British Journal of Pharmacology (1998) 125, 1172–1179 © 1998 Stockton Press All rights reserved 0007–1188/98 $12.00
http://www.stockton-press.co.uk/bjp

The central action of the 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on cardiac inotropy and vascular resistance in the anaesthetized cat

[1,3]Andrew G. Ramage & [2]M. de Burgh Daly

Departments of [1]Pharmacology and [2]Physiology, Royal Free Campus, University College London, Rowland Hill Street, London NW3 2PF

1 Experiments were carried out to determine the effects of the application of the selective 5-HT_2 receptor agonist DOI intravenously (in the presence of the peripherally acting 5-HT_2 receptor antagonist, BW501C67, 1 mg kg^{-1}, i.v.) or to the 'glycine sensitive area' of the ventral surface (30 μg each side) on the left ventricular inotropic (left ventricular dP/dt max) and vascularly isolated hindlimb responses in anaesthetized cats. For the ventral surface experiments, NMDA (10 μg each side) was applied to act as a positive control. In all experiments heart rate and mean arterial blood pressure were held constant to exclude any secondary effects caused by changes in these variables.

2 DOI ($n=6$) i.v or on the ventral surface had no effect on left ventricular dP/dt max but caused a significant increase in hindlimb perfusion pressure of 40 ± 9 and 50 ± 14 mmHg, respectively. Respiration was unaffected. NMDA ($n=6$), applied to the ventral surface, caused significant increases in both left ventricular dP/dt max and hindlimb perfusion pressure of $1{,}950\pm349$ mmHg s^{-1} and 69 ± 17 mmHg respectively, with no associated change in left ventricular end-diastolic pressure. The amplitude of respiratory movements increased.

3 It is concluded that activation of 5-HT_2 receptors at the level of the rostral ventrolateral medulla (RVLM) excites sympathetic premotor neurons and/or their antecedents controlling hindlimb vascular resistance but not those controlling the inotropic effects on the left ventricle.

Keywords: 5-HT_2 receptors; NMDA, DOI; rostral ventrolateral medulla; force of heart contraction; inotropy; left ventricular dP/dt max; hindlimb perfusion pressure; vascular resistance; anaesthetized cat

Introduction

Activation of central 5-HT_2 receptors using the selective receptor agonist DOI is generally considered to cause sympatho-excitation and a rise in blood pressure (McCall & Clement, 1994). In one of the early studies it was demonstrated that i.v. DOI caused a centrally mediated rise in left inferior cardiac nerve activity and blood pressure although, surprisingly there was no associated increase in heart rate in anaesthetized cats (McCall & Harris, 1988). This observation was confirmed and extended to apply to both the left and right inferior cardiac nerve and the left and right sympathetic outflows that runs from the stellate along the vagus (Ramage *et al*., 1993).

Further, application of DOI to the ventral surface (King & Holtman, 1990; Mandal *et al*., 1990) or by microinjection into the subretrofacial nucleus of an anaesthetized cat also caused a rise in blood pressure, associated with no change in heart rate *via* activation of 5-HT_2 receptors. Mandal *et al*., 1990 attributed this increase in blood pressure caused by DOI, applied to the ventral surface and i.v., to a selective increase in force of contraction of the heart. This led them to the intriguing conclusion that there was 'a central pathway for selective sympatho-excitation of the heart, leading to an increase in cardiac inotropy without an increase in cardiac chronotropy' which 'is mediated by activation of 5-HT_2 receptors on neurones located in the ventral lateral medulla, most likely within the subretrofacial nucleus.'

This particular area of the ventral surface is often referred to as the 'glycine-sensitive area' which was first identified by Schläfke & Loeschcke (1967); it has been shown to be important in the maintenance of sympathetic tone (Guertzenstein & Silver, 1974; Feldberg & Guertzenstein, 1976). This area is also termed the rostral ventrolateral medulla (RVLM) and the neurones from this area have been shown to lie within the subretrofacial nucleus, at least in the cat (Dampney, 1994). Further, experimental data suggest that these neurones are organized, to a large degree, topographically with respect to different vascular beds (Lovick, 1987; Dampney & McAllen, 1988; McAllen & May, 1994) and more recently to the heart as indicated by changes in left inferior cardiac nerve activity (Campos & McAllen, 1997). The experiments of Mandal *et al*., (1990) also suggest that the organization of these neurones within the ventral surface, at least those involved at controlling the sympathetic outflow to the left ventricle, may be related to the type/s of receptors present on, and/or causing activation of these so called pre-vasomotor sympathetic neurones (Dampney, 1994). Finally, the experiments of Mandal *et al*. (1990) also explain why DOI i.v. causes an increase in cardiac sympathetic outflow but no associated change in heart rate, although there is an increase in blood pressure (McCall & Harris, 1988; Ramage *et al*., 1993). However, in the experiments of Mandal *et al*. (1990) the increase in the cardiac inotropy that was observed may be indirect, due to the rise in blood pressure (von Anrep, 1912; Wallace *et al*., 1963). Therefore the present experiments have been carried out to re-examine the ability of DOI given i.v. and to the 'glycine sensitive area' of the ventral surface, to increase left ventricular inotropy in experiments in which blood pressure and heart rate were kept constant. In addition, the effects of DOI on regional vascular resistance, the latter being measured as changes in

[3] Author for correspondence.

hindlimb vascular resistance, was also determined. A preliminary account of the these observations has been given (Ramage & Daly, 1997)

Methods

The experiments were performed on cats of either sex, weighing 2.8–4.3 kg (mean 3.26±0.46 (s.d.) kg). They were anaesthetized with a mixture of α-chloralose (Sigma, 1% w/v) 70 mg kg^{-1} and pentobarbitone sodium 6 mg kg^{-1} intravenously. Rectal temperature was monitored throughout each experiment and maintained between 37° and 39°C. The bladder was continuously drained of urine *via* a catheter inserted suprapubically in female animals or per urethram in males to exclude reflexes from this organ. The essential features of the experimental preparation are shown in Figure 1. After completion of the surgical procedures and before connecting the extracorporeal circuits, heparin (Monoparin, C.P. Pharmaceuticals Ltd., Wrexham, U.K., 1000 i.u. kg^{-1} i.v.) was administered to render the blood incoagulable.

