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Pressor effects following microinjection of 5-HT_{1A} receptor agonists into the raphe obscurus of the anaesthetized rat

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1 The effects of electrical stimulation and microinjections (90 nl) of the 5-HT_{1A} receptor agonists, flesinoxan and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), and glutamate into the raphe obscurus on blood pressure, heart rate and phrenic nerve activity (central inspiratory drive) were investigated in rats anaesthetized with α-chloralose.

2 Electrical stimulation of the raphe obscurus caused a rise in blood pressure which was associated with bradycardia, while glutamate (2.7 nmol) caused only a rise in blood pressure.

3 Flesinoxan (1.3 nmol) and 8-OH-DPAT (0.7 nmol) increased blood pressure by 9 ± 1 and 14 ± 2 mmHg, respectively and did not affect heart rate. For both agonists the effect on blood pressure was shown to be dose-dependent; again no effect on the heart rate was observed over the dose-ranges chosen.

4 Microinjections of the non-selective 5-HT_{1A} receptor antagonists, (±)-pindolol (2.7 nmol) or methiothepin (5.2 nmol), into the raphe obscurus prevented the increase in blood pressure caused by microinjection of flesinoxan. However, (±)-pindolol caused a sustained rise in blood pressure of 15 ± 1 mmHg while methiothepin caused a transient rise in blood pressure. Neither drugs affected heart rate. The ability of methiothepin to attenuate the pressor effect of flesinoxan was found to be partially reversed after 30 min.

5 It is suggested that activation of 5-HT_{1A} receptors within the raphe obscurus can cause sympathoexcitation.

Introduction

The 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor agonists, flesinoxan and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), have been shown to lower arterial blood pressure in rats (Gradin *et al.*, 1985; Martin & Lis, 1985; Fozard *et al.*, 1987; Dreteler *et al.*, 1990), cats (McCall *et al.*, 1987; Ramage & Fozard, 1987; Ramage *et al.*, 1988; Wouters *et al.*, 1988b) and dogs (Laubie *et al.*, 1989) by a central action. In the rat, brain areas that contain 5-HT_{1A} binding sites are the hypothalamus, midbrain raphe nuclei, the nucleus tractus solitarius and medullary raphe nuclei (Pazos & Palacios, 1985; Vergé *et al.*, 1986; Thor *et al.*, 1990). All these areas are known to be involved in the central regulation of blood pressure (Loewy & Neil, 1981; Spyer, 1981). 5-Hydroxytryptaminergic neurones from the nucleus raphe pallidus (B_1) and raphe obscurus (B_2) project directly to the intermediolateral cell column (Dahlström & Fuxe, 1965; Loewy, 1981; Loewy *et al.*, 1981), the main site of origin of the sympathetic preganglionic neurones, which are the final site at which integration of cardiovascular control can occur within the central nervous system (Coote, 1988).

The present study was carried out to determine if the raphe obscurus is involved in the central hypotensive action of flesinoxan and 8-OH-DPAT by observing the effects on blood pressure and heart rate following microinjection of these 5-HT_{1A} receptor agonists into the raphe obscurus. Furthermore, as phrenic motoneurones in the cat have been shown to be surrounded by 5-HT immunoreactive fibres (Holtman *et al.*, 1984) and chemical and electrical stimulation of the raphe obscurus in the cat causes excitation of phrenic motoneurones i.e. increases central inspiratory drive (Holtman *et al.*, 1986), the effect of microinjection into the raphe obscurus of flesinoxan and 8-OH-DPAT was also examined on phrenic nerve activity in the rat.

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A preliminary account of some of these observations has been given (Dreteler, 1990).

