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Enhancement and depression of spinal reflexes by 8-hydroxy-2-(di-*n*-propylamino)tetralin in the decerebrated and spinalized rabbit: involvement of 5-HT_{1A}- and non-5-HT_{1A}-receptors

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1 In decerebrated, spinalized and paralyzed rabbits, intravenous administration of the 5-HT_{1A}-receptor agonists (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 3–300 nmol kg^{-1}, cumulative) and flesinoxan (22–2200 nmol kg^{-1}, cumulative) significantly increased the short latency reflex evoked in gastrocnemius medialis motoneurones by electrical stimulation of all myelinated afferents (Aβ and Aδ fibres) of the sural nerve. Reflexes increased to median values of 198% (inter-quartile range (IQR) 148–473%) and 296% (IQR 254–522%) of pre-drug values with the highest doses of 8-OH-DPAT and flesinoxan, respectively. The enhancement of reflexes induced by 5-HT_{1A}-receptor agonists was not reversed by the selective 5-HT_{1A}-receptor antagonist (**S**)WAY-100135 (2.05 μmol kg^{-1}).

2 The effects of 8-OH-DPAT were tested after pretreatment with (**S**)WAY-100135 (2.05 μmol kg^{-1}), its more potent analogue WAY-100635 (185 nmol kg^{-1}), and the 5-HT_2/5-HT_{1D}-/5-HT_7-receptor ligand ritanserin (1.67 μmol kg^{-1}). 8-OH-DPAT (300 nmol kg^{-1} single dose) significantly increased gastrocnemius reflex responses in the presence of (**S**)WAY-100135 and WAY-100635, to median values of 260% (IQR 171–295%) and 165% (IQR 136–170%) of pre-drug levels, respectively. These values were not significantly different from each other, or from the effects of 8-OH-DPAT given alone. When 8-OH-DPAT was given after ritanserin, reflexes were a median of 102% (IQR 76–148%) of pre-drug values: i.e. there was no significant increase in responses. Neither WAY-100635 nor ritanserin had any effects on reflexes *per se*.

3 WAY-100635 (185 nmol kg^{-1}) and ritanserin (1.67 μmol kg^{-1}) were given after 8-OH-DPAT (300 nmol kg^{-1}). The agonist increased reflexes to a median value of 184% (IQR 135–289%), after which WAY-100635 significantly reduced responses to 165% (IQR 130–254%) and ritanserin further decreased reflexes to a median of 107% (IQR 100–154%) of pre-drug levels, i.e. not significantly different from controls.

4 Previous studies have shown that reflexes evoked by large myelinated axons tend to be suppressed, rather than enhanced, by 5-HT_{1A}-receptor agonists. When tested against reflexes evoked by stimulation of the sural nerve at strengths between 1.5 and 2.5 times threshold, 8-OH-DPAT (3–300 nmol kg^{-1}, cumulative) and flesinoxan (22–2200 nmol kg^{-1}, cumulative) significantly reduced gastrocnemius responses to median values of 36% (IQR 15–75%) and 17% (IQR 12–38%) of pre-drug levels, respectively. This inhibition was fully reversed by (**S**)WAY-100135 (2.05 μmol kg^{-1}).

5 These data show that drugs that are agonists at 5-HT_{1A}-receptors increase polysynaptic spinal reflexes evoked by moderate to high stimulus intensities and depress responses to very low intensity stimuli. The inhibitory effects of these drugs were mediated through 5-HT_{1A}-receptors as they were abolished by a selective antagonist for these sites. However, the facilitatory effects of 8-OH-DPAT could be completely blocked only by a combination of ritanserin, which has no significant affinity for 5-HT_{1A}-receptors, with WAY-100635. It appears that the enhancement of reflexes by 8-OH-DPAT arises from a combined action at 5-HT_{1A}-receptors and other, ritanserin-sensitive, sites which could be 5-HT_{1D}- or 5-HT_7-receptors.

Keywords: 5-Hydroxytryptamine; sensorimotor integration; nociception; descending inhibition; descending facilitation; 5-HT_{1D}-receptor; 5-HT_7-receptor

Introduction

In studies of 5-hydroxytryptaminergic control of spinal cord function 5-HT_{1A}-receptors present something of an enigma, as they have been implicated in both excitatory and inhibitory events (e.g. Cesselin *et al*., 1994; Millan, 1995; Hasegawa & Ono, 1996). Recent work from this laboratory has shown that the polysynaptic reflex evoked in gastrocnemius motoneurones by electrical stimulation of the sural nerve in the decerebrated, non-spinalized rabbit is enhanced after intrathecal administration of the selective 5-HT_{1A}-receptor antagonists (**S**)WAY-100135 and WAY-100635 (Clarke *et al*., 1996). This observation suggests that 5-hydroxytryptamine (5-HT), acting through 5-HT_{1A}-receptors, tonically inhibits transmission between sural nerve afferents and gastrocnemius motoneurones. An obvious corollary of this conclusion is that activation of 5-HT_{1A}-receptors by agonist drugs should inhibit transmission through the sural-gastrocnemius pathway. However, previous work has shown that 5-HT_{1A}-agonists usually enhance polysynaptic reflexes while inhibiting monosynaptic responses (e.g. Nagano *et al*., 1988; Crick & Wallis, 1991; Hasegawa & Ono, 1996). The present study was performed to investigate the effects of the 'selective' 5-HT_{1A}-receptor agonists (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and flesinoxan (Van Wijngaarden *et al*., 1990; Hoyer *et al*., 1994) on gastrocnemius

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reflex responses in decerebrated, spinalized rabbits. On finding that reflexes evoked by combined stimulation of large and small cutaneous afferents were always enhanced by 5-HT$_{1A}$-receptor agonists, it was decided to investigate whether responses to stimulation of large axons only might be suppressed by the same drugs, as has been shown for monosynaptic reflexes (Crick & Wallis, 1991). The facilitatory effects of 5-HT$_{1A}$-receptor agonists could not be blocked by selective antagonists for the same sites, leading us to believe that other receptors might be involved. The identity of these sites was probed with ritanserin, a drug with very low affinity for 5-HT$_{1A}$-receptors but which binds to other sites for which 8-OH-DPAT has measurable affinity, including the 5-HT$_{1D}$- (Pauwels & Colpaert, 1996) and 5-HT$_7$- (Lovenberg *et al*., 1993) receptors. Some of these data have been published in abstract form (Clarke *et al*., 1994; Ogilvie & Clarke, 1996).

