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A comparison of the cardiovascular effects of centrally administered clonidine and adrenaline in the anaesthetized rat

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Intracerebroventricular (i.c.v.) injections of clonidine and adrenaline-induced hypotension and bradycardia in urethane anaesthetized spontaneous hypertensive rats. The hypotension induced by clonidine (3 μg i.c.v.) was antagonized by pretreatment with the α-antagonists piperoxan, which also antagonized clonidine-induced bradycardia, and yohimbine. The hypotension and bradycardia induced by adrenaline (10 μg i.c.v.) were unaffected by α-antagonist pretreatment, while β-antagonist pretreatment with (−)-propranolol or metoprolol was effective against adrenaline but not clonidine-induced hypotension and bradycardia. Pretreatments with the histamine H_2-receptor antagonists metiamide and cimetidine antagonized clonidine but not adrenaline-induced hypotension. These data indicate that different central mechanisms are involved in mediating the hypotension and bradycardia induced by centrally administered clonidine and adrenaline and do not, therefore, support the hypothesis that the hypotensive effects of clonidine (i.c.v.) are mediated by central adrenaline receptor activation in urethane-anaesthetized spontaneous hypertensive rats.

Central adrenaline releasing neurons were first demonstrated in rat brain by Hokfelt et al (1973). The central administration of adrenaline has been shown to exert variable effects on blood pressure and heart rate (Day & Roach 1974; Struyker Boudier et al 1975; Ozawa & Uematsu 1975) although after giving intra-cerebroventricular injections of adrenaline we have consistently obtained hypotension and bradycardia in conscious spontaneous hypertensive rats, and biphasic effects on blood pressure (associated with bradycardia) in urethane-anaesthetized rats (Borkowski & Finch 1977, 1978). In view of the suggestion that the hypotensive effects of clonidine, a potent antihypertensive agent considered to have a primarily central site of action (Schmitt & Schmitt 1969; Kobinger 1973), may be due to central adrenergic, rather than central noradrenergic stimulation (Bolme et al 1974; Bolme & Fuxe 1975) we have attempted in this study to compare the effects of α- and β-adrenoceptor blocking agents on clonidine and adrenaline-induced hypotension and bradycardia. If clonidine and adrenaline have similar central modes of action, these antagonists should be effective against both these cardiodepressive agents in the spontaneous hypertensive rat. Since a central interaction between histamine H_2-receptor antagonists and clonidine-induced hypotension has been indicated (Finch et al 1978), the effects of these antagonists on adrenaline-induced hypotension were also studied.

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MATERIALS AND METHODS

Male, spontaneous hypertensive (SH) rats, 200–250 g, which originated as a hypertensive mutant of the Japanese strain (Okamoto & Aoki 1963), were anaesthetized with urethane (1·5 g kg^{-1} i.p.) and a tracheotomy performed to facilitate spontaneous respiration. Blood pressure was measured from a catheter, tied into the right carotid artery, using a Bell and Howell L221 pressure-transducer connected to a Devices M2 chart recorder. The mean blood pressure was taken as the diastolic pressure plus one-third pulse pressure and the heart rate was derived from the blood pressure pulse-wave by a Devices 2751 cardiotachometer.

After positioning in a David Kopf stereotaxic frame, the skull was exposed and a trephine hole made 1 mm lateral to the mid-line and 1 mm posterior to the bregma. An injection unit was lowered to a depth of 3·5–4·0 mm below the surface of the skull to facilitate the intracerebroventricular (i.c.v.) administration of drugs (Finch et al 1975). Correct placement of the i.c.v. injection unit was confirmed by injecting 10 μl of trypan blue dye, via the unit, at the end of the experiments and examining brain sections histologically for complete ventricular staining. Results from animals showing incomplete staining were discarded.

All i.c.v. injections were made in a volume of 10 μl. Adrenaline hydrochloride (BDH) was dissolved in 0·01 M HCl immediately before use and clonidine hydrochloride (Boehringer Ingelheim) was dissolved in distilled water, the doses of the two

agonists being expressed in terms of the base. Piperoxan hydrochloride (Roche), yohimbine hydrochloride (Sigma), (−)-propranolol hydrochloride (ICI) and metoprolol tartrate (Astra) were dissolved in distilled water, the doses of antagonists being expressed in terms of the salt. Metiamide and cimetidine (SKF) were supplied in injection-form and were diluted to strength with distilled water. Results are given as the mean ± standard error of the mean (s.e.m.), where n is the number of observations. Significance was accepted when $P<0.05$ using Student's t-test.