Respiration

The chest was opened in the mid sternal line and positive pressure ventilation was applied by means of a Starling Ideal pump at a rate of 20 cycles min^{-1}. The tidal volume was adjusted to maintain the PCO_2 at 37–42 mmHg. An end-expiratory pressure of 1–3 cm H_2O prevented complete collapse of the lungs. In all experiments oxygen enriched air was administered resulting in an inspired O_2 fraction ($F_{I,\,O_2}$) of approximately 0.4. Respiratory movements of the lower thorax were measured qualitatively by a pneumatic method. These movements were central in origin, being unaffected by the rhythmic changes in lung volume.

Arterial blood pressure

In all experiments the mean arterial pressure, measured from a brachial artery, was maintained constant by connecting a pressurized reservoir of blood to the animal's systemic circulation *via* cannulae in the two femoral arteries pointing rostrally. The perfusion pump (Figure 1) drew blood from the reservoir which ensured continuous mixing of blood between the reservoir and the animal. The reservoir was enclosed in a water-jacket maintained at 37°C. The blood used to prime the extracorporeal system was obtained from a donor animal taken up to 7 days previously and treated as described elsewhere (Daly *et al*., 1993). In a few experiments the compensator was filled with the animal's own blood, the equivalent volume of Gelofusine being administered intravenously.

*Measurement of left ventricular d*P/d*t max*

Left ventricular pressure and, separately, the left ventricular end-diastolic pressure were measured by a catheter-tipped manometer (implantable pressure transducer, type 12cr/4F; Gaeltec Ltd., Dunvegan, Isle of Skye, Scotland) which was inserted into the left ventricle *via* the left atrial appendage. The output from the amplifier was differentiated electrically to record left ventricular dP/dt max. The manometer was calibrated statically against a mercury manometer. The frequency response was determined by applying a square-wave pressure transient as described by Fry (1960), and was found to be flat (±5%) to 400 Hz. A switched precision calibrator built into the amplifier/differentiator circuit enabled steady ramp functions to be applied to the differentiated to give calibration signals of 2000, 5000 and 10,000 mmHg s^{-1}. The frequency response of the differentiator was assessed by applying a sine-wave input voltage from a signal generator and gave a linear response (±5%) up to 130 Hz.

Cardiac pacing

Heart rate was maintained constant by electrical pacing through a platinum wire electrode applied to the left atrial appendage. The electrical parameters of stimulation were 10–15 V and 2 ms pulse duration, the frequency being set slightly higher than the natural heart rate expected during the control and test periods (Grass S88 stimulator through a stimulus–isolation unit, Grass Medical Instruments).

Perfusion of hindlimb

The right hindlimb was vascularly isolated and perfused through the femoral artery with arterial blood from the blood pressure compensator by means of an occlusive roller pump (type MHRE 200, Watson-Marlow Ltd, Falmouth, Cornwall, U.K.). The right profunda artery was tied to minimize

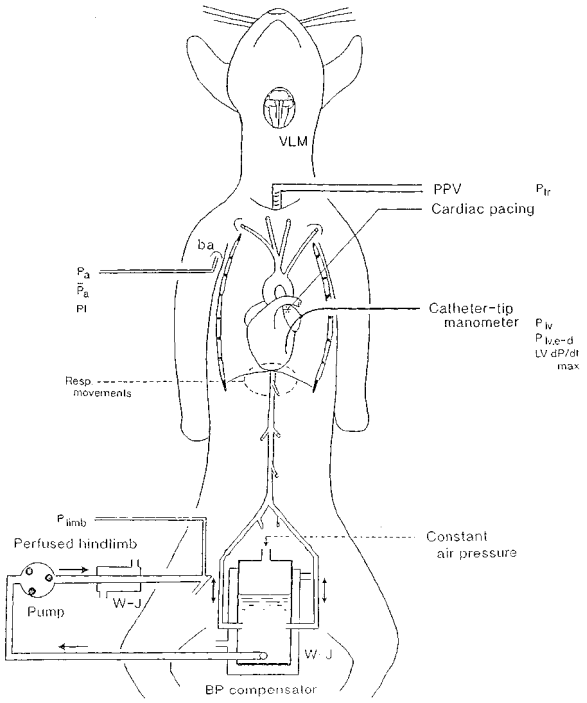

Figure 1 Diagrammatic representation of the preparation. Positive pressure ventilation (PPV) was applied, the chest being opened in the mid sternal line. A pneumatic method was used to measure qualitatively central respiratory movements. The heart was paced and a catheter-tip manometer was inserted into the left ventricle *via* the left atrial appendage. Arterial blood pressure was measured from the right brachial artery (ba). The right hindlimb was vascularly isolated and perfused at constant flow with blood from the blood pressure (BP) compensator, the pressure in which was controlled by an air-leak bypass system. The compensator was connected to cannulae inserted rostrally in the femoral arteries. It was surrounded by a water-jacket (W-J) maintained at 37°C. The ventrolateral medulla (VLM) was exposed after carrying out a laryngectomy. Measurements were made of Pa, $\overline{P}$a, phasic and mean arterial blood pressure; PI, pulse interval; Ptr, tracheal pressure; resp, respiratory movements; P lv, left ventricular pressure; P lv, e-d, left ventricular end-diastolic pressure; LV dP/dt max, maximum rate of rise of left ventricular pressure; and P-limb, hindlimb mean perfusion pressure.

collateral blood flow to the limb and this procedure provided an adequate vascular isolation (Daly & Kirkman, 1988). Perfusion was maintained at constant flow so that changes in vascular resistance were indicated as changes in mean arterial perfusion pressure from control values to the peak of the effect during the stimulus. Changes in femoral venous pressure were minimal compared to the alterations in arterial perfusion pressure, and were not therefore taken into account.

Measurements of variables

All variables were recorded on a multichannel high resolution thermal print-head recorder (model PAR 2000B, TDM Tape Services Ltd, Nottingham, U.K.) and included: intratracheal pressure, central respiratory movements, hindlimb mean perfusion pressure, arterial blood pressures (phasic and integrated mean), all of them by means of strain gauge manometers (Model P23Gb, Statham Instruments Inc., Hato Rey, Puerto Rico), left ventricular pressure, left ventricular end-diastolic pressure, left ventricular dP/dt max, and pulse interval triggered from ascending phase of anacrotic wave of the arterial blood pressure. The frequency response of the catheter–manometer system used for the measurement of arterial pressure was determined as described by Fry (1960), and was flat ($\pm 5\%$) up to 12 Hz. Zero reference pressures were obtained postmortem with the catheter tips exposed to air *in situ*.