Methods

Experiments were performed on 48 rabbits of various strains and of either sex, weighing between 1.6 and 3.2 kg. Anaesthesia was induced by intravenous administration of methohexitone sodium (10–20 mg initially and then given to effect: total exposure time 10–15 min). The trachea was cannulated and anaesthesia maintained with halothane (2–4%) delivered in a mixture of oxygen (30%) and nitrous oxide (70%). One carotid artery and one jugular vein were cannulated for recording arterial blood pressure and the administration of drugs, respectively. The spinal cord was exposed at the thoraco-lumbar junction. A complete spinal section was made at this level. All animals were decerebrated by suction to the precollicular level and anaesthesia discontinued. Paralysis was induced by gallamine triethiodide (4 mg kg^{-1} initially) and ventilation maintained, by a Harvard Bioscience Starling ideal pump, on room air supplemented with oxygen. End tidal CO_2% was monitored and maintained between 3 and 4.5%. In later experiments, blood gas analyses were performed (using a Corning 168 analyser) at intervals to ensure that blood chemistry was within normal limits. The ECG was recorded from an intra-oesophageal probe or subcutaneous electrodes and used to trigger a ratemeter (Neurolog NL253) for a record of heart rate. Arterial blood pressure was recorded (by use of a Hewlett-Packard 8805C pressure amplifier in conjunction with an 8831A blood pressure analyser) and was maintained above a mean value of 60 mmHg. In some animals this necessitated the use of slow i.v. infusion of adrenaline tartrate (10–20 μg ml^{-1}): animals requiring such treatment were excluded from analysis of cardiovascular data. Core body temperature was held between 37 and 38°C by the action of a thermostatically-controlled heating blanket (Harvard Bioscience).

The left leg was clamped securely by screws inserted into the femur and tibia. The popliteal fossa was exposed by an incision through the biceps femoris muscle and the resulting pool was filled with warmed liquid paraffin (38°C). The sural and the gastrocnemius medialis (GM) muscle nerves were cut and placed over paired platinum electrodes. A single platinum wire electrode was placed under the sural nerve at a more central location for recording afferent volleys. The threshold for the fastest sural axons (T) was established by slowly increasing the stimulus voltage until a volley was just detected at the central sural electrode. The sural nerve was stimulated with square wave pulses of 0.1 ms duration applied at strengths of between 20–40 times T (i.e. sufficient to excite Aβ and Aδ axons, high intensity), or between 1.5–2.5 times T (activating Aβ fibres only, low intensity). Usually, only one stimulus strength was used in each animal, but in 3 rabbits stimuli within each of the two intensity ranges were applied in alternate stimulus runs. A run was performed every two min throughout the experiment and in each, reflex responses to eight stimuli delivered at 1 Hz were recorded from the GM muscle nerve and averaged by either an RML 380Z microcomputer interfaced to a Gould OS4202 digital oscilloscope, or an IBM-type personal computer interfaced to a CED 1401. Short-latency reflex responses (beginning <10 ms from the stimulus, Figure 1) were quantified as voltage-time integrals (areas), which were calculated by computer. No reflexes were recorded for at least 1 h after withdrawal of anaesthesia.

Drug treatments

Reflexes were recorded for a control period of at least 30 min before any treatments were applied. All drugs were administered intravenously and flushed in with 1 ml of Ringer-Dale solution. Except where stated, drug injections were separated by intervals of 24 min. The following regimes were tested against reflexes evoked by high and low intensity stimuli: 8-OH-DPAT was given in doses of 3, 27 and 270 nmol kg^{-1}, followed by (**S**)WAY-100135 2.05 μmol kg^{-1}. Flesinoxan was given in doses of 22, 45, 156, 445, 1560 nmol kg^{-1} (total cumulative dose 2.23 μmol kg^{-1}), followed by (**S**)WAY-100135 2.05 μmol kg^{-1}. (**S**)WAY-100135 2.05 μmol kg^{-1} was administered followed by 8-OH-DPAT 300 nmol kg^{-1}.

The following regimes were tested only against reflexes evoked by high intensity stimuli: WAY-100635 185 nmol kg^{-1} was administered, followed by 8-OH-DPAT 300 nmol kg^{-1} and then WAY-100635 1.67 μmol kg^{-1}.

Some shorter intervals were allowed between drug treatments in the following experiments as the results of preliminary studies suggested that the effects of ritanserin were not long-lasting. 8-OH-DPAT 300 nmol kg^{-1} followed by WAY-100635 185 nmol kg^{-1} (24 min); ritanserin vehicle (see below) 0.8 ml (6 min) and then ritanserin 1.67 μmol kg^{-1} (6 min). Ritanserin vehicle 0.8 ml followed by ritanserin 1.67 μmol kg^{-1} (12 min); 8-OH-DPAT 300 nmol kg^{-1} (12 min) and then WAY-100635 185 nmol kg^{-1} (12 min).

Drugs

Methohexitone sodium (Brietal, Eli Lilly) was dissolved to 10 mg ml^{-1} in distilled water. Gallamine triethiodide (Flaxedil, May and Baker), was diluted to 4 mg ml^{-1} in Ringer-Dale solution. (**S**)WAY-100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide.2HCl), a gift of Wyeth Research U.K., was dissolved to a strength of 4.27 mmol l^{-1} (2 mg ml^{-1}) in 0.5% dimethyl formamide (DMF) in Ringer-Dale solution. WAY-100635 (N-[2-[4-(2-methoxyphenyl) - 1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide.3HCl), also a gift of Wyeth Research U.K., was dissolved in Ringer-Dale solution to a strength of 3.70 nmol l^{-1} (2 mg ml^{-1}). (±)-8-OH-DPAT.HBr (Research Biochemicals Inc.) and fleinoxan.HCl (a gift of Dr S.K. Long, Solvay Duphar Pharmaceuticals, Weesp, The Netherlands) were dissolved to stock strengths of 6.05 and 4.47 μmol ml^{-1} (2 mg ml^{-1} in each case), respectively, in Ringer-Dale solution. They were diluted to 0.1 and 0.01 mg ml^{-1} for the smaller i.v. injections. Ritanserin (Research Biochemicals Inc.) was solubilized in 10 μl of DMSO and suspended in warm (40°C) 5% (+)-glucose solution to a strength of 1.67 μmol kg ml^{-1} immediately before injection. The composition of the Ringer-Dale solution used in this laboratory is: (mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 0.6 and $MgCl_2$ 0.005 mmol l^{-1}.

