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Spinal 5-HT-receptors and tonic modulation of transmission through a withdrawal reflex pathway in the decerebrated rabbit

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1 In decerebrated, non-spinalized rabbits, intrathecal administration of either of the selective 5-HT_{1A}-receptor antagonists (S)WAY-100135 or WAY-100635 resulted in dose-dependent enhancement of the reflex responses of gastrocnemius motoneurones evoked by electrical stimulation of all myelinated afferents of the sural nerve. The approximate ED_{50} for WAY-100635 was 0.9 nmol and that for (S)WAY-100135 13 nmol. Intrathecal doses of the antagonists which caused maximal facilitation of reflexes in non-spinalized rabbits had no effect in spinalized preparations.

2 In non-spinalized animals, intravenous administration of (S)WAY-100135 was significantly less effective in enhancing reflexes than when it was given by the intrathecal route.

3 When given intrathecally, the selective 5-$HT_{2A/2C}$-receptor antagonist, ICI 170,809, produced a bell-shaped dose-effect curve, augmenting reflexes at low doses ($\leqslant$44 nmol), but reducing them at higher doses (982 nmol). Idazoxan, the selective α_2-adrenoceptor antagonist, was less effective in enhancing reflex responses when given intrathecally after ICI 170,809 compared to when it was given alone. Intravenous ICI 170,809 resulted only in enhancement of reflexes and the facilitatory effects of subsequent intrathecal administration of idazoxan were not compromised.

4 The selective 5-HT_3-receptor blocker ondansetron faciliated gastrocnemius medialis reflex responses in a dose-related manner when given by either intrathecal or intravenous routes. This drug was slightly more potent when given i.v. and it did not alter the efficacy of subsequent intrathecal administration of idazoxan.

5 None of the antagonists had any consistent effects on arterial blood pressure or heart rate.

6 These data are consistent with the idea that, in the decerebrated rabbit, 5-HT released from descending axons has multiple roles in controlling transmission through the sural-gastrocnemius medialis reflex pathway. Thus, it appears 5-HT tonically inhibits transmission between sural nerve afferents and gastrocnemius motoneurones by an action at spinal 5-HT_{1A}-receptors. Spinal 5-$HT_{2A/2C}$-receptors may mediate a weak inhibition of transmission in the spinal cord, but more convincing evidence was obtained for their involvement in descending facilitatory tone. Further, some of the facilitatory consequences of spinal α_2-adrenoceptor blockade may be mediated through 5-HT_2 type receptors. Spinal 5-HT_3 receptors do not appear to have a major role in tonic modulation of the sural-gastrocnemius medialis reflex.

Keywords: 5-Hydroxytryptamine; noradrenaline; somatosensory integration; nociception; withdrawal reflex; spinal cord; descending inhibition; descending facilitation

Introduction

The reflex evoked in gastrocnemius motoneurones by electrical stimulation of the sural nerve in the decerebrated rabbit is tonically suppressed by transmitters derived from spinal and supraspinal sources. Two transmitter systems contributing to this process have been identified positively: endogenous opioid peptides, released from spinal neurones and acting at μ opioid receptors (Clarke & Ford, 1987); and noradrenaline, released from the spinal terminals of descending axons and acting through α_2-adrenoceptors (Harris & Clarke, 1992; 1993). It is certain that these are not the only transmitters involved in tonic control of transmission through this withdrawal reflex pathway. Descending pathways utilizing 5-hydroxytryptamine (5-HT) are prime candidates for involvement in the control of reflexes. A substantial quantity of 5-HT is present in the spinal cord of rabbits and other mammals (Carlsson *et al.*, 1964; Fone *et al.*, 1987), most of which originates from neurones in and around the midline raphe nuclei and the immediately adjacent reticular formation of the brain stem (Dahlstrom & Fuxe, 1964; Bowker *et al.*, 1982; Skagerberg & Bjorklund, 1985). Further, 5-HT has profound effects on reflex function (e.g. Anden *et al.*, 1964; Bras *et al.*, 1990; Crick & Wallis, 1991) and crucially, there are a number of studies implicating 5-HT as a mediator of tonic descending control of spinally-mediated events (Engberg *et al.*, 1968; Rivot *et al.*, 1987, see also Duggan, 1985; Fields *et al.*, 1991).

The aim of the present study was to determine the roles of 5-HT_{1A}-, 5-$HT_{2A/2C}$-, and 5-HT_3-receptors in modulating transmission through the sural-gastrocnemius medialis (GM) reflex pathway. These particular receptors were chosen as targets because all are present in the spinal cord (see e.g. Cesselin *et al.*, 1994), and good antagonists are available for probing their functions. (S)WAY-100135 (N-tert-butyl-3,4-(2-methoxphenyl)piperazin-1-yl)-2-phenylpropionamide, Fletcher *et al.*, 1993) and its more potent and selective analogue, WAY-100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyrinidyl)cyclohexanecarboxamide, Forster *et al.*, 1995; Fletcher *et al.*, 1996) are recently developed 'silent' antagonists (i.e. little or no agonist activity) for the 5-HT_{1A}-receptor; ICI 170,809 (2-2(dimethylamino-2-methylpropylothio)-3-phenylquinoline) is a highly selective antagonist for receptors of the 5-HT_2-family, although it does not discriminate between subtypes (Blackburn *et al.*, 1988; Growcott *et al.*, 1993); and

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ondansetron is a selective and well established antagonist for 5-HT_3-receptors (Butler *et al.*, 1988). These agents have been given by the intrathecal (i.th.) and intravenous routes and their effects on the sural-gastrocnemius reflex and arterial blood pressure assessed. This experimental format is based on the assumption that intrathecal administration of drugs is likely to give rise to higher concentrations at spinal receptors than when the same agent is given by the intravenous route. Interaction between 5-hydroxytryptaminergic and adrenergic systems has also been investigated by observing the effects of intrathecal administration of the α_2-adrenoceptor antagonist idazoxan, which powerfully facilitates the sural-GM reflex (Harris & Clarke, 1992; 1993), after the 5-HT-receptor blockers. Some of these data have been published in an abstract form (Harris *et al.*, 1992; Clarke & Houghton, 1993).

