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Involvement of central α- and β-adrenoceptors in the pressor response to electrical stimulation of the rostral ventrolateral medulla in rats

[1]C. Ward-Routledge, *P. Marshall & C.A. Marsden

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG2 2UH and *Bioscience II, ICI Pharmaceutical Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

1 Electrical stimulation of the C_1 area of the rostral ventrolateral medulla in rats elicits an increase in mean arterial pressure (MAP).

2 This increase in MAP is attenuated by intra-hypothalamic and intracisternal administration of the non-selective β-adrenoceptor antagonist ($\pm$)-propranolol and the β_2-selective antagonist ICI 118551.

3 The selective β_1-antagonist atenolol and (+)-propranolol, which is inactive on β-adrenoceptors, did not alter the increase in MAP produced by stimulation of the C_1 area.

4 The α_2-adrenoceptor antagonist idazoxan enhanced the effects of C_1 stimulation on MAP when administered either into the posterior hypothalamus or intracisternally.

5 The results indicate that β_2- and α_2-receptors both have a role in the mediation of the rise in MAP during stimulation of the C_1 area and that the receptors involved are located both within the hypothalamus and the spinal cord.

Introduction

Several lines of investigation have suggested a role for adrenaline in the central regulation of blood pressure (Saavedra *et al.*, 1976; Saavedra, 1979; Howe *et al.*, 1981). It has been demonstrated that an area of the rostral ventrolateral medulla (RVL) important in the control of arterial pressure corresponds almost precisely with a region containing adrenaline-synthesizing neurones (Reis *et al.*, 1984; Ross *et al.*, 1983; 1984a). These neurones are part of the C_1 group described by Hokfelt *et al.* (1974). We have previously demonstrated that electrical stimulation of the C_1 region of the RVL increases mean arterial pressure (MAP) and simultaneously increases extracellular adrenaline in the posterior hypothalamus (Routledge & Marsden, 1987a). Other workers have shown that electrolytic lesions (Dampney & Moon, 1980), cooling (Schlarfke & See, 1980) or application of inhibitory amino acids to this area (Ross *et al.*, 1984b) lowers arterial pressure. It appears therefore that the cardiovascular responsive area of the RVL, possibly the C_1 region, may be concerned with the tonic drive to sympathetic neurones and be partly responsible for maintaining resting levels of arterial pressure.

The adrenaline-containing neurones in the C_1 region project to the hypothalamus (Hokfelt *et al.*, 1974; Swanson *et al.*, 1981) and the spinal cord where they specifically innervate the autonomic neurones of the intermediomedial and intermediolateral columns (Ross *et al.*, 1983; 1984a). Therefore, increases in MAP and in extracellular levels of hypothalamic adrenaline (Routledge & Marsden, 1987a) during electrical stimulation, suggest that the pressor response may be related to an increase in release of central adrenaline at the hypothalamic and spinal level, indicating that central adrenoceptors may be involved in mediating the increase in MAP.

Previous studies have demonstrated the existence of both α- and β-adrenoceptors in the central nervous system (Starke & Langer, 1979; Marwaha & Aghajanian, 1982; Unnerstall *et al.*, 1984). The adrenoceptors have been divided into α_1- and

[1]Present address: Institute of Pharmacology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94303, U.S.A.

α_2-subtypes (see Starke, 1981 for review) and β_1- and β_2-subtypes (Langer, 1977; Westfall, 1977). The postulated role of central adrenoceptors and central adrenaline in the C_1-stimulation-induced pressor response is in agreement with other findings demonstrating the involvement of central adrenoceptors in cardiovascular functions (Schmitt *et al.*, 1971; Kobinger & Walland, 1972; Sinha *et al.*, 1985). Other studies have demonstrated an involvement for adrenaline in cardiovascular control. Bolme *et al.* (1974), Bolme & Fuxe (1975) and Atkinson *et al.* (1986) demonstrated that the blood pressure lowering effect of clonidine is accompanied by a decreased capacity to synthesize adrenaline in the A_1/C_1 region. Consistent with these observations was the finding that the systemic injections of inhibitors of phenylethanolamine-N-methyl transferase (PNMT), the enzyme which converts noradrenaline to adrenaline, lowers MAP (Saavedra *et al.*, 1976; Lew *et al.*, 1979; Saavedra, 1979; 1980) and depletes central adrenaline (Fuller, 1982).

This study attempts to identify the central adrenoceptors involved in the C_1-stimulation-induced pressor response, by determining the effects of selective α- and β-adrenoceptor antagonists on the response. The antagonists were injected both centrally and peripherally and their effects on basal MAP and the pressor response produced by electrical stimulation of the C_1 region observed. The central injections were made both intracisternally and intrahypothalamically in an attempt to differentiate between spinal and hypothalamic effects. A radiolabelled drug diffusion study using tritiated (±)-propranolol was also done, to determine the distribution of the antagonist following its injection into specific central nervous system (CNS) sites.