Statistical analysis

Reflexes are expressed as percentages of the mean value recorded over the 24 min period immediately before the first drug injection (the pre-drug control). The normalized values are not suited for parametric analysis and are consequently expressed as medians and inter-quartile ranges (IQRs). Statistical analysis was performed by use of Friedman's repeated measures ANOVA on ranks, Wilcoxon's signed ranks or matched pairs test for paired data and Mann-Whitney U-tests for unpaired data. *P* values for the Wilcoxon and Mann-Whitney tests are based on one-tailed comparisons. Cardiovascular data were suitable for parametric analysis and are

expressed as means ± s.e.mean. They were analysed by one-way ANOVA. Tests were carried out by use of the InStat programme from GraphPad software.

Results

Reflexes evoked by stimulating the sural nerve at 20–40 times threshold

Effects of 5-HT$_{1A}$ receptor agonists Reflex responses evoked by stimuli of 20–40 T were always augmented after administration of 8-OH-DPAT (Figure 1), although the dose-response relationship was rather flat (Figure 2). A significant increase was observed after the lowest dose used (3 nmol kg^{-1}, Wilcoxon, $P=0.004$, $n=8$), but the peak effect was achieved with a cumulative dose of 30 nmol kg^{-1}, after which GM responses were a median value of 214% (IQR 147–315%, $n=8$) of pre-drug levels. The highest dose of 8-OH-DPAT had inconsistent effects on reflexes, increasing responses further in 5 animals, but decreasing them in the other 3 so that the median change in reflexes decreased slightly from the 30 to 300 nmol kg^{-1} doses (Figures 1 and 2) to 198% (IQR 148–473%) of pre-drug values. The effects of 8-OH-DPAT were not reversed by the selective 5-HT$_{1A}$-receptor antagonist (S)WAY-100135 (2.05 μmol kg^{-1}), after which reflexes were a median of 232% (IQR 148–247%) of pre-drug controls (Figures 1 and 2), still significantly higher than the pre-drug values (Wilcoxon, $P=0.007$).

Flesinoxan also significantly increased reflexes evoked by stimulation of all myelinated afferents (Figure 2, Friedman's ANOVA, $P=0.0008$). The lowest dose producing a statistically significant effect was 665 nmol kg^{-1} cumulative (Dunn's test, $P<0.05$): this value was exaggerated by the small number of subjects. After the highest dose (2.23 μmol kg^{-1} cumulative), reflexes had increased to a median value of 297% (IQR 254–522%) of pre-drug controls. Subsequent administration of (S)WAY-100135 (2.05 μmol kg^{-1}) again failed to reverse the increase in reflex responses, which remained at a median value of 302% (IQR 247–422%) of pre-drug controls (Figure 2).

The effects of 5-HT$_{1A}$-receptor antagonists given alone and subsequent administration of 8-OH-DPAT When (S)WAY-100135 was given i.v. in a dose of 2.05 μmol kg^{-1}, reflexes increased significantly to a median of 145% (IQR 114–181%, Wilcoxon, $P=0.002$, $n=9$) of pre-drug levels (Figure 3a). When 8-OH-DPAT (300 nmol kg^{-1}) was given after (S)WAY-100135, reflexes showed a further significant (Wilcoxon matched pairs, $P=0.004$) enhancement, to a median of 260% (IQR 171–295%, $n=9$) of pre-drug values (Figure 3a). The enhancement of reflexes seen after (S)WAY-100135 given alone was less than that obtained when the antagonist was given after 8-OH-DPAT or flesinoxan (Mann-Whitney U tests, $P=0.005$ and 0.002, respectively, see above).

WAY-100635 (185 nmol kg^{-1}, i.v.) alone had no significant effects on GM reflex responses so that after this drug, they were a median of 115% (IQR 112–127%, $n=5$) of pre-drug levels. Given in the presence of this antagonist, 8-OH-DPAT increased reflexes significantly over post-WAY-100635 values to a median of 165% (IQR 136–170%, $n=5$, Wilcoxon, $P=0.03$) of pre-drug controls (Figure 3b). Subsequent ad-

