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Evidence that activation of 5-HT_2 receptors in the forebrain of anaesthetized cats causes sympathoexcitation

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1 The aim of the present experiments was to determine whether the effects of lateral ventricular application of 5-HT on cardiovascular and respiratory variables in anaesthetized cats are mediated by forebrain 5-HT_2 receptors. This was carried out by determining whether the effects of 5-HT are blocked by the 5-HT_2 antagonist, cinanserin and if they are mimicked by the selective 5-HT_2 agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI).

2 Cats were anaesthetized with a mixture of α-chloralose and pentobarbitone sodium, neuromuscularly blocked and artificially ventilated. The following cardiovascular and respiratory variables were recorded: renal, splanchnic and cardiac sympathetic nerve activities, phrenic nerve activity, heart rate, arterial blood pressure, femoral arterial conductance and tracheal pressure. All drugs were administered via the lateral ventricle and the action of these agonists was restricted to forebrain sites by a cannula placed in the Aqueduct of Sylvius.

3 Cumulative doses of 5-HT (10–160 nmol kg^{-1}) and DOI (80–320 nmol kg^{-1}) injected into the lateral ventricle caused significant increases in blood pressure, heart rate, cardiac and splanchnic sympathetic nerve activity and a decrease in femoral arterial conductance. DOI and 5-HT caused a greater increase in cardiac compared with splanchnic nerve activity and failed to change renal nerve activity. 5-HT but not DOI significantly increased the magnitude and the number of phrenic bursts as well as significantly increasing tracheal pressure. The effects of 5-HT also differed from DOI in that 5-HT evoked maximal pressor and near maximal sympathoexcitatory effects after the first dose, whereas the pressor and sympathoexcitatory effects of DOI were graded over the complete dose-range.

4 The 5-HT_2 antagonist, cinanserin (265 nmol kg^{-1}, i.c.v.) caused significant falls in blood pressure, heart rate and cardiac nerve activity and an increase in femoral arterial conductance. Splanchnic and renal sympathetic nerve activity, phrenic nerve activity and tracheal pressure were unaffected by cinanserin. After pretreatment with cinanserin all cardiovascular and respiratory effects of 5-HT were significantly attenuated.

5 It is concluded that in the cat, as DOI and 5-HT have similar effects on the cardiovascular variables recorded and as the effects of 5-HT are blocked by cinanserin, 5-HT can act on 5-HT_2 receptors located in the forebrain to cause differential sympathoexcitation and a rise in arterial blood pressure. Further, the sympathoexcitatory effects mediated by 5-HT_2 receptors located in the forebrain differ from those located in the hindbrain in that they mediate increases in cardiac nerve activity and heart rate and also have no effect on renal nerve activity.

Keywords: 5-HT_2 receptors; 5-HT; DOI; cinanserin; sympathetic nerve activity; phrenic nerve activity; tracheal pressure; blood pressure

Introduction

Intravenous (i.v.) injections of 5-hydroxytryptamine$_2$ (5-HT_2) receptor agonists cause sympathoexcitation (McCall & Harris, 1988; Vaysettes-Courchay *et al.*, 1991; Ramage *et al.*, 1993). It has been suggested that this sympathoexcitatory action is due to activation of 5-HT_2 receptors located at the level of the hindbrain (King & Holtman, 1990; Mandal *et al.*, 1990; Vaysettes-Courchay *et al.*, 1991; 1992; McCall & Clement, 1994)). However, administration of the 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), to the hindbrain via the IVth ventricle indicated that the hindbrain was not the major sympathoexcitatory site of action of i..v. DOI (Shepheard *et al.*, 1991). Administration of 5-HT into the lateral ventricle, with the Aqueduct of Sylvius cannulated so as to restrict the action of 5-HT to forebrain sites, causes a rise in blood pressure and heart rate (Coote *et al.*, 1987). The receptors involved in this effect of 5-HT are unknown but it might be expected that this response is mediated via 5-HT_2 receptors (see above). Thus, it is possible that the sympathoexcitatory action of i.v. DOI is due to activation of forebrain as well as hindbrain 5-HT_2 receptors. Therefore, the present study was carried out to investigate these possibilities by examining the effects of DOI and 5-HT administered to the forebrain (via lateral ventricle with the Aqueduct of Sylvius cannulated), on regional sympathetic nerve activity, blood pressure and respiratory variables in anaesthetized cats. The effect of cinanserin, a 5-HT_2 receptor antagonist (Rubin *et al.*, 1964; Hoyer & Fozard, 1991) on the response to 5-HT was also examined. A preliminary account of these observations has been given (Anderson *et al.*, 1993).