Methods

Male Sprague-Dawley rats (250–375 g; Royal Free Hospital School of Medicine, London) were anaesthetized initially with halothane to allow the implantation of cannulae (polythene) into the right femoral artery and vein. Subsequently, α-chloralose (70 mg kg^{-1}) was given intravenously (i.v.), tracheotomy was performed and the left femoral artery and vein were cannulated so that arterial blood gases could be measured and an i.v. infusion could be given. The animals were then placed in a stereotaxic head frame. The dorsal aspect of the brainstem was exposed by retraction of the skin and muscles at the base of the skull followed by removal of the occipito-alantoid membrane and portions of the occipital bone. The animals were artificially ventilated (frequency 80 cycles min^{-1}, tidal volume 3–4 ml) with room air enriched with oxygen following neuromuscular blockade with decamethonium iodide (1 mg per animal, i.v.). Arterial blood gases and pH were monitored throughout the experiment and maintained between 90–130 mmHg for Po_2, 40–50 mmHg for Pco_2 and 7.3–7.4 for pH. Slow i.v. injections of sodium bicarbonate (1.0 M) or adjustments in respiration 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. During the experiment an i.v. infusion (6 ml kg^{-1} h^{-1}) of a solution of 50 ml gelofusine plasma substitute, 50 ml distilled water, 0.2 g glucose, 0.84 g sodium bicarbonate and 150 mg decamethonium iodide was given, to counteract the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade. Rectal temperature was monitored and maintained between 37 and 38°C by means of a homeothermic blanket system. Blood pressure

was recorded from the right femoral artery by means of a pressure transducer (Gould Statham P23XL) and heart rate was electronically derived from the blood pressure signal. Mean arterial blood pressure was calculated by adding one third of the pulse pressure to the diastolic pressure.

Phrenic nerve activity recordings

In animals in which phrenic nerve activity was recorded the nerve was exposed by deflecting the left scapula forward and dissecting the nerve free from the surrounding connective tissue. The nerve was cut and the central end was placed on bipolar silver hook electrodes for the recording of nerve activity. The number of events above the noise level was counted over a 5 s period using a spike processor (Digitimer D 130).

Microinjections into the raphe obscurus

Microinjections were made into the raphe obscurus with 5 barrel glass electrodes having a tip size of 25–35 μm. One barrel was filled with a 1:1 mixture of Wood's metal and Indium for electrical stimulation. Electrical stimuli were produced by a Digitimer DS2 stimulator (40 Hz, 100 μA, 0.5 ms, 5 s). The other four barrels were filled with the experimental drugs and saline. The pH of the saline solution was always adjusted to the pH of the test solutions. The electrode was positioned 1.0–1.5 mm rostral to the obex along the midline and advanced 1.50–1.75 mm down from the brain surface according to Paxinos & Watson (1982). Once in position, 90 nl volumes of test solutions were applied by means of positive pressure injection. In all animals the site of injection was marked by application of Pontamine sky blue (2% in saline) via one of the barrels of the electrode. At the end of each experiment the animals were perfused with fixative (10% formalin; 1% glutaraldehyde solution) and the brain was removed and stored in the fixative until it was sectioned. Brains were sectioned on a freeze microtome (100 μm). The brain sections were mounted and stained with neutral red for histological verification of the injection site.

Experimental protocols

Electrical and chemical stimulation and microinjection of single doses of flesinoxan or 8-OH-DPAT were performed in separate experiments. In experiments used to construct dose-response curves for the blood pressure effects of microinjection of flesinoxan or 8-OH-DPAT into the raphe obscurus, the time between drug injections was 15–30 min, depending on the time needed for blood pressure to return to its basal value. In the antagonist studies, a submaximal dose of flesinoxan (1.3 nmol) was microinjected initially and once the blood pressure had returned to its basal level either (±)-pindolol (2.7 nmol) or methiothepin (5.2 nmol) was microinjected, followed 5 min later by another microinjection of flesinoxan. Injections of flesinoxan were repeated at 10–15 min intervals. To investigate if the submaximal pressor response was reproducible, control experiments were performed. In these experiments only flesinoxan (1.3 nmol) was microinjected at time intervals as described for the antagonist studies.