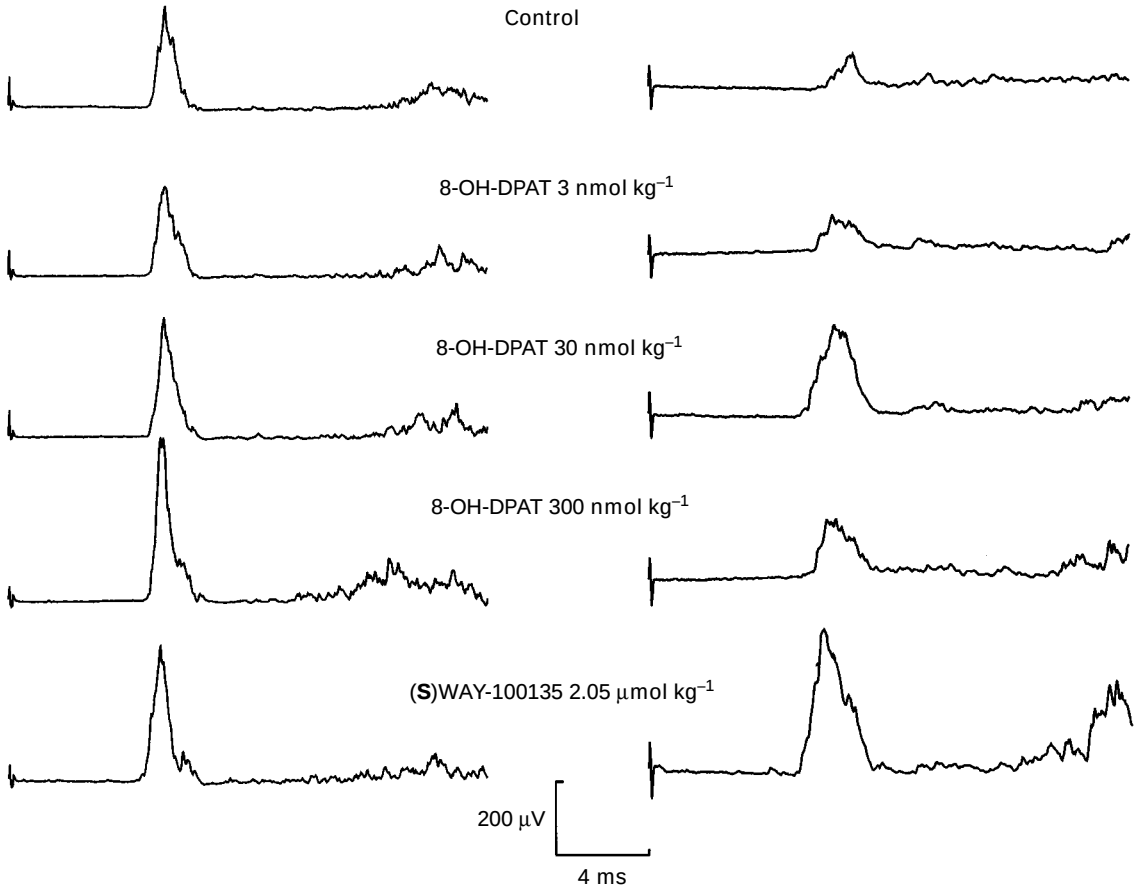

Figure 1 GM reflex responses from 2 different rabbits, evoked by stimulation of the sural nerve at 35 T (left) and 28 T (right) showing the effects of increasing doses of 8-OH-DPAT. These animals showed the two different patterns of effects seen with the highest dose of 8-OH-DPAT (300 nmol kg^{-1} cumulative) and subsequent administration of (S)WAY-100135. Only the short-latency components were measured. Each trace is the average of 8 sweeps, and the stimulus was applied at the beginning of the sweep.

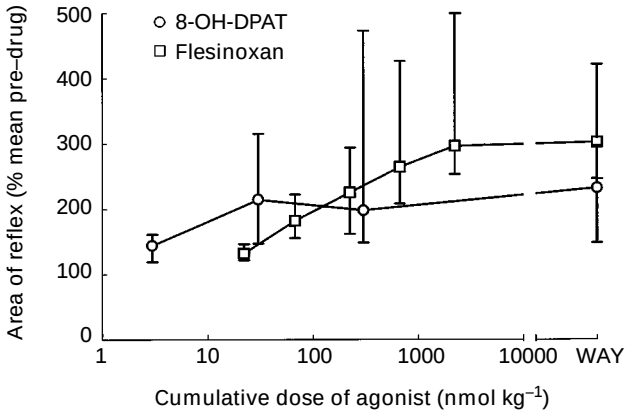

Figure 2 Dose-effect curves for 8-OH-DPAT ($n=8$) and flesinoxan ($n=4$), and subsequent administration of (**S**)WAY-100135, against the sural-GM reflex evoked by 20–40 T stimuli in spinalized animals. The effects of each agonist were statistically significant (Friedman's ANOVA, $P=0.006$ and 0.0008 for 8-OH-DPAT and flesinoxan, respectively). Each point is a median value, vertical lines indicate inter-quartile ranges.

ministration of a very large dose of WAY-100635 (1.67 μmol kg^{-1}) failed to induce any further changes in reflex responses, so that they remained at a median of 152% (IQR 108–206%) of pre-drug values (Figure 3b).

Ritanserin and 8-OH-DPAT 8-OH-DPAT, given as a single dose of 300 nmol kg^{-1}, significantly (Wilcoxon, $P=0.016$, $n=6$, Figure 4a) increased GM reflex responses to a median value of 184% (IQR 135–289%) of pre-drug values. This facilitation was slightly but significantly (Wilcoxon, $P=0.016$, Figure 4a) reduced by subsequent administration of WAY-100635 (185 nmol kg^{-1}, i.v.), so that reflexes were a median of 165% (IQR 130–254%) of pre-8-OH-DPAT levels. Under these conditions the DMSO-glucose vehicle had no further effect on reflex responses (Figure 4a), but ritanserin (1.67 μmol kg^{-1}) decreased reflexes to a level at which they were not significantly different from pre-8-OH-DPAT controls (median 107%, IQR 100–154%, $P=0.11$ vs control, $P=0.016$ vs post WAY-100635 levels, Wilcoxon tests, Figure 4a).

When given alone, neither ritanserin (1.67 μmol kg^{-1}), nor the vehicle in which it was dissolved, had any effects on reflex responses (Figure 4b). In the presence of ritanserin, 8-OH-DPAT (300 nmol kg^{-1}) caused no overall change in reflexes (Wilcoxon, $P=0.48$, $n=7$), which were a median of 102% (IQR 76–148%) of pre-drug controls (Figure 5). The overall lack of change masks a rather disparate set of responses to 8-OH-DPAT after ritanserin: reflexes increased in 3 animals, decreased in 2 and showed no change in the remainder. The variability of responses to this drug combination is reflected in the large IQR (Figure 4b). In these experiments, administration of WAY-100635 (185 nmol kg^{-1}) reversed any effects of 8-OH-DPAT in individual animals, resulting in no overall change in reflex responses but a reduction in the IQR (median value after WAY-100635 99% of pre-drug controls, IQR 91–114%; Figure 4b).

Reflexes evoked by stimulating the sural nerve at 1.5–2.5 times threshold

It was noted in the introduction that 5-HT$_{1A}$-receptor antagonists enhance reflexes in non-spinalized rabbits, a result which implies an inhibitory role for 5-HT$_{1A}$-receptors in controlling spinal transmission. The inconsistent effects of high doses of 8-OH-DPAT indicate a possible mixed excitatory-inhibitory effect of the drug. Previous workers have found that monosynaptic reflexes, evoked by large myelinated axons, are suppressed by 8-OH-DPAT. Consequently,