Exposure of the ventrolateral surface of the medulla

This was carried out as described by Guertzenstein (1973), the dura being opened early in the experiment to obtain haemostasis before administering heparin. Disks of filter paper, 3 mm diameter, were soaked in sodium chloride solution (154 mM), for control observations, or in a chemical agent in a volume of 2.5 μl, and were applied bilaterally to the 'glycine-sensitive' area (Guertzenstein & Silver, 1974), that is, caudal to the trapezoid body and rostral to the rootlets of the hypoglossal nerves. It was found that application of drugs outwith this area had no overt effects on the measured cardiovascular and respiratory variables. In some experiments, particularly those in which glycine was not applied, an injection of pontamine sky blue (approximately 500 nl of a 2% solution in 154 mM sodium chloride) was made into the brain area under one of the disks at the end of the experiment to determine at postmortem if the area matched that of the 'glycine-sensitive' area. In two animals, in which the ventrolateral medulla had been exposed, both adrenal glands were exposed *via* a retroperitoneal approach and ligatured. CSF and any applied fluid between tests were removed from the ventral surface by continuous suction through two bilaterally located small-bore catheters.

Blood gas analysis

At intervals during each experiment, arterial blood PO_2, PCO_2, pH and haematocrit were determined. Metabolic acidosis was corrected with an intravenous infusion of 1 M sodium bicarbonate solution. In every animal the PO_2 was greater than 100 mmHg. The mean values ($\pm$s.d.) were: PCO_2 37.4 ± 6.1 mmHg, pH 7.39 ± 0.046, and haematocrit $35.0 \pm 3.1\%$.

Experimental protocols and analysis of results

For DOI given intravenously all these animals received (i.v.) the peripherally acting 5-HT$_2$ receptor antagonist BW501C67 (Mawson & Whittington, 1970) in two doses of 1 mg kg^{-1}, 3 min apart to prevent the activation of peripheral 5-HT$_2$ receptors (Ramage *et al*., 1993). DOI was administered i.v. 3 min after the last dose of BW501C67. Baseline values for each variable were compared to the actual values 3 min later after giving DOI i.v. (the time the changes in hindlimb perfusion had plateaued) using Student's paired t-test. The changes caused by BW501C67 were analysed similarly. Measurements were taken, however, when left ventricular dP/dt max had reached maximum. For the ventral surface experiments, control disks of filter paper soaked in sodium chloride solution (154 mM) were applied initially and left in position for between 5 and 10 min and then removed. At least 5 min later, disks of filter paper containing either 30 μg DOI or 10 μg NMDA, were applied and the response was followed for up to 5 min. In some experiments, NMDA was applied after DOI or vice versa and then glycine. At least 20 min were allowed to elapse between each drug application. Between removal of the disks and the application of the next set of disks the ventral surface was flushed with warm (37°C) sodium chloride solution (154 mM) and drained. Measurements of all variables were taken when the changes in hindlimb perfusion pressure had reached maximum, for DOI this was after approximately 3 min while for NMDA this was after 2 min. These changes were compared with those caused by sodium chloride solution (154 mM) at the appropriate time interval using a Student's unpaired t-test. Changes caused by NMDA after DOI were compared with NMDA alone, and DOI after NMDA with DOI alone using a Student's unpaired t-test. Onset time was measured when hindlimb perfusion pressure had changed by 10% after administration of the drug and compared using a Student's unpaired t-test. Differences were considered significant when $P < 0.05$. All values are expressed as mean $\pm$ s.e.mean unless otherwise stated.

Drugs

The following drugs were used: N-methyl-D-aspartic acid (NMDA) and gylcine from Sigma Chemical Co., Dorset; BW501C67 (-anilino-N-2-m-chlorophenoxypropylacetamide), a gift from Wellcome Research Laboratories, Beckenham, Kent, U.K.; 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) from Research Biochemicals Inc., Semat Technical Ltd, St. Albans, Hertsfordshire, U.K.; pentobarbitone sodium (Sagatal) from RMB Animal Health Ltd, Dagenham Green, Essex, U.K.; α-chloralose from Sigma Chemical Co., Poole, Dorset; Gelofusine from Consolidated Chem., Wrexham, Clwyd U.K. All drugs were dissolved in the sodium chloride solution.

Results

Baseline values from which changes in all variables were measured are shown in Table 1. All the results were obtained under conditions of constant heart rate and mean arterial blood pressure.

Effects of i.v. DOI in the presence of BW501C67

BW501C67 (1 mg kg^{-1} i.v.; $n = 7$) caused a transient (15 s) and significant ($P < 0.01$) increase in left ventricular dP/dt max from 6167 ± 658 mmHg s^{-1} to 7471 ± 482 mmHg s^{-1} ($+1329 \pm 228$ mmHg s^{-1}) associated with a transient and significant increase in hindlimb perfusion from 106 ± 6 mmHg to 120 ± 10 mmHg ($+15 \pm 5$ mmHg; $P < 0.05$) but no change

Table 1 Baseline values of left ventricular end-diastolic pressure (Plv, e-d) left ventricular (LV) dP/dt max, hindlimb perfusion pressure (P-limb), mean arterial blood pressure ($\overline{P}$a) and respiratory frequency (resp. freq.)

Drug (dose; route)	n	*Plv, e-d* mmHg	*LV* dP/dt mmHg s^{-1}	*P limb* mmHg	$\overline{P}a$ mmHg	*Resp. freq.* min^{-1}
DOI i.v. 300 μg kg^{-1}	6	2.3±0.8	5267±176	125±6	144±9	14±2
Saline vlm 2.5 μl per side	12	2.7±0.8	5709±317	107±5	129±4	12±1.5
DOI vlm 30 μg kg^{-1} per side	6	1.8±0.7	5567±286	107±5	139±6	10±1
NMBA vlm 10 μg kg^{-1} per side*	6	3.8±1.3	5783±403	105±4	120±7	15±2
NMDA after DOI vlm	6	1.9±0.9	5583±335	120±13	143±9	9±1
DOI after NMDA vlm	4	4.3±2.0	5400±873	95±13	108±11	16±4
Glycine vlm 500 μg kg^{-1} per side after DOI and NMDA	7	2.8±1.4	5071±428	134±10	114±12	10±2

α-chloralose anaesthetized cats. Heart rate and mean arterial blood pressure maintained constant. (vlm – ventrolateral medulla). *Includes data from two animals in which the adrenal glands had been bilaterally ligated

in mean arterial pressure (3.1±1.4 mmHg). The second dose of BW501C67 (n=6) also caused a transient and significant increase in dP/dt max from 5560±232 mmHg s^{-1} to 7040±531 (+1480±496 mmHg s^{-1}; P<0.05) but no change in hindlimb perfusion pressure (6±8 mmHg) or mean arterial pressure (0.2±2.5 mmHg).

