Central administration of 5-HT activates 5-HT_{1A} receptors to cause sympathoexcitation and 5-HT₂/5-HT_{1C} receptors to release vasopressin in anaesthetized rats

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1 The effects of intracerebroventricular injections to the right lateral ventricle (i.c.v.) of 5-hydroxytryptamine (5-HT, 40 and 120 nmol kg⁻¹), N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT; 3 nmol kg⁻¹), 5-carboxamidotryptamine (5-CT; 3 nmol kg⁻¹), 8-hydroxy-2-(di-N-propylamino) tetralin (8-OH-DPAT; 3, 40 and 120 nmol kg⁻¹) and 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI; 40 and 120 nmol kg⁻¹) on renal sympathetic nerve activity, blood pressure, heart rate and phrenic nerve activity were investigated in normotensive rats anaesthetized with α -chloralose.

2 5-HT caused a long lasting pressor response which was associated with an initial bradycardia and renal sympathoinhibition followed by a tachycardia and renal sympathoexcitation. Pretreatment with the 5-HT₂/5-HT_{1C} receptor antagonists, cinanserin (300 nmol kg⁻¹, i.c.v.) or LY 53857 (300 nmol kg⁻¹, i.c.v.) reversed the initial bradycardia and sympathoinhibition to tachycardia and sympathoexcitation. Combined pretreatment with LY 53857 (300 nmol kg⁻¹, i.c.v.) and the 5-HT_{1A} antagonist, spiroxatrine (300 nmol kg⁻¹, i.c.v.), blocked the effects of 5-HT on all the above variables.

3 Pretreatment with the vasopressin V₁-receptor antagonist, β -mercapto- β , β -cyclopentamethylpropionyl¹, O-Me-Tyr², Arg⁸-vasopressin [(d(CH₂)₅Tyr(Me)AVP, 10 μ g kg⁻¹, i.v.)] did not affect the magnitude but reduced the duration of the pressor response produced by i.c.v. 5-HT and reversed the initial bradycardia and renal sympathoinhibition to tachycardia and sympathoexcitation.

4 1-(2,5-Di-methoxy-4-iodophenyl)-2-aminopropane (DOI) caused a pressor effect which was associated with a bradycardia and sympathoinhibition. These effects were blocked by pretreatment with BW501C67 (0.1 mg kg⁻¹, i.v.), a peripherally acting 5-HT₂/5-HT_{1C} receptor antagonist. However, BW501C67 (0.1 mg kg⁻¹, i.v.) failed to block the effects of i.c.v. 5-HT.

5 DP-5-CT, 5-CT and 8-OH-DPAT (3 nmol kg⁻¹, i.c.v.) caused sympathoexcitation, tachycardia and a rise in blood pressure. Pretreatment with methiothepin (1 mg kg⁻¹, i.v.) or spiroxatrine (300 nmol kg⁻¹, i.c.v.) attenuated the response to i.c.v. DP-5-CT.

6 It is concluded that i.c.v. administration of 5-HT activates 5-HT_{1A} receptors to cause sympathoexcitation and 5-HT₂ or 5-HT_{1C} receptors to cause the release of vasopressin.

Keywords: 5-HT_{1A} receptors; 5-HT₂ or 5-HT_{1C} receptors; vasopressin V₁-receptors; 8-OH-DPAT; DP-5-CT; 5-HT; DOI; blood pressure; sympathetic nerve activity; anaesthetized rat

Introduction

Intracerebroventricular (i.c.v.) injections of 5-hydroxytryptamine (5-HT) in anaesthetized rats cause a rise in blood pressure and variable effects on heart rate (Lambert *et al.*, 1975; 1978; Kristic & Djurkovic, 1976; 1980). In conscious rats i.c.v. administration of 5-HT also causes a pressor effect but in these animals consistently produces bradycardia (Sukamoto *et al.*, 1984; Dalton, 1986). More recently, Inoue & Buñag (1989) were able to demonstrate that i.c.v. 5-HT induced a pressor response which was associated with a consistent bradycardia and sympathoinhibition in the anaesthetized rat. These authors also demonstrated that the pressor effect was attenuated by pretreatment with a vasopressin V₁-receptor antagonist. The precise nature of the 5-HT receptor involved in this central release of vasopressin was not determined, although (as in previous studies) the pressor effect of i.c.v. 5-HT could be antagonized by the non-selective 5-HT receptor antagonists, methysergide or bromo-lysergic acid diethylamide (see Ramage, 1985; Hoyer, 1991). In addition, it has also been reported that the rise in blood pressure produced by i.c.v. administration of 5-HT is

attenuated by cervical transection of the spinal cord, adrenalectomy, adrenergic neurone blocking agents and α -adrenoceptor antagonists (Kristic & Djurkovic, 1980), suggesting that there is an additional sympathoexcitatory component to the response of 5-HT. However, Inoue & Buñag (1989) were unable to demonstrate such an action when recording from the splanchnic nerve. We therefore decided to investigate further the mechanism and nature of the receptors involved in effects of i.c.v. administration of 5-HT on blood pressure, heart rate and renal sympathetic nerve activity in rats anaesthetized with α -chloralose using more selective agonists and antagonists for the different 5-HT receptor subtypes. In addition respiratory variables were monitored. A preliminary account of these observations has been presented to the British Pharmacological Society (Anderson, 1991; Anderson *et al.*, 1992b).

Methods

Experiments were performed on male Sprague-Dawley normotensive rats (250–350 g). Anaesthesia was induced with halothane (2.5% in oxygen) and maintained with α -chloralose (80 mg kg⁻¹, i.v.). Supplementary doses of α -

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chloralose ($10\text{--}20\text{ mg kg}^{-1}$, i.v.) were given as required. Depth of anaesthesia was assessed by the stability of cardiovascular and respiratory variables being recorded. The left carotid artery was cannulated for the measurement of blood pressure and for sampling arterial blood for analysis of pH and blood gases. Blood pressure was measured with a pressure transducer (Gould Statham P23XL) and the heart rate was derived electronically from the blood pressure signal (Gould Biotach Amplifier). The left jugular vein was cannulated for drug administration and a tracheal cannula was implanted. Body temperature was monitored by a rectal probe and maintained at $36\text{--}38^\circ\text{C}$ with a homeothermic blanket system (Harvard). The animals were artificially ventilated (rate 50 min^{-1} , stroke volume 8 ml kg^{-1}) with oxygen-enriched room air by use of a positive pressure pump (Harvard Rodent Ventilator 683) and neuromuscular blockade was produced with decamethonium (3 mg kg^{-1} , i.v.). Blood samples were taken from a T-piece on the carotid arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were maintained between $90\text{--}130\text{ mmHg } P_{O_2}$, $40\text{--}50\text{ mmHg } P_{CO_2}$ and pH $7.3\text{--}7.4$. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused ($6\text{ ml kg}^{-1}\text{ h}^{-1}$) via the jugular vein with a solution comprising 10 ml plasma substitute (gelofusine), 10 ml distilled water, 0.04 g glucose, 0.168 g sodium bicarbonate and 30 mg decamethonium. This was to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade.

