Comparison of the effects of IVth ventricular administration of some tryptamine analogues with those of 8-OH-DPAT on autonomic outflow in the anaesthetized cat

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1 The present study compares the effects on representative autonomic outflows of IVth ventricular application of tryptamine analogues which act at 5-HT₁ receptors with 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT).

2 Cumulative doses of 8-OH-DPAT, N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT) and 5-carboxamidotryptamine (5-CT, 2.5–40 nmol kg⁻¹), sumatriptan (10–160 nmol kg⁻¹), indorenate (100–800 nmol kg⁻¹), 5-hydroxytryptamine (5-HT, 20–640 nmol kg⁻¹) both alone and in the presence of cinanserin (0.1 mg kg⁻¹) were given into the IVth ventricle of cats which were anaesthetized with a mixture of α -chloralose and pentobarbitone sodium, neuromuscularly blocked and artificially ventilated. Recordings were made of arterial blood pressure, heart rate, renal, cardiac, splanchnic and phrenic nerve activities, femoral arterial flow, tracheal and intragastric pressures.

3 Central application of each of the agonists evoked significant falls in arterial blood pressure. In addition 8-OH-DPAT, DP-5-CT, 5-CT and 5-HT all evoked a differential inhibition of sympathetic nerve activities, with renal nerve activity being the most sensitive and cardiac nerve activity the least sensitive. In the dose-ranges used, administration of sumatriptan evoked reductions only in renal and splanchnic nerve activities whilst indorenate reduced activity in all three sympathetic nerves to a similar extent.

4 The effect of the agonists on heart rate was more inconsistent than the effects on sympathetic outflow. IVth ventricular application of 5-CT and sumatriptan were without effect on heart rate whilst 8-OH-DPAT, DP-5-CT, indorenate and 5-HT alone and in the presence of cinanserin all evoked significant bradycardias. However, whilst atropine partially reversed the bradycardias evoked by 8-OH-DPAT and only slightly reversed those caused by indorenate, atropine was without effect on those evoked by DP-5-CT or 5-HT.

5 None of the analogues tested had significant effects on gut motility, phrenic nerve discharge or tracheal pressure. 8-OH-DPAT, DP-5-CT, indorenate and 5-HT were without effect on femoral arterial conductance. However, following pretreatment with cinanserin, 5-HT evoked a significant reduction in femoral arterial conductance. At its highest dose, sumatriptan evoked a significant increase in femoral arterial conductance as did 5-CT at the 20 nmol kg⁻¹ dose.

6 It is concluded that the present data support the view that 5-HT_{1A} receptors at the level of the brainstem are involved in the central sympathoinhibitory effects caused by intravenous administration of 5-HT_{1A} agonists. Further, brainstem 5-HT_{1A} receptors play an important role in the control of renal sympathetic outflow while brainstem 5-HT₂ receptors are involved in the control of skeletal muscle and/or skin blood flow. Selective tryptamine agonists for 5-HT_{1A} receptors differ from non-tryptamine agonists in that they do not cause an increase in central cardiac vagal tone.

Keywords: 5-HT_{1A} receptors; 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT); N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT); 5-carboxamidotryptamine (5-CT); sumatriptan; indorenate; 5-hydroxytryptamine (5-HT); cinanserin; sympathetic nerve activity; blood pressure

Introduction

Determination of the *in vivo* function of particular receptors is often made by studying the deficit produced by application of selective antagonists for the receptor. However, such determination of the functions of the many subtypes of the 5-HT₁ receptor has been particularly difficult due to the lack of selective antagonists (see Hamon *et al.*, 1990). Another method for investigating the specific role of different receptor subtypes *in vivo* is the use of agonists for that particular subtype. Although in the case of the 5-HT_{1A} receptor subtype these agonists are more selective than the available antagonists, they may still not be totally selective and

therefore *in vivo* it is sometimes difficult to assign all the observed induced changes to activation of a particular receptor subtype. However, if other structurally different agonists also produce the same range of effects, it is then much more likely that these effects are mediated through activation of one particular receptor subtype. In investigating the function of 5-HT_{1A} receptors in cardiovascular regulation the most commonly used agonist is the aminotetralin 8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT) a simplified ergot congener (Hjorth *et al.*, 1982). In addition, a small amount of data exists on other agonists, such as flesinoxan, an N-substituted phenylpiperazine analogue (Wouters *et al.*, 1988). Using such information it has been concluded that the sympathoinhibition, increase in vagal drive to the heart and fall in blood pressure observed with these compounds is due to activation of 5-HT_{1A} receptors (see Ramage, 1990). It has recently been suggested that if agonists must be used alone to

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determine the function of a particular receptor then it may be preferable to use agonists which are structurally-related to the natural transmitter (Leff & Martin, 1988). Analogues of 5-HT which are selective for 5-HT₁ receptors such as N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT; highly selective for the 1_A subtype), 5-carboxamidotryptamine maleate (5-CT; highly potent at the 1_A, 1_B and 1_D receptor subtypes) and sumatriptan (with 30 times higher affinity for 1_D than 1_A; see Hoyer & Fozard, 1991) do exist. However, little is known of their central effects on autonomic regulation, although it has been suggested that indorenate, a tryptamine analogue, causes its central hypotensive action by activation of 5-HT₁ receptors (Safdy *et al.*, 1982). One probable reason for this lack of information is that these substances do not cross the blood-brain barrier well (Mir *et al.*, 1987; Humphrey *et al.*, 1990). In addition, the wide distribution of 5-HT_{1A} receptors within the central nervous system (Pazos & Palacios, 1985) forestalls the use of local injection of these compounds into the central nervous system, since this may not produce the complete range of autonomic effects as observed for intravenous 8-OH-DPAT.