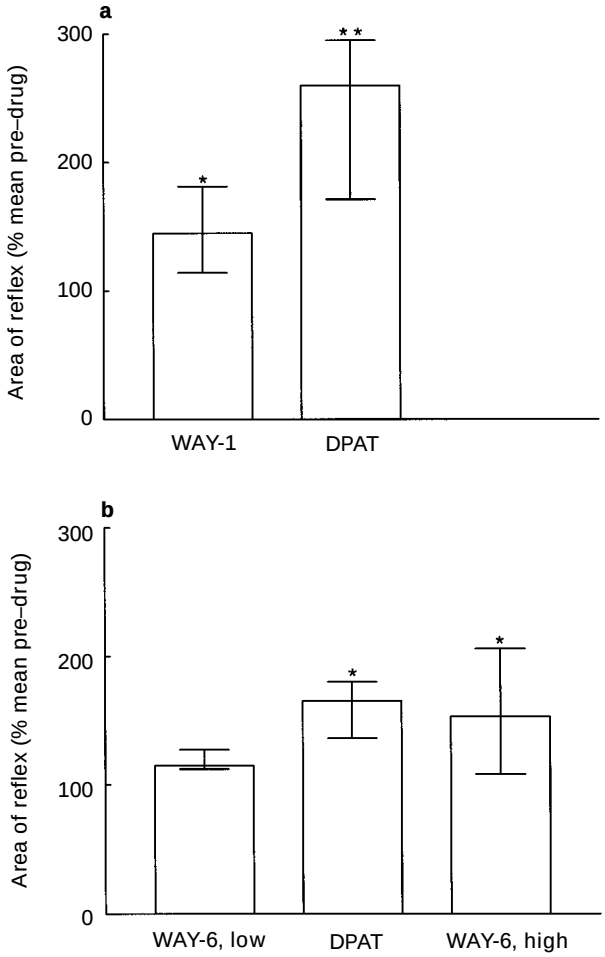

Figure 3 Histograms showing median values for reflexes evoked by high intensity stimuli after sequential intravenous administration of: (a) WAY-100135 (WAY-1, 2.05 μmol kg^{-1}, $n=7$) and 8-OH-DPAT (DPAT, 300 nmol kg^{-1}). (b) WAY-100635 (WAY-6 low, 185 nmol kg^{-1}, $n=5$), 8-OH-DPAT (DPAT, 300 nmol kg^{-1}) and WAY-100635 (WAY-6 high, 1.67 μmol kg^{-1}). Friedman's ANOVA indicated a significant change in reflexes across these treatments ($P=0.028$). The height of each column represents the median value reached after each treatment, and the vertical lines indicate the inter-quartile ranges. *Significantly greater than pre-drug control; **significantly greater than post-(**S**)WAY-100135 or WAY-100635 values. Details of tests are given in the text.

we surmised that reflexes evoked by selective stimulation of large myelinated sural nerve fibres might be more likely to show inhibition by 5-HT$_{1A}$-agonists. In the experiments described in this part of the results, the sural nerve was stimulated at just above threshold for eliciting measurable reflex responses in the GM motor nerve. Animals were deliberately selected on the basis of having low thresholds for eliciting reflexes and the stimuli used were in the range 1.5 to 2.5 times threshold for the sural nerve.

8-OH-DPAT (Figures 5 and 6) caused a dose-related inhibition of reflexes evoked by low intensity stimuli. After a cumulative dose of 300 nmol kg^{-1}, the median response was 36% (IQR 15–75%, $n=10$) of pre-drug values. The effect overall was statistically significant (Friedman's ANOVA, $P=0.012$) and the minimum effective dose was 3 nmol kg^{-1} (Wilcoxon test, $P=0.008$). Administration of (**S**)WAY-100135 (2.05 μmol kg^{-1}) completely reversed the inhibitory effects of 8-OH-DPAT (Figures 5 and 6), so that after the antagonist reflexes were a median of 155% (IQR 123–390%, $n=10$) of pre-drug values, significantly higher than post-8-OH-DPAT levels (Wilcoxon, $P=0.0001$), but not quite greater than pre-drug controls (Wilcoxon, $P=0.08$).

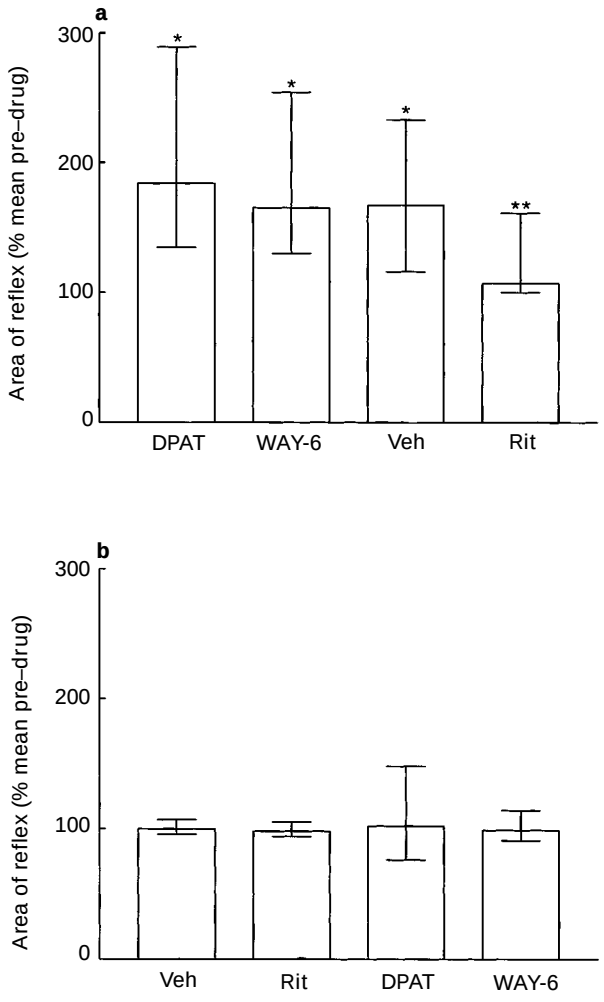

Figure 4 Histograms showing median values for reflexes evoked by high intensity stimuli after sequential intravenous administration of: (a) 8-OH-DPAT (DPAT, 300 nmol kg^{-1}, $n=6$); WAY-100635 (WAY-6, 185 nmol kg^{-1}); DMSO-glucose vehicle (Veh, 0.8 ml including 10 μl DMSO) and ritanserin (Rit, 1.67 μmol kg^{-1}). Friedman's ANOVA indicated a significant difference across these treatments ($P=0.0009$). (b) DMSO-glucose vehicle (Veh, $n=7$), ritanserin (Rit, 1.67 μmol kg^{-1}), 8-OH-DPAT (DPAT, 300 nmol kg^{-1}) and WAY-100635 (WAY-6, 185 nmol kg^{-1}). There were no significant differences between any of these treatments (Friedman's ANOVA, $P=0.9$). The height of each column represents the median value reached after each treatment, and the vertical lines indicate the inter-quartile ranges. *Significantly greater than pre-drug control; **not significantly different from pre-drug control. Details of tests are given in text.