Drugs

α-Chloralose (Sigma); L-glutamate, (Sigma); flesinoxan ((+)-(R)-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)- 1-piperazinyl]ethyl]-4- fluorobenzamide · HCl, Duphar B.V.); 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin · HBr, Research Biochemicals Inc.) and methiothepin monomethanesulphonate (Jilek *et al.*, 1980; Pharmaceutical Biochem. Research Inst., Prague, Czechoslovakia) were dissolved in saline. (±)-Pindolol (Sandoz) was dissolved in 0.01 N HCl and saline and buffered to pH 7.4.

Statistical analysis

Data are expressed as mean ± s.e.mean. Paired Student's t test was used to estimate the significance between mean values; $P < 0.05$ was considered statistically significant.

Results

Effect of electrical and glutamate stimulation of the raphe obscurus

The baseline values for blood pressure and heart rate as well as the effects of the different treatments on blood pressure, heart rate and phrenic nerve activity are presented in Table 1.

Electrical stimulation of the raphe obscurus caused an immediate rise in mean arterial blood pressure (Figure 1a). This rise in blood pressure (30 ± 6 mmHg) was accompanied by a decrease in heart rate (37 ± 9 beats per minute (b.p.m.)). In two experiments there was a small increase in phrenic nerve activity (< 15%), but in the other experiments there was no effect. To examine the effects of stimulating only the cell bodies in the raphe obscurus and avoiding stimulation of fibres of passage, the excitatory amino acid L-glutamate was microinjected into this nucleus. Injection of L-glutamate (2.7 nmol) into the raphe obscurus (Figure 1b) caused an increase in blood pressure of 22 ± 6 mmHg, had hardly any effect on heart rate and caused an increase in phrenic nerve activity (53 ± 10%). The increase in phrenic nerve activity consisted of an increase in the number of bursts (Figure 1b) and not of an increase in the number of events in each burst. The glutamate effects on blood pressure lasted 1–2 min. Electrical stimulation or chemical stimulation with glutamate outside the raphe obscurus (Figure 1a and b) had no, or slight depressor effects. Injection of 90 nl of saline into the raphe obscurus never affected any of the variables measured.

Effects of microinjection of flesinoxan or 8-OH-DPAT into the raphe obscurus

The baseline values for blood pressure and heart rate and the effects of microinjection of flesinoxan and 8-OH-DPAT into

Table 1 Effects of electrical stimulation (ES) and microinjection of glutamate, flesinoxan, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) or saline into the raphe obscurus of the anaesthetized rat on mean arterial blood pressure (MAP), heart rate (HR) and phrenic nerve activity (PNA)

Treatment	*Dose* (nmol)	n	*Baseline MAP* (mmHg)	*MAP* (mmHg)	*Baseline HR* (b.p.m.)	*HR* b.p.m.	% PNA
ES		7	129 ± 5	30 ± 6*	404 ± 14	−37 ± 9*	NE
Glutamate	2.7	7	98 ± 6	22 ± 6*	410 ± 14	NE	53 ± 10*
Flesinoxan	1.3	9	101 ± 6	9 ± 1*	400 ± 14	NE	NE
8-OH-DPAT	0.7	7	98 ± 7	14 ± 2*	407 ± 19	NE	NE
Saline		6	104 ± 4	NE	407 ± 18	NE	NE

Data are expressed as mean ± s.e.mean. * $P < 0.05$ versus baseline values. NE: no effect.