Methods

Experiments were performed on 20 male adult cats (2.5–3.5 kg) 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 as assessed by the cardiovascular and respiratory responses to paw pinch and the state of the pupil. Following a tracheotomy low in the neck, the animals were

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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 neuromuscular blockade with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings were made of right inferior cardiac, splanchnic and renal sympathetic nerve activities and left phrenic nerve activity using bipolar silver hook electrodes. Arterial blood pressure, heart rate, right femoral arterial flow (from which conductance was calculated) and tracheal pressure were also recorded as previously described (Shepheard *et al.*, 1991). Body temperature, arterial blood gases and pH were monitored. 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 μg kg^{-1}, i.v.). A constant infusion of a solution comprising of 500 ml plasma substitute (Gelofusine), 500 ml H_2O, 8.4 g of $NaHCO_3$ 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; Pa CO_2 41–48 mmHg and PaO_2 112–130 mmHg by varying the rate and tidal volume of the ventilator or by a slow infusion i.v. of 1M sodium bicarbonate.

To give microinjections into the lateral ventricle (i.c.v.) the cats were placed in a stereotaxic frame, the dorsal surface of the skull was exposed and a hole (1 cm^2) made in the bone, the centre of which was 13.5 mm anterior from the inter-aural line and 3 mm lateral from midline. A stainless steel guide cannula (22 gauge) was implanted unilaterally into the right lateral cerebral ventricle. The co-ordinates used were 13.5 mm anterior from the intra-aural line, 3 mm lateral (right) from midline and 8 mm dorsal to stereotaxic zero (10 mm ventral from the surface of the dura). These co-ordinates were determined by use of the stereotaxic atlas of Snider & Niemer (1961). When the ventricle was successfully cannulated, cerebrospinal fluid filled the cannula and pulsed in time with heart rate and respiration and there was no resistance to injection of artificial cerebrospinal fluid. Drug and vehicle solutions were administered in a volume of 20 μl over a 1 min period via an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to an Agla micrometer syringe. To prevent the passage of drug from the lateral ventricle to the fourth ventricle a cannula was placed in the Aqueduct of Sylvius. This was achieved by removing the atlanto-occipital membrane and a portion of the occipital bone and advancing a cannula (external diameter 3 mm, internal diameter 1 mm) along the midline of the dorsal surface of the medulla, under the cerebellum. When the Aqueduct of Sylvius was successfully cannulated cerebrospinal fluid flowed down the cannula. At the end of each experiment, the placement of these cannulae was confirmed by the administration of 20 μl of 2% pontamine sky blue dye. Only experiments in which dye was seen to flow down the Aqueduct of Sylvius cannula and in which dye was observed in the lateral and third ventricles were included in the mean result.

All variables were recorded for a 20 min stabilization period. An initial microinjection of saline (of similar pH to the test compound; see below) was then given to flush the ventricles. 10 min later the test compound or saline (vehicle control) was administered i.c.v. Cumulative dose-response curves were constructed for 5-HT (10, 40 160 nmol kg^{-1}) and DOI (80, 160, 320 nmol kg^{-1}). For 5-HT the dose interval was 10 min while for DOI the dose interval was 5 min and the highest dose was monitored for 10 min. These times were chosen to allow the response of each dose to reach a maximum before the next injection. In separate control experiments saline was administered at 5, 5, 10 and 10 min. Measurements were taken 1, 3, 5 and 10 min (where appropriate) after each dose of agonist or saline. In DOI experiments, all animals were pretreated with the peripherally acting 5-HT_2 receptor antagonist, BW501C67 (Mawson & Whittington, 1970; Fuller *et al.*, 1986). BW501C67 (1 mg kg^{-1}) was administered i.v. 15 min before the addition of the first dose of DOI (5 min before the initial i.c.v. injection of saline). This was to prevent the activation of peripheral 5-HT_2 receptors following i.c.v. administration of DOI (see Shepheard *et al.*, 1991). In six experiments, animals were pretreated with cinanserin (265 nmol kg^{-1} i.c.v.) and in 3 of these experiments 5-HT was administered 10 min later.