Methods

Blood pressure measurements

Male Alderley Park Wistar rats (270–320 g) were anaesthetized with an alphaxalone/alphadalone mixture ('Saffan', Glaxo) 18 mg kg^{-1} i.v. for induction and 0.3–1.4 mg kg^{-1} min^{-1} i.v. for maintenance via a venous cannula. An arterial catheter containing heparinised saline 1000 units ml^{-1} was inserted into the left common carotid artery to facilitate continuous monitoring of blood pressure via a Bell and Howell blood pressure transducer connected to a Lectromed (Multitrace 4) heat sensitive recorder. The mean arterial blood pressure was calculated as diastolic pressure + 1/3 pulse pressure (systolic pressure minus diastolic pressure).

Implantation of central cannulae

Drugs were administered by four different routes, for each route a different group of rats was used. The administration routes were:- intrahypothalamic (i.h.), intracerebroventricular (i.c.v.), intracisternal (i.c.) and intravenous (i.v.). For implantation of i.h. and i.c.v. cannulae the rats were placed in a stereotaxic frame (David Kopf Instruments) incisor bar set at 5 mm above the intraaural line) and the cranium exposed and cleaned. Two holes were drilled in the skull and injection cannulae (consisting of a 15 mm length of 25 gauge stainless steel hypodermic needle) were implanted into the posterior hypothalamus (rostral-caudal −1.0; sagittal +0.5 vertical −8.0 mm) and lateral ventricle (rostral-caudal 0.0; sagittal +0.7; vertical −3.0 mm). The cannulae were kept in position with dental cement.

For i.c. injection the rats were placed in the stereotaxic frame with the incisor bar lowered to −5.0 mm flexing the head downwards allowing better exposure to the atlanto-occipital membrane. An incision was made in the back of the neck at the base of the skull and the intracisternal membrane exposed. The cannula (consisting of a 10 mm length of 26 gauge stainless steel hypodermic needle) was inserted into the atlanto-occipital membrane so that the tip protruded into the foramen magnus. Polythene tubing was connected to the exposed end of the cannula to facilitate drug administration.

For i.v. administration of drugs, the i.v. cannula for administration of anaesthetic was disconnected and drugs were injected via this cannula, which was then reconnected to the anaesthetic infusion system.

Implantation of stimulating electrodes

Saffan anaesthetized male Alderley Park Wistar rats (280–320 g) were placed in a stereotaxic frame and a modified SNE 100 (Clarke Electromedical Equipment) concentric needle electrode (100 μm o.d.) was implanted into the C_1 region of the RVL (rostral-caudal −2.7; sagittal +1.8; vertical −7.0). As the electrode was lowered into and through the C_1 region a stimulating current (see below) was passed and the final position of the electrode was that at which stimulation produced the greatest increase in MAP. The electrode remained in position throughout the experiment, and its position was verified histologically at the end of each experiment.

General experimental procedure

A diagram of the general protocol is given in Figure 1. The C_1 region of the RVL was electrically stimulated with square wave pulses of 1 ms duration, 250 μA were passed at 10 Hz, for 10 s every 3 min.

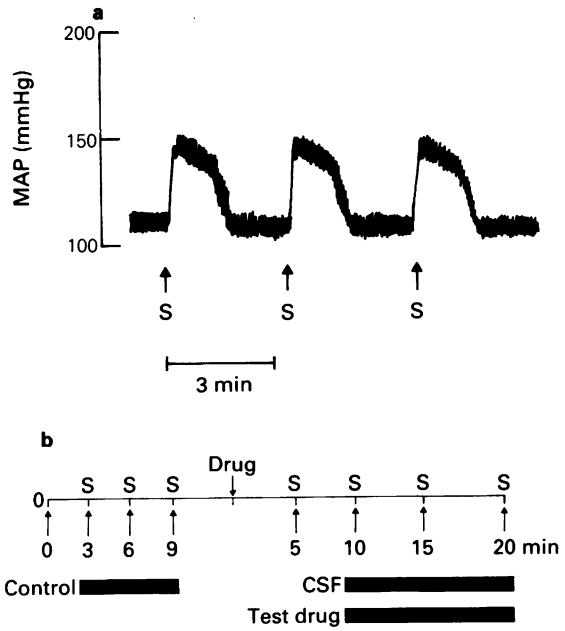

Figure 1 Diagram demonstrating (a) a typical blood pressure trace obtained during 3 control stimulation(s) to the C_1 region of the Saffan anaesthetized rat (stimulation parameters were square wave pulses of 1 ms, 250 μA at 10 Hz for 10 s every 3 or 5 min) and (b) the experimental protocol used in the antagonist studies (see Methods for details). MAP = mean arterial pressure.