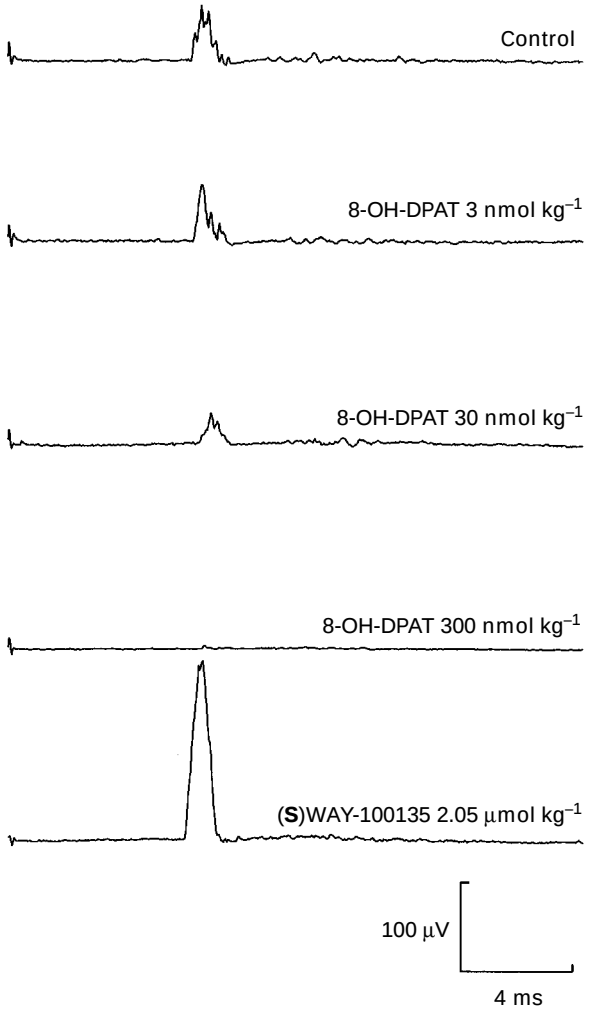

Figure 5 GM reflex responses evoked by stimulation of the sural nerve at 1.6 times threshold, showing the effects of increased doses of 8-OH-DPAT followed by (S)WAY-100135. The data are from the same animal as the left hand panel of Figure 1. Each trace is the average of 8 sweeps and the stimulus was applied at the beginning of each sweep.

Flesinoxan also suppressed reflexes to low intensity sural stimuli (Figure 6), so that after the highest dose used (2.23 μmol kg^{-1}), responses were a median value of 17% (IQR 12–38%, $n=4$) of pre-drug levels. This effect was statistically significant (Friedman's ANOVA, $P=0.021$, minimum effective dose 665 nmol kg^{-1}, Dunn's test, $P<0.05$), and was reversed by (S)WAY-100135 (2.05 μmol kg^{-1}, i.v.), after which GM reflexes were 116% (IQR 106–180%) of pre-drug values (Figure 6). Again, the potency of flesinoxan is underestimated as a result of the low number of experiments.

Given alone, (S)WAY-100135 (2.05 μmol kg^{-1}) had no significant effect on reflexes evoked by low intensity stimuli. After the antagonist, responses were a median value of 122% (IQR 107–158%, $n=8$, $P=0.12$, Wilcoxon) of pre-drug values. Subsequent administration of 8-OH-DPAT had no consistent or significant effects on GM reflexes: responses were a median of 163% (IQR 58–211%, $P=0.14$ vs pre-drug levels) of pre-drug values after both agents had been given.

Cardiovascular effects of 5-HT$_{1A}$-agonists

A most surprising observation of these experiments was that neither 8-OH-DPAT nor flesinoxan had any consistent effects on arterial blood pressure (ANOVA, $P>0.05$), although both agents reduced heart rate (Table 1). The bradycardic effects of the 5-HT$_{1A}$-agonists were statistically significant (ANOVA, $P<0.005$ in both cases), and were fully reversed by (S)WAY-100135. The lowest doses at which significant effects were seen were 300 nmol kg^{-1} for 8-OH-DPAT and 668 nmol kg^{-1} for flesinoxan (Student-Newman-Keuls test).

8-OH-DPAT had no effects on blood pressure in the presence of (S)WAY-100135 or WAY-100635 (Table 2), but did reduce heart rate significantly in the presence of (S)WAY-100135 (ANOVA followed by Student-Newman-Keuls test, $P<0.01$) by a mean of 24 beats min^{-1} compared to pre-drug levels. There was a tendency for heart rate to decrease when 8-OH-DPAT was given after WAY-100635, but this did not reach significance (ANOVA, $P>0.05$). In the presence of ritanserin, 8-OH-DPAT caused a significant increase in mean arterial blood pressure (ANOVA followed by Student-Newman-Keuls test, $P<0.05$, Table 2), by a mean of 9 mmHg over control. In these conditions the agonist still caused a significant decrease in heart rate (mean change 32 ± 2 beats min^{-1}, ANOVA followed by Student-Newman-Keuls test, $P<0.01$). These effects were reversed by WAY-100635 (185 nmol kg^{-1}).

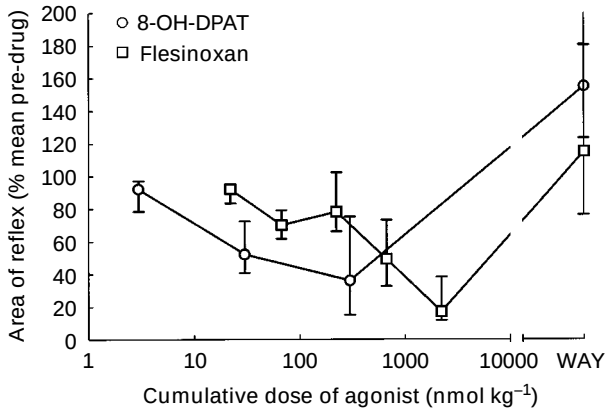

Figure 6 Dose-effect curves for 8-OH-DPAT and flesinoxan, and subsequent administration of (**S**)WAY-100135, against the sural-GM reflex evoked by 1.5–2.5 T stimuli in spinalized animals. (○) Data for 8-OH-DPAT ($n=10$); (□) data for flesinoxan ($n=4$). Friedman's ANOVA indicates that effects of each drug were statistically significant, $P=0.012$ and 0.021 for 8-OH-DPAT and flesinoxan, respectively. Each point is a median value, vertical lines indicate inter-quartile ranges. The ordinate scale has been abbreviated for reasons of clarity.