Analysis of data

All sympathetic nerve activities were quantified by rectifying and integrating signals above background noise over 5 s periods with 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 with a solid state electronic integrator, the output of which was also displayed on the Grass polygraph in arbitrary units (see Shepheard *et al.*, 1991). This 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 settings 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 is reported as the mean level over 1 min in arbitrary units. The frequency of bursts of the phrenic nerve was measured by counting the bursts in 1 min. In all experiments, baseline values were the average over a 1 min period and just before the first injection of 5-HT, DOI or vehicle. 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 time matched changes in the appropriate control by two way analysis of variance and the least significant difference test was used to compare the means (Sokal & Rohlf, 1969). Values from the saline experiments were takan at time intervals which corresponded to the application of the drugs. Changes in baseline values caused by cinanserin and BW501C67 were statistically analysed with Student's *t* test for paired data. All values are expressed as the mean ± s.e.mean; differences were considered significant when $p < 0.05$.

Drugs and solutions

The following drugs were used:- 5-hydroxytryptamine creatinine sulphate (5-HT; BDH, Poole, Dorset); 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI; Research Biochemicals Inc., Semat Technical Ltd, St. Albans, Herts); BW501C67 (2-anilino-N-(2-(3-chlorophenoxy)propyl) acetamide HCl; a gift from Wellcome Research Laboratories, Beckenham, Kent); cinanserin HCl (Squibb Inc., Princeton, U.S.A.); α-chloralose (Sigma Chemical Co., Poole, Dorset); Gelofusine (Consolidated Chem., Wrexham, Clwyd); pentobarbitone sodium (May & Baker Ltd.); sodium nitroprusside (Sigma Chemical Co., Poole, Dorset); vecuronium bromide (Organon Teknika Ltd, Cambridge, U.K.). All drugs were dissolved in 0.9% w/v saline. The pH of the drug solutions to be given i.c.v. were tested and back-titrated to pH 7.4 where possible. Solutions of DOI and cinanserin were found to have pH values between 5.5–6.0. Therefore, for DOI and cinanserin, saline with the same pH as the drug solution was initially injected to flush the ventricle. Administration of acidified saline did not affect any of the variables being recorded. The composition of the artificial cerebrospinal fluid used to check the position of the i.c.v. cannula was (mM): KH_2PO_4 2.2, $MgSO_4.7H_2O$ 1.2, KCl 2.0, glucose 10, $NaHCO_3$ 25, NaCl 115 and $CaCl_2.2H_2O$ 2.5. All doses refer to the salts of the compounds.

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

	n	*HR* (beats min^{-1})	*MAP* (mmHg)	*FAC* (ml min^{-1} $mmHg^{-1} \times 10^{-3}$)	*TP* (cmH_2O)	*Insp rate* (burst min^{-1})
Control saline	5	229±11	135±7	69±11	4.3±0.4	5.4±1.1
5-HT	5	202±12	116±8	73±13	4.1±0.2	5.4±1.4
DOI	4	181±9	128±8	75±18	5.0±0.2	5.8±0.8
Cinanserin	6	209±9	136±3	54±16	5.0±0.5	8.3±0.9
Cinanserin/5-HT	3	202±10	132±3	123±27	4.2±0.4	7.0±1.0

Results

Baseline values for all experimental groups are shown in Table 1.