Following 3 control stimuli the drugs were administered through one of the four different injection routes and the response to further C_1 stimulation, 5, 10, 15 and 20 min post-drug injection was measured. Blood pressure was recorded throughout this period. For control experiments artificial cerebrospinal fluid (CSF) was administered by each of the injection routes and its effects on further C_1 stimulation 5, 10, 15 and 20 min post-injection were monitored. The increases following 3 control stimuli were meaned and compared with the mean increases at 10, 15 and 20 min post-drug. The mean of the 3 control stimuli was also compared with the mean increases in blood pressure 10, 15 and 20 min post CSF injection.

Intracisternal injections of adrenaline: effects of pretreatment with (±)-propranolol

To show that the dose of (±)-propranolol used was sufficient to block the effects of released adrenaline, the response to adrenaline injection i.c. in the presence and absence of (±)-propranolol was studied. In one group of rats intracisternal cannulae were inserted and following a 5 min control period adrenaline (33 μg) was injected. The effects on MAP were monitored for the following 60 min. In a second group of rats (±)-propranolol (18 μg) was administered i.c. followed by adrenaline (33 μg) after a 20 min period. Again the effects were monitored for 60 min post-adrenaline injection.

(±)-[^3H]-propranolol diffusion study

To determine how far (±)-propranolol diffused from the site of injection, (±)-[^{3}H]-propranolol was administered via the previously described routes and the extent of its diffusion measured.

Saffan anaesthetized male Alderley Park Wistar rats (270–320 g) were placed in a stereotaxic frame. In one group of rats injection cannulae were implanted into the posterior hypothalamus, in a second group into the lateral ventricles, and in a third group into the cisterna magna (co-ordinates given in previous section). (±)-[^{3}H]-propranolol (92.5 MBq i.c.; 37 MBq i.c.v. and i.h.) was injected through the cannulae in a volume of 5 μl for i.c. injections and 1 μl for i.c.v. and i.h. injections. Unlabelled (±)-propranolol was used to make up the correct drug concentration. The rats were killed 5 and 20 min post-drug administration, decapitated and the brain and spinal cord removed. The different brain regions were dissected out and the thoracic and lumbar regions of the spinal cord removed, weighed and then sonicated in 1 ml of 0.1 M perchloric acid containing 0.02% sodium metabisulphite. This procedure was repeated for control rats injected with unlabelled (±)-propranolol. The radioactivity in the sonicated tissue was counted by liquid scintillation spectroscopy using Fisofluor (15 ml) as scintillant. Plasma activity was also counted, following the collection of blood (5 ml) into lithium heparin tubes; the tubes centrifuged for 25 min at 3000 r.p.m. and 1 ml of the plasma retained and counted by liquid scintillation. The results were expressed as d.p.m. and background radioactivity obtained from unlabelled (±)-propranolol injected rat brain tissue subtracted from each brain region.

Drugs

Drugs used were:- (±)-adrenaline bitartrate (Sigma Chemical Co.), erythro-DL-1(7-methylindan-4-yloxyl-3-isopropylamin) butan-2-ol (ICI 118551), (±)-propranolol, (+)-propranolol hydrochloride and atenolol (ICI p.l.c.), idazaxon hydrochloride (Reckitt & Coleman) and (±)-[4-^{3}H]-propranolol hydrochloride (Amersham International plc).

Drugs for central administration were dissolved in artificial cerebrospinal fluid (CSF – aqueous solution of (in mM): NaCl 27.65, KCl 2.6, $MgCl_2$ 0.93, $CaCl_2$

Table 1 Distribution of (±)-[4-^{3}H]-propranolol following intracisternal administration

CNS region	Time 5 min	Time 20 min
Forebrain	1.6	2.6
Striatum	1.2	0.8
Hypothalamus	1.8	2.0
Hind brain	2.3	2.5
Rostral brain stem	28.3	0.7
Caudal brain stem	100.0	25.5
Thoracic spinal cord	39.9	100.0
Lumber spinal cord	1.0	14.5
Blood plasma	168.0	296.0

Levels of radiolabel found in specific brain regions and blood plasma 5 and 20 min following intracisternal administration of (±)-[4-^{3}H]-propranolol. The values are: % of the radioactivity found in the CNS region where highest amount of radiolabel was found (100%). Note the relatively high diffusion of label into peripheral blood (plasma value) compared to data in Figure 2.

1.26, $NaHCO_3$ 237, NaH_2PO_4 1.5 and pH 7.0). For drugs administered i.h. and i.c.v. a 1 μl volume was injected over 1 min. For i.c. administration a 5 μl volume was injected over 1 min. Drugs given i.v. were dissolved in 0.9% saline and given in a volume of 2 ml kg^{-1}.

Analysis of results

The C_1 stimulation data were analysed by use of Student's paired t test. Mean increase in MAP of 3 pre-drug control stimuli were compared to the mean increases in MAP to further C_1 stimulation 10, 15 and 20 min post-drug and post-CSF (see Figure 1) for each group of rats. There was no significant difference in pre-drug control values for each group of animals and so the values on the figures are the mean values of all the groups.