Cannulation of the lateral cerebral ventricle

The rats were placed in a stereotaxic head holder and a stainless steel guide cannula (22 gauge) was implanted into the right lateral cerebral ventricle. The co-ordinates used from bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. Drug and vehicle solutions were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a $100\text{ }\mu\text{l}$ syringe (Hamilton). At the end of the experiment, the cannula placement was confirmed by the administration of $5\text{ }\mu\text{l}$ of 2% pontamine sky blue dye.

Recording of phrenic nerve and renal nerve activity

The right phrenic nerve was exposed by deflecting the scapula forwards and dissecting the nerve clear of overlying muscle and connective tissue. The nerve was cut peripherally and placed on a bipolar silver hook electrode as described previously (Dreteler *et al.*, 1991). Phrenic nerve activity was quantified by counting the number of action potentials above the noise level over 5 s with a spike processor (Digitimer D130). To maintain phrenic nerve activity, a measure of central inspiratory drive, the blood P_{CO_2} values in these animals were maintained at a slightly higher ($40\text{--}50\text{ mmHg}$) level than the physiological norm ($35\text{--}49\text{ mmHg}$). This usually locked the rate of phrenic nerve firing to the rate of the animals chest movements caused by the respiration pump and changes in phrenic nerve activity were the result of changes in the size of each inspiratory burst. The right kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the renal artery and nerve. Renal nerve activity was recorded as previously described (Ramage & Wilkinson, 1989). Renal nerve activity was quantified by integrating the signal above background noise over 5 s with a solid state integrator (Medical Electronics workshop, Royal Free Hospital School of Medicine). The noise levels were verified at the end of the experiment after the administration of pentobarbitone sodium (20 mg per animal).

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether renal nerve activity and heart rate were reduced by a rise in blood

pressure caused by noradrenaline (25 ng per animal , i.v.) and were raised by a reduction in blood pressure caused by sodium nitroprusside ($0.6\text{ }\mu\text{g per animal}$, i.v.). Only preparations with an intact baroreceptor reflex were used.

Experimental protocols

The preparation was allowed to stabilize for 30 min before the administration of saline ($5\text{ }\mu\text{l}$ i.c.v.). After a 5 min control period a single dose of test compound or saline control was given i.c.v. and the response was followed for at least 30 min . In antagonist studies, antagonists administered i.c.v. were given 10 min before the injection of test drug. However, both LY 53857 and spiroxatrine were administered in two doses of 150 nmol kg^{-1} alone or combined, 5 min apart and the test drug administered 5 min after the last dose of these antagonists. Vehicle for spiroxatrine (0.01 N HCl) was administered in 2 volumes of $5\text{ }\mu\text{l}$ 5 min apart and the test agonist was given 5 min after the last dose of vehicle. When antagonists were given i.v. the test drug was administered 5 min later. However, for methiothepin the test drug was administered following a stabilization period of 20 min . These pretreatment times were chosen to allow stabilization of any changes in the variables being recorded caused by administration of the antagonists. In each rat the cardiovascular response of a single dose of the test drug was recorded.

Analysis of results

Baseline values were taken 1 min before the addition of drug or vehicle. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 1 min in arbitrary units and was expressed as the percentage change from baseline. Changes in mean blood pressure, heart rate, renal and phrenic nerve activity caused by the test drug were compared with time-matched vehicle controls by two-way analysis of variance and were subsequently analysed by the least significant difference test (Sokal & Rohlf, 1969). Biphasic responses in some variables were observed following i.c.v. administration of 5-HT and these phases were analysed separately. Thus, the maximum change for each phase of the 5-HT response was measured and compared to the maximum change in vehicle controls during the same period by Student's *t* test for unpaired data. Changes in variables caused by antagonist or vehicle pretreatments were compared to the pre-dose baseline by Student's *t* test for paired data. All values are expressed as the mean $\pm$ s.e.mean, differences in the mean were taken as significant when $P < 0.05$.

Drugs and solutions

The drugs used were BW501C67, (2-anilino-N-(2-(3-chlorophenoxy)propyl) acetamide HCl), 5-carboxamidotryptamine maleate (5-CT), N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT; these were gifts from Wellcome Laboratories, Beckenham, Kent), α -chloralose (Sigma Chemical Co., Poole, Dorset); DOI, 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (Research Biochemicals Inc., Semat, St. Albans); decamethonium iodide (Koch-Light, Haverhill, Suffolk); Gelofusine (Consolidated Chem., Wrexham, Clwyd); 8-hydroxy-2-(di-N-propylamino)tetralin HBr (8-OH-DPAT; Research Biochemicals Inc., Semat, St. Albans); 5-hydroxytryptamine creatinine sulphate, 5-HT (BDH, Poole, Dorset); halothane (ICI Pharmaceuticals Ltd, Macclesfield); hexamethonium bromide (Koch-Light, Haverhill, Suffolk); $[\beta\text{-mercapto-}\beta\text{-}\beta\text{-cyclopentamethylenepropionyl}^1\text{,O-Me-Tyr}^2\text{,Arg}^8\text{-}]$ -vasopressin, (d(CH₂)₅Tyr(Me)AVP; Sigma Chemical Co., Poole, Dorset); methiothepin mesylate (Research Biochemicals Inc., Semat, St. Albans); noradrenaline acid tartrate (Winthrop, Guildford, Surrey); sodium nitroprusside (Sigma Chemical Co., Poole, Dorset) and spiroxatrine (a gift from Janssen, Wantage, Oxon). Drugs given i.c.v. were dis-

solved in 0.9% w/v saline except for spiroxatrine and the combination of spiroxatrine and LY 53857 which were dissolved in 0.01 N hydrochloric acid (HCl). Solutions were administered in a dose volume of 5 μ l over a 20 s period. All drugs given i.v. were dissolved in saline.

Results

Effect of i.c.v. administration of saline

Saline i.c.v. (5 μ l; $n = 6$) had little effect on blood pressure, heart rate, renal or phrenic nerve activity and these variables remained stable for the duration of the experiment (see Figure 1). Baseline values for blood pressure and heart rate for this group of experiments were 122 ± 6 mmHg and 417 ± 13 beats min^{-1} (mean $\pm$ s.e.mean).