Methods

Experiments were performed on 70 rabbits of various strains and of either sex, weighing between 1.6 and 3.5 kg. Anaesthesia was induced by intravenous administration of methohexitone sodium (Brietal, Eli Lilly) administered at 10–20 mg initially and then given to effect. The trachea was cannulated and anaesthesia maintained with halothane (2–4%) delivered in oxygen : nitrous oxide (30 : 70). One carotid artery and one jugular vein were cannulated for recording of arterial blood pressure and administration of drugs, respectively. The spinal cord was exposed at the thoraco-lumbar junction. Where it was required, a complete spinal section was made at this level. A fine polythene cannula (o.d. 0.63 mm) was inserted through an opening in the dura and threaded down so that its tip lay near segments L7 and S1. All animals were decerebrated by suction to the pre-collicular level and anaesthesia discontinued. Paralysis was induced by gallamine triethiodide (Flaxedil, May & Baker, 4 mg kg^{-1} initially) and ventilation maintained 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 at intervals to ensure that blood chemistry was within normal limits. The ECG was recorded from an intra-oesophageal probe and used to trigger a ratemeter for a record of heart rate. Arterial blood pressure was recorded and maintained above a mean value of 60 mmHg. In some animals this necessitated the use of a slow i.v. infusion of adrenaline tartrate (10–20 $\mu g\ ml^{-1}$): animals requiring such treatment were excluded from analysis of cardiovascular data. Core temperature was held between 37 and 38°C by the action of a thermostatically-controlled heating blanket.

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 filled with warmed liquid paraffin (38°C). The sural nerve and the gastrocnemius medialis (GM) muscle nerve 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 sural nerve was stimulated with square wave pulses of 0.1 ms duration applied at stimulus strengths of between 20–40 times threshold, i.e. sufficient to excite all myelinated axons. Reflexes were recorded every 2 min throughout the experiment by averaging the GM reflex responses to eight stimuli delivered at 1 Hz. The ensuing neurograms were quantified by integrating the short-latency component of the signal (see Figure 1) with respect to time to provide the 'area' of the response. 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. Drugs were usually given in increasing doses with intervals of 24 min between each injection.

Non-spinalized animals

Intrathecal injections (S)WAY-100135 was given by the intrathecal route to 9 rabbits in doses of 1, 2, 10, 21, 103, 205 and 410 nmol, giving a cumulative total of 752 nmol. WAY-100635 was also administered intrathecally to 9 animals in doses of 0.09, 0.09, 0.7, 2, 9 and 18 nmol for a total dose of 30 nmol. Four of these rabbits received further injections of 92 and 184 nmol to bring the total administered to 306 nmol. Idazoxan (415 nmol i.th.) was given in a single dose after the last injection of WAY-100635. The animals were then subject to a spinal section at the thoraco-lumbar junction. Eight rabbits received i.th. injections of ICI 170,809 in quantities of 1, 3, 13, 27, 134, 268 and 536 nmol, giving a cumulative dose of 982 nmol, followed by idazoxan and spinalization as described for WAY-100635. Ondansetron was given to 9 animals: the doses used were 2, 3, 17, 34, 171, 341 and 683 nmol, accumulating to a total of 1251 nmol. Six of these rabbits received a subsequent injection of idazoxan (415 nmol i.th.) and were then spinalized.

Intrathecal injections of all drugs were flushed through the cannula with 50–60 μl of Ringer-Dale solution.

Intravenous injections (S)WAY-100135 was given intravenously to 11 animals at 2, 18, 184 and 1848 nmol kg^{-1} i.v., giving a cumulative total of 2.05 $\mu mol\ kg^{-1}$). Six rabbits received ICI 170,809 i.v. in doses of 3, 5, 19, 54, 187, 536 and 1870 nmol kg^{-1}, giving a total cumulative dose of 2.68 $\mu mol\ kg^{-1}$ (1 mg kg^{-1}). Ondansetron was administered to 6 animals by the i.v. route in injections of 3, 7, 24, 68, 239, 683 and 2390 nmol kg^{-1} (cumulative total 3.41 $\mu mol\ kg^{-1}$, 1 mg kg^{-1}). All animals receiving ICI 170,809 and ondansetron i.v. were subsequently treated with idazoxan (415 nmol i.th.), and were then subjected to complete spinal transection.

Spinalized animals

(S)WAY-100135 was given to 7 and WAY-100635 to 5 spinalized rabbits as a control. The doses used were 123 nmol - i.th. for (S)WAY-100135 and 37 nmol i.th. for WAY-100635, i.e. approximately those doses at which the largest effects were achieved with these drugs in non-spinalized animals. These treatments were followed by intravenous administration of the same antagonist: the doses used were 2.05 $\mu mol\ kg^{-1}$ for (S)WAY-100135 and 185 nmol kg^{-1} for WAY-100635.

Drugs

(S)WAY-100136 (*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- pyrinidyl)cyclo- hexanecarboxamide.-3HCl), also a gift of Wyeth Research U.K., was dissolved in Ringer-Dale solution to strengths of 3.70, 0.37 and 0.04 mmol l^{-1} (2, 0.2 and 0.02 mg ml^{-1}). ICI 170,809 ((2-2(dimethylamino-2 - methylpropylothio) - 3 - phenylquinoline.HCl) was dissolved in 5% D-glucose solution to a strength of 5.4 mmol l^{-1} (2 mg ml^{-1}). Ondansetron was dissolved in 1% dimethyl sulphoxide (DMSO) – 5% D-glucose solution to 6.8 mmol l^{-1} (2 mg ml^{-1}). Naloxone HCl (DuPont U.K.) was dissolved in Ringer-Dale solution to a strength of 6.1 mmol l^{-1}). The Ringer-Dale solution used in this laboratory is (concentration in mmol l^{-1}): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 0.6, $MgCl_2$ 0.005. Neither this solution, nor any of the vehicles employed in the present study, have any effects on any measured parameter (Harris & Clarke, 1992).

Statistical analysis

Reflexes are expressed as percentages of the mean value recorded over the 24 min immediately before the first drug in-

jection (the pre-drug control). These data are not suitable for parametric analysis and are consequently expressed as medians and ranges. Statistical analysis was performed by use of Friedman's repeated measures ANOVA on ranks, Wilcoxon's signed ranks or matched pairs tests for paired data and Mann–Whitney U-tests for unpaired data. The P values given for the Wilcoxon and Mann–Whitney tests are 1-tailed. Cardiovascular data were suitable for parametric analysis and are expressed as means ± s.e.mean. They were analysed by 1-way ANOVA for repeated measures. Tests were carried out with the InStat programme from GraphPad software.