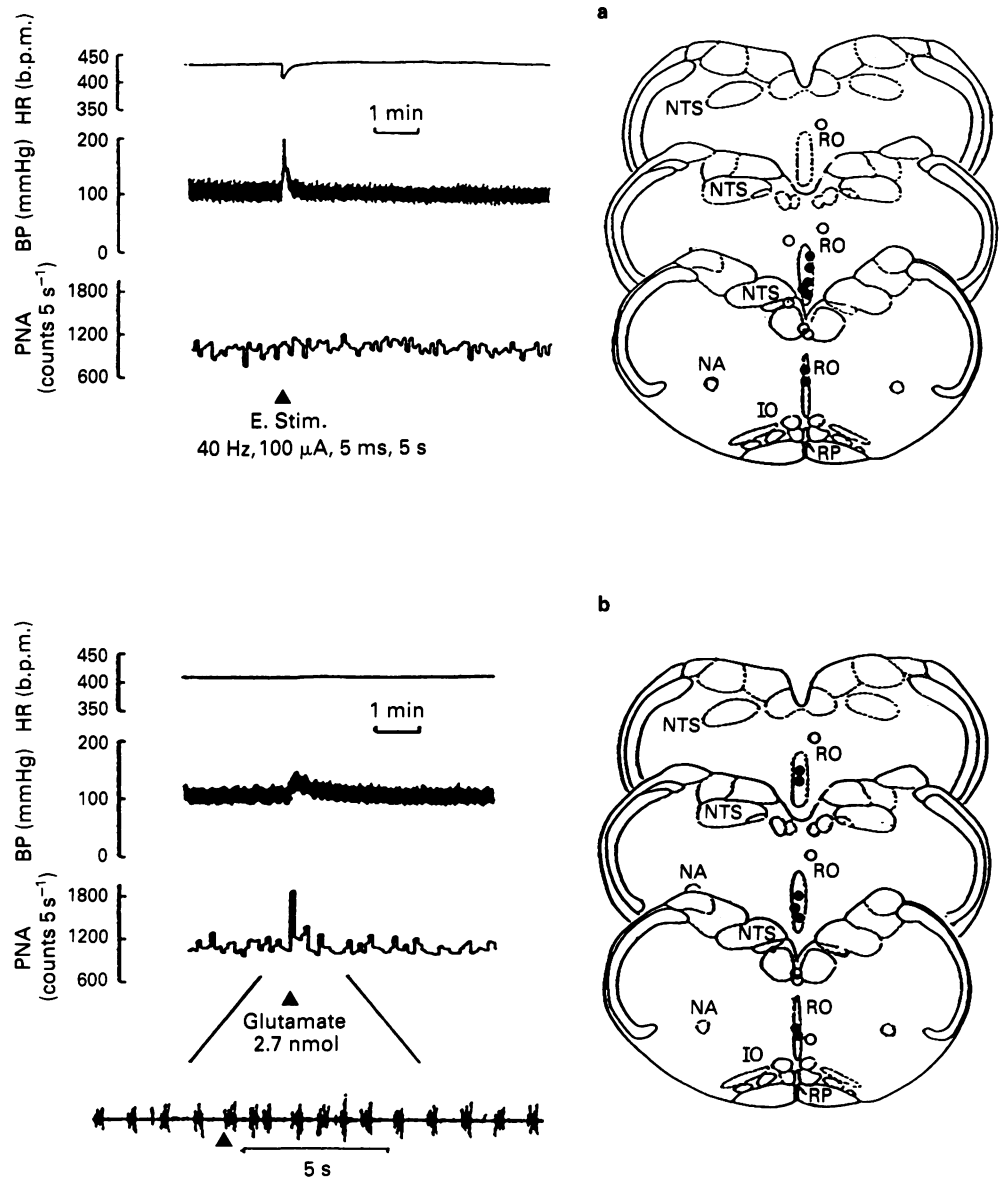

Figure 1 Left: Effect of electrical stimulation (a) and chemical stimulation with glutamate (b) of the raphe obscurus on heart rate (HR), blood pressure (BP) and phrenic nerve activity (PNA). Right: Injection sites in rat brainstem at the level 1.0–2.0 mm rostral to the obex. RO, nucleus raphe obscurus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; RP, nucleus raphe pallidus; IO, inferior olive; (●) injection sites where positive effects were found; (○) injection sites where no or different effects were found.

the raphe obscurus on blood pressure, heart rate and phrenic nerve activity are shown in Table 1.

