Br. J. Pharmacol. (1991), **104**, 466–470

Microinjections of 5-HT_{1A} agonists into the dorsal motor vagal nucleus produce a bradycardia in the atenolol-pretreated anaesthetized rat

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1 The effects of microinjections (100 nl) into the dorsal motor vagal nucleus of the 5-HT_{1A} receptor agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and flesinoxan, the 5-HT_2 receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), the 5-HT_3 receptor agonist phenylbiguanide (PBG), the α_2-adrenoceptor agonist clonidine and the excitatory amino acid glutamate on heart rate, blood pressure, tracheal pressure and phrenic nerve activity were investigated in atenolol-pretreated rats anaesthetized with sodium pentobarbitone.

2 Microinjections of glutamate (2.5 nmol) caused decreases in blood pressure, heart rate and phrenic nerve activity. In contrast, microinjections of 5-HT (1.2 nmol), 8-OH-DPAT (1.2 nmol) and flesinoxan (1.3 nmol) all caused a bradycardia but had no effect on blood pressure. In addition, 8-OH-DPAT and flesinoxan caused an increase in phrenic nerve activity.

3 Microinjections of DOI, PBG and clonidine had no significant effect on any of the variables recorded. None of the drugs used had any significant effect on tracheal pressure.

4 These results support the hypothesis that activation of 5-HT_{1A} receptors causes excitation of cardiac vagal motoneurones and suggest that these receptors are also important in the control of central respiratory drive.

Keywords: Cardiac vagal motoneurones; phrenic nerve activity; 5-HT_{1A} receptors; 8-OH-DPAT; flesinoxan; brainstem microinjections; anaesthetized rats

Introduction

It has previously been demonstrated that intravenous injections of the 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor agonists, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), ipsapirone and flesinoxan in the anaesthetized cat cause a large increase in central vagal drive to the heart (Ramage & Fozard, 1987; Ramage *et al.*, 1988). This led to the suggestion that central 5-HT pathways, via 5-HT_{1A} receptors, are involved in the control of vagal tone to the heart. In the cat, the cardiac vagal motoneurones (CVMs) are located mainly in the nucleus ambiguus (see Hopkins, 1987) and microinjection of 8-OH-DPAT into this nucleus produces a bradycardia (Izzo *et al.*, 1988). In addition, autoradiographic studies have shown 8-OH-DPAT binding sites in the nucleus ambiguus (Dashwood *et al.*, 1988) and immunohistochemical studies have demonstrated that 5-HT-immunoreactive boutons make synaptic contact with CVMs in this nucleus (Izzo *et al.*, 1988). Together, these observations strongly support the view that in the cat, there is an excitatory 5-HT-containing pathway that innervates cardiac vagal motoneurones and involves the activation of 5-HT_{1A} receptors.

There is similar evidence for such a pathway in the rat. 5-HT immunoreactivity has been demonstrated in the nucleus ambiguus and the dorsal motor vagal nucleus (DMVN) (Steinbusch, 1981). Both regions contain CVMs in this species (Nosaka *et al.*, 1982) and have been demonstrated to contain 5-HT_{1A} binding sites (Pazos & Palacios, 1985; Manaker & Verderame, 1990). Both intravenous administration of 5-HT_{1A} receptor agonists (Gradin *et al.*, 1985; Cherqui *et al.*, 1988) and intracerebroventricular administration of 5-HT (Dalton, 1986) produces a bradycardia which has been shown to be partly due to an increase in central vagal drive. However, Wolf *et al.* (1981) demonstrated variable changes in heart rate when 5-HT was microinjected into the NTS/DMVN region.

Thus the aim of the present study was to determine by microinjecting the selective and equipotent 5-HT_{1A} receptor agonists 8-OH-DPAT (Middlemiss & Fozard, 1983) and flesinoxan (Wouters *et al.*, 1988; Wijngaarden *et al.*, 1990) into the DMVN whether 5-HT acting via 5-HT_{1A} receptors plays a role in the control of CVMs in the rat. Excitation of CVMs was assessed by measuring changes in heart rate in atenolol-pretreated anaesthetized rats. Atenolol was chosen to block the effects of any changes in sympathetic drive to the heart caused by microinjections of these agonists, as it is a β-adrenoceptor antagonist which does not bind to 5-HT receptors (Middlemiss *et al.*, 1977) and poorly penetrates the central nervous system (Street *et al.*, 1979). Furthermore, as 5-HT_2 (Pazos *et al.*, 1985), 5-HT_3 (See Pratt *et al.*, 1990) and α_2-adrenoceptor (Unnerstall *et al.*, 1984) binding sites have also been demonstrated in the region of the DMVN in the rat, the effects of microinjections of selective agonists at these receptors were also investigated. Finally, the effects of microinjection of these agonists on phrenic nerve activity, a measure of central inspiratory drive, were also investigated since this drive is known to modulate cardiovascular responses and the excitability of CVMs (see Daly, 1986) and 5-HT pathways have been demonstrated to be involved in central respiratory control (Holtman *et al.*, 1986; 1990).