Results

5-HT$_{1A}$-receptor antagonists

Intrathecal (S)WAY-100135 When administered by the intrathecal route, (S)WAY-100135 caused a significant, dose-dependent increase in the sural-GM reflex (Friedman's ANOVA, $P=0.0025$, Figures 1 and 2, Table 1), although enhancement of responses was seen in only 8 of the 9 animals tested. Responses were significantly larger than control after the lowest dose (median 108%, range 97–896% of pre-drug values, $P=0.01$, Wilcoxon test). The ED_{50} was approximately 13 nmol and the maximum increase was seen with a cumulative dose of 136 nmol, after which the reflex was a median of 373% (range 78–5809%) of pre-drug controls. Administration of (S)WAY-100135 above this dose had variable effects, causing the reflex to increase further in 4 animals, but to decrease in the remainder. After the highest dose (752 nmol cumulative), the response was a median of 135% (range 1–8474%, Figure 2, Table 1) of pre-drug controls, which was not significantly different from controls (Wilcoxon, $P>0.05$).

Intravenous (S)WAY-100135 Given intravenously, (S)WAY-100135 significantly increased reflex responses (Friedman's ANOVA on ranks, $P=0.0043$, Figures 1 and 2, Table 1). The minimum effective dose was 20 nmol kg^{-1}, after which the responses were augmented to 120% (range 24–250%, $n=11$) of pre-drug levels ($P=0.021$, Wilcoxon test). This was also the peak increase seen with i.v. administration. It was significantly less than the potentiation seen after the 13, 34 or 136 nmol cumulative i.th. doses (equivalent approximately to 5.4, 13 and 57 nmol kg^{-1} in the whole rabbit) of (S)WAY-100135 (Mann–Whitney, $P=0.015$ at least). Higher intravenous doses of the 5-HT$_{1A}$-antagonist produced no further significant changes in reflexes (Figures 1 and 2).

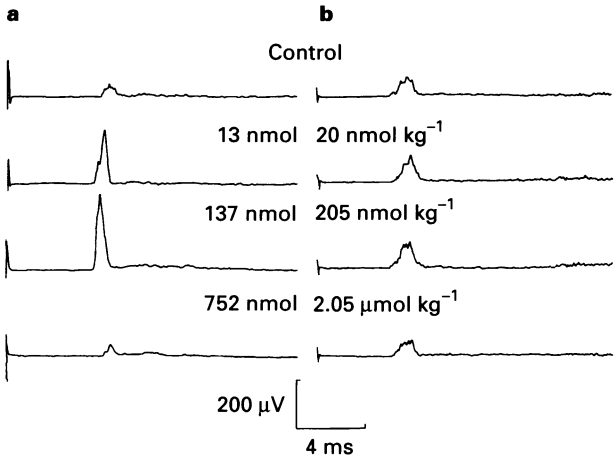

Figure 1 Gastrocnemius medialis reflex responses to sural nerve stimulation from decerebrated, non-spinalized rabbits, showing the effects of intrathecal (a) and intravenous (b) administration of (S)WAY-100135. Doses are indicated by each trace. Records are averages of 8 sweeps, and the stimulus was applied at the beginning of each trace.

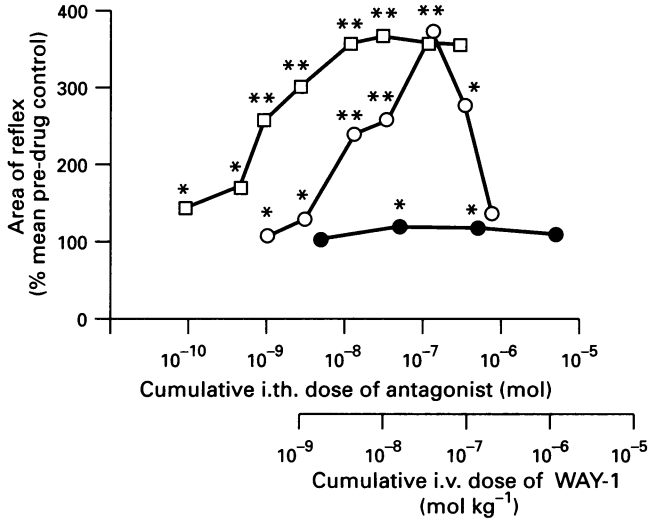

Figure 2 Dose-effect curves for intrathecal (○) and intravenous (●) (S)WAY-100135 and intrathecal WAY-100635 (□) on the sural-gastrocnemius medialis reflex in non-spinalized rabbits. Each point is a median value from 9 (both i.th.) or 11 (i.v.) experiments. Selected ranges are given in the text. The abscissae are positioned such that each point on the intrathecal scale corresponds to the equivalent intravenous dose, i.e. the amount given (mol) divided by the average weight of rabbits used in these groups (2.4 kg). *Indicates significant difference from pre-drug level; **significant difference from peak i.v. effect (at 20 nmol kg^{-1}, see text for details of statistical tests).

Table 1 The effects on the sural-G.M. reflex of a range of doses of 5-HT$_{1A}$- receptor antagonists and subsequent administration of intrathecal idazoxan (415 nmol)

Drug	*Route*		*Treatment* *Lowest dose*	*Middle dose*	*Highest dose*	*Idazoxan*
(S)WAY-100135	I.th.	Cumulative dose (nmol)	1	34	752	415
		Median (range) change in reflex	108[1]	257[1]	135[2]	147[3]
		(% mean pre-drug)	(97–896)	(82–5080)	(1–8474)	(41–8894)
	I.v.	Cumulative dose (nmol kg^{-1})	2	205	2005	NA
		Median (range) change in reflex	104	119[4]	110[2]	–
		(% mean pre-drug)	(67–208)	(101–286)	(59–319)	
WAY-100635	I.th.	Cumulative dose (nmol)	0.09	3	30	415
		Median (range) change in reflex	144[1]	301[1]	368[1]	381[3]
		(% mean pre-drug)	(70–256)	(124–658)	(133–5638)	(141–7257)

[1]Significantly greater than pre-drug control; [2]significantly lower than peak effect of (S)WAY-100135; [3]not significantly different from post-(S)WAY-100135 or post-WAY-100635 values, or the effects of idazoxan (686 nmol) given alone; [4]significantly greater than pre-drug control but less than maximum increase with i.th. (S)WAY-100135. See text for details of statistical tests.

Intrathecal WAY-100635 WAY-100635 also caused a dose-dependent increase in the GM reflex responses (Friedman's ANOVA, $P<0.0001$, Figure 2, Table 1), but in this case all 9 animals used responded in the same way. Enhancement of reflexes over control was significant at the 0.09 nmol dose (median value 144% of control, range 70–256%, $P=0.01$, Wilcoxon test), the ED_{50} value was approximately 0.9 nmol and the maximum increase was to a median of 368% (range 133–5638%) of controls at the 30 nmol cumulative dose. This was not significantly different from the maximum effect observed after intrathecal (S)WAY-100135 ($P>0.05$, Mann–Whitney). Doses above 30 nmol produced no further changes in the GM reflex responses: after a cumulative dose of 308 nmol the reflex was a median value of 355% (range 149–7224%, $n=4$) of pre-drug values. The marked decrease in reflexes which was seen with the largest intrathecal doses of (S)WAY-100135 was not evident with WAY-100635 (Figure 2, Table 1).