DOI, 300 μg kg^{-1} i.v., (n=6) had no significant effect on left ventricular dP/dt max (50±263 mmHg s^{-1}) or mean arterial pressure (2.5±1.7 mmHg) but caused a significant increase in hindlimb perfusion pressure of 40±9 mmHg (Figure 2). Frequency of respiratory movements tended to decrease (−3±2 min^{-1}). The mean onset time for the increase in hindlimb perfusion was 15.0±2.0 s.

Effects of sodium chloride solution, DOI, NMDA and glycine applied bilaterally to the ventrolateral medulla

The sodium chloride solution, 154 mM (n=12) applied to the ventral surface, had little effect on the variables being monitored. The changes in left ventricular dP/dt max after 2 and 3 min were only 66±53 and 50±68 mmHg s^{-1} respectively, while for hindlimb perfusion pressure the changes were 4±2 and 0±2 mmHg (Figure 2). There was no effect on spontaneous respiratory movements.

Application of DOI, 30 μg each side (n=6), had no significant effect on left ventricular dP/dt max (233±263 mmHg s^{-1}), mean arterial pressure (5±3 mmHg) or on the frequency of respiratory movements (−1±0.5 min^{-1}) but caused a significant (P<0.05) increase of 50±13 mmHg in hindlimb perfusion pressure (Figure 2). Onset time for the increase in hindlimb perfusion was 64.5±17 s. Traces from one of these experiments are shown in Figure 3.

However, application of NMDA, 10 μg each side (n=6), caused a significant increase, after 2 min, in left ventricular dP/dt max of 1950±349 mmHg s^{-1} with no change in left ventricular end-diastolic pressure and arterial blood pressure (0±3 mmHg). In addition, NMDA caused a significant increase in hindlimb perfusion pressure of 69±17 mmHg (see Figure 2). These changes were accompanied by a considerable increase in the amplitude of respiratory movements but no change in respiratory frequency. Traces from one of these experiments are also shown in Figure 3. In two experiments, in which the adrenal glands on both sides had been ligated, NMDA caused similar changes to that observed in animals with intact adrenals. Data from these animals was included in the mean data on all variables for the NMDA alone experiments, as the changes did not fall outside the range of the effects of NMDA in animals with intact adrenals. The mean onset time for these effects was 30±7 s and this did

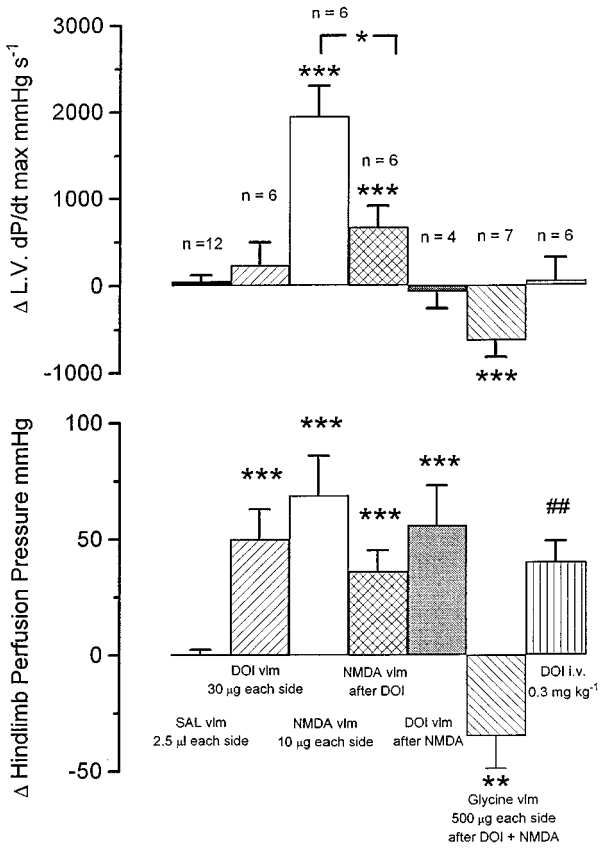

Figure 2 Anaesthetized cats: histograms showing the change (Δ) in left ventricular (L.V.) dP/dt max (mmHg s^{-1}) and hindlimb perfusion pressure (mmHg) caused by bilateral application on disks of filter paper 3 mm in diameter of sodium chloride (NaCl 154 mM) 2.5 μl each side; DOI 30 μg each side; N-methyl-D-aspartic acid (NMDA) 10 μg each side (these data include data from two animals in which the adrenal glands had been bilaterally ligated); NMDA after DOI; DOI after NMDA; glycine 500 μg each side after the application of DOI and NMDA; and DOI 300 μg kg^{-1} i.v. Each histogram represents the mean change and the bars show the s.e.mean. Changes caused by the test substances are compared with those caused by sodium chloride solution or as indicated using a Student's unpaired t-test *P<0.05 **P<0.01, ***P<0.001. For i.v. DOI actual values before and after drug were compared using Student's paired t-test. ##P<0.01.

significantly differ from that observed for DOI. Traces from one of these experiments are shown in Figure 4.

In six experiments, NMDA applied after DOI resulted in a significant increase in left ventricular dP/dt max (667±242 mmHg s^{-1}) with no associated change in left