Effect of i.c.v. administration of 5-HT

5-HT [40 ($n = 6$); 120 ($n = 8$) nmol kg^{-1}] caused immediate, dose-related increases in arterial blood pressure (Figures 1 and 2) which reached maxima, between 1 and 5 min after injection, of 6 ± 1 and 19 ± 2 mmHg respectively. The rise in

blood pressure remained elevated for 5 min after the low dose and 20 min after the high dose. In addition, 5-HT (40 and 120 nmol kg^{-1}) caused initial significant ($P < 0.05$) falls in heart rate of 17 ± 5 and 16 ± 2 beats min^{-1} and in renal nerve activity of $23 \pm 8\%$ and $41 \pm 8\%$ respectively. The initial decrease in these variables (first phase) was followed by an increase (second phase). Maximum increases in heart rate and renal nerve activity produced by 5-HT (40 and 120 nmol kg^{-1}) were 19 ± 5 and 63 ± 14 beats min^{-1} and 47 ± 15 and $137 \pm 32\%$ respectively and occurred between 10–20 min. These biphasic changes in heart rate and renal nerve activity were temporally matched. 5-HT (120 nmol kg^{-1}) caused an initial (1–5 min) significant reduction in phrenic nerve activity of $22 \pm 5\%$. In 3 animals this was followed by a secondary rise in phrenic nerve activity ($227 \pm 84\%$, 10–20 min). However, in the remaining animals phrenic nerve activity returned to baseline levels after 5 min. Baseline values for blood pressure and heart rate in the low and high dose groups were 112 ± 6 mmHg and 417 ± 31 beats min^{-1} and 112 ± 4 mmHg and 417 ± 18 beats min^{-1} respectively.

The effect of pretreatment (i.c.v.) with either cinanserin or LY 53857 on the response to 5-HT

Cinanserin (300 nmol kg^{-1} ; $n = 6$) or LY 53857 (300 nmol kg^{-1} ; $n = 6$) had no significant effect on blood pressure, heart rate, renal nerve activity or phrenic nerve activity. Baseline values for blood pressure and heart rate were 102 ± 4 mmHg and 398 ± 10 beats min^{-1} and 102 ± 7 mmHg and 417 ± 21 beats min^{-1} respectively. Neither drug prevented the rise in blood pressure caused by 5-HT (120 nmol kg^{-1}) but the initial bradycardia and renal sympathoinhibition were reversed to tachycardia and sympathoexcitation (Figures 2 and 3). The 5-HT induced changes in phrenic nerve activity were not significantly altered (Figure 3).

Effect of i.v. pretreatment with BW501C67 on the response to i.c.v. 5-HT

5-HT (120 nmol kg^{-1}) administered i.c.v. in animals pretreated with BW501C67 (0.1 mg kg^{-1} , i.v.; $n = 4$) caused similar effects to those observed in non-pretreated animals; there was an immediate increase in blood pressure and biphasic changes in heart rate and renal nerve activity (Figure 3). However, the duration of the bradycardia and the renal sympathoinhibition was significantly prolonged. Baseline values for blood pressure and heart rate were 115 ± 11 mmHg and 420 ± 22 beats min^{-1} .

The effect of combined pretreatment (i.c.v.) with LY 53857 and spiroxatrine on the response to 5-HT

Combined pretreatment with LY 53857 (300 nmol kg^{-1}) and spiroxatrine (300 nmol kg^{-1} ; $n = 4$) did not significantly change baseline values (baseline values for blood pressure and heart rate were 98 ± 7 mmHg and 380 ± 7 beats min^{-1}). However, the effect of 5-HT (120 nmol kg^{-1} , i.c.v.) on all variables was significantly reduced in animals pretreated with this combination compared to animals pretreated with LY 53857 (300 nmol kg^{-1} , i.c.v.) alone (Figure 4). The combination of LY 53857 and spiroxatrine was dissolved in 0.01 N HCl (vehicle for spiroxatrine) whereas previously LY 53857 had been dissolved in saline. Therefore, in 2 separate experiments, animals were pretreated with LY 53857 dissolved in 0.01 N HCl. In these experiments the response to 5-HT (120 nmol kg^{-1} , i.c.v.; data not shown) was similar to that observed previously in animals pretreated with LY 53857 alone dissolved in saline.

Figure 1 Anaesthetized rats: (a) a comparison of the changes from baseline values over time (min) caused by i.c.v. saline ($\bullet$; $n = 6$), 5-HT 40 nmol kg^{-1} (Δ ; $n = 6$) and 5-HT 120 nmol kg^{-1} ($\square$; $n = 8$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. (b) Histograms showing the maximal changes in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA) during the first phase (1–5 min) and the second phase (10–20 min) of the response to i.c.v. 5-HT (40 nmol kg^{-1} , hatched columns; 120 nmol kg^{-1} , open columns). Each point represents the mean value and the vertical bars show s.e.mean. * $P < 0.05$ and ** $P < 0.01$ compared to i.c.v. saline over the same periods.

Figure 2 Traces showing the effects of i.c.v. 5-HT (120 nmol kg⁻¹) in absence (a) and presence (b) of cinanserin (300 nmol kg⁻¹, i.c.v.) and (c) i.c.v. N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT, 3 nmol kg⁻¹, i.c.v.) on arterial blood pressure (BP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA) in anaesthetized rats.

Figure 3 Anaesthetized rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (120 nmol kg⁻¹, i.c.v.) in the presence of saline (□; 5 μl; n = 8), cinanserin (●; 300 nmol kg⁻¹, i.c.v.; n = 6), LY 53857 (○; 300 nmol kg⁻¹, i.c.v.; n = 6), BW501C67 (■; 0.1 mg kg⁻¹, i.v.; n = 4) and d(CH₂)₅Tyr(Me)AVP (▲; 10 μg kg⁻¹, i.v.; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean *P < 0.05 and **P < 0.01 compared to 5-HT saline pretreatment. Statistical significance for the effects of 5-HT on HR and RNA in animals pretreated with cinanserin, LY 53857 and d(CH₂)₅Tyr(Me)AVP between 1–5 min are the same and are illustrated by a single symbol for the sake of clarity.

The effect of pretreatment (i.v.) with d(CH₂)₅Tyr(Me)-AVP on the response to 5-HT

d(CH₂)₅Tyr(Me)AVP (10 μg kg⁻¹ i.v.; n = 6) had no significant effect on blood pressure, heart rate, renal or phrenic nerve activity. Baseline values for blood pressure and heart rate were 104 ± 4 mmHg and 388 ± 8 beats min⁻¹. Pretreatment with d(CH₂)₅Tyr(Me)AVP did not prevent the rise in blood pressure caused by 5-HT (120 nmol kg⁻¹, i.c.v.) but the duration of the pressor rise was significantly attenuated, see Figure 3. The 5-HT-induced bradycardia and renal sympathoinhibition were reversed to an immediate tachycardia and sympathoexcitation in the presence of d(CH₂)₅Tyr(Me)-AVP (Figure 3). Changes in phrenic nerve activity caused by 5-HT were unaffected.

Effect of i.c.v. and i.v. administration of 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI) in the absence and presence of BW501C67

DOI 12 nmol kg⁻¹ (i.c.v.) had no effect on blood pressure, heart rate, renal and phrenic nerve activity (data not shown). DOI (40 nmol kg⁻¹, n = 4; 120 nmol kg⁻¹, n = 6; i.c.v.) produced maximum increases in blood pressure of 7 ± 1 and 10 ± 2 mmHg respectively and decreases in heart rate and

300 nmol kg⁻¹, i.c.v.; n = 6), BW501C67 (■; 0.1 mg kg⁻¹, i.v.; n = 4) and d(CH₂)₅Tyr(Me)AVP (▲; 10 μg kg⁻¹, i.v.; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean *P < 0.05 and **P < 0.01 compared to 5-HT saline pretreatment. Statistical significance for the effects of 5-HT on HR and RNA in animals pretreated with cinanserin, LY 53857 and d(CH₂)₅Tyr(Me)AVP between 1–5 min are the same and are illustrated by a single symbol for the sake of clarity.