Effects of lateral ventricular (i.c.v.) injections of saline and i.v. BW501C67

Administration of saline ($n=5$; Figure 4) over a 30 min period produced no significant change in any of the variables being measured (Figure 4). Further, BW 501C67 (1 mg kg^{-1}; i.v., $n=4$) pretreatment also caused no significant changes in any of the variables being measured (see Figure 3).

Effects of cumulative doses of 5-HT and DOI (in the presence of BW501C67) injected into the lateral ventricle on cardiovascular variables

Both 5-HT (10–160 nmol kg^{-1}; $n=5$) and DOI (80–320 nmol kg^{-1}; $n=4$) caused significant ($p<0.05$, when compared with saline) rises in blood pressure, heart rate, cardiac and splanchnic nerve activity and a decrease in femoral arterial conductance. Representative traces from a typical experiment for 5-HT and DOI are shown in Figures 1 and 3. 5-HT caused a maximum rise in blood pressure of 16±3 mmHg at the dose of 10 nmol kg^{-1} while DOI caused a maximum rise of 22±2 mmHg at the dose of 320 nmol kg^{-1} (Figures 2 and 4). These rises were associated with maximum increases in heart rate of 30±7 and 32±2 beats min^{-1} and decreases in femoral arterial conductance of 11±5 and 12±4 ml min^{-1} $mmHg^{-1}$ $\times 10^{-3}$, respectively. The rise in blood pressure caused by 5-HT

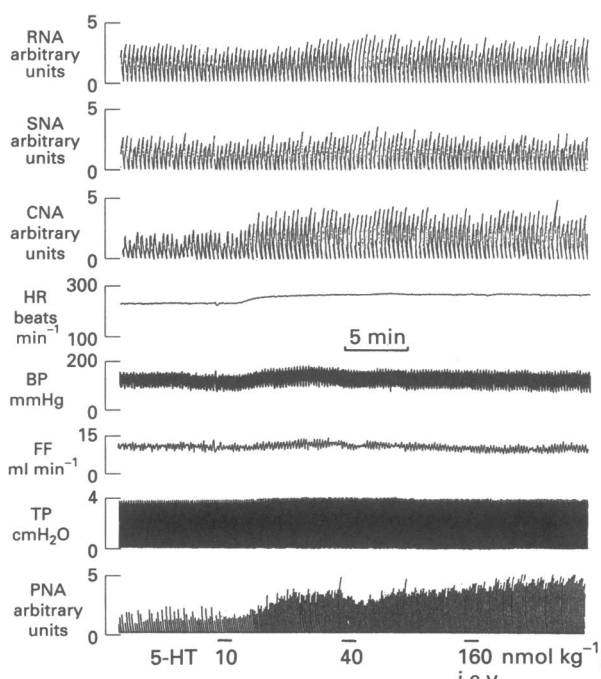

Figure 1 Traces showing the effects of cumulative i.c.v. doses of 5-HT (10, 40, 160 nmol kg^{-1}) on recordings of integrated renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), blood pressure (BP), femoral flow (FF), tracheal pressure (TP) and integrated phrenic nerve activity (PNA) in an anaesthetized cat.