Microinjection of flesinoxan (1.3 nmol; Figure 2a) or 8-OH-DPAT (0.7 nmol; Figure 2b) into the raphe obscurus caused an instantaneous significant increase in blood pressure of 9 ± 1 mmHg and 14 ± 2 mmHg, respectively which lasted between 5 and 10 min. However, the rise in blood pressure caused by these drugs was not associated with any changes in heart rate or phrenic nerve activity. Microinjection of either flesinoxan or 8-OH-DPAT outside the raphe obscurus nucleus (Figure 2a and b) had no effects on blood pressure, heart rate or phrenic nerve activity.

In separate experiments dose-response curves were constructed (Figure 3) for the effects of flesinoxan and 8-OH-DPAT on blood pressure. Successive microinjections of increasing doses of flesinoxan (0.22–2.6 nmol) caused dose-dependent increases in blood pressure (baseline value 93 ± 4 mmHg) which at the highest dose used reached 27 ± 8 mmHg. Administration of 8-OH-DPAT (0.09–1.4 nmol) into the raphe obscurus also produced a dose-dependent rise in blood pressure (baseline value 91 ± 8 mmHg) reaching a maximum of 21 ± 6 mmHg at the highest dose given. Again, even at the highest doses administered, both flesinoxan and 8-OH-DPAT failed to affect heart rate (baseline values 437 ± 12 and 425 ± 19 b.p.m., respectively) or phrenic nerve activity.

Effect of pretreatment with (±)-pindolol or methiothepin on repeated microinjections of submaximal pressor doses of flesinoxan into the raphe obscurus

In control experiments the pressor responses caused by microinjection of successive doses of 1.3 nmol flesinoxan, were reproducible. Microinjection of (±)-pindolol (2.7 nmol; $n = 4$) caused a maintained rise in blood pressure of 15 ± 1 mmHg with no change in heart rate (baseline values 101 ± 3 mmHg and 394 ± 14 b.p.m., respectively). In these experiments the pressor response to flesinoxan was reduced from 10 ± 2 mmHg before to 2 ± 1 mmHg 5 min after pretreatment with (±)-pindolol. Microinjection of methiothepin (5.2 nmol; $n = 5$) caused an increase in blood pressure of 7 ± 2 mmHg (baseline value 104 ± 4 mmHg) with no change in heart rate (baseline value 439 ± 4 b.p.m.). Blood pressure returned to