Figure 4 Anaesthetized rats: a comparison of the changes from post-pretreatment values over time (min) caused by 5-HT (120 nmol kg^{-1} , i.c.v.) in the presence of LY 53857 (300 nmol kg^{-1} , i.c.v.; $\circ$; $n=6$) with the combined pretreatment of LY 53857 (300 nmol kg^{-1} , i.c.v.) and spiroxatrine (300 nmol kg^{-1} , i.c.v.; $\blacktriangle$; $n=4$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. $*P<0.05$ and $**P<0.01$ compared to 5-HT in the presence of LY 53857

renal nerve activity of 18 ± 6 and $19 \pm 9 \text{ beats min}^{-1}$ and 31 ± 9 and $53 \pm 7\%$ respectively, 5 min after injection. There was no change in phrenic nerve activity (Figure 5). Baseline values for blood pressure and heart rate were $126 \pm 5 \text{ mmHg}$ and $424 \pm 21 \text{ beats min}^{-1}$ and $122 \pm 2 \text{ mmHg}$ and $406 \pm 20 \text{ beats min}^{-1}$ respectively. Pretreatment with BW501C67 (0.1 mg kg^{-1} , i.v.), which had no effect *per se*, significantly attenuated the response to i.c.v. DOI (120 nmol kg^{-1} ; $n=4$; Figure 5) on these variables. Baseline values for blood pressure and heart rate in animals pretreated with BW501C67 were $112 \pm 2 \text{ mmHg}$ and $406 \pm 20 \text{ beats min}^{-1}$.

DOI, 120 nmol kg^{-1} ($n=3$), given i.v. produced a rise in blood pressure of $22 \pm 2 \text{ mmHg}$ which was maintained over 20 min. Again bradycardia and renal sympathoinhibition were observed and reached maxima of $21 \pm 3 \text{ beats min}^{-1}$ and $54 \pm 6\%$ respectively 1 min following injection. The bradycardia was not maintained and had returned to baseline by 10 min. However, the renal sympathoinhibition was maintained for 20 min. Phrenic nerve activity was not measured in these animals. Baseline values for blood pressure and heart rate were $117 \pm 4 \text{ mmHg}$ and $429 \pm 22 \text{ beats min}^{-1}$. Pretreatment with BW501C67 (0.1 mg kg^{-1} , i.v.; $n=2$) abolished the rise in blood pressure caused by i.v. DOI (data not illustrated).

Figure 5 Anaesthetized rats: a comparison of the changes with over time (min) from baseline or post-pretreatment values caused by i.c.v. 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI) 40 nmol kg^{-1} ($\square$; $n=6$) and i.c.v. DOI 120 nmol kg^{-1} in the absence ($\circ$; $n=6$) and presence of BW501C67 (0.1 mg kg^{-1} , i.v.; $\bullet$; $n=4$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. $*P<0.05$ and $**P<0.01$ compared to i.c.v. saline (not illustrated for the sake of clarity). $\dagger P<0.05$ and $\dagger\dagger P<0.01$ comparing the effects of DOI (120 nmol kg^{-1} , i.c.v.) in the absence and presence of BW501C67.

Effect of i.c.v. administration of 5-CT, DP-5-CT and 8-OH-DPAT

DP-5-CT (3 nmol kg^{-1} ; $n=8$) caused an immediate and significant rise in blood pressure, heart rate and renal nerve activity reaching a maximum by 5 min of $9 \pm 3 \text{ mmHg}$, $39 \pm 5 \text{ beats min}^{-1}$ and $83 \pm 15\%$, respectively (Figures 2 and 6). These changes were maintained for at least 30 min. There was no change in phrenic nerve activity (Figure 6). Baseline values for blood pressure and heart rate were $114 \pm 4 \text{ mmHg}$ and $391 \pm 11 \text{ beats min}^{-1}$.

5-CT (3 nmol kg^{-1} ; $n=5$) also caused an immediate significant rise in blood pressure of $17 \pm 5 \text{ mmHg}$ after 2 min. This rise in blood pressure was associated with significant increases in heart rate of $48 \pm 7 \text{ beats min}^{-1}$ and in renal nerve activity of $77 \pm 35\%$ (Figure 6). Both the rise in heart rate and in renal nerve activity were well maintained returning to near baseline values by 30 min. However, blood pressure remained elevated for only 2 min (Figure 6) and in three experiments, between 3 and 5 min after injection, the rise in blood pressure was followed by a substantial fall of $21 \pm 3 \text{ mmHg}$ below baseline. 5-CT caused no significant changes in phrenic nerve activity. Baseline values for blood

Figure 6 Anaesthetized rats: a comparison of the changes with time (min) from baseline values induced by i.c.v. N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT, ▲; 3 nmol kg^{-1} ; $n = 8$), 5-carboxamidotryptamine (5-CT, ■; 3 nmol kg^{-1} ; $n = 5$) and 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT, ●; 3 nmol kg^{-1} ; $n = 6$) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. * $P < 0.05$ and ** $P < 0.01$ compared to i.c.v. saline (not illustrated for the sake of clarity).

pressure and heart rate were $114 \pm 4 \text{ mmHg}$ and $442 \pm 2 \text{ beats min}^{-1}$.

8-OH-DPAT (3 nmol kg^{-1} ; $n = 6$) caused significant increases in blood pressure, heart rate and renal nerve activity (Figure 6). The onset of these changes was immediate and was maximal at 10 min, reaching $7 \pm 3 \text{ mmHg}$, $49 \pm 11 \text{ beats min}^{-1}$ and $57 \pm 17\%$ respectively. Phrenic nerve activity was also significantly increased ($75 \pm 24\%$, 15 min), however the onset of this response was delayed, see Figure 6. Baseline values for blood pressure and heart rate were $96 \pm 4 \text{ mmHg}$ and $391 \pm 11 \text{ beats min}^{-1}$. Higher doses, 40 and 120 nmol kg^{-1} , of 8-OH-DPAT produced small reductions in blood pressure and these were associated with dose-related tachycardia of 21 ± 5 and $38 \pm 14 \text{ beats min}^{-1}$, respectively, after 10 min. These higher doses of 8-OH-DPAT did not significantly alter renal or phrenic nerve activity (data not illustrated). Baseline values were for blood pressure 122 ± 5 and $108 \pm 8 \text{ mmHg}$ and for heart rate 428 ± 10 and $411 \pm 16 \text{ beats min}^{-1}$ respectively.