The present experiments were carried out to compare the action of tryptamine analogues DP-5-CT, 5-CT, sumatriptan and indorenate with that of 8-OH-DPAT on sympathetic nerve activity, blood pressure and heart rate by administering these compounds into the IVth ventricle. This method of administration will allow substances to reach the ventral surface of the brainstem, an area which has recently been identified as a site for the central cardiovascular effects of 8-OH-DPAT (Gillis *et al.*, 1989; Laubie *et al.*, 1989; King & Holtman, 1990). In addition, the effects of 5-HT alone and in the presence of the 5-HT₂ antagonist cinanserin were also investigated, since it has previously been reported that 5-HT applied to the ventral surface of the brain stem failed to affect sympathetic nerve activity and blood pressure (Coote *et al.*, 1987), though effects could be observed if the 5-HT were applied in the presence of a 5-HT₂ receptor antagonist (Gillis *et al.*, 1989).

Preliminary accounts of some of these observations have been given (Shepherd *et al.*, 1989; 1990).

Methods

Experiments were performed on male adult cats anaesthetized with a mixture of α -chloralose (70 mg kg⁻¹) and pentobarbitone sodium (6 mg kg⁻¹) i.v.; supplementary doses of α -chloralose (10–15 mg kg⁻¹) were given as required. Following a tracheotomy low in the neck, the animals were intubated and artificially ventilated (rate 30 per min, tidal volume 17–20 ml) with oxygen-enriched room air using a positive pressure ventilator (Harvard 665A) after neuromus-

cular blockade with vecuronium bromide (200 μ g kg⁻¹). Arterial blood pressure, heart rate, body temperature and arterial blood gases and pH were monitored as previously described (Shepherd *et al.*, 1991b). A constant infusion of a solution comprising 500 ml plasma substitute (Gelofusine), 500 ml H₂O, 8.4 g NaHCO₃ and 2 g of glucose was given at a rate of 6 ml kg⁻¹ h⁻¹ into the brachial vein to maintain blood volume and to counteract the development of non-respiratory acidosis. During the experiments, pH and arterial blood gases were kept within the following ranges, pH 7.24–7.35; PaCO₂ 41–48 mmHg and PaO₂ 112–130 by varying the rate and tidal volume of the ventilator or by a slow infusion i.v. of 1M sodium bicarbonate.

Simultaneous recordings were made of right inferior cardiac, splanchnic and renal nerve activities and left phrenic nerve activity. The right inferior cardiac nerve was exposed retropleurally by deflecting the scapula and removing the second rib. The splanchnic and renal nerves were exposed by a retroperitoneal approach through the right flank. The left phrenic nerve was exposed low down in the neck at the level of the 4th and 5th spinal nerves. Whole nerve activity was recorded from the intact nerves by use of bipolar silver hook electrodes as previously described (Shepherd *et al.*, 1991b). In all experiments sympathetic nerve activity was tested to see if it was under baroreceptor modulation by checking that activity in the nerves increased during a fall in blood pressure induced by sodium nitroprusside (2 μ g kg⁻¹, i.v.) or decreased during a rise in blood pressure induced by noradrenaline (0.5 μ g per animal, i.v.). In addition, femoral arterial flow, from which conductance was calculated, was measured with an electromagnetic flow probe placed on the right femoral artery. Tracheal pressure was monitored by a pressure transducer connected to a side arm of the tracheal cannula. Gastric motility was measured by inserting a rubber balloon into the stomach via the oesophagus. This balloon was then filled with 30 ml of saline and connected to a pressure transducer.

To give microinjections into the IVth ventricle the animal's head was placed in a stereotaxic frame and a cannula placed so that its tip lay in the IVth ventricle as described previously (Shepherd *et al.*, 1991b). The position was confirmed at the end of the experiment by injecting pontamine sky blue dye (20 μ l over 1 min). When the cannula was correctly placed it was found that the dye was distributed over the floor of the IVth ventricle from its anterior end to the level of the lateral recesses and from there through the lateral recesses, down on to the ventral surface of the medulla.

All variables were recorded for a 20 min stabilization period. In each animal a microinjection of vehicle ('initial' vehicle) was then given into the IVth ventricle (i.c.v.) followed 10 min later by cumulative doses of either a test drug or vehicle, only one drug or vehicle being tested per animal.