For the adrenaline i.c. administered group Student's unpaired t test was used to compare MAP in adrenaline-injected (±)-propranolol-pretreated rats with MAP in adrenaline-injected control rats.

Results

Drug diffusion study

The extent of diffusion of (±)-propranolol caudally from the point of infusion into the foramen magnum is shown in Table 1, and following injection into the hypothalamus and lateral ventricles in Figure 2.

Diffusion of i.c. injected radiolabel was relatively localized, the highest amount of radiolabel being found in the thoracic spinal cord 20 min post-injection. Substantial amounts were also measured in the caudal brain stem and the lumbar spinal cord 20 min post-injection, 25.5 and 14.5% respectively of that found in the thoracic spinal cord. Negligible levels of radioactivity were detected throughout the rostral regions of the brain both 5 and 20 min post-injections, but appreciable amounts were found in plasma (Table 1).

Injections of radiolabel into the hypothalamus were found to be very localized (Figure 2a), with the majority of radiolabel found in this area 20 min post-injection. Negligible amounts of radiolabel were detected in other brain regions, including those contralateral to the injection site, and in blood plasma (Figure 2).

Diffusion of radiolabel following i.c.v. injections was more widespread (Figure 2b). The highest amount of radiolabel was found in the striatum but there were substantial amounts in most brain areas ipsilateral and contralateral to the drug injection site (Figure 2b). A high percentage of radioactivity was also found in blood plasma 5 and 20 min post-injection.

Effects of electrical stimulation to the C_1 area of the RVL on MAP

Following electrical stimulation of the C_1 area of the RVL a reproducible increase in MAP of 28 ± 5 mmHg ($P < 0.01$) was observed, following each 10 s stimulation period MAP returned to basal levels.

Effects of adrenoceptor antagonists on the C_1 pressor response

Propranolol The non-specific β-adrenoceptor antagonist (±)-propranolol (18 μg i.c., i.c.v., or i.h.) decreased the C_1 stimulation-induced pressor response by 14 ± 1, 14 ± 1 and 10 ± 2 mmHg, respectively ($P < 0.05$) when compared to pre-stimulation controls. A smaller decrease was observed at a lower dose (8 μg) (Figure 3). (±)-Propranolol (18 μg) i.c. and i.h. also decreased basal pre-stimulation levels of MAP (3 ± 1 mmHg, $P < 0.05$) as did 8 μg i.h. (8 ± 3 mmHg, $P < 0.05$). Neither (±)-propranolol administered i.v. at the highest control dose (18 μg) nor artificial CSF (i.c., i.c.v. or i.h.) altered basal MAP or the C_1 pressor response. The inactive isomer (+)-propranolol (i.c., i.c.v., i.h., i.v.) also had no effect on either basal MAP or the C_1 stimulated pressor response (Figure 3).

Atenolol Atenolol (33 μg), a selective β_1-antagonist, had no effect on either basal MAP or the C_1 stimulation-induced pressor response when administered i.c., i.c.v., i.h. or i.v.