The effect of pretreatment with either methiothepin or spiroxatrine on the response to DP-5-CT

Methiothepin (1 mg kg^{-1} i.v.; $n = 4$) caused a significant reduction in blood pressure of $32 \pm 2 \text{ mmHg}$ 5 min following

injection. This was associated with an initial increase in renal nerve activity, reaching a maximum between 1 and 5 min of $64 \pm 14\%$ and then returning to near baseline levels after 20 min. Methiothepin did not cause any significant changes in heart rate or phrenic nerve activity. Baseline values for blood pressure and heart rate were $114 \pm 5 \text{ mmHg}$ and $424 \pm 10 \text{ beats min}^{-1}$. The response produced by DP-5-CT (3 nmol kg^{-1}) administered i.c.v. 20 min after methiothepin was significantly attenuated (Figure 7).

The effect of DP-5-CT (3 nmol kg^{-1} , i.c.v.) on all variables was significantly attenuated in spiroxatrine (300 nmol kg^{-1} , i.c.v.; $n = 6$) pretreated animals compared to vehicle (0.01 N HCl i.c.v.; $n = 6$) pretreated animals, see Figure 7. Spiroxatrine pretreatment did not alter baseline values *per se* (blood pressure $109 \pm 4 \text{ mmHg}$ and heart rate $404 \pm 8 \text{ beats min}^{-1}$). Vehicle pretreatment caused a significant rise in heart rate of $19 \pm 4 \text{ beats min}^{-1}$ and in renal nerve activity of $28 \pm 10\%$. Baseline values for blood pressure and heart rate were $109 \pm 4 \text{ mmHg}$ and $404 \pm 8 \text{ beats min}^{-1}$ respectively.

Discussion

In the present experiments in α -chloralose anaesthetized rats, treated with a neuromuscular blocking agent, i.c.v. injection of DP-5-CT, 5-CT, 8-OH-DPAT (low dose) and 5-HT [in

Figure 7 Anaesthetized rats: histogram comparing changes from post-treatment values 5 min after i.c.v. administration of N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT, 3 nmol kg^{-1}) in the presence of saline (open columns; $5 \mu\text{l}$ i.c.v.; $n = 8$), methiothepin (solid columns; 1 mg kg^{-1} , i.v.; $n = 4$), 0.01 N HCl (vehicle control for spiroxatrine; hatched columns; $10 \mu\text{l}$ i.c.v.; $n = 6$) and spiroxatrine (cross hatched columns; 300 nmol kg^{-1} , i.c.v.; $n = 6$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). All values are the mean with s.e.mean shown by the vertical bars. * $P < 0.05$ and ** $P < 0.01$. Comparisons are made against the appropriate control.

animals pretreated with the 5-HT₂/5-HT_{1C} receptor antagonists cinanserin or LY 53857 (Rubin *et al.*, 1964; Cohen *et al.*, 1983; 1985; see Hoyer, 1991)], caused renal sympathoexcitation, tachycardia and a rise in blood pressure. As DP-5-CT is a highly selective agonist for 5-HT_{1A} receptors (Mir *et al.*, 1987; Doods *et al.*, 1988; see Schoeffter & Hoyer, 1988) and the sympathoexcitation, tachycardia and rise in blood pressure caused by i.c.v. administration of DP-5-CT are blocked by the 5-HT_{1A} receptor antagonists, methiothepin (Fozard *et al.*, 1987; Schoeffter & Hoyer, 1988) and spiroxatrine, a selective antagonist for this subtype of the 5-HT receptor (Nelson & Taylor, 1986; see Hoyer, 1991), it is concluded that such responses are due to the activation of 5-HT_{1A} receptors. Furthermore, 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist (Middlemiss & Fozard, 1983; Fozard *et al.*, 1987) and 5-CT, a non-selective 5-HT₁ receptor agonist (Schoeffter & Hoyer, 1988; see Hoyer, 1991) also caused sympathoexcitation, tachycardia and a rise in blood pressure supporting the above conclusion. The observation that the rise in blood pressure, sympathoexcitation and tachycardia caused by 5-HT in the presence of a 5-HT₂/5-HT_{1C} antagonist could be blocked by the addition of spiroxatrine, demonstrates that the sympathoexcitation caused by 5-HT is also due to activation of 5-HT_{1A} receptors.

The above data indicating the activation of 5-HT_{1A} receptors, reached by i.c.v. administration, causes sympathoexcitation contrast with the findings from previous studies using 8-OH-DPAT and other non-structurally related 5-HT_{1A} agonists. These agonists given i.v. have been demonstrated to cause a centrally mediated decrease in blood pressure and sympathoinhibition in rats, cats, rabbits and dogs (see Ramage, 1990). However, in the rat there is some evidence that activation of 5-HT_{1A} receptors can also cause a pressor response and/or sympathoexcitation. In conscious spontaneously hypertensive rats i.v. 8-OH-DPAT caused an initial tachycardia and rise in blood pressure which was then followed by a bradycardia and hypotension (Fozard *et al.*, 1987). 8-OH-DPAT (i.v.) in conscious and anaesthetized rats mediates the release of adrenaline by central sympathoexcitation of the adrenal glands (Chaouloff & Jeanrenaud, 1987; Chaouloff *et al.*, 1990a,b; Bagdy *et al.*, 1989; Bouhelal & Mir, 1990). Microinjection of 5-HT_{1A} agonists into the raphe obscurus causes a pressor response (Dreteler *et al.*, 1991). Furthermore, i.c.v. administration of low doses of 8-OH-DPAT in conscious rats also causes a pressor response which is attenuated by methiothepin (Dedeoglu & Fisher, 1991). These combined data, at the least in the rat, demonstrate that activation of 5-HT_{1A} receptors can cause sympathoexcitation as well sympathoinhibition. In the present study and that of Dedeoglu & Fisher (1991), higher doses of 8-OH-DPAT administered i.c.v. tended to cause falls in blood pressure. A possible explanation for this observation is that the sympathoexcitation is masked by the sympathoinhibitory action of 8-OH-DPAT. A high dose of 8-OH-DPAT when given i.c.v. may diffuse to the mid and hind brain of the rat where activation of 5-HT_{1A} receptors is known to cause a fall in blood pressure and/or sympathoinhibition, in areas such as the dorsal raphe (Connor & Higgins, 1990), raphe magnus and pallidus (Valenta & Singer, 1990) and the rostral ventrolateral medulla (Nosjean & Guynet, 1991). However, it is possible that high doses of 8-OH-DPAT could have a non-selective action at receptors other than 5-HT_{1A} receptors (see Dedeoglu & Fisher, 1991).