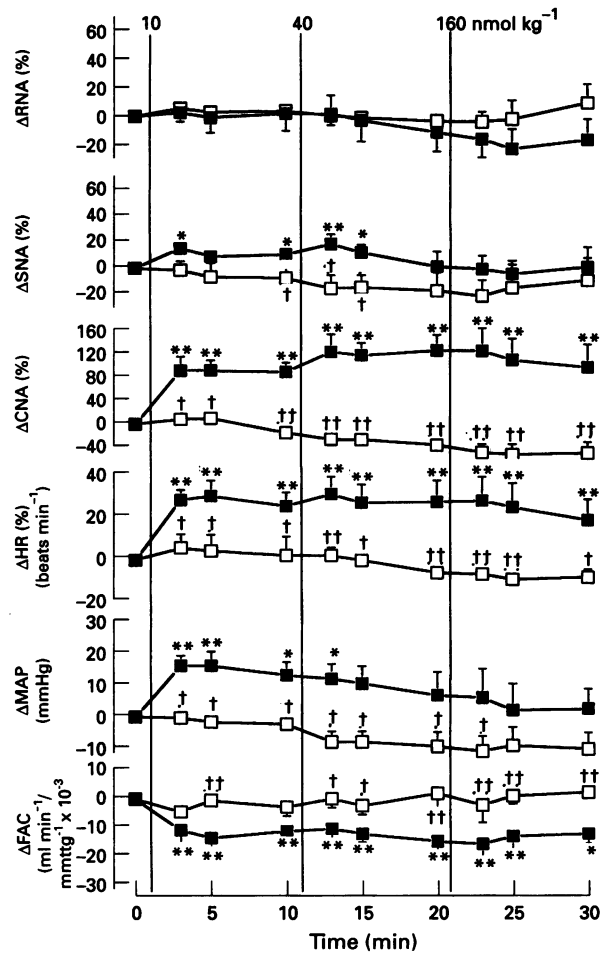

Figure 2 Anaesthetized cats: a comparison of the changes (Δ) from baseline values over time caused by cumulative doses (10–160 nmol kg^{-1}) of 5-HT (■; $n=5$) alone and in the presence of cinanserin (□; 265 nmol kg^{-1}, i.c.v.; $n=3$) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value with s.e.mean. The above changes caused by 5-HT were compared to changes caused by the saline (not illustrated for the sake of clarity, see Figure 4) or 5-HT in the presence of cinanserin using two way analysis of variance and the least significant difference test to compare the means. 5-HT compared to saline; *$p<0.05$; **$p<0.01$. 5-HT compared to 5-HT in the presence of cinanserin; †$p<0.05$; ††$p<0.01$. The vertical lines show the points at which 5-HT was injected.

was not maintained and began to decline after the second dose as did renal and splanchnic nerve activity (Figure 2).

Both 5-HT and DOI evoked greater mean increases in cardiac than splanchnic nerve activity (Figure 2 c.f. Figure 4): neither drug affected renal nerve activity. For 5-HT, the maximum increases in cardiac and splanchnic nerve activity were 129±27 and 20±7%. However, in the first 3 min after the low dose of 5-HT cardiac nerve activity had risen by 94±18%, while splanchnic nerve activity had only increased by 16±6% (see Figure 2). For DOI, the increases in cardiac and splanchnic nerve activity were more gradual, reaching maxima of 154±24 and 49±15%, respectively, by the highest dose (Figure 4).

Effect of DOI (in the presence of BW501C67) and 5-HT on phrenic nerve activity and tracheal pressure

5-HT caused a significant increase in the magnitude (153±58%) and rate (inspiratory rate; 1.2±0.4 burst min^{-1}) of phrenic nerve activity as well as an increase in tracheal pressure (0.20±0.06 cmH_2O). These changes were near maximal 5 min after administration of 10 nmol kg^{-1} 5-HT and were maintained with higher doses of 5-HT. DOI failed to cause significant changes in phrenic nerve activity or tracheal pressure. Figures 1 and 3 show traces of the above effects however combined data has not been illustrated.

Effect of cinanserin alone and on the response to 5-HT on all variables

Cinanserin (265 nmol kg^{-1}; i.c.v.; $n=6$) caused a significant fall in blood pressure, heart rate and cardiac nerve activity of 12±2 mmHg, 23±3 beats min^{-1} and 32±9%, respectively. These changes were also associated with a significant increase in femoral arterial conductance of 27±12 ml min^{-1} $mmHg^{-1}$ $\times 10^{-3}$. Splanchnic and renal nerve activity were unchanged even though blood pressure had fallen. Respiratory variables also were unchanged by cinanserin.

In the presence of cinanserin ($n=3$) the response to 5-HT (10–160 nmol kg^{-1}) was significantly attenuated on all cardiovascular variables (Figure 2). The increases in phrenic nerve activity and tracheal pressure caused by 5-HT were abolished (data not illustrated).