A preliminary account of some of these observations has been given (Sporton *et al.*, 1989).

Methods

Experiments were performed on male Sprague-Dawley rats (250–385 g) anaesthetized with pentobarbitone sodium (75–90 mg kg^{-1}, i.p.). A tracheotomy was performed and both femoral arteries and veins were cannulated. Blood pressure was measured from the left femoral artery by means of a pressure transducer (Gould Statham P23XL) and heart rate was derived electronically from the blood pressure signal. Mean arterial pressure was calculated by adding one third of the pulse pressure to the diastolic pressure. Tracheal pressure was also measured. The animals were artificially ventilated (rate 50 min^{-1}, volume 3–5 ml) with oxygen enriched room air by use of a positive pressure pump and neuromuscular blockade

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was produced with decamethonium iodide (1 mg per animal). Blood samples were taken from the right femoral artery and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were maintained between 90–130 mmHg Po_2, 40–50 mmHg Pco_2 and pH 7.3–7.4. Slow i.v. injections of sodium bicarbonate (1.0 M) or adjustments of the respiratory pump were made as necessary to maintain pH and blood gases within this range. The Pco_2 was kept high in order to produce activity in the phrenic nerve. Once ventilated, the animals were infused (6 ml kg^{-1} h^{-1}) via a femoral vein with a solution containing 50 ml of a plasma substitute (Gelofusine), glucose (2 mg ml^{-1}), $NaHCO_3$ (8.4 mg ml^{-1}), decamethonium iodide (1.5 mg ml^{-1}) and pentobarbitone sodium (3 mg ml^{-1}) made up to a total volume of 100 ml with distilled water. This was to counteract the development of non-respiratory acidosis and to maintain blood volume, anaesthesia and neuromuscular blockade. The other femoral vein was used for the i.v. administration of drugs.

Recordings of phrenic nerve activity

The phrenic nerve was exposed on the left side by retracting the shoulder blade. The nerve was cut or crushed and the central end was placed on bipolar silver hook electrodes for the recording of phrenic nerve activity (PNA). PNA was quantified by counting in 5 s periods the number of events above the noise with a spike processor (Digitimer D 130). In all experiments the bursts of phrenic nerve activity were synchronised with the chest movements caused by the respiratory pump.

Microinjection into the dorsal motor vagal nucleus (DMVN)

The animals were placed in a stereotaxic frame and the dorsal surface of the brainstem was exposed. Microinjections (100 nl) were made into the DMVN on the right or the left sides of the brainstem with five barrelled glass microelectrodes, tip diameter 25–35 μm. Electrode penetrations were made 0–1 mm caudal to obex, 0.1–0.5 mm lateral to the midline and to a depth of 600–1000 μm below the dorsal surface of the brainstem. The electrode tip was judged to be in the DMVN if an instantaneous bradycardia was elicited by microinjection of glutamate. In most animals injection sites were marked with a 100 nl injection of pontamine sky blue dye (2% in 0.15 M sodium chloride (0.9%) solution). At the end of the experiment the brainstem was removed and fixed in a solution of 10% formalin and 1% glutaraldehyde in saline. 50 μm sections were cut and stained with neutral red so that the position of the injection site could be verified histologically.

Drug solutions used in the microelectrode barrels were:- artificial CSF, saline, glutamate (4.3 mg ml^{-1}), 5-HT creatine sulphate (5.0 mg ml^{-1}), 8-OH-DPAT (3.0 mg ml^{-1}), flesinoxan (6.0 mg ml^{-1}), DOI HCl (4.4 mg ml^{-1}), PBG (2.2 mg ml^{-1}) and clonidine HCl (0.5 mg ml^{-1}). Glutamate, 5-HT, and 8-OH-DPAT were dissolved in artificial CSF whereas flesinoxan, DOI, PBG and clonidine were dissolved in saline. In each case control injections were performed with the appropriate vehicle. Drug solutions were adjusted to pH 7.4 where possible, however when using flesinoxan, DOI or PBG, solutions were at pH 4.2–5.6.