Table 1 Baseline values of heart rate (HR), mean arterial blood pressure, (BP), femoral arterial conductance (FAC), gastric motility (GM), tracheal pressure (TP) and inspiratory rate (Insp rate)

	n	HR (beats min ⁻¹)	BP (mmHg)	FAC (ml min ⁻¹ mmHg ⁻¹ × 10 ⁻³)	GM (line length mm min ⁻¹)	TP (cmH ₂ O)	Insp rate (burst min ⁻¹)
Control CSF	5	205 ± 12	150 ± 7	48 ± 4	58 ± 11	7.4 ± 0.4	8.6 ± 0.9
Control saline	5	197 ± 8	117 ± 10	67 ± 9	49 ± 4	6.9 ± 0.3	9.7 ± 0.8 (n = 4)
8-OH-DPAT	5	211 ± 10	120 ± 12	96 ± 18	47 ± 7	9.1 ± 1.1	10.0 ± 1.1 (n = 3)
DP-5-CT	5	218 ± 11	137 ± 10	53 ± 12	36 ± 7	7.6 ± 0.5	9.5 ± 0.5 (n = 4)
5-CT	5	221 ± 15	116 ± 11	63 ± 11	42 ± 8	8.2 ± 0.6	7.6 ± 1.0
Indorenate	5	197 ± 12	117 ± 8	77 ± 14	60 ± 6	7.4 ± 0.4	7.2 ± 1.6
Sumatriptan	5	224 ± 11	103 ± 4	77 ± 19	43 ± 6	8.2 ± 0.3	6.8 ± 0.2
5-HT	5	215 ± 7	126 ± 5	65 ± 22	30 ± 7	9.0 ± 0.4	8.0 ± 1.0 (n = 2)
Cinanserin	5	202 ± 8	107 ± 11	74 ± 4	57 ± 14	8.1 ± 0.4	5.2 ± 0.8 (n = 4)
Cinanserin/5-HT	5	200 ± 8	104 ± 11	88 ± 7	55 ± 6	8.1 ± 0.4	5.5 ± 0.9 (n = 4)

8-OH-DPAT, 8-hydroxy-2-(di-n-propylaminotetralin); DP-5-CT, N,N-di-n-propyl-5-carboxamidotryptamine; 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine.

Test drugs were administered cumulatively at 10 min intervals except for 5-HT alone, 5-HT in the presence of cinanserin and sumatriptan which were given at 15 min intervals. In all experiments atropine methylnitrate (0.1 mg kg^{-1} , i.v.) was administered at least 10 min after the last dose of the test drug. The times between injections represent the time taken for the changes in blood pressure and nerve activities to stabilize. All i.c.v. injections were given in a volume of $20 \mu\text{l}$ over a period of 1 min.

Two sets of control experiments were performed. The first set were controls for drugs dissolved in CSF. Test i.c.v. injections of CSF were given at 10 min intervals which corresponded in time to the i.c.v. injections of 8-OH-DPAT and 5-CT. However, after the time of the final 5-CT injection, the CSF injections were continued at 10 min intervals so that they could be used as controls for the 5-HT, 5-HT in the presence of cinanserin and sumatriptan experiments in which the drugs were administered at 15 min intervals. In these latter controls, values were taken at time intervals which corresponded to the application of the drugs, though this did not necessarily match an injection of CSF. The second set of controls were for drugs dissolved in saline. Test i.c.v. injections of saline were given at 10 min intervals to correspond to the time of i.c.v. injections of DP-5-CT and indorenate.

Analysis of data

All sympathetic nerve activities were quantified by rectifying and integrating the signals above background noise over 5 s periods using solid state electronic integrators. The outputs were then displayed on a Grass polygraph recorder and were calibrated in arbitrary units. Phrenic nerve activity was quantified by integrating the amplitude and frequency of the action potentials in each inspiratory burst or, if continuous, by integrating the amount of activity in a 5 s period, again using a solid state electronic integrator, the output of which was displayed on the Grass polygraph in arbitrary units (see Shephard *et al.*, 1991b). The first method of quantifying phrenic nerve activity gives an indication of both the amount of activity in each inspiratory burst and the frequency of inspiratory bursts. The validity of the threshold setting used to quantify the nerve activities was verified at the end of each experiment after administration of pentobarbitone sodium (60 mg per animal) or by crushing the nerves centrally to block all activity. All nerve activity was measured as the mean level over 1 min in arbitrary units.

Intragastric pressure was quantified by displaying the output from the pressure transducer on the Grass polygraph recorder. The total line length of the ink trace was measured with an IBAS IPS image analyser (Kontron U.K.) and converted to give mean line length in mm min^{-1} . Total line length gives an indication of changes in both frequency and amplitude of gastric contractions (see Shephard *et al.*, 1991b). Tracheal pressure was continually monitored throughout each experiment but as no obvious changes were observed no further analysis was carried out.