Effects of (S)WAY-100135 and WAY-100635 in spinalized rabbits Intrathecal (S)WAY-100135 (123 nmol) had no effect on reflexes in spinalized rabbits. After the drug, reflexes were a median value of 107% (range 59–166%, $n=7$) of pre-drug values. This value was significantly less than those obtained after cumulative doses of 34 or 136 nmol were given to non-spinalized rabbits (Mann–Whitney, $P=0.01$ at least). In contrast, 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% (range 102–192%, Wilcoxon, $P=0.002$, $n=9$) of pre-drug levels. This was not significantly different from the effects of the same dose given cumulatively to non-spinalized animals (Mann–Whitney, $P>0.05$).

WAY-100635 had no effect on reflexes in spinalized rabbits when given i.th. at 37 nmol, or i.v. at 185 nmol kg^{-1}. After these treatments, reflexes were median values of 102% (range 88–114%, $n=5$) and 115% (range 84–132%) of pre-drug controls, respectively. Neither value was significantly different from controls ($P>0.05$, Wilcoxon), and both were significantly less than the increases seen with the 30 nmol cumulative i.th. dose of WAY-100635 in non-spinalized animals ($P<0.001$ in both cases, Mann–Whitney).

ICI 170,809

Intrathecal administration The 5-$HT_{2A/2C}$-receptor blocker, ICI 170,809 had biphasic effects on reflexes when administered intrathecally (Figure 3, Table 2), which were statistically significant compared to controls (Friedman's ANOVA, $P=0.008$). Low doses induced increases in the sural-GM reflex so that after a cumulative dose of 44 nmol, responses were a median of 199% (range 62–321%, $n=8$) of pre-drug values (significant compared to control, $P=0.02$, Wilcoxon). The approximate ED_{50} for this effect was between 5 and 10 nmol (equivalent to roughly 2.1–4.2 nmol kg^{-1}). Higher doses caused the reflex to decrease towards control values. After the 982 nmol cumulative dose, reflexes were a median value of 50% (range 25–226%) of controls. This decrease was significant relative to the peak values obtained with lower doses of ICI 170,809, but not with respect to the pre-drug levels (Wilcoxon tests, $P=0.02$ and <0.05 respectively).

To ensure that the spinal cord was operating normally after intrathecal administration of ICI 170,809, naloxone was given in a dose of 780 nmol kg^{-1} i.v. after spinalization. After this treatment, the sural-GM reflex increased to a median value of 411% (range 78–1087%) of pre-drug controls. This is entirely consistent with the effects of naloxone given in normal spinalized preparations (Clarke *et al.*, 1988).

Intravenous administration Intravenous ICI 170,809 also caused increases in the sural-GM reflex when administered at low doses (Figure 3, Table 2). At 8 nmol kg^{-1}, the reflex was augmented to a median of 121% (range 77–358%) of pre-drug levels. This was not significantly different from the largest increase seen after intrathecal administration (Mann–Whitney, $P>0.05$). The peak effect was achieved with a cumulative i.v. dose of 79 nmol kg^{-1}, after which GM responses were a median of 185% (range 77–335%) of controls, and the approximate ED_{50} was 27 nmol kg^{-1}. Increasing the dose of ICI 170,809 above 79 nmol kg^{-1} induced no further significant changes in reflexes. The decrease in responses that had been seen with higher intrathecal doses was not evident even after the largest intravenous injections. After the 2.68 μmol kg^{-1}, reflexes were at a significantly higher level relative to pre-drug controls, compared to the effects of the highest intrathecal dose (Mann–Whitney, $P=0.02$).

Ondansetron

Intrathecal administration Intrathecal administration of the 5-HT_3-receptor antagonist ondansetron caused reflexes to increase dose-dependently in most animals so that reflexes were a median value of 201% (range 6–902%, $n=9$) of pre-drug controls after the highest dose given (Figure 4, Table 3). The increases over control values were statistically significant (Friedman's ANOVA, $P=0.0001$), and became so at a cumulative dose of 227 nmol (Wilcoxon, $P=0.02$). This dose, equivalent to about 95 nmol kg^{-1} in the whole rabbit, was also the approximate ED_{50}.

Intravenous administration The effects of intravenous ondansetron were statistically indistinguishable from those induced by dosing through the intrathecal route (Figure 4, Table 3). Significant increases were seen with the lowest dose used (3 nmol kg^{-1}, Wilcoxon, $P=0.03$), and the peak increase was to 189% (range 117–272%) of pre-drug levels after a cumulative dose of 1.01 μmol kg^{-1}. This was not significantly different from the maximum effect seen with intrathecal administration (Mann–Whitney, $P>0.05$). Indeed, there are no statistically significant differences between the i.v. and i.th. dose-effect curves. The approximate ED_{50} was 34 nmol kg^{-1}, i.e. slightly lower than that for intrathecal application.

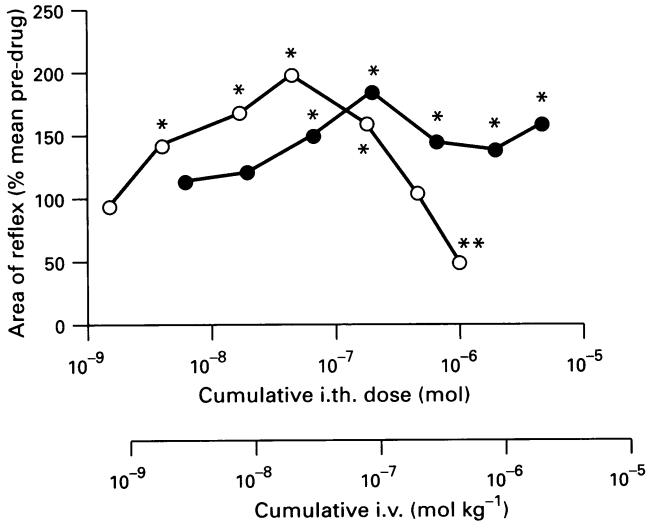

Figure 3 Cumulative dose-effect curves for the action of intrathecal (○) and intravenous (●) administration of ICI 170,809 on the sural-gastrocnemius medialis reflex in non-spinalized rabbits. Each point represents a median value from 8 (i.th.) or 6 (i.v.) experiments. The abscissae are positioned such that each point on the intrathecal scale corresponds to the equivalent intravenous dose (see Figure 2). *Denotes a significant difference from the pre-drug control; **a significant difference from the peak increase seen with i.th. ICI 170,809.