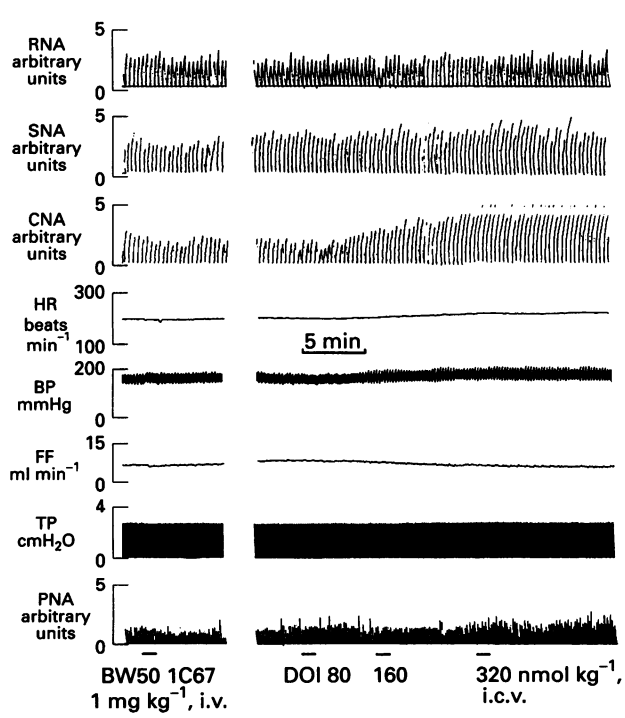

Figure 3 Traces showing the effects of cumulative i.c.v. doses of DOI (80, 160, 320 nmol kg^{-1}) on recordings of integrated renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), blood pressure (BP), femoral flow (FF), tracheal pressure (TP) and integrated phrenic nerve activity (PNA) in an anaesthetized cat pretreated with BW501C67 (1 mg kg^{-1}, i.v.). The time between the BW501C67 injection (i.v.) and the first DOI injection (i.c.v.) is 15 min.

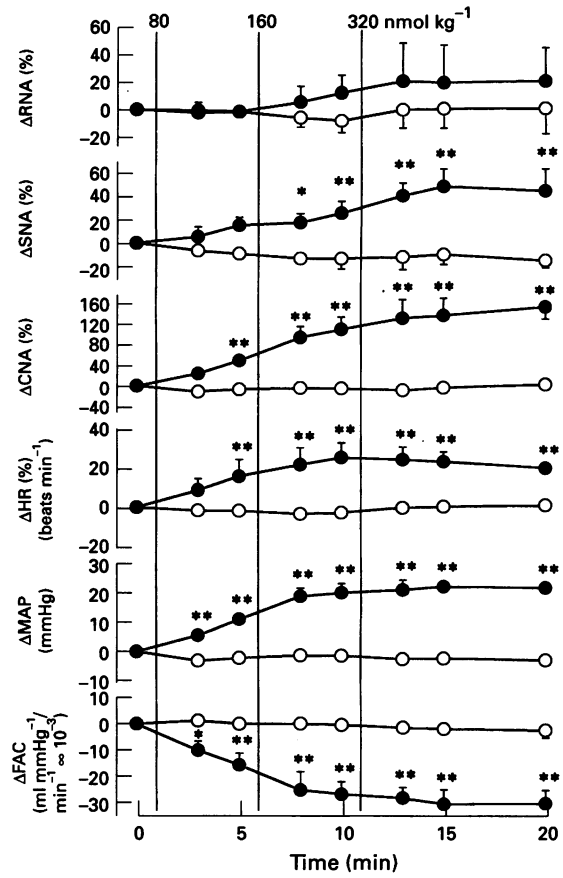

Figure 4 Anaesthetized cats: a comparison of the changes (Δ) from baseline values over time caused by cumulative doses of DOI (●; 80–320 nmol kg^{-1}; $n=4$) and saline (○, 3×20 µl; $n=5$) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value with s.e.mean. The above changes caused by DOI were compared to changes caused by the saline using two way analysis of variance and the least significant difference test to compare the means, *$p<0.05$; **$p<0.01$. The vertical lines show the points at which DOI and saline were injected.