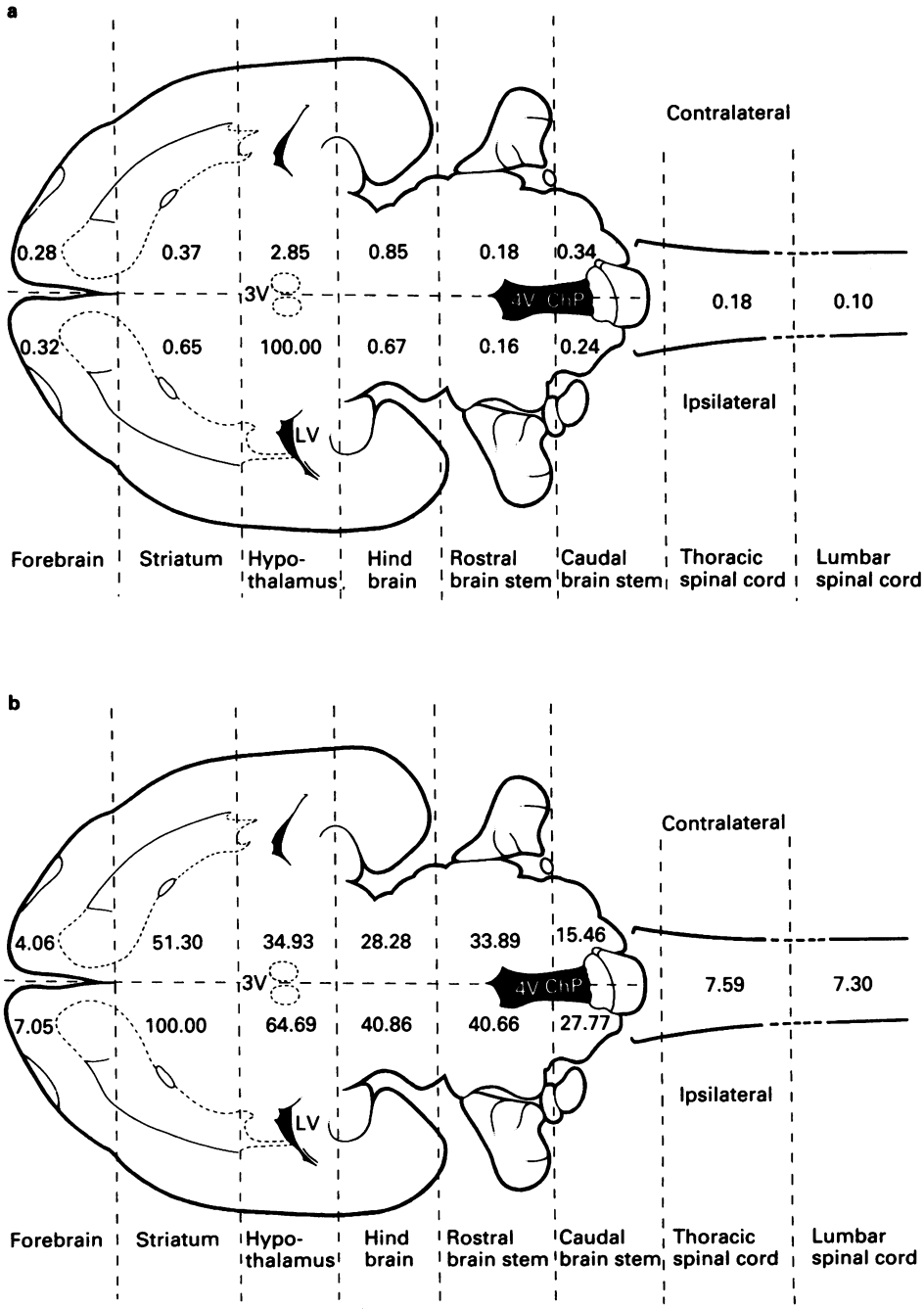

Figure 2 Diagram illustrating the extent of diffusion of radiolabel following injection of (±)-[4-^{3}H]-propranolol into the hypothalamus (a) or into a lateral ventricle (b). The rats were killed 20 min after injection and brain regions dissected out from both sides of the brain, sonicated and counted for radioactivity by liquid scintillation spectroscopy. Results are expressed as % of radiolabel found in the injected hypothalamus (a) or the ipsilateral striatum (b) (these being the areas containing the most radiolabel 20 min, following injection). The plasma value following intrahypothalamic injection was 15% and following intracerebroventricular injection 54%. LV = lateral ventricle, 3V = 3rd ventricle and 4V = 4th ventricle.

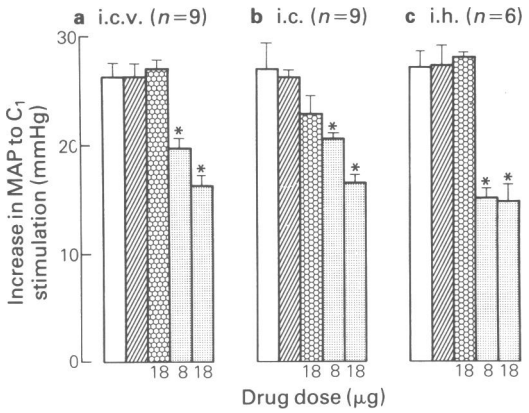

Figure 3 Effects of the β-adrenoceptor antagonist ($\pm$)-propranolol (stippled columns) and its inactive isomer (+)-propranolol (hexagonally-hatched columns) on the C_1 stimulation-induced increase in mean arterial pressure (MAP) (open columns) compared to controls injected by the same routes with CSF (diagonally-hatched columns). The drugs were administered (a) intracerebroventricularly (i.c.v.), (b) intracisternally (i.c.) and (c) intrahypothalamically (i.h.). Values for the increase in MAP are expressed as mmHg; vertical lines indicate s.e. mean. Total drug doses are given in μg. Values following drug or CSF administration were compared with pre-stimulation control values by use of Student's paired t test, $^*P < 0.05$. For reasons of clarity, and because there was no significant difference in pre-stimulation values for each group within each administration route, the values for pre-stimulation controls in the figure are the mean values.