Table 2 The effects on the sural-gastrocnemius medialis reflex of a range of doses of ICI 170,809, and subsequent administration of intrathecal idazoxan (415 nmol)

Drug	Route		Treatment: Lowest dose	Middle dose	Highest dose	Idazoxan
ICI 170,809	I.th.	Cumulative dose (nmol)	1	44	982	415
		Median (range) change in reflex	97	199[1]	50[2]	97[3]
		(% mean pre-drug)	(89–107)	(62–321)	(25–226)	(48–366)
	I.v.	Cumulative dose (nmol kg^{-1})	3	81	2680	415
		Median (range) change in reflex	114	185[1]	159[4]	432[5]
		(% mean pre-drug)	(81–207)	(77–335)	(63–394)	(55–824)

[1]Significantly greater than pre-drug control; [2]significantly lower than peak effect of ICI 170,809; [3]significantly greater than post-ICI 170,809 values but significantly less than the effects of idazoxan (281 nmol) given alone; [4]significantly greater than the effects of the highest dose of ICI 170,809 given by the i.th. route; [5]significantly greater than post-ICI 170,809 values but not significantly different from the effects of idazoxan (686 nmol) given alone. See text for details of statistical tests.

Table 3 The effects on the sural-gastrocnemius medialis reflex of a range of doses of ondansetron, and subsequent administration of intrathecal idazoxan (415 nmol)

Drug	Route		Treatment: Lowest dose	Middle dose	Highest dose	Idazoxan
Ondansetron	I.th.	Cumulative dose (nmol)	2	56	1251	415
		Median (range) change in reflex	106	128	201[1]	347[2]
		(% mean pre-drug)	(73–145)	(39–1709)	(6–902)	(180–2687)
	I.v.	Cumulative dose (nmol kg^{-1})	3	102	3410	415
		Median (range) change in reflex	113	167[1]	187[1]	450[2]
		(% mean pre-drug)	(98–123)	(153–259)	(96–306)	(183–3254)

[1]Significantly greater than pre-drug control; [2]significantly greater than post-ondansetron levels and not significantly different from the effects of idazoxan (686 nmol) given alone. See text for details of statistical tests.

Effects of idazoxan post 5-HT-receptor antagonists

Intrathecal idazoxan causes a large increase in the GM reflex responses (Harris & Clarke, 1992). In the present study, the effects of idazoxan (415 nmol) given after 5-HT-receptor antagonists have been compared to those of 281 and 686 nmol cumulative doses of the α_2-adrenoceptor antagonist given alone, using data from the study of Harris and Clarke (1992). After these doses of idazoxan, reflexes were a median value of 503% (range 126–2772%) and 592% (range 140–4679%) of pre-drug controls, respectively ($n=24$).

α_2-adrenoceptor blockade and spinalization after WAY-100635 Administration of idazoxan (415 nmol, i.th.) after WAY-100635 had no effect on reflex responses over and above those induced by WAY-100635 itself. After the two drugs, reflexes were a median value of 381% (range 141–7257%) of pre-drug controls ($P>0.05$ versus 30 nmol dose, Wilcoxon test, Figure 5, Table 1). This was not significantly different from the effects of a 686 nmol dose of idazoxan given alone (Mann–Whitney, $P>0.05$). Spinalization in the presence of WAY-100635 and idazoxan had mixed effects: in four animals GM reflexes increased, in four others reflexes decreased and in the ninth animal no change was observed. The effects of idazoxan and spinalization did not depend on the dose of WAY-100635 present.

ICI 170,809 Given after intrathecal ICI 170,809, idazoxan increased reflexes significantly over post-ICI 170,809 levels from 50% to a median value of 97% (range 48–366%) of pre-drug levels (Wilcoxon, $P=0.004$, Figure 5, Table 2), a value which was not significantly different from pre-drug controls (Wilcoxon, $P>0.05$). The effect of combined administration of ICI 170,809 and idazoxan was much less than when idazoxan was given alone at 281 nmol (Mann–Whitney, $P<0.0001$, Figure 5). On spinalization in the presence of ICI 170,809 plus idazoxan, reflexes decreased in 4 animals, increased in 1 and did not change in the remaining 3 preparations.

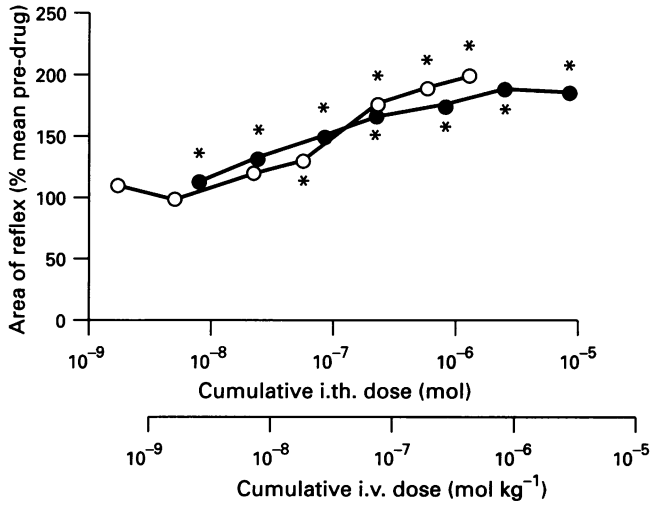

Figure 4 Cumulative dose-effect curves for the actions of intrathecal (○) and intravenous (●) administration of ondansetron on the sural-gastrocnemius medialis reflex in non-spinalized rabbits. Each point represents a median value from 9 (i.th.) or 6 (i.v.) experiments. The abscissae are positioned such that each point on the intrathecal scale corresponds to the equivalent intravenous dose (see Figure 2). *Denotes a significant difference from the pre-drug control.

After intravenous ICI 170,809, idazoxan increased reflexes significantly over the levels reached after 5-HT$_2$-receptor blockade, to a median value of 432% (range 55–824%) of pre-drug controls (Wilcoxon, $P=0.008$, Figure 5, Table 2). This increase was not statistically distinguishable from that observed after idazoxan given alone at 686 nmol (Mann–Whitney, $P>0.05$). Spinalization in the presence of this drug milieu always led to a decrease in the size of reflexes.