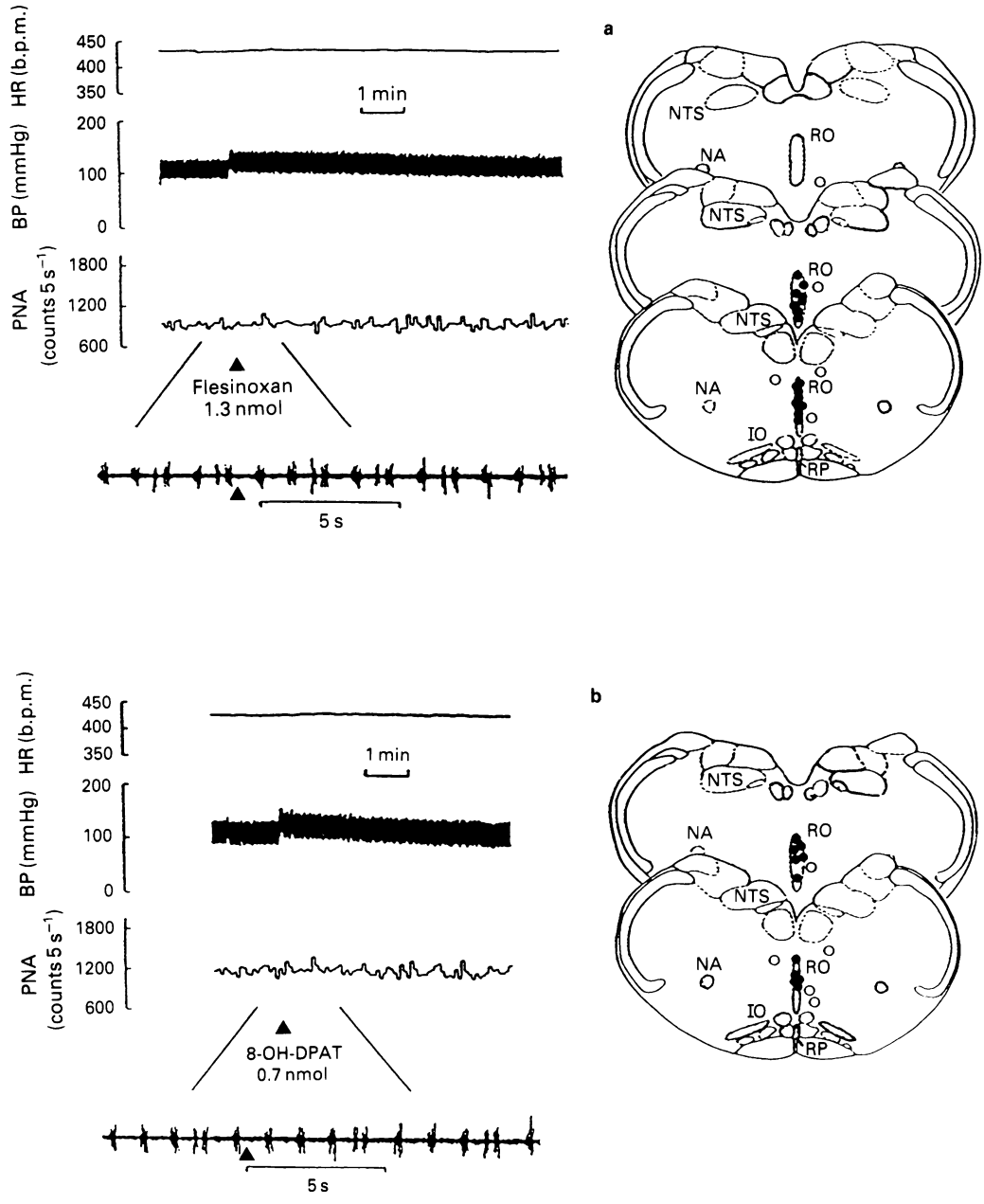

Figure 2 Left: effect of microinjection of flesinoxan (a) and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (b) into the raphe obscurus on heart rate, blood pressure and phrenic nerve activity. Right: injection sites in rat brainstem at the level 1.0–2.0 mm rostral to the obex. RO, nucleus raphe obscurus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; RP, nucleus raphe pallidus; IO, inferior olive; (●) injection sites where positive effects were found; (○) injection sites where no effects were found.

near the baseline value (108 ± 9 mmHg) between 3–4 min after the injection of methiothepin. The increase in blood pressure produced by flesinoxan (13 ± 2 mmHg) was abolished 5 min after the microinjection of methiothepin. However, in 3 experiments 30 min after the microinjection of methiothepin the pressor response to flesinoxan had returned to 6 ± 1 mmHg.

Discussion

In the present study both electrical stimulation and chemical stimulation with glutamate and the selective 5-HT_{1A} receptor agonists, flesinoxan (Wouters *et al.*, 1988a,b) and 8-OH-DPAT (Middlemiss & Fozard, 1983), consistently caused a pressor response when stimuli were applied within, but not outside, the brain region known as the raphe obscurus. In the case of electrical stimulation the pressor effect was accompanied by a decrease in heart rate whereas the pressor effect caused by chemical stimulation failed to cause any change in heart rate. As stimulation with glutamate does not produce any changes in heart rate, the bradycardia caused by electrical stimulation may be due to stimulation of axons of passage. However, the rise in blood pressure caused by electrical stimulation of the raphe obscurus may have initiated a baroreceptor-mediated reflex bradycardia while the pressor response after chemical stimulation with glutamate and also the 5-HT_{1A} receptor agonists may have caused a change in baroreceptor reflex preventing the expected bradycardia. The reason for the failure to observe reflex bradycardia to the pressor effect of chemical stimulation with glutamate or the 5-HT_{1A} receptor agonists of the raphe obscurus remains to be determined.