5-HT has previously been shown to regulate a number of neuroendocrine responses including the release of vasopressin in conscious rats (Steardo & Iovino, 1986; see Van de Kar, 1991). Furthermore, in anaesthetized rats the pressor response to i.c.v. 5-HT is blocked by pretreatment with the vasopressin V₁-receptor antagonist d(CH₂)₅Tyr(Me)AVP (Inoue & Buñag, 1989), although the associated bradycardia is only attenuated in the first 5 min. In the present study, pretreatment with d(CH₂)₅Tyr(Me)AVP, at a dose which has previously been shown to abolish the pressor response to

injected vasopressin (Buñag & Miyajima, 1984), only attenuated the duration of the rise in blood pressure caused by i.c.v. 5-HT. This difference may be explained by the different anaesthetic used, in the present study α -chloralose was used while in that of Inoue & Buñag (1989) urethane was used. Interestingly, in the present study the bradycardia and sympathoinhibition caused by 5-HT were reversed to tachycardia and sympathoexcitation in the presence of d(CH₂)₅Tyr(Me)AVP. A similar observation has been made in conscious rats (Pergola & Alper, 1991) and in that study the pressor response to i.c.v. 5-HT was completely blocked by combined α_1 -adrenoceptor and vasopressin V₁-receptor blockade. In conscious rats in which the sinoaortic nerves had been cut (Pergola & Alper, 1991) i.c.v. 5-HT, although causing a pressor response, produced a marked tachycardia. Taken together these data indicate that i.c.v. 5-HT in conscious and anaesthetized rats causes the release of vasopressin. The peripherally mediated pressor response induces a baroreceptor mediated sympathoinhibition and bradycardia which masks the ability of 5-HT to cause sympathoexcitation and tachycardia through activation of 5-HT_{1A} receptors. It is of interest that a low dose of 5-HT (4 nmol kg⁻¹) given i.c.v. caused a pressor response associated with a tachycardia in conscious rats, whereas a higher dose (120 nmol kg⁻¹) produced a pressor response and a biphasic effect on heart rate, bradycardia followed by tachycardia (Dedeoglu & Fisher, 1991; Anderson *et al.*, 1992a). Presumably the low dose of 5-HT produced only the sympathoexcitatory component of the 5-HT response. Therefore the pattern of response caused by i.c.v. administration of 5-HT is dependent on the dose of 5-HT given, and in anaesthetized animals, the anaesthetic used.

The present data show that the release of vasopressin by i.c.v. 5-HT is mediated by 5-HT₂ or 5-HT_{1C} receptors, as a similar effect is obtained by pretreatment with the 5-HT₂/5-HT_{1C} receptor antagonists, cinanserin and LY 53857 (i.c.v.) to that with the vasopressin V₁-receptor antagonist. This conclusion is supported by other studies in conscious rats (Brownfield *et al.*, 1988; Pergola & Alper, 1991; see Van de Kar, 1991).

The selective 5-HT₂/5-HT_{1C} receptor agonist DOI given i.c.v. or i.v. caused a rise in blood pressure, bradycardia and sympathoinhibition. However, these effects were attenuated by the peripherally acting 5-HT₂/5-HT_{1C} receptor antagonist BW501C67 (Mawson & Whittington, 1970; Fuller *et al.*, 1986; BW501C67 has a pK_D of 9.5 at 5-HT₂ and a pK_D of 8.5 at 5-HT_{1C} receptors, G.R. Martin unpublished observations), whereas the response produced by i.c.v. administration of 5-HT on these variables was essentially unaffected by i.v. BW501C67. Therefore, the effects of DOI on blood pressure, heart rate and renal nerve activity can be attributed to activation of peripheral 5-HT₂ receptors on vascular smooth muscle (Dabire *et al.*, 1989; Alper, 1990) and not by activation of central 5-HT₂ or 5-HT_{1C} receptors to release vasopressin. In this context, DOI given i.v. has been shown not to cause the release of vasopressin (Bagdy *et al.*, 1992; see Van de Kar, 1991). Interestingly, in the anaesthetized cat, activation of central 5-HT₂ or 5-HT_{1C} receptors has been shown to cause sympathoexcitation (McCall & Harris, 1988; Vayssettes-Courchay *et al.*, 1991; Shephard *et al.*, 1991; Ramage *et al.*, 1991). In the present study and in previous studies (Alper, 1990; Vayssettes-Courchay *et al.*, 1990) there is no evidence for a centrally mediated increase in sympathetic tone in the rat. Therefore, it appears that the cat and rat are different in this respect.

In previous studies activation of 5-HT pathways has been demonstrated to increase central respiratory drive (Holtman *et al.*, 1986a,b; Dreteler *et al.*, 1991). Furthermore, central administration of 8-OH-DPAT has been shown to increase respiratory rate (Gillis *et al.*, 1989) and phrenic nerve activity (Sporton *et al.*, 1991), a measure of central inspiratory drive. The present results also demonstrate that 8-OH-DPAT can cause an increase in central inspiratory drive. However, the

involvement of 5-HT_{1A} receptors in this action of 8-OH-DPAT is doubtful as both DP-5-CT and 5-CT failed to have any effect on phrenic nerve activity.

The precise site/sites in the brain where 5-HT is acting to cause these cardiovascular effects remain to be determined but the rapid onset of response would suggest a brain area close to the lateral or 3rd ventricles. Angiotensin II administered i.c.v. has been shown to cause a rise in blood pressure and bradycardia in conscious rats and this has been attributed to the release of vasopressin through activation of angiotensin receptors located in the subfornical organ (Iovino & Steardo, 1984; see Hartle & Brody, 1984). Therefore, it is possible that the release of vasopressin caused by 5-HT may occur following activation of 5-HT receptors located in the subfornical organ. Smits & Struyker-Boudier (1976) have demonstrated that microinjection of 5-HT into the anterior hypothalamus/pre-optic area can cause an increase in blood pressure. This area is situated close to the 3rd ventricle and

5-HT containing neurones have been shown to project to this site from the dorsal raphe nucleus (see Coote, 1990). Thus, 5-HT may be acting at the level of the hypothalamus to produce the cardiovascular response observed following i.c.v. injection. Further microinjection studies are required to determine the precise site of action of 5-HT.

In conclusion, the present study demonstrates that i.c.v. administration of 5-HT causes sympathoexcitation by activation of 5-HT_{1A} receptors and the release of vasopressin through activation of 5-HT₂ or 5-HT_{1C} receptors.