Ondansetron After intrathecal ondansetron, idazoxan augmented GM reflexes to a median of 347% (range 180–2687%, $n=6$) of pre-drug controls. The corresponding value obtained when giving idazoxan after intravenous ondansetron was an increase to 450% (range 183–3254%) of pre-drug values. The effects of idazoxan were in both cases significant relative to the post-ondansetron values (Wilcoxon tests, $P=0.02$ in both cases, Figure 5, Table 3), but were not significantly different from the effects of idazoxan given alone at 686 nmol (Mann–Whitney, $P>0.05$ in both cases).

Spinalization in the presence of intrathecal or intravenous ondansetron plus idazoxan always led to a decrease in the size of reflexes.

Cardiovascular effects of 5-HT-receptor antagonists

Table 4 shows the mean arterial blood pressures and heart rates recorded before and after i.th. and i.v. administration of the 5-HT-receptor antagonists used in this study. None of them had any significant or consistent effects in this respect (ANOVA, $P>0.05$ in all cases). Idazoxan had no effects on blood pressure when given after any of the other drugs.

Discussion

5-HT$_{1A}$-receptors and tonic descending inhibition

In the present experiments the largest effects were observed with intrathecal administration of the two 5-HT$_{1A}$-receptor antagonists, both of which increased GM reflex responses to sural nerve stimulation. A reasonable interpretation of this observation is that 5-HT, acting at spinal 5-HT$_{1A}$-receptors, tonically suppress transmission through the sural-GM reflex pathway.

The properties of (S)WAY-100135 and WAY-100635 (S)WAY-100135 and WAY-100635 are selective antagonists for 5-HT$_{1A}$-receptors with negligible affinity for all other sites against which they have been tested, with the exception of α_1-adrenoceptors. All other proposed 5-HT$_{1A}$-receptor antagonists are also partial agonists (Fletcher *et al.*, 1993; Lejeune *et al.*, 1993; Mundey *et al.*, 1994), or have poor selectivity against other receptor types (Fletcher *et al.*, 1993; Routledge, 1996). (S)WAY-100135 and WAY-100635 have roughly 100 times higher affinity at 5-HT$_{1A}$- than at α_1-receptors (Fletcher *et al.*, 1993; 1996; Forster *et al.*, 1995). Blockade of α_1-adrenoceptors is associated with a reduction in descending facilitation of the sural-GM reflex (Harris & Clarke, 1992), so the effects of (S)WAY-100135 and WAY-100635 are unlikely to have arisen from interaction with adrenoceptors. Furthermore, the higher potency of WAY-100635 to (S)WAY-100135 in increasing reflexes in the present study is in concordance with published values for the affinities of these two compounds at 5-HT$_{1A}$-receptors (Fletcher *et al.*, 1996). The combination of these facts with the efficacy of very low intrathecal doses of (S)WAY-100135 and WAY-100635, provides good evidence that the enhancement of reflexes resulting from the use of these drugs was due to an interaction with 5-HT$_{1A}$-receptors.

Some authors have suggested that (S)WAY-100135 may have partial agonist activity at post-synaptic 5-HT$_{1A}$-receptors in the cat (Escandon *et al.*, 1994), and in the rat, where the agonist-like action is very weak indeed (Fletcher *et al.*, 1993). In view of the facilitatory effects of 5-HT$_{1A}$-agonists on reflexes in spinalized rabbits (Clarke *et al.*, 1994), it is just feasible that the effects of (S)WAY-100135 obtained in the present study were due to an agonist-type action. However, intrathecal doses of the drugs which were effective in increasing reflexes in non-spinalized animals had no effect when administered to spinalized preparations. Further, there is no evidence that WAY-100635 has any partial agonist activity (Fletcher *et al.*, 1996). Thus, it is most likely that the enhancement of reflexes seen after intrathecal (S)WAY-100135 and WAY-100635 in non-spinalized rabbits was the result of blockade of 5-HT$_{1A}$-receptors.

Actions specific to (S)WAY-100135 (S)WAY-100135 had two effects which were not shared by WAY-100635 and were thus presumably not due to interaction with 5-HT$_{1A}$-receptors: it decreased reflexes at high doses (>136 nmol) in non-spinalized animals, and increased reflexes when given at 2.05 μmol kg^{-1}, i.v., in spinalized rabbits. The first of these actions may have been the result of interaction with excitatory receptors for which the agent has lower affinity than 5-HT$_{1A}$ (e.g. α_1-adrenoceptors), or due to spread of the drug to the brain (see below). The second effect was presumably peripherally-mediated since it was not obtained with intrathecal injection.

5-HT$_{1A}$-receptors and descending control of spinal reflexes - Neither (S)WAY-100135 nor WAY-100635 augmented reflexes when given intrathecally to spinalized rabbits, showing that the potentiation produced by these compounds is dependent on the integrity of descending 5-hydroxytryptaminergic

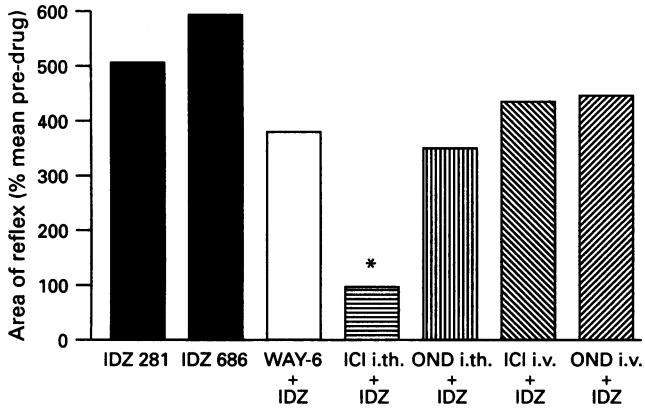

Figure 5 The effects on the sural-gastrocnemius medialis reflex of idazoxan (IDZ) given alone at 281 and 686 nmol intrathecal (i.th.) (solid columns, $n=24$, data from Harris & Clarke, 1992), and idazoxan 415 nmol i.th. given after intrathecal WAY-100635 (WAY-6, open column, 30 or 306 nmol, $n=9$), ICI 170,809 (ICI, horizontally hatched column, 982 nmol, $n=9$) and ondansetron (OND, vertically hatched column, 1251 nmol, $n=6$), and intravenous ICI 170,809 (left-rising hatched column, 2.68 μmol kg^{-1}, $n=6$) and ondansetron (right-rising hatched column, 3.41 μmol kg^{-1}, $n=6$). *Denotes a significant difference from the effects of idazoxan given alone (281 nmol).