The effects of electrical stimulation on phrenic nerve activity varied from a small increase to no change in activity, whereas stimulation with glutamate caused an increase in phrenic nerve activity. However, microinjections of flesinoxan or 8-OH-DPAT had no effect on phrenic nerve activity. This difference between glutamate and flesinoxan and 8-OH-DPAT presumably reflects activation of different receptors. Flesinox-

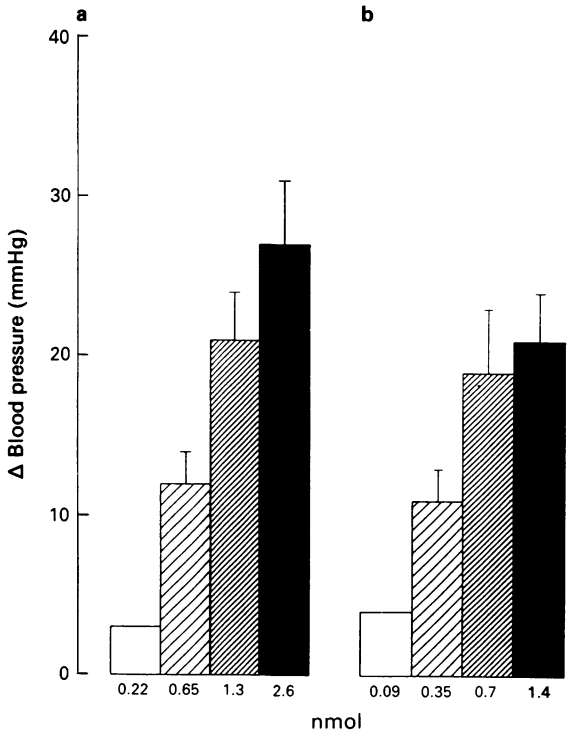

Figure 3 Effect of microinjection of consecutive doses of (a) flesinoxan (0.22, 0.65, 1.3 and 2.6 nmol) or (b) 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.09, 0.35, 0.7 and 1.4 nmol) into the raphe obscurus of the anaesthetized rat on mean arterial blood pressure. Data are expressed as mean (columns) with s.e.mean shown by vertical bars ($n = 5$).

an and 8-OH-DPAT, although structurally unrelated, are selective agonists with a comparable high affinity for the 5-HT$_{1A}$ receptor (Schoeffter & Hoyer, 1988; Hoyer, 1988; van Wijngaarden *et al.*, 1990). Both drugs proved to be equipotent in eliciting a dose-dependent pressor response after microinjection into the raphe obscurus supporting the view that the 5-HT$_{1A}$ receptor might mediate these blood pressure effects. Indeed, the non-selective 5-HT$_1$ receptor antagonist, methiothepin (Schoeffter & Hoyer, 1988; Hoyer, 1988), which inhibits the cardiovascular effects of 8-OH-DPAT and flesinoxan in the anaesthetized rat (Fozard *et al.*, 1987; Dreteler *et al.*, 1990), appeared to block reversibly the action of flesinoxan microinjected into the raphe obscurus. Moreover, (±)-pindolol, which also displays an antagonist action at 5-HT$_{1A}$ receptors (Schoeffter & Hoyer, 1988) and is known to block the cardiovascular effects of flesinoxan in the cat (Wouters *et al.*, 1988b) and in the rat (although in this species some involvement of functional antagonism could not be excluded; Dreteler *et al.*, 1990), also attenuated the pressor effect of flesinoxan in the present study. Again, functional antagonism could not be excluded as (±)-pindolol caused a sustained rise in blood pressure similar to that observed for flesinoxan and 8-OH-DPAT. This increase in blood pressure observed with (±)-pindolol may reflect an agonist action at 5-HT$_{1A}$ receptors (Hjorth & Carlsson, 1986). Methiothepin also caused a transient rise in pressure. However, an explanation for this action of methiothepin remains to be determined. Taken together, these observations suggest that the pressor effect elicited by flesinoxan and 8-OH-DPAT is mediated via 5-HT$_{1A}$ receptor activation in the raphe obscurus. 5-HT$_{1A}$ receptors do not seem to be involved in the increase in phrenic nerve activity caused by glutamate activation of the raphe obscurus. An increase in phrenic nerve activity has also been observed for injection of glutamate into the raphe obscurus in the cat (Holtman *et al.*, 1986) but the present results differ in that glutamate caused an increase in the number of bursts indicating an increase in frequency of respiration and not, as seen in the cat, an increase in the number of events in each burst indicating a greater depth of inspiration.