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References

- ALPER, R.H. (1990). Hemodynamic and renin responses to ($\pm$)-DOI, a selective 5-HT₂ receptor agonist, in conscious rats. *Eur. J. Pharmacol.*, **175**, 323–332.
- ANDERSON, I.K. (1991). Evidence to suggest that activation of forebrain 5-HT_{1A} receptors causes sympathoexcitation in anesthetized rats. *Br. J. Pharmacol.*, **104**, 65P.
- ANDERSON, I.K., GARDINER, S.M., WIDDOP, R.E., BENNETT, T., MARTIN, G.R. & RAMAGE, A.G. (1992a). Regional haemodynamic effects of 5-HT i.c.v. in conscious Long-Evans and Brattleboro rats. *Br. J. Pharmacol.*, **107**, 14P.
- ANDERSON, I.K., MARTIN, G.R. & RAMAGE, A.G. (1992b). Evidence to suggest that activation of central 5-HT_{2/1C} receptors causes the release of vasopressin in anaesthetized rats. *Br. J. Pharmacol.*, **105**, 30P.
- BAGDY, G., SVED, A.F., MURPHY, D.L. & SZEMEREDI, K. (1992). Pharmacological characterization of serotonin receptor subtypes involved in vasopressin and plasma renin activity responses to serotonin agonists. *Eur. J. Pharmacol.*, **210**, 285–289.
- BAGDY, G., SZEMEREDI, K. & MURPHY, D.L. (1989). Marked increases in plasma catecholamine concentrations preceded hypotension and bradycardia caused by 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) in conscious rats. *J. Pharm. Pharmacol.*, **41**, 270–272.
- BOUHELAL, R. & MIR, A.K. (1990). Role of the adrenal gland in the metabolic and cardiovascular effects of 8-OH-DPAT. *Eur. J. Pharmacol.*, **181**, 173–179.
- BROWNFIELD, M.S., GREATHOUSE, J., LORENS, S.A., ARMSTRONG, J., URBAN, J.H. & VAN DE KAR, L.D. (1988). Neuropharmacological characterization of serotonergic stimulation of vasopressin secretion in conscious rats. *Neuroendocrinology*, **47**, 277–283.
- BUÑAG, R.D. & MIYAJIMA, E. (1984). Sympathetic hyperactivity elevates blood pressure during acute cerebroventricular infusions of hypertonic salt in rats. *J. Cardiovasc. Pharmacol.*, **6**, 844–851.
- CHAOULOFF, F., BAUDRIE, V. & LAUBIE, D. (1990a). Evidence that the 5-HT_{1A} receptor agonists buspirone and ipsapirone activate adrenaline release in the conscious rat. *Eur. J. Pharmacol.*, **177**, 107–110.
- CHAOULOFF, F. & JEANRENAUD, B. (1987). 5-HT_{1A} and α -2 adrenergic receptors mediate the hyperglycaemic and hypotensinemic effects of 8-hydroxy-2-(di-n-propylamino)-tetralin in conscious rats. *J. Pharmacol. Exp. Ther.*, **243**, 1159–1166.
- CHAOULOFF, F., LAUBIE, D. & BAUDRIE, V. (1990b). Ganglionic transmission is a prerequisite for the adrenaline-releasing and hyperglycaemic effects of 8-OH-DPAT. *Eur. J. Pharmacol.*, **185**, 11–18.
- COHEN, M.L., FULLER, R.W. & KURZ, K.D. (1983). LY 53857, a selective and potent serotonergic (5-HT₂) receptor antagonist, does not lower blood pressure in the spontaneously hypertensive rat. *J. Pharmacol. Exp. Ther.*, **227**, 327–332.
- COHEN, M.L., KURZ, K.D., MASON, N.R., FULLER, R.W., MARZONI, G.P. & GARBRECHT, W.L. (1985). Pharmacological activity of the isomers of LY 53857, potent and selective 5-HT₂ receptor antagonists. *J. Pharmacol. Exp. Ther.*, **235**, 319–323.
- CONNOR, H.E. & HIGGINS, G.A. (1990). Cardiovascular effects of 5-HT_{1A} receptor agonists injected into the dorsal raphe nucleus of conscious rats. *Eur. J. Pharmacol.*, **182**, 63–72.
- COOTE, J.H. (1990). The central antihypertensive action of 5-hydroxytryptamine: the location of site of action. In *Cardiovascular Pharmacology of 5-Hydroxytryptamine; Prospective Therapeutic Applications*. ed. Saxena, P.R., Wallis, D.I., Wouters, W. & Bevan, P. pp. 259–266. The Netherlands: Kluwer.
- DABIRE, H., CHAUCHE-TEYARA, K., CHERQUI, C., FOURNIER, B. & SCHMITT, H. (1989). DOI is a mixed agonist-antagonist at postjunctional 5-HT₂ receptors in the pithed rat. *Eur. J. Pharmacol.*, **170**, 109–111.
- DALTON, D.W. (1986). The cardiovascular effects of centrally administered 5-hydroxytryptamine in the conscious normotensive and hypertensive rat. *J. Auton. Pharmacol.*, **6**, 67–75.
- DEDEOGLU, A. & FISHER, L.A. (1991). Central nervous actions of serotonin and a serotonin_{1A} receptor agonist: Cardiovascular excitation at low doses. *J. Pharmacol. Exp. Ther.*, **257**, 425–432.
- DOODS, H.N., BODDEKE, H.W.G.M., KALKMAN, H.O., HOYER, D., MATHY, M.-J. & VAN ZWIETEN, P.A. (1988). Central 5-HT_{1A} receptors and the mechanism of the central hypotensive effect of (+) 8-OH-DPAT, DP-5-CT, R28935, and urapidil. *J. Cardiovasc. Pharmacol.*, **11**, 432–437.
- DRETELER, G.H., WOUTERS, W., SAXENA, P.R. & RAMAGE, A.G. (1991). Pressor effects following microinjection of 5-HT_{1A} receptor agonists into the raphe obscurus of the anaesthetized rat. *Br. J. Pharmacol.*, **102**, 317–322.
- FOZARD, J.R., MIR, A.K. & MIDDLEMISS, D.N. (1987). Cardiovascular response to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT): Site of action and pharmacological analysis. *J. Cardiovasc. Pharmacol.*, **9**, 328–347.
- FULLER, R.W., KURZ, K.D., MASON, N.R. & COHEN, M.L. (1986). Antagonism of a peripheral vascular but not an apparently central serotonergic response by xylamide and BW501C67. *Eur. J. Pharmacol.*, **125**, 71–77.
- GILLIS, R.A., HILL, K.J., KIRBY, J.S., QUEST, J.A., HOMASH, P., NORMAN, W.P. & KELLAR, K.J. (1989). Effect of activation of central nervous system serotonin 1A receptors on cardiorespiratory function. *J. Pharmacol. Exp. Ther.*, **248**, 851–857.
- HARTLE, D.K. & BRODY, M.J. (1984). The angiotensin II pressor system of the rat forebrain. *Circ. Res.*, **54**, 355–366.
- HOLTMAN, J.R., ANASTASI, N.C., NORMAN, W.P. & DRETCHEN, K.L. (1986a). Effect of electrical and chemical stimulation of the raphe obscurus on phrenic nerve activity in the cat. *Brain Res.*, **362**, 214–220.
- HOLTMAN, J.R., DICK, T.E. & BERGER, A.J. (1986b). Involvement of serotonin in the excitation of phrenic motoneurons evoked by stimulation of the raphe obscurus. *J. Neuroscience*, **6**, 1185–1193.
- HOYER, D. (1991). The 5-HT receptor family: ligands, distribution and receptor-effector coupling. In *5-HT_{1A} Agonists, 5-HT₂ Antagonists and Benzodiazepines: their Comparative Behavioural Pharmacology*. ed. Rodgers, R.J. & Cooper, S.J. pp. 31–57. Chichester: John Wiley & Sons Ltd.