Discussion

Excitatory effects of 5-HT$_{1A}$-receptor agonists

The present experiments were conducted in the expectation that, as 5-HT$_{1A}$-receptor antagonists increase the sural-GM reflex in non-spinalized rabbits, then selective 5-HT$_{1A}$-receptor agonists would suppress that reflex in spinalized preparations. However, when reflexes were evoked by the same stimulus intensities used in the experiments in non-spinalized animals (i.e. sufficient to excite all myelinated axons), the opposite was the case. Both 8-OH-DPAT and flesinoxan augmented reflex responses and in neither case could this effect be reversed by the selective but not very potent 5-HT$_{1A}$-receptor antagonist (**S**)WAY-100135. The mild reflex-enhancing actions of this antagonist were not sufficient to explain its failure to reverse the effects of 8-OH-DPAT or flesinoxan. Furthermore, the excitatory effects of 8-OH-DPAT could not be prevented by pretreatment with (**S**)WAY-100135 or its more potent analogue WAY-100635 in doses which should have reversed 5-HT$_{1A}$-mediated actions of the agonist (Fletcher *et al.*, 1996; Mundey *et al.*, 1996). The results cannot be explained by the antagonists not gaining sufficient access to the nervous system as the inhibitory effects of 5-HT$_{1A}$ agonists on reflexes, and their bradycardic actions were completely reversed by (**S**)WAY-100135 and/or WAY-100635 (see below). WAY-100635 was able to reduce reflexes already augmented by 8-OH-DPAT (Figure 4a), but it was only possible to block completely the excitatory effects of 8-OH-DPAT by combined administration of the 5-HT$_{1A}$ antagonist with ritanserin, which has very low affinity for 5-HT$_{1A}$-receptors (Lovenberg *et al.*, 1993).

Table 2 The effects of 8-OH-DPAT (DPAT, 300 nmol kg^{-1}) on cardiovascular variables when given in the presence of (**S**)WAY-100135 (2.05 μmol kg^{-1}), WAY-100635 (185 nmol kg^{-1}) or ritanserin (1.67 μmol kg^{-1})

	BP (mmHg)		*HR* (beats min^{-1})	
Treatment	*Pre-drugs*	*Post-drugs*	*Pre-drugs*	*Post-drugs*
(**S**)WAY-100135 + DPAT ($n=11$)	69±3	72±4	268±14	249±20
WAY-100635 + DPAT ($n=5$)	68±5	69±4	271±15	259±17
Ritanserin + DPAT ($n=7$)	62±4	71±6	280±7	248±10

Values are means±s.e.mean.

Thus, it appears that both 5-HT$_{1A}$- and non-5-HT$_{1A}$-receptors are involved in the facilitatory actions of 8-OH-DPAT. Ritanserin is best known as a 5-HT$_{2A/2C}$-receptor blocker, but it also has significant affinity for α_1-adrenoceptors (Van Wijngaarden *et al.*, 1990) and 5-HT$_{1D}$- (i.e. human 5-HT$_{1D\alpha}$, Zgombick *et al.*, 1995) and 5-HT$_7$-receptors (Lovenberg *et al.*, 1993). 8-OH-DPAT has very low affinity for α_1- or 5-HT$_{2A/2C}$-sites, but recent work has shown that this agent has moderate affinity for cloned 5-HT$_7$- (Lovenberg *et al.*, 1993) and 5-HT$_{1D}$-receptors (Pauwels & Colpaert, 1996). Furthermore, a preliminary study has revealed functional 5-HT$_{1D}$-receptor-mediated effects of 8-OH-DPAT in rabbits (Dando *et al.*, 1996). Thus, one way of explaining the present results is to propose that the facilitatory effect of 8-OH-DPAT is mediated by 5-HT$_{1A}$-receptors and by one or both of the 5-HT$_{1D}$- or 5-HT$_7$-receptor types. There are no grounds for excluding either of these receptors as possible mediators of the non-5-HT$_{1A}$-receptor effects of 8-OH-DPAT. It might be considered that the ability of 8-OH-DPAT to inhibit 5-HT uptake could explain some of the present findings, but such an action is seen only with very high systemic doses and is not shared by flesinoxan (Assie & Koek, 1996).

Little information is available on the effects of 5-HT$_{1D}$-receptor ligands on polysynaptic withdrawal reflexes, but it has been shown that 5-HT$_{1B/1D}$-receptor agonists depress the tail-flick in the rat (e.g. Alhaider & Wilcox, 1993, see also Millan, 1995) and attenuate the clasp-knife reflex in the decerebrated cat (Miller *et al.*, 1995). A non-5-HT$_{1A}$-receptor activated by 8-OH-DPAT and with characteristics similar to the 5-HT$_{1D}$-receptor has been shown to suppress monosynaptic reflexes in rat isolated spinal cord (Manuel *et al.*, 1995). No firm information is available on the possible functional effects of 5-HT$_7$-receptors in the spinal cord, although they are thought to be present (Gustafson *et al.*, 1996).

Increased responsiveness of polysynaptic reflex pathways after treatment with 8-OH-DPAT or other 5-HT$_{1A}$-receptor agonists is a common finding (see Millan, 1995). Thus, in the rat, polysynaptic ventral root reflexes (Nagano *et al.*, 1988; Hasegawa & Ono, 1996) and tail flick reflexes (Zemlan *et al.*, 1988; Ali *et al.*, 1994, but see Eide & Hole, 1991) are augmented after 8-OH-DPAT. Indeed, 5-HT$_{1A}$-agonists induce

Table 1 The effects of 5-HT$_{1A}$-receptor agonists and subsequent (**S**)WAY-100135, on arterial blood pressure (BP) and heart rate (HR) in decerebrated, spinalized rabbits

	BP (mmHg)			*HR* (beats min^{-1})		
Drug	*Control*	*Post agonist*	*Post (**S**)WAY-100135*	*Control*	*Post agonist*	*Post (**S**)WAY-100135*
8-OH-DPAT ($n=12$)	69±3	66±4	68±3	262±7	244±12	276±11
Flesinoxan ($n=7$)	71±6	75±5	75±3	255±9	218±9	246±10

Post-drug values were taken after the highest dose of each agonist. Data are means±s.e.mean.