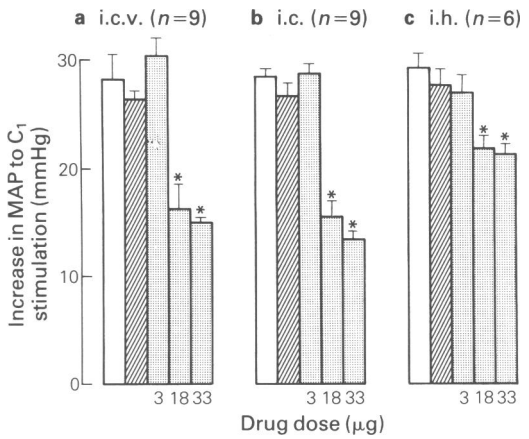

Figure 4 Effects of ICI 118551 (stippled columns) on the increase in mean arterial pressure (MAP) to C_1 stimulation (open columns) when administered (a) intracerebroventricularly (i.c.v.), (b) intracisternally (i.c.) and (c) intrahypothalamically (i.h.) compared to controls injected similarly with CSF (hatched columns). Values for increase in MAP are expressed as mmHg; vertical lines indicate s.e. mean. Total drug doses are given in μg. Values following CSF or drug-administration were compared with pre-stimulation control values by use of Student's paired t test, $^*P < 0.05$. For reasons of clarity, and because there were no significant differences between the pre-stimulation values for each group within each administration route, the pre-stimulation values in the figures are the mean values.

ICI 118551 Administration of ICI 118551 (3 μg) i.c., i.c.v. or i.h. had no effect on basal MAP. However, at the higher dose of 33 μg it significantly decreased MAP when administered i.c. or i.c.v. (12 ± 5 and 3 ± 1 mmHg, respectively, $P < 0.05$). When injected into the hypothalamus, ICI 118551 (3 or 33 μg) had no effect on basal MAP.

ICI 118551 (18 and 33 μg i.c., i.c.v. or i.h.) significantly decreased the C_1 pressor response while 3 μg had no effect (Figure 4). There was a significantly greater attenuation ($P < 0.05$) following administration of ICI 118551, (18 and 33 μg) i.c. and i.c.v. than following i.h. administration (Figure 4). Administration of ICI 118551 i.v. at the highest central dose (33 μg) was without effect on basal MAP or the C_1 pressor response.

Idazoxan Idazoxan (66 μg) enhanced the C_1 pressor response, regardless of the central route of administration, compared to pre-stimulation control values. This effect was more pronounced ($P < 0.05$) following hypothalamic administration than following i.c. or i.c.v. administration (Figure 5). The facilitation was also observed at a lower dose (33 μg i.c., i.c.v. and i.h.) and again the effect was more pronounced in the hypothalamus ($P < 0.05$) (Figure 5). There was no significant change in basal MAP following administration of idazoxan 33 or 66 μg i.c.v. and 33 μg i.c., but at the 66 μg dose i.c. there was a significant decrease in basal MAP (-10 ± 3 mmHg, $P < 0.05$). When administered i.v. at the highest central dose (66 μg), idazoxan increased basal MAP (6 ± 2 mmHg, $P < 0.05$) but had no effect on the C_1 pressor response.

Intracisternal administration of adrenaline on MAP and the effect of ($\pm$)-propranolol

Following intracisternal injection of adrenaline (33 μg) a biphasic response in blood pressure was observed. An immediate pressor response ($+59 \pm 4$ mmHg, $P < 0.01$) was followed by a prolonged depressor effect (-15 ± 5 mmHg, $P < 0.05$) (Figure 6). Pretreatment with ($\pm$)-propranolol (18 μg), a dose without effect on basal MAP, abol-

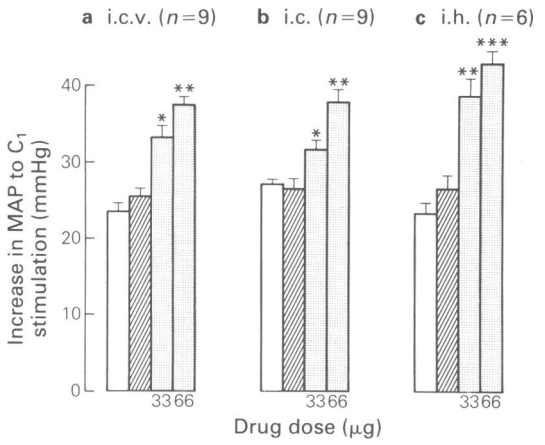

Figure 5 Effects of idazoxan (stippled columns) on the increase in mean arterial pressure (MAP) due to C_1 stimulation (open columns) when administered (a) intracerebroventricularly (i.c.v.), (b) intracisternally (i.c.) and (c) intrahypothalamically (i.h.) compared to CSF injected control (hatched columns). Values for the increase in MAP are expressed in mmHg; vertical lines indicate s.e. mean. The total dose of idazoxan is given in μg. In the figure the pre-stimulation values from all groups within each injection area have been meaned as have values following administration of CSF. Statistical analysis was carried out by use of Student's paired t test comparing pre-stimulation controls with values following CSF and drug administration for each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$.