Statistical analysis

Measurements of all variables where made just prior to the microinjection of the test substance and then when the fall in heart rate was maximum. For all variables, statistical analysis was performed to compare values before and after the microinjection of drugs by use of Student's paired t test. Values were considered significant at $P < 0.05$.

Drugs

The following drugs were purchased from the companies indicated in parentheses; sodium pentobarbitone and decamethonium HBr (May & Baker Ltd); Gelofusine (Consolidated Chemicals Ltd); L-glutamate and 5-hydroxytryptamine creatine sulphate (Sigma), 8-hydroxy-2-(di-n-propylamino) tetralin HBr (8-OH-DPAT); (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride, (DOI HCl) (Semat Tech., U.K.) and phenylbiguanide (K K Labs. U.S.A.). The following compounds were kindly donated by the companies indicated in parentheses; flesinoxan (Duphar B.V.) and clonidine HCl (Boehringer Ingelheim). All drug concentrations are the salts of the drug except for 8-OH-DPAT and flesinoxan.

Results

Baseline values

Baseline values for heart rate, mean arterial blood pressure and phrenic nerve activity are given in Table 1. Microinjections of vehicle solutions had no effects on any of these variables. Tracheal pressure data has not been quantified as no drug had any obvious effect on this parameter.

Effects of injections of glutamate into the dorsal motor vagal nucleus DMVN

Microinjections of glutamate (2.5 nmol, $n = 26$) into the region of the dorsal motor vagal nucleus caused significant mean decreases in heart rate of -21 ± 3 beats min^{-1}, blood pressure of -32 ± 2 mmHg and phrenic nerve activity of -54 ± 8% (Table 1). An example of the effects of an injection of glutamate is shown in Figure 1a. The effects of glutamate

Table 1 Baseline values and changes (mean ± s.e.mean) in heart rate (HR, beats min^{-1}), mean arterial blood pressure (BP, mmHg) and phrenic nerve activity (PNA, spikes 5 s^{-1}) following microinjection of drugs into the dorsal motor vagal nucleus

		HR (beats min^{-1})		*BP* (mmHg)		*PNA* (spikes 5 s^{-1})		
	n	*Control*	*Change*	*Control*	*Change*	*Control*	*Change*	(%)
Glutamate	26	319 ± 4	−21 ± 3***	100 ± 2	−32 ± 2***	1187 ± 74	−714 ± 104***	(−54 ± 8%)
5-HT	5	328 ± 4	−6 ± 2*	91 ± 6	0 ± 0	838 ± 81	−26 ± 49	(−3 ± 6%)
8-OH-DPAT	14	324 ± 7	−12 ± 1***	100 ± 2	0 ± 0	1048 ± 123	+169 ± 37**	(+20 ± 5%)
Flesinoxan	6	329 ± 4	−10 ± 1***	98 ± 3	−3 ± 2	923 ± 171	+261 ± 49**	(+30 ± 6%)
DOI	7	316 ± 8	+2 ± 1	99 ± 3	−2 ± 1	1611 ± 126	−11 ± 26	(−1 ± 2%)
PBG	5	308 ± 6	+1 ± 2	97 ± 3	0 ± 0	1296 ± 334	−20 ± 18	(−3 ± 3%)
Clonidine	6	334 ± 8	0 ± 1	102 ± 4	−1 ± 1	1453 ± 81	+7 ± 28	(+1 ± 2%)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
8-OH-DPAT = 8-hydroxy-2-(di-n-propylamino)tetralin; DOI = (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl; PBG = phenylbiguanide.