Except for gastric motility, measurements of all variables were made in the minute before each injection of test substance. Because of its slow periodicity, measurements of gastric motility were made in the 3 min period before each injection of test substance. In all experiments baseline values were those values measured just before the first injection of test drug or vehicle. This was usually 10 min after the 'initial' vehicle. However, in the cinanserin pretreatment experiments, cinanserin was administered 10 min after 'initial' vehicle followed 10 min later by 5-HT. Baseline values for these experiments were taken as those values prior to the first injection of 5-HT. All results are expressed as changes from baseline values. In order to normalize the data, changes in integrated nerve activity are given as percentage changes from baseline. The changes in all other variables are presented as actual changes.

The changes in response to test drugs were compared with

the responses to the appropriate vehicle injections at matched time intervals by two way analysis of variance and the least significant difference for comparisons between the means (Sokal & Rohlf, 1969). Changes from baseline values caused by cinanserin were statistically analysed by Student's paired *t* test. Differences were considered significant when $P < 0.05$.

Drugs and solutions

The following drugs were used:- 8-hydroxy-2-(di-n-propyl-amino)tetralin HBr (8-OH-DPAT), N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT), 5-carboxamidotryptamine maleate (5-CT, Research Biochemicals Inc., Semat Technical Ltd, St. Albans, Herts); sumatriptan HCl (a gift from the Wellcome Research Laboratories, Kent);

Figure 1 Anaesthetized cats: the effect of cumulative doses ($2.5\text{--}40 \text{ nmol kg}^{-1}$) of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, ●), N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT, ▼) and 5-carboxamidotryptamine (5-CT, ■) administered into the IVth ventricle on changes (Δ) from baseline values in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (BP) and femoral arterial conductance (FAC). Each point represents the mean value ($n = 5$) with s.e.mean. The above changes were compared to changes caused by the appropriate vehicle administration using two way analysis of variance and the least significant difference test to compare the means, * $P < 0.05$; ** $P < 0.01$. The data for the appropriate vehicle control has been omitted for the sake of clarity.

Figure 2 Traces showing recordings of gastric balloon pressure (G bal P), tracheal pressure (TP), integrated phrenic nerve activity (PNA), femoral arterial flow (FF), arterial blood pressure (BP), heart rate (HR), integrated cardiac (CNA), splanchnic (SNA) and renal (RNA) nerve activities in an anaesthetized cat. The three panels show the effects of CSF and then the cumulative doses of 10 and 40 mmol kg⁻¹ of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) administered into the IVth ventricle. In the third panel the effect of an i.v. bolus injection of atropine methylnitrate (0.1 mg kg⁻¹) is illustrated.

indorenate HCl, (a gift from Miles Laboratories, Inc., Elkhart, Indiana, U.S.A.); Gelofusine (Consolidated Chem., Wrexham, Clwyd); 5-hydroxytryptamine creatinine sulphate (5-HT, BDH, Poole, Dorset); noradrenaline acid tartrate (Winter, Guildford, Surrey); cinanserin HCl (a gift from Squibb & Sons, Inc., Princeton, N.J., U.S.A.); α -chloralose, sodium nitroprusside and atropine methylnitrate (Sigma Chemical Co., Poole, Dorset). Drugs given i.c.v. were dissolved in artificial CSF (8-OH-DPAT, 5-CT, 5-HT, sumatriptan) or 0.9% w/v saline (DP-5-CT, indorenate, cinanserin). The composition of the artificial CSF used was (mM): KH₂PO₄ 2.2, MgSO₄·7H₂O 1.2, KCl 2.0, glucose 10, NaHCO₃ 25, NaCl 115 and CaCl₂·2H₂O 2.5. All doses except noradrenaline refer to the salts of the drug.

Results

The baseline values for all experimental groups are shown in Table 1.

Controls: effects of i.c.v. injections of CSF and saline

Injection of CSF ($n = 5$) every 10 min over a 90 min period caused only small changes from baseline in mean blood pressure (-6 ± 4 mmHg), heart rate ($+6 \pm 5$ beats min⁻¹), femoral arterial conductance ($+2 \pm 3$ ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$), renal ($+12 \pm 13\%$) and splanchnic ($+17 \pm 15\%$)

nerve activity whereas cardiac nerve activity increased from baseline by $+55 \pm 19\%$. Data for 75 min of this control are shown in Figure 5. The rate of bursting and magnitude of each phrenic burst showed no significant change ($+1.0 \pm 0.5$ burst min⁻¹ and $+22 \pm 11\%$ respectively) from baseline values). Tracheal pressure and gastric motility ($+16 \pm 6$ mm min⁻¹) changes little over this period.

Injections of saline ($n = 5$) at 10 min intervals over 60 min caused only small changes from baseline values in blood pressure (-3 ± 1 mmHg), heart rate ($+4 \pm 3$ beats min⁻¹), femoral arterial conductance ($+7 \pm 3$ ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$), in splanchnic ($+3 \pm 10\%$) and renal ($-6 \pm 4\%$) nerve activity. Cardiac nerve activity showed a small increase from baseline of $15 \pm 2\%$. The rate of bursting and magnitude of each phrenic burst showed no significant change ($+1.3 \pm 0.5$ burst min⁻¹ and $+3 \pm 36\%$, respectively) from baseline values. Again tracheal pressure and gastric motility ($+2 \pm 6$ mm min⁻¹) changes little over this period. Data for 40 min of this control are shown in Figure 4.