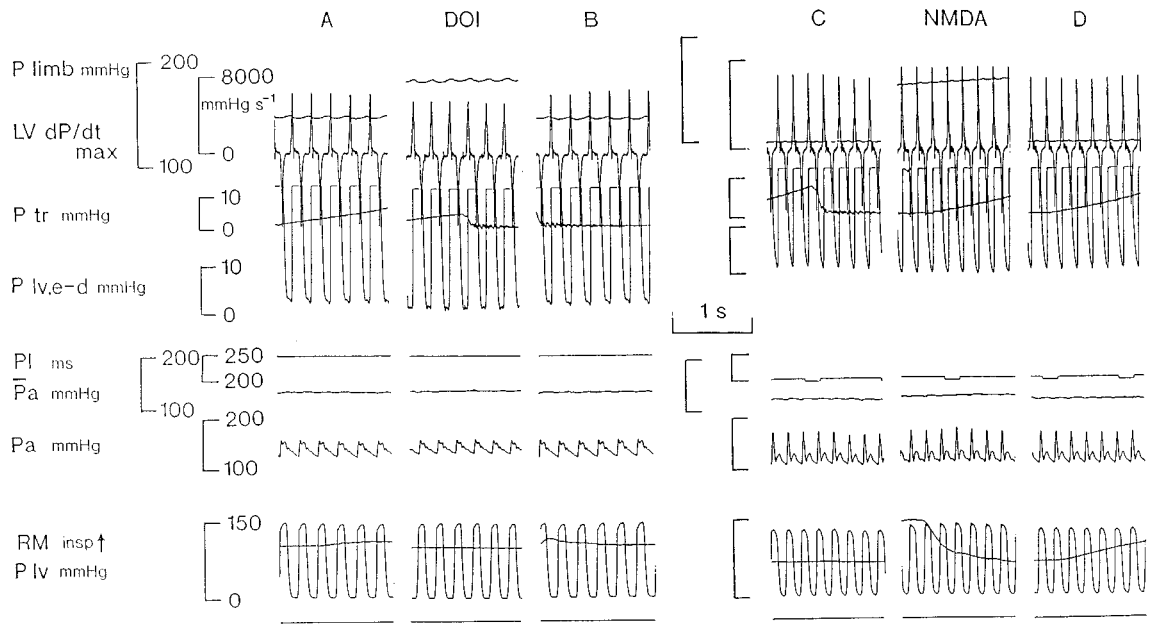

Figure 3 Artificially ventilated anaesthetized cats: traces showing the effects of topical application to the 'glycine-sensitive area' of the ventrolateral medulla of DOI, 30 μg each side, from one experiment, and from another, NMDA, 10 μg each side. In each experiment the heart was electrically paced and the mean arterial blood pressure was maintained constant. Panels A and C, initial control states 1 min before the application of the drugs to the medulla; panels B and D, final control states after rinsing the medulla with sodium chloride solution, 154 mM. Panel DOI was taken 3 min after the application of the drug, panel B 5 min after rinsing the area with sodium chloride solution. Panel NMDA was taken 2 min after the application of the drug, panel D 8 min after rinsing the area with the sodium chloride solution (154 mM). Records from above downwards: P-limb, hindlimb mean perfusion pressure; LV (left ventricular) dP/dt max; Ptr, tracheal pressure; P lv, e-d, left ventricular end-diastolic pressure; PI, pulse interval; Pa, $\bar{P}$a, phasic and mean arterial blood pressure; RM, respiratory movements (inspiration upwards); P lv, left ventricular pressure. Time calibration, 1 s.

ventricular end-diastolic pressure and mean arterial pressure (0.05 ± 0.05 mmHg), a significant increase in hindlimb perfusion pressure of 36 ± 9 mmHg and again no changes in the frequency of respiratory movements (Figure 2). Both the changes in left ventricular dP/dt max and hindlimb perfusion pressure were reduced compared with the responses to NMDA before DOI. However, only the change in left ventricular dP/dt max was significantly different to that observed with NMDA after the sodium chloride solution (Figure 2), whereas the effects of DOI after NMDA were unchanged (Figure 2).

Glycine (500 μg each side) following DOI or NMDA caused significant decreases in left ventricular dP/dt max (-629 ± 195 mmHg s^{-1}) and hindlimb perfusion pressure (-35 ± 14 mmHg) after 2 min (Figure 2) but no change in mean arterial pressure (1.4 ± 0.1 mmHg). The effects of glycine on respiratory movements were variable with no overall change in the frequency. The mean onset time for these effects was 32.5 ± 7.2 s and this did significantly differ from that observed for DOI 64.5 ± 17.0 s. Traces from one of these experiments are shown in Figure 5.

Discussion

The present experiments show that DOI given by i.v. administration or topical application to an area of the ventral surface of the brainstem, does not cause an increase in left ventricular dP/dt max but does cause an increase in regional vascular resistance, as indicated by the increase in hindlimb perfusion pressure. The position of this area on the ventral surface and the ability of glycine applied to this site to cause a decrease in left ventricular dP/dt max and vasodilatation in the hindlimb, are consistent with that which is known as the 'glycine sensitive area' or the RVLM (Dampney, 1994). Thus the present observations contradict the conclusion of Mandal *et al.* (1990), that the rise in blood pressure evoked by activation of central 5-HT$_2$ receptors with DOI applied intravenously, topically to the 'glycine sensitive area' or by microinjection into the subretrofacial nucleus was due to a selective increase in sympathetic activity to the left ventricle. The present experiments, where blood pressure was maintained constant, imply that the increase in force of contraction observed by Mandal *et al.* (1990) was secondary to the rise in blood pressure (von Anrep, 1912; Wallace *et al.*, 1963) and that the rise in blood pressure observed by Mandal *et al.* (1990) was due to an increase in vascular resistance. Further, in the present experiments, no increase in left ventricular dP/dt max was observed in response to i.v. DOI, at a dose previously demonstrated to cause increase in activity in cardiac sympathetic nerves, particularly the left inferior cardiac nerve (McCall & Harris, 1988; Ramage *et al.*, 1993). Since there was no change in heart rate this indicates that these cardiac sympathetic nerves must also be innervating other structures, such as the pulmonary, bronchial and/or coronary vasculature. This is an especially important observation, in that it again confirms that changes in the function of a specific organ cannot be attributed to alteration in the activity of a particular sympathetic nerve unless the end-organ response is also measured. However, the present experiments do demonstrate that, at the level of the ventral surface of the brainstem, pathways controlling hindlimb vascular resistance and those that control the sympathetic outflow to the left ventricle of the heart differ in their response to activation of central 5-HT$_2$ receptors.