In the rat, stimulation of the midline region of the rostral medulla with glutamate has been reported to cause a small decrease in blood pressure (Minson *et al.*, 1986). In the present study, stimulation of neurones in the posterior part of the raphe obscurus by glutamate elicited a pressor response. This disparity in effects observed in the two studies may be due to the stimulation of different anatomical areas within the raphe obscurus. In this respect, Yusoff & Coote (1988) have shown that electrical stimulation of different areas of the raphe obscurus in the rat, though increasing skeletal muscle and skin sympathetic nerve activity, either increased or decreased renal sympathetic nerve activity. In the rat, microinjections of 5-HT$_{1A}$ receptor agonists into other raphe nuclei e.g. raphe magnus and pallidus (Valenta & Singer, 1990) or the dorsal raphe nucleus (Connor & Higgins, 1990), in doses equivalent to the doses used in the present study, caused a decrease in blood pressure and heart rate, while microinjections into the median raphe had no effect (Connor & Higgins, 1990). The falls in blood pressure and heart rate observed after injection of the 5-HT$_{1A}$ receptor agonists into the dorsal raphe were blocked by N-methylatropine, suggesting that these cardiovascular effects were vagally mediated (Connor & Higgins, 1990). These results, together with the results from the present study, suggest that the various raphe nuclei have complex effects on autonomic outflow. In this respect, electrical and chemical stimulation of the raphe obscurus in the rat has been reported to enhance gastric motility (McCann *et al.*, 1989).

It is now well established that 5-HT$_{1A}$ receptor agonists lower blood pressure via a reduction in sympathetic output (Ramage & Fozard, 1987; Ramage *et al.*, 1988; Saxena & Villalon, 1990). Activation of central 5-HT$_{1A}$ receptors causes inhibition (Colino & Halliwell, 1987; Sprouse & Aghajanian, 1987) of neuronal activity, which in the dorsal raphe is thought to be mediated via a somatodendritic autoreceptor (Vergé *et al.*, 1985; Sprouse & Aghajanian, 1987). In contrast, the present results indicate that in the raphe obscurus, activation of 5-HT$_{1A}$ receptors, as well as neuronal excitation by glutamate or electrical stimulation, elicits sympathoexcitation. However, it is not known whether the 5-HT$_{1A}$ receptors in the raphe obscurus are located postsynaptically causing direct excitation of the neurones or presynaptically causing inhibition of an inhibitory input to these cells.

In conclusion, microinjection of the 5-HT$_{1A}$ receptor agonists flesinoxan and 8-OH-DPAT into the raphe obscurus of the anaesthetized rat dose-dependently increases blood pressure without any change in heart rate. Since these drugs lower blood pressure following systemic administration, the consequences of the activation of raphe obscurus activation are apparently overruled by the sympathoinhibition resulting from the activation of the other sites, such as the dorsal raphe nucleus and raphe magnus and pallidus. However, the pathways that underlie the blood pressure effects caused by microinjection of the 5-HT$_{1A}$ agonists into the raphe obscurus and the way in which this nucleus might be involved in central cardiovascular regulation need further investigation.

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