Effects of cumulative doses (2.5–40 nmol kg⁻¹) of 8-OH-DPAT, DP-5-CT and 5-CT injected into the IVth ventricle

All three compounds caused dose-related decreases in sympathetic nerve activity and blood pressure (Figure 1). Blood pressure fell from baseline values by 45 ± 11 , 34 ± 4 and 38 ± 6 mmHg for 8-OH-DPAT, DP-5-CT and 5-CT respec-

Figure 3 Traces showing recordings of gastric balloon pressure (G bal P), tracheal pressure (TP), integrated phrenic nerve activity (PNA), femoral arterial flow (FF), arterial blood pressure (BP), heart rate (HR), integrated cardiac (CNA), splanchnic (SNA) and renal (RNA) nerve activities in an anaesthetized cat. The three panels show the effects of CSF and then the cumulative doses of 20 and 80 nmol kg⁻¹ of N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT) administered into the IVth ventricle. In the third panel the effect of an i.v. bolus injection of atropine methylnitrate (0.1 mg kg⁻¹) is illustrated.

tively by the highest dose. The decrease in sympathetic nerve activity in the sympathetic outflows was not uniform. For 8-OH-DPAT ($n = 5$) the reductions in renal and splanchnic nerve activity became significant ($P < 0.05$), compared with control, at the 5 nmol kg⁻¹ dose (Figure 1) reaching $-92 \pm 4\%$ and $-90 \pm 3\%$ respectively by the highest dose. The reduction in cardiac nerve activity was not significant until the 20 nmol kg⁻¹ dose and only reached $-62 \pm 9\%$ at the highest dose. Representative traces from a typical experiment are shown in Figure 2.

In the DP-5-CT experiments ($n = 5$) the decrease in renal nerve activity became significant by the 10 nmol kg⁻¹ dose reaching $-67 \pm 10\%$ by the highest dose while the reduction in splanchnic nerve activity became significant by the 20 nmol kg⁻¹ dose, reaching $-66 \pm 2\%$ by the highest dose. Again the reduction in cardiac nerve activity was smaller, only becoming significant at 40 nmol kg⁻¹ reaching $-42 \pm 16\%$. Representative traces from one of these experiments is shown in Figure 3.

5-CT ($n = 5$) caused a similar profile of sympathoinhibition to that observed for 8-OH-DPAT and DP-5-CT, renal nerve activity being most sensitive to the sympathoinhibitory actions of 5-CT. A significant reduction in renal nerve activity was observed by the lowest dose and reached $-78 \pm 15\%$ by the highest dose of 5-CT. The reduction in splanchnic nerve activity became significant at the 10 nmol kg⁻¹ dose and reached $-62 \pm 13\%$ while the reduction in cardiac nerve activity was not significantly different from control values until the highest dose reaching $-25 \pm 27\%$.

Surprisingly, the reductions in sympathetic outflow and blood pressure caused by all three drugs were not associated with changes in femoral arterial conductance. Only 5-CT, at the dose of 20 nmol kg⁻¹, caused a small but significant increase in femoral arterial conductance of 14 ± 8 ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$ (Figure 1).

The reductions in sympathetic outflow and blood pressure evoked by the two tryptamine analogues were associated with only small changes in heart rate (Figure 1). For DP-5-CT there was a significant reduction in heart rate of -11 ± 4 at 40 nmol kg⁻¹ and for 5-CT there was an increase in heart rate of 22 ± 11 beats min⁻¹ at the highest dose. 8-OH-DPAT caused dose-related decreases in heart rate reaching -55 ± 13 beats min⁻¹ at the highest dose. In some cats (Figure 2) a profound sinus arrhythmia was also observed by the highest dose. Injection of atropine methylnitrate (0.1 mg kg⁻¹, i.v.) after the highest dose of 8-OH-DPAT caused the heart rate to return towards baseline levels (see Figure 2) producing a mean increase of 46 ± 14 beats min⁻¹. In contrast, intravenous injection of atropine methylnitrate did not affect the changes in heart rate caused either by DP-5-CT or by 5-CT.

Effects of cumulative doses of indorenate (100–800 nmol kg⁻¹) injected into the IVth ventricle

Indorenate ($n = 5$) caused dose related decreases in blood pressure, heart rate and activity in all three sympathetic nerves (Figure 4). The reduction in all the sympathetic nerve

activities was significant at all four doses. Although the decrease in cardiac nerve activity at the 100 nmol kg⁻¹ dose ($-25 \pm 11\%$) was slightly less than that observed in renal ($-46 \pm 17\%$) and splanchnic ($-45 \pm 17\%$) nerve activity, by the highest dose the reduction in cardiac nerve activity ($-80 \pm 8\%$) was similar to that observed in splanchnic ($-87 \pm 11\%$) and renal ($-90 \pm 7\%$) nerve activities. This sympathoinhibition was associated with a decrease in blood pressure of -23 ± 8 mmHg at the highest dose but there was no significant change in femoral arterial conductance. There was however, a significant fall in heart rate which reached a maximum of -33 ± 10 beats min⁻¹ by the highest dose. Subsequent administration of atropine methylnitrate caused a small increase in heart rate in 3 out of the 5 experiments of between 10–15 beats min⁻¹.