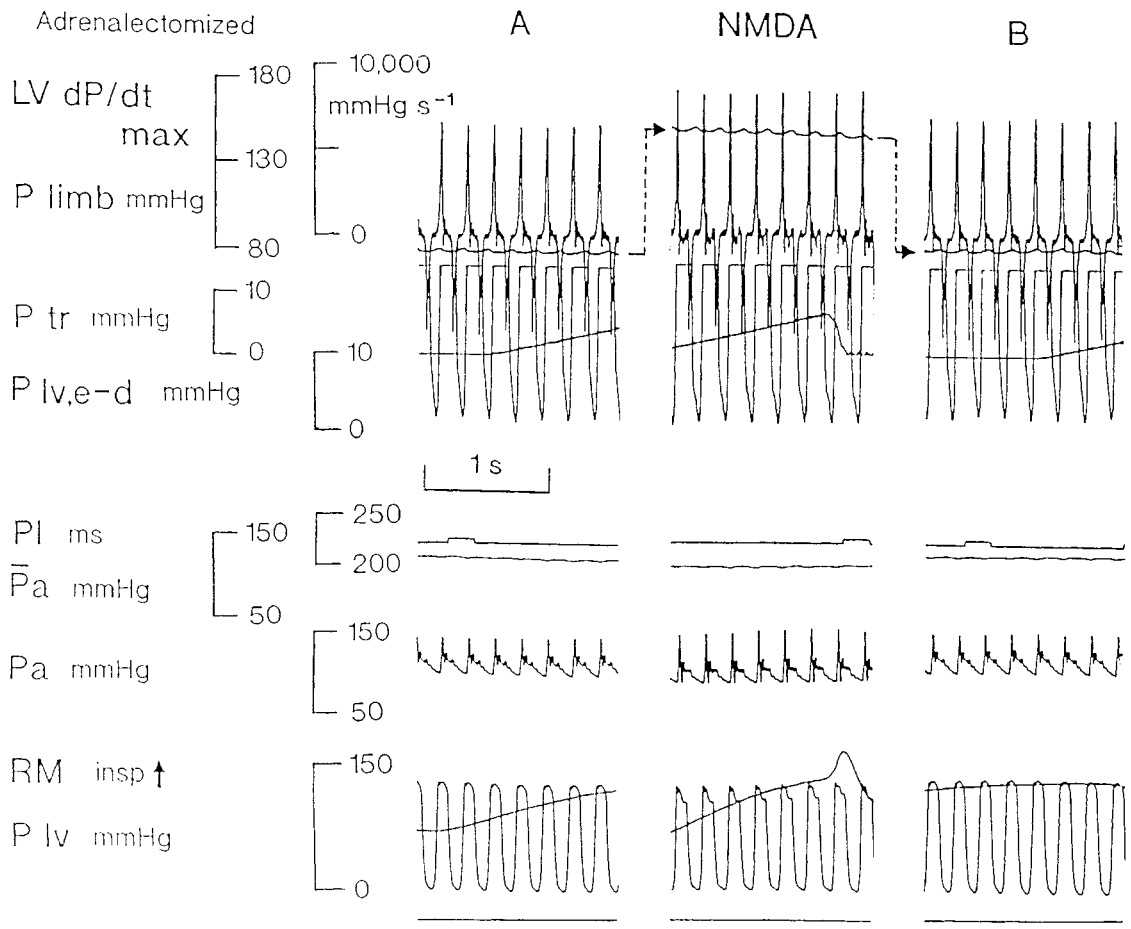

Figure 4 Artificially ventilated anaesthetized cat: traces showing the effects of topical application to the 'glycine-sensitive area' of the ventrolateral medulla of NMDA, 10 μg each side, in which the adrenal glands had been ligated. The heart was electrically paced and the mean arterial blood pressure was maintained constant. Control state panel A was taken 1 min before the application of NMDA. Panel NMDA was taken 2 min into the test, panel B 5 min after rinsing the ventral medulla with sodium chloride solution (154 mM). Records from above downwards: LV (left ventricular) dP/dt max; P-limb, hindlimb mean perfusion pressure; Ptr, tracheal pressure; P lv, e-d, left ventricular end-diastolic pressure; PI, pulse interval; Pa, $\bar{P}$a, phasic and mean arterial blood pressure; RM, respiratory movements (inspiration upwards); P lv, left ventricular pressure. Time calibration, 1 s. For clarity, the arrows show the changes in the positions of the P limb trace between panels.

The question arises as to why DOI, applied to the 'glycine sensitive area' of the ventral surface, causes an increase in hindlimb perfusion pressure but has no effect on the inotropic state of the ventricle. It may be because DOI spreads to different areas of the ventral surface and to the spinal cord affecting neurons other than the sympathetic premotor neurons located beneath the 'glycine sensitive area' (Dampney, 1994), which might prevent DOI causing an increase in inotropy. It is unlikely that neurons in the spinal cord are being affected, as DOI applied outside the glycine-sensitive area was without effect. It cannot be assumed, however, that the drugs are not affecting these other neurons within and around the brain area underneath the pledgets. Thus whether the effects of DOI are the result of direct or indirect action on the premotor sympathetic neurons remains to be determined, but they must be a result of an action indirectly/directly on neurons within and around the 'glycine sensitive area'. Further, application of the excitatory amino acid NMDA to this area, which would activate the NMDA subtype of the glutamate receptor on or in the vicinity of neurons near the site of application, causes vasoconstriction in the hindlimb confirming that neurons in this area are involved in the control of sympathetic activity to the vasculature (Dampney, 1994). NMDA also increased the inotropic state of the left ventricle. As blood pressure and heart rate were kept constant and there was no associated change in left ventricular end-diastolic pressure, the observed increase in the inotropic state, of the left ventricle in response to topical application of NMDA must be a consequence of an increase in sympathetic drive and/or release of adrenaline and noradrenaline. The ability of NMDA to cause a positive inotropic response in animals in which both adrenals had been ligated, demonstrates for the first time, that sympathetic premotor neurons and/or their antecedents in this area also control the sympathetic supply to the cardiac ventricles.

Furthermore, it follows that, as blood pressure was maintained constant, the cardiovascular changes observed in the present experiments are the effect of excitation of these neurons alone without the interference of any feedback systems due to changes in blood pressure evoked by activation of these central neurons, i.e. baroreceptor-mediated sympatho-inhibition. Therefore the failure of DOI to evoke any inotropic effect is not because this area is devoid of neurons which are involved in the control of the sympathetic supply to the left ventricle, but rather to an inability of DOI to excite these neurons. This could be due to DOI failing to gain access to