Table 4 The effects of (a) intrathecal and (b) intravenous administration of 5-HT receptor antagonists on mean arterial blood pressure and heart rate in decerebrated rabbits

	Mean arterial pressure		*Heart rate*	
Drug	*Control*	*Treated*	*Control*	*Treated*
	(mmHg)		*(beats min^{-1})*	
a Intrathecal administration of antagonists				
(S)WAY-100135	89±4	87±3	262±10	273±8
WAY-100635	78±3	81±2	260±8	265±7
ICI 170,809	96±4	93±3	257±7	251±12
Ondansetron	107±5	109±5	265±9	259±10
b Intravenous administration of antagonists				
(S)WAY-100135	99±15	98±13	269±13	263±15
ICI 170,809	89±4	88±3	254±8	257±10
Ondansetron	88±6	88±6	262±7	268±3

The 'treated' values were those obtained after the highest dose of each drug (or 30 nmol for WAY-100635). Values are mean±s.e.mean.

systems. Assuming that i.th. administration of (S)WAY-100135 gave rise to higher spinal concentrations of the drug than equivalent doses given i.v., the fact that the antagonist was more potent by the i.th. route suggests that the effects of this drug were mediated at receptors in the spinal cord. This conclusion can be extended to the actions of i.th. WAY-100635, which was more potent than (S)WAY-100135 in a ratio compatible with the binding of the two agents at 5-HT_{1A}-receptors (Fletcher *et al.*, 1996). It is not clear why the maximum enhancement obtained with i.v. (S)WAY-100135 was so much lower than that seen with i.th. administration of the antagonists. It is possible that allowing the drug freer access to the brain stem resulted in disinhibition of other descending inhibitory systems which counteracted the effects mediated by the drug in the spinal cord.

There is much debate on the roles of 5-HT receptors in general, and 5-HT_{1A}-receptors in particular, in controlling transmission through the spinal cord. Most of the evidence supporting a role for 5-HT in tonic descending inhibition is based on the effects of the non-selective 5-$HT_{1/2}$-receptor antagonist/partial agonist drug methysergide (Engberg *et al.*, 1968; Rivot *et al.*, 1987; see Duggan & Morton, 1988). It is not possible to make a precise identification of the receptors involved in the process on the basis of such observations. More recently, Tjolsen *et al.* (1993) showed in a preliminary study that the 5-HT_{1A}-receptor antagonist/partial agonist NAN-190 increased the responses of dorsal horn neurones in the rat, and Zemlan *et al.* (1994) presented evidence to suggests that inhibition of dorsal horn cells from stimulation within the rostral ventromedial medulla in rat is similar to that from 5-HT_{1A}-receptor agonists. These findings are in full agreement with those of the present study.

However, 5-HT_{1A}-receptor agonists have been shown to have both inhibitory and excitatory effects on spinally-mediated events (Nagano *et al.*, 1988; Zemlan *et al.*, 1988; Bras *et al.*, 1990; Jackson & White, 1990; Wang & Dun, 1990; Crick & Wallis, 1991; Bervoets *et al.*, 1993; Alhaider & Wilcox, 1993; Ali *et al.*, 1994; Clarke *et al.*, 1994; Hasegawa & Ono, 1996; see also Cesselin *et al.*, 1994; Millan, 1995). Inhibitory effects of 5-HT_{1A}-receptor agonists have been most commonly observed on responses evoked by stimulation of low-threshold afferent fibres. It is feasible that 5-HT_{1A}-receptors may influence spinal transmission by having opposing (i.e. inhibitory and excitatory) actions at different sites in the cord (Clarke *et al.*, 1994), although other explanations are possible (Millan, 1995). The present data suggest that only inhibitory 5-HT_{1A}-receptors are tonically active in the spinal cord of decerebrated rabbits. Studies on the spinal distribution of 5-HT_{1A}-receptor sites show that they are particularly dense in the superficial laminae of the dorsal horn (Marlier *et al.*, 1991; Laporte *et al.*, 1995; Kia *et al.*, 1996), but they are also found in many other parts of the grey matter, including on the somata of motoneurones (Kheck *et al.*, 1995).

It should be noted that the invasive surgery performed in preparing animals for experiments of the type described here would have a profound influence on the central nervous system (see Clarke, 1994). The extent to which this type of sensory barrage might influence the function of 5-hydroxytryptaminergic neurones is uncertain, but it is possible that such inputs may be responsible for activating some types of tonic descending inhibition of spinal circuits.

Receptors of the 5-HT_2 family and descending control of reflexes

Intrathecal ICI 170,809 increased GM reflexes at low doses but higher doses returned reflexes to control levels. When given i.v. this drug produced only the former of these effects, and this with an ED_{50} 2 to 5 times higher than that seen with i.th. administration. These data suggest that spinal 5-HT_2-type receptors might make a small contribution to tonic inhibition of reflexes and that they may also be involved in descending facilitation.

The properties of ICI 170,809 ICI 170,809 is a pure antagonist with close to nanomolar affinity at 5-HT_{2A} and 5-HT_{2C} receptors (Blackburn *et al.*, 1988; Cox *et al.*, 1988; Growcott *et al.*, 1993). It does not differentiate between these sub-types and is a potent but non-surmountable antagonist at the 5-HT_2-receptors in rat fundus (which are presumably 5-HT_{2B}, Growcott *et al.*, 1993). The great advantage of ICI 170,809 for the purposes of the present study is the fact it has more than 400 times higher affinity for 5-HT_2-type sites than for any other receptors (Blackburn *et al.*, 1988; Cox *et al.*, 1988). Ketanserin and ritanserin, the most widely used of the 'selective' 5-HT_2-receptor antagonists, both have significant affinity for α_1-adrenoceptors (Van Wijngaarden *et al.*, 1990), which are themselves involved in control of the sural-GM reflex (Harris & Clarke, 1992).

5-HT_2-receptors and descending inhibition ICI 170,809 increased reflexes when given by either the i.th. or i.v. routes, although slightly lower intrathecal doses were needed to achieve this effect. It is therefore possible that 5-HT_2-type receptors contribute to tonic inhibition of transmission in the sural-GM pathway. However, the difference between the i.th. and i.v. dose routes was very small and it is not possible to be confident of this conclusion. Some workers have suggested that some inhibitory effects of spinal application of 5-HT may be mediated through 5-HT_2-receptors (Solomon & Gebhart, 1988; Crisp *et al.*, 1991), but this family of receptors is more often associated with excitatory effects in the spinal cord (e.g. Eide & Hole, 1991).

