduced the effects of i.v. fentanyl. They concluded that the bulbospinal and direct spinal actions of fentanyl may occur together to produce overall inhibition of the reflex.¹³

Other studies have claimed interaction of serotonergic receptors with other neurotransmitter systems in the spinal cord. It was reported that a 5-HT₃ receptor selective agonist (2-methyl serotonin) caused antinociceptive effects that involved spinal opioid and GABA receptor systems.²⁷ When this drug was injected intracerebroventricularly no antinociception was observed. It was therefore concluded that the antinociception after intrathecal injection was a spinal cord effect. However, 2-methyl serotonin does have affinity for 5-HT₁ receptors.²⁸ Selectivity of drugs for 5-HT receptor subtypes is often not high. Frequently, pKᵢ values for a drug binding to different receptor subtypes differ by one log unit or less.²⁸ ²⁹ Thus, Giordano²⁷ may have been describing the system described in this paper—the activation of 5-HT₁A receptors in the spinal cord. However, more confidence may be placed in the findings of the present paper because the 5-HT₁₅₄A-selective drugs that were used have pKᵢ values for the 5-HT₁A receptor subtype that are two or three log units greater than values for other receptors.²⁸ ²⁹

Clearly, the involvement of 5-HT₁ receptors other than 5-HT₁₅₄A receptors and of 5-HT₂ and 5-HT₃ receptors needs to be investigated because of the many reports of antinociceptive effects after intrathecal drugs reported to be selective for these 5-HT receptor subtypes. Furthermore, more detailed dose–response studies must await the arrival of more selective agonist and antagonist drugs that are also water-soluble, so that it will be possible to inject these drugs intrathecally and to study actions confined to the spinal cord. These issues are clearly important if there is to be development of a drug designed to target the spinal cord 5-HT receptors involved in antinociception. For example, an intrathecal drug selective for a 5-HT receptor subtype that is only involved in antinociception in the brain will work only if a high dose of the drug is given intrathecally and the drug subsequently spreads to the brain. Such an approach to therapy will inevitably lead to side-effects. We conclude that 5-HT₁₅₄A receptors in the spinal cord are involved in nociceptive mechanisms that can be assessed by measuring responses to noxious electrical stimuli. Other 5-HT₁ receptors (non-5-HT₁₅₄A receptors) are involved in spinally mediated antinociception that can be assessed by measuring responses to thermal noxious stimuli.

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