Effects of cumulative doses of sumatriptan (10–160 nmol kg⁻¹) injected into the IVth ventricle

Sumatriptan ($n = 5$, Figure 5), like 8-OH-DPAT, DP-5-CT and 5-CT caused sympathoinhibition and a fall in blood pressure. As with the other agonists, the renal nerve activity was the most sensitive to administration of sumatriptan, sympathoinhibition being observed at a dose of 10 nmol kg⁻¹ whereas sympathoinhibition in the splanchnic nerve became apparent only at the 20 nmol kg⁻¹ dose. These reached near maxima at 20 min and were $-45 \pm 8\%$ and $-34 \pm 17\%$, respectively at the highest doses. Sumatriptan was without significant effect on both cardiac nerve activity and heart rate. The sympathoinhibitory action of sumatriptan was associated with a maximum fall in blood pressure of 24 ± 5 mmHg at the highest dose, becoming significant at the 20 nmol kg⁻¹ doses. The maximum fall in blood pressure was associated with a significant increase in femoral conductance of 13 ± 9 ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$.

Effects of cumulative doses of 5-hydroxytryptamine (20–640 nmol kg⁻¹) injected into the IVth ventricle alone and in the presence of cinanserin (0.1 mg kg⁻¹)

Pretreatment with cinanserin (0.1 mg kg⁻¹, $n = 5$) had little effect on the parameters being recorded except femoral arterial conductance in which there was a significant increase of 14 ± 4 ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$ (Table 1). Cumulative doses of either 5-HT alone or in the presence of cinanserin caused significant falls in blood pressure by the highest dose of 19 ± 5 and 22 ± 3 mmHg respectively. Again, this was associated with a non-uniform sympathoinhibition (Figure 6). For 5-HT alone ($n = 5$) splanchnic nerve activity had declined significantly compared to control values at the dose of 80 nmol kg⁻¹ while significant sympathoinhibition was not observed in the renal nerve until the dose of 320 nmol kg⁻¹ both reaching a maximum of $-47 \pm 15\%$ and $-47 \pm 21\%$ respectively by the highest dose. Cardiac nerve activity was unaffected by cumulative doses of 5-HT alone. In the presence of cinanserin, 5-HT ($n = 5$) caused significant sympathoinhibition initially in the renal nerve at the dose of 80 nmol kg⁻¹ whereas significant sympathoinhibition was not observed in the splanchnic nerve until the following dose of 160 nmol kg⁻¹ declining to a maximum of $-43 \pm 24\%$ while renal declined by $-60 \pm 21\%$ in both cases by the highest dose. Furthermore, cardiac nerve activity also fell by $-9 \pm 24\%$. Although this was a small fall, it was significant compared to control data because in the control CSF alone experiments, cardiac nerve activity increased quite markedly over this time period (see above). In fact, cardiac nerve activity was significantly different from control values by the dose of 160 nmol kg⁻¹. In addition 5-HT in the presence of cinanserin caused a significant decrease in femoral arterial conductance at a dose of 160 nmol kg⁻¹ reaching a maximum fall of -13 ± 9 ml min⁻¹ mmHg⁻¹ $\times 10^{-3}$ by the highest dose, whereas 5-HT alone caused no significant change.

Figure 4 Anaesthetized cats: comparison of the effect of cumulative doses (100–800 nmol kg⁻¹) of indorenate (●), with time matched saline control values (▼) administered into the IVth ventricle on changes (Δ) from baseline values in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (BP) and femoral arterial conductance (FAC). Each point represents the mean value ($n = 5$) with s.e.mean. Comparisons of the changes evoked by indorenate with those produced by saline were made by two way analysis of variance and the least significant difference test to compare the means; * $P < 0.05$; ** $P < 0.01$.

Finally, 5-HT both alone and in the presence of cinanserin caused a significant decline in heart rate reaching, by the highest dose, -18 ± 8 and -9 ± 5 beats min⁻¹ respectively. These falls in heart rate were unaffected by atropine methylnitrate.

Effect of all drugs on the magnitude or rate of phrenic nerve activity, tracheal pressure and gastric motility

None of the drugs had significant effects on the above variables. Data are illustrated only for single experiments with 8-OH-DPAT and DP-5-CT, (Figures 2 and 3) and the mean changes on gastric motility were $+16 \pm 6$ mm min⁻¹ and $+2 \pm 4$ mm min⁻¹, respectively.