5-HT_2-receptors and tonic facilitation of reflexes Intrathecal but not intravenous application of ICI 170,809 produced a biphasic dose-response curve, so it is reasonable to suggest that the inhibitory effects of high intrathecal doses of the drug were due to interaction with spinal receptors. This result is consistent with the view that 5-HT_2-type receptors mediate tonic facilitation of transmission through the sural-GM pathway. Nagano *et al.* (1988) showed that ketanserin inhibited polysynaptic ventral root reflexes in the rat, an effect they were able to attribute to a combined action of the drug at 5-HT_2-receptors and α_1-adrenoceptors. Many others have found facilitatory effects of 5-HT_2-receptor activation on motoneurone responses (Connell & Wallis, 1989; Jackson & White, 1990; Zhang, 1991; Yamazaki *et al.*, 1992), and it is believed that depolarization of motoneurones is a function of 5-HT_2-type receptors. It has been noted that the 5-HT_{2B}-receptor has pharmacological characteristics similar to those of the motoneurone 5-HT-receptor (see Hoyer *et al.*, 1994). The distribution of 5-HT_2-type receptors supports their putative role in the control of motor systems, as they are rather sparse in the dorsal grey matter, but densely localized near somatic and autonomic motoneurone pools (Thor *et al.*, 1993). Thus, it is reasonable to surmise that the inhibitory action of ICI 170,809 seen in the present study was due to the blockade of 5-HT_2-type receptors in the ventral horn, although a dorsal site of action cannot be discounted.

It is tempting to speculate that the biphasic action of ICI 170,809 results from interactions with different members of the 5-HT_2-receptor family. This is a question which could readily be addressed with the development of sub-type selective antagonists.

5-HT_3-receptors and tonic control of reflexes

Ondansetron is a selective, potent and well-researched 5-HT_3-receptor antagonist (Butler *et al.*, 1988). In the present study the intrathecal and intravenous dose-response curves for this drug were statistically indistinguishable. Indeed, the effects of intravenous ondansetron became significant at a lower dose that when it was given intrathecally. It seems that the effects of intrathecal ondansetron could have resulted from leakage of the material from the site of injection and therefore that spinal 5-HT_3-receptors do not make a major contribution to tonic

control of the sural-GM reflex. The dose-response curves for the two routes are so similar that it is not possible to discount a small contribution from spinal receptors. The non-spinal effects of ondansetron may have been exerted in the brainstem or peripherally: our experimental design does not permit us to distinguish between these possible sites of action.

It is important to emphasize that our results do not mean that 5-HT_3 receptors are not important in the control of spinal function. They are present in large numbers in the dorsal grey matter (Laporte *et al.*, 1992; Kia *et al.*, 1995), and it is thought that 5-HT_3-receptors are responsible for a significant part of the antinociceptive actions of exogenously applied 5-HT (Glaum *et al.*, 1988; Alhaider *et al.*, 1991; Millan, 1995).

Interactions between 5-hydroxytryptaminergic and adrenerigc systems

5-HT_{1A}-receptors Administration of idazoxan after WAY-100635 gave rise to no further increases in reflexes, suggesting that the reflex pathway was saturated after 5-HT_{1A}-receptor blockade. However, spinalization in the presence of the two antagonists produced a different pattern of effects compared to that seen after idazoxan alone. When the α_2-adrenoceptor antagonist is present, spinalization always results in a decrease in reflex responses, indicating the presence of descending facilitation (Harris & Clarke, 1992). The mixture of effects seen on spinal section when both WAY-100635 and idazoxan were present indicates that the dominance of descending faciliation normally revealed after α_2-adrenoceptor blockade was somehow compromised. The nature of this interaction between adrenergic and 5-hydroxytryptaminergic controls remains to be elucidated.

5-HT_2-receptors Administered after ICI 170,809, idazoxan did increase reflexes over the post-ICI 170,809 level, but the combined effect of the two drugs was to produce no overall effect. Idazoxan plus ICI 170,809 had less effect than a lower dose of idazoxan given alone. This result was only seen after i.th. administration of the 5-HT_2-receptor antagonist, so it appears that blockade of spinal 5-HT_2-receptors reduces some of the reflex enhancement resulting from blockade of spinal α_2-adrenoceptors. It has been noted previously that α_2-adrenoceptor antagonists reduce descending inhibition and increase descending facilitation of the sural-GM reflex (Harris & Clarke, 1992; 1993). The present data suggest that a component of the latter effect is mediated through 5-HT_2-receptors. This may result from the blockade of inhibitory adrenoceptors located on the spinal terminals of descending 5-hydroxytryptaminergic neurones projecting to motor nuclei; such a control has been shown to operate at 5-hydroxytryptaminergic terminals in rabbit brain (Feuerstein *et al.*, 1985) and in rat hippocampus (Mongeau *et al.*, 1993). Not all of the descending facilitation released by idazoxan is mediated through 5-HT_2-receptors, as in most animals reflexes still decreased on spinalization in the presence of idazoxan and ICI 170,809. We have already shown that α_1-adrenoceptors mediate some of the facilitatory effects resulting from α_2-adrenoceptor blockade (Harris & Clarke, 1992).

5-HT_3-receptors Ondansetron did not affect the responses to intrathecal idazoxan when given by the i.v. or by the i.th. routes. There does not appear to be any form of interaction between 5-HT_3-receptor and α_2-adrenoceptor-mediated events in the rabbit spinal cord.

Spinal 5-HT-receptors and cardiovascular control

None of the 5-HT-receptor antagonists used in this study affected blood pressure or heart rate. It seems that neither 5-HT_{1A}-, 5-HT_2, nor 5-HT_3-receptors exerts any sort of tonic control over cardiovascular parameters in the decerebrated, unanaesthetized rabbit. This is surprising in view of the rich innervation by 5-hydroxytryptaminergic terminals of spinal sympathetic preganglionic neurones in the rabbit (Jensen *et al.*, 1995), and the dense accumulation of 5-HT_{1A}- and 5-HT_2-receptors near autonomic nuclei in the spinal cord (Thor *et al.*, 1993).

Conclusions

The data presented above show that selective blockade of spinal 5-HT_{1A}-receptors augments transmission in a hindlimb withdrawal reflex pathway and provide the first evidence for the involvement of a specific 5-HT-receptor sub-type in tonic descending control of spinal function. It is possible that selective activation of the 5-hydroxytryptaminergic pathway involved may have an antinociceptive action but more studies on the source and actions of the inhibitory pathway are needed to substantiate this idea. The effects of 5-HT_2-receptor blockade were complex, but provided further evidence that these receptors contribute to descending facilitation of spinal function. 5-HT_3-receptors do not appear to make a substantial contribution to tonic control of reflexes in the preparation used in the present experiments.

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