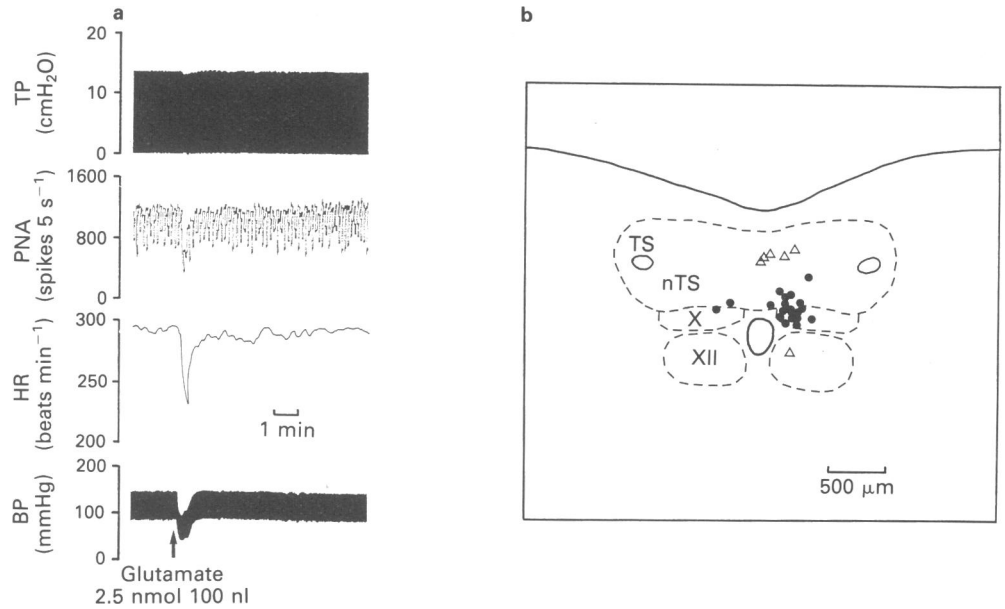

Figure 1 (a) The effects of a microinjection (2.5 nmol, 100 nl) of glutamate into the dorsal motor vagal nucleus on tracheal pressure (TP, cmH_2O), phrenic nerve activity (PNA, spikes $5\,s^{-1}$), heart rate (HR, beats min^{-1}) and blood pressure (BP, mmHg). (b) Diagram showing the position of glutamate injection sites that were successfully labelled on a representative transverse section through the brainstem at a level just caudal to obex. TS = tractus solitarius, nTS = nucleus tractus solitarius, X = dorsal motor vagal nucleus, XII = hypoglossal nucleus. (●) positive glutamate injection sites, (△) negative glutamate injection sites.

were rapid in onset, the mean time to the maximum bradycardia was 16 ± 3 s, and were of short duration (30–60 s). Later histological verification of the injection sites showed that, for sites that were successfully labelled, nearly all were in, or close to, the dorsal motor vagal nucleus (Figure 1b). The effects of glutamate described above were termed a 'positive response' and subsequent injection sites were estimated to be in the region of the DMVN if a positive glutamate response could be obtained. Injections of glutamate ($n = 6$) in areas outside of the DMVN (Figure 1b) had insignificant effects on heart rate, blood pressure and phrenic nerve activity.

Effects of injections of 5-hydroxytryptamine into the dorsal motor vagal nucleus

Microinjections of 5-HT (1.2 nmol, $n = 5$) into the region of the dorsal motor vagal nucleus caused a small but significant