Discussion

In the present study, application of either 5-HT or the selective 5-HT_2 receptor agonist DOI (Shannon *et al.*, 1984; Wijngaarden *et al.*, 1990) into the lateral ventricle has been shown to produce rises in blood pressure, heart rate and sympathetic nerve activity and a decrease in femoral arterial conductance. This rise in blood pressure is not due to a generalized sympathoexcitation but to a differential excitation of sympathetic outflows. Significant increases in activity occurred in both the inferior cardiac and splanchnic nerves while there was no increase in renal nerve activity. Furthermore, the increase in activity in the inferior cardiac nerve was greater than that in the splanchnic nerve. These effects are similar to the observations of Coote *et al.* (1987) who also found that lateral ventricular administration of 5-HT caused a rise in blood pressure

and heart rate associated with no change in renal nerve activity. These effects of 5-HT are mediated by activation of 5-HT_2 receptors as they were attenuated by pretreatment with the 5-HT_2 receptor antagonist cinanserin (Rubin *et al.*, 1964; Hoyer & Fozard, 1991).

However, the effects of 5-HT on the above cardiovascular variables differed from those of DOI, in that larger doses of DOI caused further increases in these variables while with 5-HT the increases in these variables were observed to be near maximal after the first dose. In fact, arterial blood pressure began to decline after the second dose of 5-HT while splanchnic and renal nerve activity declined after the third dose. Again these observations are consistent with those of Coote *et al.* (1987) in that only the low doses of 5-HT produced a rise in blood pressure whilst high doses in separate experiments produced a delayed fall in blood pressure associated with renal nerve sympathoinhibition. Therefore these observations suggest that the failure of the high cumulative dose of 5-HT, in the present experiments, to cause further increases in the above cardiovascular variables, is not the result of tachyphylaxis but is, presumably, due to 5-HT activating different receptor subtypes to oppose the sympathoexcitatory and pressor effects caused by activation of 5-HT_2 receptors.

Data from other experiments in which DOI (King & Holtman, 1990; Mandal *et al.*, 1990) or the mixed 5-HT_2/5-HT_3 receptor agonist, quipazine (Vayssettes-Courchay *et al.*, 1991; 1992) were applied to the rostral ventrolateral medulla led to the suggestion that 5-HT_2 receptors in this region are responsible for the sympathoexcitatory effect of i.v. administration of DOI or quipazine (see McCall & Clement, 1994). The present results would suggest that 5-HT_2 receptors located within the forebrain also make a major contribution to sympathoexcitatory effects of i.v. DOI (or quipazine). However, the present experiments indicate that the renal sympathoexcitation caused by i.v. DOI (Ramage *et al.*, 1993) is not due to activation of forebrain 5-HT_2 receptors. Presumably, 5-HT_2 receptors located in the rostral ventrolateral medulla mediate renal sympathoexcitation (Vayssettes-Courchay *et al.*, 1992).

In the context of the present results it is surprising that i.v. DOI causes sympathoexcitation but no associated tachycardia (see Ramage *et al.*, 1993). A possible explanation for this failure is that the expected tachycardia due to activation of forebrain 5-HT_2 receptors by i.v. DOI is being overridden by the activation of 5-HT_2 receptors at other sites within the brain. In this respect, activation of 5-HT_2 receptors located in the caudal ventrolateral medulla and the nucleus tractus solitarii have been demonstrated, indirectly, to cause sympathoinhibition (Vayssettes-Courchay *et al.*, 1992). Further, application of DOI into the IVth ventricle (which presumably activated all the brainstem sites) caused no change in cardiac, splanchnic and renal sympathetic nerve activity or heart rate, although there was an associated increase in femoral arterial conductance and a rise in blood pressure (Shepheard *et al.*, 1991). This latter observation suggests that the sympathoinhibitory sites in the brainstem are capable of overriding some of the sympathoexcitatory effects caused by activation of 5-HT_2 receptors located in the rostral ventrolateral medulla, such as renal nerve sympathoexcitation (see above). This may therefore also apply to the effects produced by activation of forebrain 5-HT_2 receptors. However, data fom i.v. administration of 5-HT_2 agonists indicates that the central hindbrain sympathoexcitatory effects such as the increases in renal nerve activity are not completely overridden by these brainstem inhibitory sites, as observed with IVth ventricle application. This difference possibly reflects the accessibility afforded by the different routes (i.v. versus IVth ventricular) of administration of DOI to these various central sites.