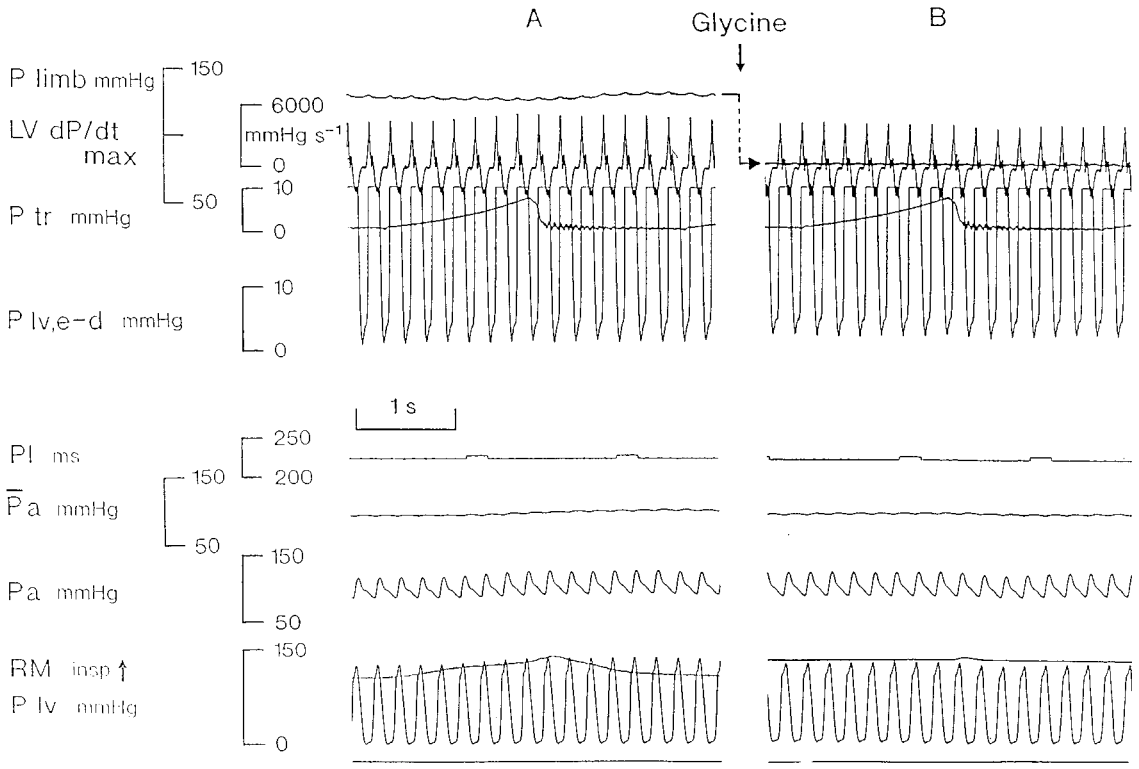

Figure 5 Artificially ventilated anaesthetized cat: traces showing the effects of topical application to the 'glycine-sensitive area' of the ventrolateral medulla of glycine, 500 μg each side, after previous application of DOI and NMDA. The heart was electrically paced and the mean arterial blood pressure was maintained constant. Control panel A was taken 1 min before the application of glycine, panel B 3 min into the test. Records from above downwards: P-limb, hindlimb mean perfusion pressure; LV (left ventricular) dP/dt max; Ptr, tracheal pressure; P lv, e-d, left ventricular end-diastolic pressure; PI, pulse interval; Pa, $\overline{\text{P}}$a, phasic and mean arterial blood pressure; RM, respiratory movements; P lv, left ventricular pressure. Time calibration, 1 s. For clarity, the change in position of the P limb trace between panels A and B is shown by the arrow.

these neurons. In this respect, in the present experiments DOI applied to the surface of the RVLM significantly reduced the increase in left ventricle dP/dt max evoked by NMDA. This observation suggests that the inability of DOI to increase the inotropic state of the left ventricle is not due to lack of accessibility to these neurons. Further, Mandal *et al.* (1990) have shown that injection of DOI into the subretrofacial nucleus gives a similar effect, a rise in blood pressure associated with no change in heart rate, to that observed with application of DOI to the 'glycine sensitive area'. This would be consistent with the view that the neurons DOI is accessing when applied to this area are found in the subretrofacial nucleus. It has been shown that there is considerable overlap in the positions of sympathetic premotor neurons in the subretrofacial nucleus controlling inferior cardiac nerve activity with those controlling hindlimb skeletal muscle sympathetic outflow (Campos & McAllen, 1997). This supports the view that the difference observed in the present experiments is not due to lack of accessibility of DOI to the neurons controlling left ventricular inotropic state.

Interestingly, analysis of the published data does provide evidence that DOI affects cardiac premotor neurons in this area, at least those controlling chronotropy, albeit not by excitation. If DOI did not affect chronotropic sympathetic premotor neurons, then a sympatho-withdrawal mediated bradycardia should be observed in response to the rise in blood pressure caused by activation of vascular sympathetic premotor neurons. Furthermore, this rise in blood pressure would also be expected to cause a vagally-mediated as well as a sympatho-withdrawal mediated bradycardia. Previous work has shown that the application of DOI to the RVLM or to the subretrofacial nucleus, although increasing blood pressure, had no effect on heart rate (King & Holtman, 1990) even after vagotomy (Mandal *et al.*, 1990). Thus the published data indicate that DOI can exert an effect on chronotropic sympathetic premotor neurons by interfering with the baroreceptor-mediated inhibitory input to these neurons. Further the RVLM is also known to contain sympathetic premotor neurons that control the sympathetic supply to the adrenal medulla involved in the release of adrenaline (McAllen, 1986). Estimations from the data provided by Furnival *et al.* (1971) and McAllen (1986) indicate that activation of neurons within the subretrofacial nucleus causes the release of enough adrenaline and noradrenaline to activate cardiac β-adrenoceptors. Consequently, the failure of DOI to affect left ventricular dP/dt max in the present experiments also suggests that DOI does not cause excitation of sympathetic premotor neurons controlling the release of adrenaline from the adrenal medulla. However, activation of 5-HT$_2$ receptors in the RVLM is known to evoke an increase in splanchnic (Vayssettes-Courchay *et al.*, 1991) and renal (Vayssettes-Courchay *et al.*, 1992) sympathetic nerve activity. The combined data indicate, therefore, that sympathetic premotor neurons controlling cardiac chronotropic effects, the inotropic effects on the left ventricle and the release of adrenaline/noradrenaline from the adrenal medulla differ from those controlling the vasculature in their response to the 5-HT$_2$ receptor agonist DOI.

The application of DOI to the ventral surface of the medulla (King & Holtman, 1990), and to the fourth ventricle (Shepheard *et al*., 1991), and the i.v. administration of this agonist (unpublished data) all cause a decrease in respiratory rate as measured by the phrenic nerve electroneurogram. However, this effect was not observed in the present experiments as DOI failed to cause any overall change in respiration, while NMDA, on the other hand, only increased the amplitude of respiratory movements. This may be due to the difference in experimental conditions in the present experiments, the chest being open, and/or to the blood pressure being maintained constant. It would seem unlikely that this difference is due to the different methods of assessing respiratory activity in the two groups of experiments.

In the present experiments, it was found that the i.v. administration of the peripheral 5-HT$_2$ receptor antagonist BW501C67 caused consistent transient positive inotropic responses and increases in hindlimb perfusion pressure. This is the first report of any cardiovascular effects of this antagonist. It is very doubtful that it is a consequence of blockade of 5-HT$_2$ receptors as this would be expected to cause a decrease in hindlimb perfusion pressure and also, probably a negative inotropic effect. The mechanism underlying this action of BW501C67 remains to be determined.