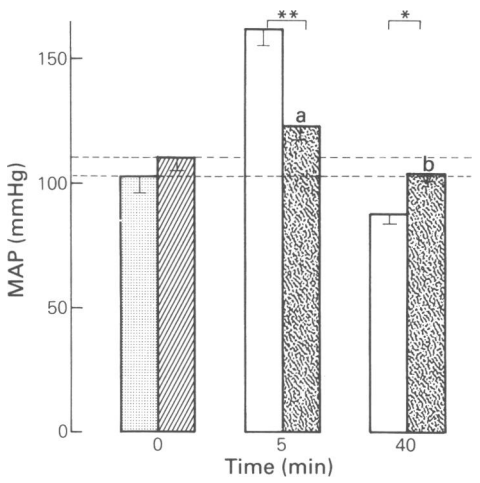

Figure 6 Histogram showing the adrenaline (33 μg i.c.) (open columns)-induced changes in mean arterial pressure (MAP) in Saffan anaesthetized rats 5 and 40 min following administration and the effects of 5 min pretreatment with (±)-propranolol (18 μg, i.v.) (columns a and b) on the adrenaline-induced pressor and depressor response. Stippled column represents basal MAP and hatched column effects of (±)-propranolol alone on basal MAP. MAP values expressed as mmHg; vertical lines indicate s.e. mean. The effects of adrenaline following pretreatment with (±)-propranolol were compared with the effects of adrenaline alone by use of Student's unpaired t test. $n = 6$ in each group. * $P < 0.05$, ** $P < 0.01$.

ished the hypotensive effect of adrenaline (33 μg) and greatly reduced the hypertensive effect (Figure 6).

Discussion

The results obtained in this study demonstrate the ability of the non-selective β-adrenoceptor antagonist (±)-propranolol and the selective β_2-adrenoceptor antagonist ICI 118551 (Bilski *et al.*, 1983) to attenuate, and the α_2-adrenoceptor antagonist idazoxan to enhance the C_1-stimulation-induced pressor response. However, before discussing the pharmacological effects of these antagonists, the possible sites of action following central injection via various routes need to be defined.

There was little diffusion of (±)-[4-^{3}H]-propranolol following i.h. and i.c. injections, but widespread distribution was found following i.c.v. administration. This study therefore demonstrates that drugs administered i.h. and i.c. are principally exerting their effects in the hypothalamus and thoracic spinal cord, respectively. However, the possibility that drugs given i.c. also exert an effect at the lower brain stem level cannot be ruled out as a substantial amount of radiolabel was found in this brain region. This finding is in agreement with a recent study using intrathecal injections of [^{125}I]-TRH which found the ^{125}I to be very localized, with only a small amount of diffusion of drug back into the brain as well as further down the spinal cord (Fone *et al.*, 1987).

The site of action of drugs following i.c.v. injection is not so well defined as there was widespread diffusion 20 min post-injection. However, it can be assumed that the antagonists used in the study following i.c.v. administration are able to exert effects at those sites shown to be important in blood pressure regulation (Van der Gugten, 1976; Ross *et al.*, 1983; 1984b) namely the hypothalamus, brain stem and spinal cord.

In common with other studies (Myers & Hoch, 1978; Wolfson & Brown, 1983) the radioactivity recovered in the CNS in the present experiments was only a small proportion of the total injected. A partial explanation for the discrepancy is that label is

diffusing into the peripheral circulation. In the present study significant amounts of label were found in plasma regardless of the route of central injection and label could also have bound to blood vessels, fat deposits and other peripheral sites.

The penetration of the labelled drug into the peripheral systems after central injection suggests an alternative site of action of the antagonists used in this study. To examine the possibility that the effects observed related to a peripheral rather than central site of action each of the antagonists was administered i.v. at its highest central dose and its effects on the C_1 pressor response examined. However, none of the drugs administered i.v. altered the C_1 response, indicating that the antagonists used exerted their effects centrally at the hypothalamic, spinal and possibly brain stem level.

The decrease in basal MAP produced by (±)-propranolol (i.c. or i.h.) is in agreement with other studies where (±)-propranolol has been administered centrally (Lewis & Haeusler, 1975; Randall *et al.*, 1985), and with the reported central hypotensive effects of propranolol (Day & Roach, 1974). The lack of effect of the inactive isomer (+)-propranolol, which has mainly local anaesthetic activity while retaining only minimal β-blocking activity (Barrett & Cullum, 1968), indicated that the central effects of (±)-propranolol are a consequence of β-adrenoceptor antagonism rather than membrane stabilization.

The lack of effect of the selective β-adrenoceptor antagonist atenolol on basal MAP, but the decrease in basal MAP following the selective β_2-adrenoceptor antagonist ICI 118551 suggest that the hypotensive effects of (±)-propranolol and ICI 118551 are due to their action at central β_2-adrenoceptors. Recently, adrenaline has been shown to be a potent β_2-adrenoceptor agonist (Ueda *et al.*, 1983; 1985; Goshima *et al.*, 1985), therefore the central hypotensive effect of (±)-propranolol and ICI 118551 could be the result of modulation of adrenaline release via presynaptic β_2-adrenoceptors, in agreement with studies by Ueda *et al.* (1983) and Goshima *et al.* (1985), or antagonism of postsynaptic β_2-receptors. The failure of ICI 118551 administered into the hypothalamus to modify basal MAP suggests that the β_2-adrenoceptors involved are located in the medulla and/or spinal cord.