The present experiments also demonstrate that activation of forebrain 5-HT_2 receptors can cause a decrease in femoral arterial conductance indicating that vasoconstriction has occurred in the hindlimb skeletal muscle and/or skin vascular beds. A similar effect was also evoked by DOI when administered i.v. (Ramage *et al.*, 1993) or into the IVth ventricle (Shepheard *et al.*, 1991). These combined observations suggest that 5-HT_2 receptors at all levels of the brain are capable of activating sympathetic pathways that control skeletal muscle and/or skin blood flow. This presumed sympathetic-vasoconstrictor pathway to skeletal muscle appears to be tonically active because i.v. (Ramage, 1985; 1988), IVth ventricular (Shepheard *et al.*, 1994) or lateral ventricular administration (present study) of 5-HT_2 receptor antagonists causes an increase in femoral arterial conductance. However, only with lateral ventricular administration was this increase in femoral arterial conductance associated with a fall in blood pressure which was presumably due to the additional cardiac sympathoinhibition and bradycardia caused by cinanserin at this site. This latter observation indicates that there is a tonic activation of forebrain 5-HT_2 receptors located on a pathway controlling heart rate; however, the failure to expose this pathway by i.v. administration of a 5-HT_2 antagonist remains to be elucidated.

Activation of forebrain 5-HT_2 receptors by 5-HT also produced changes in respiratory variables; a recruitment of fibres and an increase in the rate of respiratory drive. This contrasts with the effects of activation of 5-HT_2 receptors located in the IVth ventricle (Shepheard *et al.*, 1991) and the ventral surface of the medulla (King & Holtman, 1990) which cause a decrease in respiratory rate. Furthermore, the present experiments indicate that 5-HT_2 receptors are located on a forebrain pathway which is capable of exciting bronchial vagal motoneurones because the 5-HT-induced increase in tracheal pressure was blocked by pretreatment with cinanserin. It is surprising that DOI, a selective 5-HT_2 agonist, did not increase these respiratory variables. A possible explanation is that these respiratory effects are mediated by 5-HT_2 receptors when they are in their low affinity state (guanyl nucleotide-insensitive) as DOI only binds to the high affinity state (guanyl nucleotide-sensitive) of the 5-HT_2 receptor (Glennon *et al.*, 1988; Teitler *et al.*, 1990).

It is concluded from the present results that the sympathoexcitation along with the rise in blood pressure and the respiratory effects evoked by lateral ventricular administration of 5-HT are mediated by the activation of 5-HT_2 receptors located in the forebrain. Further, the ability of cinanserin to cause reductions in cardiac nerve activity and heart rate and an increase in femoral arterial conductance suggests that the 5-HT_2 receptors mediating these effects are under tonic activation. On the other hand those receptors involved in the control of splanchnic nerve activity and central respiratory drive are not under tonic activation. The precise nature of the 5-HT_2 receptors involved in the control of central respiratory activity seems to differ from those which mediate sympathoexcitation, as DOI fails to have any effect on phrenic nerve activity or tracheal pressure. Further, the sympathoexcitatory effects mediated by 5-HT_2 receptors located in the forebrain differ from those located in the hindbrain, in that they mediate increases in cardiac nerve activity and heart rate, and also have no effect on renal nerve activity. Overall, the present data support the view that it is too simplistic to assume that the effects of i.v. DOI are due solely to a stimulation of 5-HT_2 receptors located in the rostral ventrolateral medulla.

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