Discussion

IVth ventricular application of the tryptamine analogues DP-5-CT, 5-CT, indorenate and sumatriptan and the simplified

Figure 5 Anaesthetized cats: comparison of the effect of cumulative doses (10–160 nmol kg⁻¹) of sumatriptan (●), with time matched CSF control values (▼) administered into the IVth ventricle on changes (Δ) from baseline values in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (BP) and femoral arterial conductance (FAC). Each point represents the mean value ($n = 5$) with s.e.mean. Comparison of the changes evoked by sumatriptan with those produced by CSF were made by two way analysis of variance and the least significant difference test to compare the means; * $P < 0.05$; ** $P < 0.01$.

ergot congener, 8-OH-DPAT, all caused significant falls in sympathetic nerve activity and blood pressure. These falls were associated with little change in femoral arterial conductance. The sympathoinhibition was not uniform in the three different sympathetic outflows that were monitored, renal nerve activity was much more sensitive to the sympathoinhibitory actions of these compounds than cardiac nerve activity. Such a profile of action has been previously observed in anaesthetized cats with i.v. administration of 8-OH-DPAT, ipsapirone and flesinoxan (Ramage & Fozard, 1987; Ramage *et al.*, 1988; Ramage & Wilkinson, 1989). It has also been demonstrated that the sympathoinhibitory action of i.v. 8-OH-DPAT and the hypotensive action of DP-5-CT, administered via the vertebral artery of anaesthetized cats, can be reversed by spiperone (McCall *et al.*, 1987) or is blocked by pretreatment with (–)-pindolol (Doods *et al.*, 1988), respectively. As both drugs act as antagonists at 5-HT_{1A} receptors, and since all the above compounds bind to 5-HT_{1A} receptors (see Hoyer & Fozard, 1991) it can be concluded that the central hypotensive action

Figure 6 Anaesthetized cats: the effect of cumulative doses (20–640 nmol kg⁻¹) of 5-hydroxytryptamine alone (●), and in the presence of 0.1 mg kg⁻¹ cinanserin (▼) administered into the IVth ventricle on changes (Δ) from baseline values in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (BP) and femoral arterial conductance (FAC). Each point represents the mean value ($n = 5$) with s.e.mean. The above changes were compared with changes caused by the CSF vehicle alone by two way analysis of variance and the least significant difference test to compare the means; * $P < 0.05$; ** $P < 0.01$. The data for the CSF vehicle control has been omitted for the sake of clarity.

of all the above compounds can be explained, at least in part, by activation of 5-HT_{1A} receptors at the level of the brain-stem.

Both DP-5-CT and 5-CT showed a similar potency in their sympathoinhibitory actions when compared to the potency of 8-OH-DPAT. The potency of these compounds in causing sympathoinhibition is in line with their comparative affinities for 5-HT_{1A} receptors (DP-5-CT and 5-CT pK_D 9.5 c.f. 8-OH-DPAT pK_D 8.7, see Hoyer & Fozard, 1991). Further, as all agonists tested caused a decrease in renal nerve activity it may be suggested that reduction in this variable is related to the affinity of these compounds for 5-HT_{1A} receptors. Sumatriptan, which, depending on the species, is also considered to be an agonist at 5-HT_{1D} or 5-HT_{1B} receptors (Peroutka & McCarthy, 1989) was found to cause a decrease in renal nerve activity. However the selectivity of sumatriptan for 5-HT_{1D} compared to 5-HT_{1A} receptors is not particularly large since it has about a 30 times higher affinity for the former compared with the latter (see Hoyer & Fozard, 1991). Thus, an agonist action at 5-HT_{1A} receptor could explain the

ability of sumatriptan to cause renal sympathoinhibition. As the sumatriptan dose-response curve for renal sympathoinhibition is very shallow it may be that there is an interaction between 5-HT_{1A} and 5-HT_{1D} receptors at this level. In this respect, addition of 8-OH-DPAT after sumatriptan failed to have any further sympathoinhibitory action (unpublished observation). 5-CT however, has no greater selectivity between 5-HT_{1A} and 5-HT_{1D} receptors than sumatriptan but has a much higher affinity for both subtypes (see Hoyer & Fozard, 1991) but causes a fairly steep dose-related reduction in renal nerve activity. Therefore attributing any effects observed in the present study to activation of 5-HT_{1D} is impossible due to the lack of a selective agonist for this receptor type. The failure of indorenate to cause an overt differential sympathoinhibition may be related to the large dose chosen as at high doses of all 5-HT_{1A} agonists there is a large reduction in cardiac as well as renal nerve activity. Interestingly enough, even at the dose chosen, there was no increase in femoral arterial conductance. However little is known of the binding profile of indorenate to 5-HT receptor subtypes and the failure to cause differential sympathoinhibition may be related to indorenate interacting with other 5-HT receptors. In this respect indorenate, when given i.v. to anaesthetized cats, can cause an increase in tracheal pressure which is indicative of an agonist at 5-HT₂ receptors (see Ramage, 1990).