(±)-Propranolol and ICI 118551 i.h., i.c. and i.c.v. also attenuated, in a dose-dependent manner, the increase in MAP due to electrical stimulation. Again these effects appear to be due primarily to a β_2-adrenoceptor action since atenolol (i.h., i.c. and i.c.v.) was without effect on the pressor response. In view of the selective increase in hypothalamic adrenaline release during C_1 stimulation (Routledge & Marsden, 1987a), the effects of (±)-propranolol and ICI 118551 could relate to inhibition of the effects produced by released adrenaline. Electrical stimulation to the C_1 region increases the extracellular levels of adrenaline in the posterior hypothalamus with no change in that of noradrenaline (Routledge & Marsden, 1987a). Neurones originating in the C_1 region also innervate the intermediolateral column (IML) of the thoracic spinal cord (Hokfelt *et al.*, 1984; Armstrong *et al.*, 1982; Ross *et al.*, 1981; 1984a), thus it is probable that extracellular levels of adrenaline in the spinal cord are also increased during C_1 stimulation. As the C_1 pressor response was attenuated by both i.h. and i.c. administration of (±)-propranolol and ICI 118551, these results again suggest that β_2-adrenoceptors involved in the modulation of MAP are located both in the brain (hypothalamus) and spinal cord (IML). The doses of (±)-propranolol used in this study were sufficient to antagonize the effects of locally administered adrenaline, as the pressor response produced by i.c. administration of adrenaline was antagonized by pretreatment with (±)-propranolol.

Administration of the α_2-adrenoceptor antagonist idazoxan given i.h., i.c.v. and i.c., did not alter basal MAP but enhanced the pressor response produced by C_1 stimulation, this was most pronounced following hypothalamic administration. There is considerable evidence for the occurrence of α_2-adrenceptors in the CNS (Starke, 1977; Langer, 1977; Unnerstall *et al.*, 1984) and, more specifically, their occurrence in regions of the CNS involved in cardiovascular control (Rouot & Snyder, 1979; Unnerstall, 1984; Giron *et al.*, 1985) and for their involvement in cardiovascular control (Bhargava *et al.*, 1972; Day *et al.*, 1980).

Recent studies have demonstrated that central presynaptic α_2-adrenoceptors may be important in the modulation of adrenaline release via negative feedback inhibition both *in vitro* (Scatton *et al.*, 1979; Goshima *et al.*, 1985) and *in vivo* (Routledge & Marsden, 1987b). The increase in the pressor response following the electrical stimulation of the C_1 area supports the postulated role of adrenaline in the autoregulation of its own release. The observations that idazoxan increases hypothalamic extracellular adrenaline and *in vitro* release of adrenaline, combined with the postulated role of adrenaline in cardiovascular regulation, suggest that increased extracellular levels of adrenaline produced by idazoxan may potentiate the pressor effects of C_1 stimulation.

Although the increase in extracellular hypothalamic adrenaline during stimulation of the C_1 area strongly argues in favour of an adrenergic pathway from the C_1 area to the hypothalamus (Routledge & Marsden, 1987a), the counter argument has been made that adrenaline in the brain is

principally a postsynaptic metabolite of noradrenaline (Mefford, 1987). In this situation changes in extracellular adrenaline would reflect altered noradrenaline release and metabolism. However, Routledge & Marsden (1987a) found that C_1 area stimulation increased extracellular hypothalamic adrenaline but not noradrenaline, which supports the evidence for an adrenergic pathway derived from cell bodies in the medulla (Hokfelt *et al.*, 1974). Whether all adrenaline in the brain is derived from specific adrenergic neurones remains to be determined.

In summary, these results indicate that central adrenaline may have a pressor role in the central regulation of blood pressure, and that the central adrenoceptors modulating the action of adrenaline are β_2- and α_2-adrenoceptors. This modulation occurs both at the spinal and the hypothalamic level. However, from these experiments it is not possible to elucidate the exact mechanism of the actions of adrenaline or the involvement of central adrenoceptors in blood pressure regulation. It is important to note that the β_2-antagonist did not prevent the C_1 area-induced pressor response completely, suggesting the involvement of one or more other neurotransmitters. Neuropeptide Y coexists with adrenaline in C_1 neurones (Hokfelt *et al.*, 1984) and substance P is found in neurones projecting to the IML (Lorenz *et al.*, 1985). Such results raise the possibility that on C_1 stimulation both an amine and a peptide are released; the mechanism by which these substances act to produce the final receptor-mediated response needs to be determined.

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