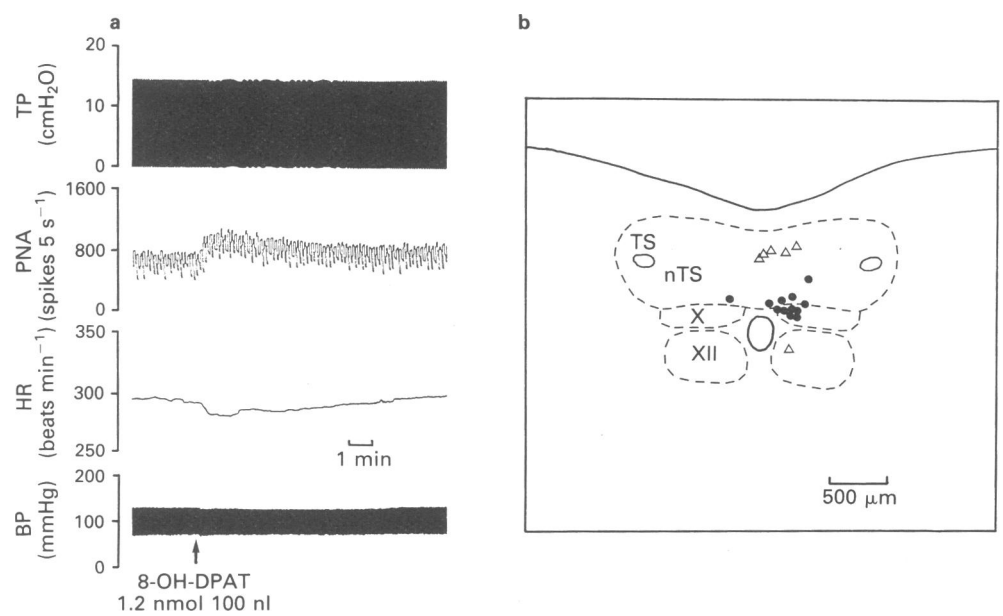

Figure 2 (a) The effects of a microinjection (1.2 nmol, 100 nl) of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) into the dorsal motor vagal nucleus on tracheal pressure (TP, cmH_2O), phrenic nerve activity (PNA, spikes $5\,s^{-1}$), heart rate (HR, beats min^{-1}) and blood pressure (BP, mmHg). (b) Diagram showing the position of 8-OH-DPAT injection sites that were successfully labelled on a representative transverse section through the brainstem at a level just caudal to obex. TS = tractus solitarius, nTS = nucleus tractus solitarius, X = dorsal motor vagal nucleus, XII = hypoglossal nucleus. (●) positive 8-OH-DPAT injections sites, (△) negative 8-OH-DPAT injection sites.

decrease in mean heart rate of -6 ± 2 beats min^{-1} but had no effects on blood pressure or phrenic nerve activity (Table 1). This decrease in heart rate was of long duration; time to the maximum bradycardia was 8.0 ± 0.5 min.

Effects of injections of 8-OH-DPAT and flesinoxan into the dorsal motor vagal nucleus

Microinjections of 8-OH-DPAT (1.2 nmol, $n = 14$) and flesinoxan (1.3 nmol, $n = 6$) into the region of the dorsal motor vagal nucleus caused significant decreases in mean heart rate of -12 ± 1 and -10 ± 1 beats min^{-1} respectively. There were no accompanying effects on blood pressure but there were significant increases in mean phrenic nerve activity of $20 \pm 5\%$ and $30 \pm 6\%$ respectively (Table 1). An example of the effects of injection of 8-OH-DPAT is shown in Figure 2a. For both drugs the effects on heart rate were immediate in onset and mean times to the maximum bradycardia were 6.2 ± 1.0 and 2.0 ± 1.5 min respectively. Since phrenic nerve activity was synchronized with the chest movements caused by the respiratory pump the increase in this activity was seen as an increase in the number of spikes produced during each inspiratory burst, rather than as an increase in inspiratory rate.

Injections ($n = 6$) of 8-OH-DPAT made into areas outside the region of the DMVN (Figure 2b) had no effects on heart rate, blood pressure or phrenic nerve activity. These sites were also those at which glutamate did not produce a positive response (Figure 1b).

Effects of injections of DOI, phenylbiguanide and clonidine into the dorsal motor vagal nucleus

Microinjections of DOI (1.2 nmol, $n = 7$), PBG (1.2 nmol, $n = 5$) and clonidine (0.2 nmol, $n = 6$) into the region of the dorsal motor vagal nucleus had no effects on any of the variables recorded (Table 1). Microinjection of glutamate at these same sites before and following the injection of these compounds produced a positive response. Histology confirmed that these sites were all within or close to the dorsal motor vagal nucleus.

Discussion

These results show that microinjections into the dorsal motor vagal nucleus of glutamate, 5-HT and the structurally unrelated 5-HT$_{1A}$ agonists, 8-OH-DPAT, and flesinoxan produce a decrease in heart rate in the atenolol pretreated rat. In addition, the selective agonists for 5-HT$_2$ and 5-HT$_3$ receptors DOI and phenylbiguanide (see Wijngaarden *et al.*, 1990) respectively, failed to have any effect on heart rate. These results, taken together with previous observations (see introduction) and the ability of 5-HT$_{1A}$ antagonists to block synaptic activation of cardiac vagal motoneurones via cardiopulmonary afferents (Bogle *et al.*, 1990), strongly support the hypothesis that 5-HT causes increased activity in cardiac vagal motoneurones by an action on 5-HT$_{1A}$ receptors. Although both 8-OH-DPAT and flesinoxan are known to be highly selective for 5-HT$_{1A}$ receptors (Middlemiss & Fozard, 1983; Wouters *et al.*, 1988) at increased concentrations all drugs tend to lose their selectivity. Evidence from binding studies suggests that at higher concentrations, 8-OH-DPAT may have some action on α_2-adrenoceptors (Wijngaarden *et al.*, 1990) and these receptors are known to be present in the region of the DMVN (Unnerstall *et al.*, 1984). However, flesinoxan has no affinity for α_2-adrenoceptors (Wouters *et al.*, 1988; Wijngaarden *et al.*, 1990) but had an identical effect to 8-OH-DPAT in the present experiments indicating that it is highly likely that these drugs are causing the above action by activating 5-HT$_{1A}$ receptors.