In conclusion, the results of the present experiments taken together with those of other studies indicate that sympathetic premotor neurons in the RVLM involved in cardiac regulation, and possibly adrenaline/noradrenaline release, differ from those involved in the regulation of the hindlimb vasculature, in that activation of 5-HT$_2$ receptors directly/indirectly causes excitation of vascular but not left ventricular inotropic or adrenaline/noradrenaline-releasing sympathetic premotor neurons. The mechanism for this difference, compared with sympathetic premotor neurons controlling vascular sympathetic outflows, remains to be elucidated.

We wish to express our thanks to Miss T. J. Lewis and Mr S. Wilkinson for expert technical assistance. The work was supported by a grant from the British Heart Foundation to M.deB.D.

References

CAMPOS, R.R. & MCALLEN, R.M. (1997). Cardiac sympathetic premotor neurones. *Am. J. Physiol.,* **272,** R615 – R620.

DALY, M. DE B. & KIRKMAN, E. (1988). Cardiovascular responses to stimulation of pulmonary C fibres in the cat: their modulation by changes in respiration. *J. Physiol.,* **402,** 43 – 63.

DALY, M. DE B., WARD, J. & WOOD, L.M. (1993). Effects of distension of the urinary bladder on the cardiovascular reflexes from carotid baroreceptors in the dog. *J. Physiol.,* **463,** 545 – 564.

DAMPNEY, R.A.L. (1994). The subretrofacial vasomotor nucleus; anatomical, chemical and pharmacology properties and role in cardiovascular regulation. *Prog. Neurobiol.,* **42,** 197 – 227.

DAMPNEY, R.A.L. & MCALLEN, R.M. (1988). Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. *J. Physiol.,* **395,** 41 – 56.

FELDBERG, W. & GUERTZENSTEIN, P.G. (1976). Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. *J. Physiol.,* **258,** 337 – 355.

FRY, D.L. (1960). Physiological recording by modern instruments with particular reference to pressure recording. *Physiol. Rev.,* **40,** 753 – 788.

FURNIVAL, C.M., LINDEN, R.J. & SNOW, H.M. (1971). The inotropic and chronotropic effects of catecholamines on the dog heart. *J. Physiol.,* **214,** 15 – 28.

GUERTZENSTEIN, P.G. (1973). Blood pressure effects obtained by drugs applied to the ventral surface of the brain stem. *J. Physiol.,* **229,** 395 – 408.

GUERTZENSTEIN, P.G. & SILVER, A. (1974). Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *J. Physiol.,* **242,** 489 – 503.

KING, K.A. & HOLTMAN, J.R. (1990). Characterisation of the effects of activation of ventral medullary serotonin receptor subtypes on cardiovascular activity and respiratory motor outflows to the diaphragm and larynx. *J. Pharmacol. Exp. Ther.,* **252,** 665 – 674.

LOVICK, T.A. (1987). Differential control of cardiac and vasomotor activity by neurones in nucleus paragigantocellularis lateralis in the cat. *J. Physiol.,* **389,** 23 – 35.

MANDAL, A.K., KELLAR, K.J. NORMAN, W.P. & GILLIS, R.A. (1990). Stimulation of serotonin$_2$ receptors in the ventrolateral medulla of the cat results in non-uniform increases in sympathetic outflow. *Circ. Res.,* **67,** 1267 – 1280.

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.

MCALLEN, R.M. (1986). Action and specificity of ventral medullary vasopressor neurones in the cat. *Neurosci.,* **18,** 51 – 59.

MCALLEN, R.M. & MAY, C.N. (1994). Differential drives from the rostral ventrolateral medullary neurons to three identified sympathetic outflows. *Am. J. Physiol.,* **267,** R935 – R944.

MCCALL, R.B. & HARRIS, L.T. (1988). 5-HT$_2$ receptor agonists increase spontaneous sympathetic nerve discharge. *Eur. J. Pharmacol.,* **151,** 113 – 116.

MCCALL, R.B. & CLEMENT, M.E. (1994). Role of serotonin$_{1A}$ and serotonin$_2$ receptors in the central regulation of the cardiovascular system. *Pharmacol Rev.,* **46,** 231 – 243.

RAMAGE, A.G. & DALY, M. DE B. (1997). Central effects of the 5-HT$_2$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and NMDA on left ventricular dP/dt max in anaesthetized cats. *Br. J. Pharmacol.,* **120,** 27P.

RAMAGE, A.G., SHEPHEARD, S.L., JORDAN, D. & KOSS, M.C. (1993). Can the 5-HT$_{2/1C}$ agonist DOI cause differential sympathoexcitation in nerves supplying the heart in anaesthetized cats? *J. Auton. Nerv. Syst.,* **42,** 53 – 62.

SHEPHEARD, S.L., JORDAN, D. & RAMAGE, A.G. (1991). Investigation of the effects of IVth ventricular administration of the 5-HT$_2$ agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on autonomic outflow in the anaesthetized cat. *Br. J. Pharmacol.,* **104,** 367 – 372.

SCHLÄFKE, M. & LOESCHKE, H.H. (1967). Lokalisation eines an de Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen oberfläche der Medulla oblongata durch Kälterblockade. *Pflüg. Arch.,* **297,** 201 – 220.

VAYSSETTES-COURCHAY, C.V., BOUYSSET, F., VERBEUREN, T.J., LAUBIE, M. & SCHMITT, H. (1991). Quipazine-induced hypertension in anaesthetized cats is mediated by central and peripheral 5-HT$_2$ receptors: role of the ventrolateral pressor area. *Eur. J. Pharmacol.,* **192,** 389 – 395.

VAYSSETTES-COURCHAY, C.V., BOUYSSET, F., VERBEUREN, T.J., SCHMITT, H. & LAUBIE, M. (1992). Cardiovascular effects of microinjections of quipazine-into nuclei of the medulla oblongata in anaesthetized cats: comparison with L-glutamate. *Eur. J. Pharmacol.,* **211,** 243 – 250.

VON ANREP, G. (1912). On the part played by the suprarenals in the normal vascular reactions of the body. *J. Physiol.,* **45,** 307 – 317.

WALLACE, A.G., SKINNER JR N.S. & MITCHELL, J.H. (1963). Hemodynamic determinants of the maximal rate of rise of left ventricular pressure. *Am. J. Physiol.,* **205,** 30 – 36.

(Received May 20, 1998
Revised August 15, 1998
Accepted August 20, 1998)