The finding that 8-OH-DPAT applied to the IVth ventricle caused an increase in cardiac vagal drive in the present study confirms previous observations with i.v. administration of 8-OH-DPAT, flesinoxan and ipsapirone (Ramage & Fozard, 1987; Ramage *et al.*, 1988) in anaesthetized cats and is consistent with the large amount of evidence for a role of 5-HT_{1A} receptors in the control of cardiac vagal motoneurone excitability (Bogle *et al.*, 1990; Sporton *et al.*, 1991; Chitravanshi & Calaresu, 1992). However, the tryptamine analogues, except indorenate which caused only a small increase in cardiac vagal tone, failed to increase cardiac vagal tone in the present study. Even a very high dose of DP-5-CT (80 nmol kg⁻¹, see Figure 3) failed to cause an increase in vagal tone to the heart. This is somewhat surprising since the tryptamine analogues DP-5-CT and 5-CT have comparable effects to 8-OH-DPAT on central sympathetic drive, especially renal nerve activity, which it is possible (see above) to attribute to activation of 5-HT_{1A} receptors. Further it is interesting that none of the agonists used in the present study, even 8-OH-DPAT, increased the excitability of other central parasympathetic motoneurons, such as those that control the airways and gastric motility, although central 5-HT pathways have been implicated in the control of gastric motility (Hornby *et al.*, 1990). This suggests that the properties of cardiac vagal motoneurons may differ from those of other vagal motoneurons.

The effects of IVth ventricular administration of 5-HT in the present study confirm those of Coote *et al.* (1987), in that administration of 5-HT caused a decrease in renal nerve activity. However, in the present experiments, administration of cinanserin into the IVth ventricle, to block 5-HT₂ receptors, which have been demonstrated to have a sympathoexcitatory action (Gillis *et al.*, 1989; Shephard *et al.*, 1991b), did not unmask a greater sympathoinhibitory action and hypotensive effect of 5-HT as might be expected from the

suggested opposing action of 5-HT_{1A} and 5-HT₂ receptors on sympathetic tone (Gillis *et al.*, 1989). The present data only demonstrated that the fall in blood pressure was of a similar magnitude but the changes in the various variables which were causing the fall in blood pressure differed, suggesting that the relationship between 5-HT_{1A} and 5-HT₂ receptors in control of sympathetic outflow is not simply opposing at the level of the brainstem.

The fact that IVth ventricular application of cinanserin caused femoral arterial vasodilatation with little effect on blood pressure and central sympathetic outflow supports previous reports that this is a characteristic effect of 5-HT₂ antagonists in anaesthetized cats (Ramage, 1988). Furthermore, activation of 5-HT₂ receptors at the level of the brainstem also causes femoral arterial vasoconstriction (Shephard *et al.*, 1991b). It is therefore surprising that 5-HT in the presence of cinanserin also causes femoral arterial vasoconstriction, since this is not a characteristic effect of activation of 5-HT_{1A} receptors and suggests that another type of 5-HT receptor is involved in the control of the femoral bed as well as the 5-HT₂ receptor. This could be a 5-HT₃ receptor, as the 5-HT₃ receptor agonist, phenylbiguanide, given i.c.v., causes a decrease, while the 5-HT₃ receptor antagonist, granisetron causes an increase in femoral arterial conductance (Shephard *et al.*, 1991a).

The present results also confirm that the brainstem is the major site of action for the sympathoinhibitory effect of 5-HT_{1A} agonists administered i.v. The rostral ventrolateral medulla (RVLM) has been suggested as the main site within the brainstem at which 5-HT_{1A} agonists act to cause sympathoinhibition (Gillis *et al.*, 1989; King & Holtman, 1990; Laubie *et al.*, 1990). Neurones in this area are known to project to preganglionic sympathetic neurones in the intermediolateral cell column and many 5-HT-containing neurones are also found in this area (see Ciriello *et al.*, 1986). In addition, stimulation of neurones in the RVLM is known to cause differential effects on sympathetic nerve outflow to the kidney compared with outflow to skeletal muscle (McAllen & Dampney, 1992). Whether the differential effects on sympathetic outflow seen in the present study by administering 5-HT_{1A} agonists into the IVth ventricle can be explained by an action at the RVLM alone or by an interaction of the RVLM with other brain areas remains to be determined.

It is concluded that at present, tryptamine analogues do not confer any advantages over non-tryptamine analogues in investigating the function of brainstem 5-HT_{1A} receptors in the control of central autonomic function. However, the present data support the view that 5-HT_{1A} receptors do play an important role in brainstem control of renal and splanchnic sympathetic outflow while 5-HT₂ receptors play a role in the control of skeletal muscle and/or skin blood flow. Further, selective tryptamine agonists for 5-HT_{1A} receptors differ from non-tryptamine agonists in that they do not cause an increase in central cardiac vagal tones.

We would like to thank Mr S. Wilkinson for technical assistance. This work is supported by the Wellcome Trust. We wish to thank Drs P.L.R. Andrews and T. Cowen for help with analysis of gut motility.

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(Received September 10, 1993

Revised October 18, 1993

Accepted October 21, 1993)