The precise anatomical site at which these 5-HT$_{1A}$ receptors are located is more difficult to assess. Although the injection sites were either in the DMVN, or were very close to the DMVN in the commissural region of the nucleus tractus solitarius (NTS), it would seem likely that the injected drugs may have spread to, and possibly had their actions at, sites surrounding the DMVN. An indication of the spread of these drugs can be assessed by the size of the dye spots produced by 100 nl injections of pontamine sky blue though the tissue volume receiving an effective concentration of drug cannot be determined. However, these recovered dye spots were all fairly compact, usually only up to 100 μm in diameter. Injections of 8-OH-DPAT into the hypoglossal nucleus and the dorsal part of the NTS had no effects on heart rate but injections into the NTS/DMVN border were effective (see Figure 2). Histological studies in rats have shown that vagal motoneurones in the DMVN which innervate the stomach have extensive dendritic fields which spread into the medial and commissural NTS (Shapiro & Miselis, 1985). If cardiac vagal motoneurones have a similar morphology then it is possible that injections into these regions of the NTS may still directly activate cardiac vagal motoneurones if there are 5-HT$_{1A}$ receptors on the dendrites of these motoneurones. However, the present results do not rule out the possibility that the effects of 5-HT$_{1A}$ agonists are mediated via local interneurones. In this respect Manaker & Verderame (1990) have shown 5-HT$_{1A}$ binding in the NTS as well as the DMVN of the rat.

These results also show that microinjections of 8-OH-DPAT and flesinoxan into the DMVN caused an increase in phrenic nerve activity suggesting that activation of 5-HT$_{1A}$ receptors in the DMVN results in an increase in central respiratory drive. In the rat, the ventral and ventrolateral subnuclei of the NTS are involved in respiratory control (see Feldman, 1986). Electrical stimulation of the ventral regions of the caudal NTS in the rat have been shown to produce hypopneas (Barraco & El-Ridi, 1989) similar to those produced by injections of glutamate in the present study. However, 8-OH-DPAT and flesinoxan both produced an increase in central respiratory drive. In this respect, in the cat (Holtman *et al.*, 1986) and the rat (Dreteler *et al.*, 1991), chemical stimulation of the raphe obscurus causes a similar increase in phrenic nerve activity and raphe neurones containing 5-HT are known to project to the NTS (Schaffar *et al.*, 1988; Holtman *et al.*, 1990). The present results would suggest that these 5-HT pathways activate 5-HT$_{1A}$ receptors to cause an increase in central inspiratory drive. The failure of 5-HT to have any effect on phrenic nerve activity may be due to the fact that the 5-HT is unable to spread to the respiratory regions of the NTS due to rapid neuronal uptake.

DOI and PGB failed to have any effect when given at the same molar concentrations as 8-OH-DPAT and flesinoxan. Clonidine, at the concentration used, is known to cause a sympathetically mediated decrease in heart rate when injected into the NTS (Gurtu *et al.*, 1982). The lack of effects demonstrated in this present study suggests that activation of 5-HT$_2$ and 5-HT$_3$ receptors and α_2-adrenoceptors in the region of DMVN does not result in the activation of CVM's or affect central inspiratory drive.

The present results support the view that 5-HT pathways produce excitation of CVMs and phrenic motoneurones through the activation of 5-HT$_{1A}$ receptors.

This work was supported by the Wellcome Trust. S.C.E.S. was a Wellcome Vacation Scholar. We wish to thank Mr S. Wilkinson for technical assistance.

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(Received April 12, 1991
Revised June 11, 1991
Accepted June 18, 1991)