spontaneous tail-flicks in the rat (Bervoets *et al*., 1993). Furthermore, systemic but not iontophoretic application of 8-OH-DPAT augments the glutamate-evoked activity of lumbar motoneurones in the rat (Jackson & White, 1990), while high concentrations of the drug have been shown to depolarize spinal motoneurones in rat (Takahashi & Berger, 1990) and cat (Zhang, 1991). The depolarizing effect of 8-OH-DPAT in rat motoneurones has been attributed to activation of a cation current with characteristics similar to I_h (Takahashi & Berger, 1990; Pape, 1996). One would expect these motor-related actions to be mediated by receptors in the ventral horn. However, some workers have shown excitatory actions of 8-OH-DPAT on dorsal horn neurones (El-Yassir *et al*., 1988; Ali *et al*., 1994; but see Zemlan *et al*., 1994; Gjerstad *et al*., 1996; Lopez-Garcia & King, 1996). In addition, Zhuo and Gebhart (1991) have described descending facilitation of withdrawal reflexes in the rat from stimulation of brain stem reticular tissue, which they attributed to activation of 5-HT$_1$-like receptors in the dorsal horn. Thus, excitatory effects of 8-OH-DPAT may arise from actions at more than one point in a polysynaptic reflex pathway.

Inhibitory effects of 5-HT$_{1A}$-receptor agonists

Both 8-OH-DPAT and flesinoxan depressed reflexes evoked by very weak stimulation of the sural nerve. This inhibition was always reversed by (**S**)WAY-100135, and is thus very likely to have been mediated by 5-HT$_{1A}$-receptors. It has been suggested that some spinal inhibitory actions of 8-OH-DPAT are mediated through α_2-adrenoceptors (Millan & Colpaert, 1991; Millan, 1994). However, the 'antinociceptive' effects described in these papers were achieved with doses several times greater than those found to be effective in the present study. Furthermore, (**S**)WAY-100135, which completely abolished 5-HT$_{1A}$-agonist mediated inhibition in the present experiments, has negligible affinity at α_2-receptors (Fletcher *et al*., 1993).

8-OH-DPAT has been shown consistently to depress monosynaptic reflexes in anaesthetized rats (Nagano *et al*., 1988; Hasegawa & Ono, 1996) and in neonatal rat spinal cord (Crick & Wallis, 1991; Wallis *et al*., 1993). These results and those of the present experiments raise the possibility that spinal inhibition mediated by 5-HT$_{1A}$-receptors is directed only at inputs from large myelinated axons. An increase in reflexogenic drive from large diameter afferents could explain the enhancement of reflexes seen with 5-HT$_{1A}$-receptor antagonists in non-spinalized rabbits (Clarke *et al*., 1996). Another possibility is that inputs generating relatively long-lasting excitatory postsynaptic potentials in spinal interneurones (e.g. those from nociceptive afferents) may be selectively enhanced by facilitatory 5-HT$_{1A}$-receptors. We favour the latter explanation over the former.

How is it possible to reconcile the inhibitory and excitatory actions of 5-HT$_{1A}$-receptor agonists? As explained above, some but not all of the excitatory effects of these drugs can be attributed to interaction with non-5-HT$_{1A}$-receptors. One has to conclude that 5-HT$_{1A}$-receptors can exert facilitatory and inhibitory effects on transmission between cutaneous afferents and motoneurones, presumably at different points in the reflex pathway. The overall effect of 8-OH-DPAT appears to be the result of an admixture of excitatory and inhibitory influences in which excitation usually predominates. It is possible that the inhibitory action is effected at the terminals of primary afferent fibres (see Marlier *et al*., 1991; Cesselin *et al*., 1994; Laporte *et al*., 1995), and thereby results in restriction of the flow of afferent information into the spinal cord. The excitatory action, which we believe involves amplification of the afferent signal reaching the motoneurone pool, would be mediated at a more ventral location in the grey matter, but probably not at the motoneurones themselves (Jackson & White, 1990; Wang & Dun, 1990). To account for the actions of 5-HT$_{1A}$-antagonists in non-spinalized rabbits (Clarke *et al*., 1996), it is necessary to propose that only the 5-hydroxytryptaminergic pathway to the inhibitory 5-HT$_{1A}$-receptors is tonically active in decerebrated animals.

Cardiovascular effects of 5-HT$_{1A}$-receptor ligands

We were surprised that neither 8-OH-DPAT nor flesinoxan had any inconsistent effects on arterial blood pressure, although both caused bradycardia. This effect was apparent even though vagal drive to the heart was reduced by the presence of gallamine. A fall in blood pressure, probably mediated by receptors located in the medulla, is one of the most widely observed actions of 5-HT$_{1A}$-receptor agonists (e.g. McCall *et al*., 1987). This effect has also been seen in the anaesthetized rabbit (Shepheard *et al*., 1990). It would appear that the decerebrated preparation used in the present study is able to maintain arterial pressure in the face of the bradycardic actions of 5-HT$_{1A}$-agonists, presumably by increasing peripheral resistance. Interestingly, 8-OH-DPAT increased arterial blood pressure in the presence of ritanserin. It appears then that non-5-HT$_{1A}$-receptors may contribute to the depressor effects of this drug.

Conclusions

The present experiments provide clear evidence that not all of the effects of 8-OH-DPAT in the decerebrated rabbit can be attributed to activation of 5-HT$_{1A}$-receptors. It is notable that non-5-HT$_{1A}$-mediated actions of the agonist were obtained with moderate i.v. doses (100 μg kg^{-1}), so the use of this drug as a tool for probing 5-HT$_{1A}$-receptor function must be carefully re-examined. Notwithstanding these observations, it is clear that 5-HT$_{1A}$-receptors have more than one function in controlling transmission through spinal reflex pathways (Millan, 1995). Many apparently conflicting results have been obtained from studies in which 5-HT$_{1A}$-agoinsts have been tested in models of nociception (e.g. Zemlan *et al*., 1988; Eide & Hole, 1991; Ali *et al*., 1994; Millan, 1994; Cesselin *et al*., 1994; Gjerstad *et al*., 1996). In such experiments, the behaviours observed are the result of activity in multi-neuronal systems, providing many possible sites of drug action. The current confusion over the role of 5-HT$_{1A}$-receptors in pain processing almost certainly arises from the multi-functionality of these receptors and the lack of specificity of 8-OH-DPAT, as demonstrated by the present study and others. With 5-HT$_{1A}$-receptors mediating opposing effects within the spinal cord, almost any action or combination of actions of 5-HT$_{1A}$-receptor agonists could be obtained in any given experiment, depending on the balance of inhibitory and excitatory effects achieved.

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