- INOUE, A. & BUÑAG, R.D. (1989). Sympathetic inhibition and vasopressin mediation during centrally induced responses to serotonin in rats. *J. Cardiovasc. Pharmacol.*, **13**, 902–907.
- IOVINO, M. & STEARDO, L. (1984). Vasopressin release to central and peripheral angiotensin II in rats with lesions of the subfornical organ. *Brain Res.*, **322**, 365–368.
- KRISTIC, M.K. & DJURKOVIC, D. (1976). Hypertension mediated by activation of the rat brain 5-hydroxytryptamine receptor sites. *Experientia*, **32**, 1187–1189.
- KRISTIC, M.K. & DJURKOVIC, D. (1980). Analysis of cardiovascular responses to central administration of 5-hydroxytryptamine in rats. *Neuropharmacol.*, **19**, 455–463.
- LAMBERT, G.A., FRIEDMAN, E. & GERSHON, S. (1975). Centrally mediated cardiovascular responses to 5-HT. *Life Sci.*, **17**, 915–920.
- LAMBERT, G.A., FRIEDMAN, E., BUCHWEITZ, E. & GERSHON, S. (1978). Involvement of 5-hydroxytryptamine in the central control of respiration, blood pressure and heart rate in the anaesthetized rat. *Neuropharmacol.*, **17**, 807–813.
- MAWSON, C. & WHITTINGTON, H. (1970). Evaluation of the peripheral and central antagonistic activities against 5-hydroxytryptamine of some new agents. *Br. J. Pharmacol.*, **39**, 223P.
- MCCALL, R.B. & HARRIS, L.T. (1988). 5-HT₂ receptor agonists increase spontaneous sympathetic nerve discharge. *Eur. J. Pharmacol.*, **151**, 113–116.
- MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-hydroxy-2-(di-n-propylamino) tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- MIR, A.K., HIBERT, M. & FOZARD, J.R. (1987). Cardiovascular effects of N,N-dipropyl-5-carboxamidotryptamine, a potent and selective 5-HT_{1A} receptor ligand. In *Neuronal Messengers in Vascular Function*. ed. Nobin, A., Owman, C. & Arneko-Nobin, B. pp. 21–29. Amsterdam: Elsevier.
- NELSON, D.L. & TAYLOR, E.W. (1986). Spiroxitrine: a selective serotonin_{1A} receptor antagonist. *Eur. J. Pharmacol.*, **129**, 307–314.
- NOSJEAN, A. & GUYENET, P.G. (1991). Role of the ventral lateral medulla in the sympatholytic effect of 8-OH-DPAT in rats. *Am. J. Physiol.*, **260**, R600–R609.
- PERGOLA, P.E. & ALPER, R.H. (1991). Vasopressin and autonomic mechanisms mediate cardiovascular actions of central serotonin. *Am. J. Physiol.*, **260**, R1188–R1193.
- PERGOLA, P.E. & ALPER, R.H. (1992). Effects of central serotonin on autonomic control of heart rate in intact and baroreceptor deficient rats. *Brain Res.*, **582**, 215–220.
- RAMAGE, A.G. (1985). The effects of ketanserin, methysergide and LY 53857 on sympathetic nerve activity. *Eur. J. Pharmacol.*, **113**, 295–303.
- RAMAGE, A.G. (1990). Influence of 5-HT_{1A} receptor agonists on sympathetic and parasympathetic nerve activity. *J. Cardiovasc. Pharmacol.*, **15**, (suppl. 7), S75–S85.
- RAMAGE, A.G., SHEPHEARD, S.L., JORDAN, D. & KOSS, M.C. (1991). Examination of the sympathoexcitatory action of the 5-HT₂ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on cardiac sympathetic nerve activity in the anaesthetized cat. *J. Physiol.*, **438**, 84P.
- RAMAGE, A.G. & WILKINSON, S.J. (1989). Evidence that different regional sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of putative 5-HT_{1A} and α_2 adrenoceptor agonists in anaesthetized cats. *Br. J. Pharmacol.*, **98**, 1157–1164.
- RUBIN, B., PIALA, J.J., BURKE, J.C. & CRAVER, B.N. (1964). A new potent and specific serotonin inhibitor (SQ 10,643) 2'-(3-dimethylaminopropylthio) cinnamanilide hydrochloride: anti-serotonin activity on the uterus and on gastrointestinal, vascular and respiratory systems of animals. *Arch. Int. Pharmacodyn.*, **152**, 132–143.
- SCHOEFFTER, P. & HOYER, D. (1988). Centrally acting hypotensive agents with affinity for 5-HT_{1A} binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. *Br. J. Pharmacol.*, **95**, 975–985.
- SHEPHEARD, S.L., JORDAN, D. & RAMAGE, A.G. (1991). Investigation of the effects of IVth ventricular administration of the 5-HT₂ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on autonomic outflow in the anaesthetized cat. *Br. J. Pharmacol.*, **104**, 367–372.
- SMITS, J.F. & STRUYKER-BOUDIER, H.A. (1976). Intrahypothalamic serotonin and cardiovascular control in the rat. *Brain Res.*, **111**, 422–427.
- SOKAL, R.R. & ROHLF, F.J. (1969). *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco, CA: Freeman.
- SPORTON, S.C.E., SHEPHEARD, S.L., JORDAN, D. & RAMAGE, A.G. (1991). Microinjections of 5-HT_{1A} agonists into the dorsal motor vagal nucleus produce a bradycardia in the atenolol-pretreated anaesthetized rat. *Br. J. Pharmacol.*, **104**, 466–470.
- STEARDO, L. & IOVINO, M. (1986). Vasopressin release after enhanced serotonergic transmission is not due to activation of the renin-angiotensin system. *Brain Res.*, **382**, 145–148.
- SUKAMOTO, T., YAMAMOTO, T., WATANABE, S. & UEKI, S. (1984). Cardiovascular responses to centrally administered serotonin in conscious normotensive and spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **100**, 173–179.
- VALENTA, B. & SINGER, E.A. (1990). Hypotensive effects of 8-hydroxy-2-(di-n-propylamino)tetralin and 5-methylurapidil following stereotaxic microinjection into the ventral medulla of the rat. *Br. J. Pharmacol.*, **99**, 713–716.
- VAN DE KAR, L.D. (1991). Neuroendocrine pharmacology of serotonergic (5-HT) neurons. *Annu. Rev. Pharmacol. Toxicol.*, **31**, 289–320.
- VAYSSETTES-COURCHAY, C., BOUYSET, F., VERBEUREN, T.J., LAUBIE, M. & SCHMITT, H. (1990). The cardiovascular effects of quipazine are mediated by peripheral 5-HT₂ and 5-HT₃ receptors in anaesthetized rats. *Eur. J. Pharmacol.*, **192**, 389–395.
- VAYSSETTES-CHOURCHAY, C., BOUYSET, F., VERBEUREN, T.J., LAUBIE, M. & SCHMITT, H. (1991). Quipazine-induced hypotension in anaesthetized cats is mediated by central and peripheral 5-HT₂ receptors: role of the ventrolateral pressor area. *Eur. J. Pharmacol.*, **192**, 389–395.

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