RESULTS

Clonidine (3 μg i.c.v.) induced falls, in both blood pressure and heart rate, which were maximal at 10–20 min after injection (Table 1). Adrenaline (10 μg i.c.v.) also induced a fall in blood pressure and heart rate which was maximal at 20–40 min after injection (Table 1). However, the hypotensive response induced by adrenaline (10 μg i.c.v.) was always preceded by a short-lasting hypertensive effect (Table 1).

Resting blood pressure and heart rate were not significantly affected by pretreatment with either piperoxan (25–100 μg i.c.v.) or yohimbine (25–100 μg i.c.v.) 30 min after injection. However, while neither pretreatment had any significant effect on the maximal falls in blood pressure and heart rate induced by adrenaline (10 μg i.c.v.; Fig. 1) both piperoxan (25–100 μg i.c.v.) and yobimbine (25–100 μg i.c.v.) pretreatments significantly antagonized the maximal hypotension induced by clonidine (3 μg i.c.v.; Fig. 1), piperoxan (50 and 100 μg i.c.v.) and yohimbine (50 μg i.c.v.) pretreatments also significantly antagonized the maximal bradycardia induced by clonidine (Fig. 1).

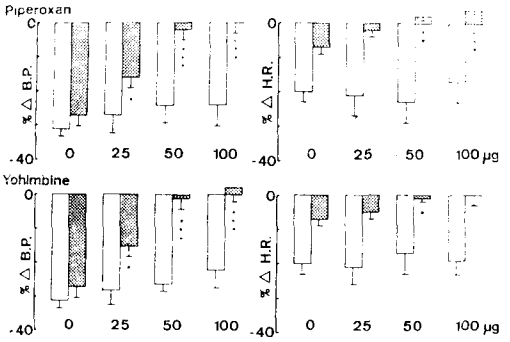

FIG. 1. The percentage changes in blood pressure and heart rate induced by adrenaline (10 μg i.c.v.) (open columns) and clonidine (3 μg i.c.v.) (stippled columns) before and after a 30 min pretreatment with piperoxan (25–100 μg i.c.v.) and yohimbine (25–100 μg i.c.v.). 7 rats were used at each dose level and the mean maximal response is shown, the vertical bars indicating the standard error of the mean. Values of significance shown are: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Neither (−)-propranolol (25–100 μg i.c.v.) nor metoprolol (25–100 μg i.c.v.) significantly affected the resting blood pressure (measured 30 min after injection) although the resting heart rate was reduced by a maximum of approximately 20% (a fall in heart rate of 60–70 beats min^{-1}). After central β-blockade, the subsequent administration of clonidine (3 μg i.c.v.) still induced falls in blood pressure and heart rate (Fig. 2), but the maximal hypotension and bradycardia induced by adrenaline (10 μg i.c.v.) were significantly antagonized by pretreatment with (−)-propranolol (50–100 μg i.c.v.) and metoprolol (25–100 μg i.c.v.; Fig. 2).

The resting blood pressure and heart rate were not significantly affected 30 min after pretreatment with either metiamide (100–400 μg i.c.v.) or cimetidine (100–400 μg i.c.v.). However, while neither

Table 1. The hypotension and bradycardia induced by adrenaline (10 μg i.c.v.) and clonidine (3 μg i.c.v.) in urethane-anaesthetized spontaneous hypertensive rats.

			% Change from basal value (mean ±s.e.m.)					
			Time (min)					
	Parameter	Basal value	1	5	10	20	40	60
Adrenaline (10 μg i.c.v.)	(n=8)							
	B.P.	126 ± 4	+6 ± 7	+12 ± 3	−1 ± 4	−27 ± 3	−35 ± 2	−25 ± 4
	H.R.	329 ± 6	−6 ± 4	−6 ± 3	−12 ± 3	−20 ± 3	−20 ± 3	−13 ± 2
Clonidine (3 μg i.c.v.)	(n=6)							
	B.P.	121 ± 5	−2 ± 2	−20 ± 1	−30 ± 4	−24 ± 3	−24 ± 2	−20 ± 4
	H.R.	322 ± 15	0 ± 2	−6 ± 1	−8 ± 2	−6 ± 3	−4 ± 2	−3 ± 3

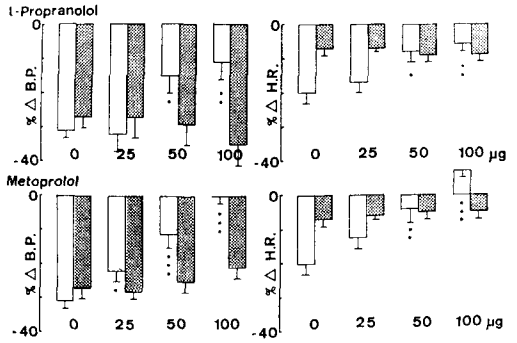

FIG. 2. The percentage changes in blood pressure and heart rate induced by adrenaline (10 μg i.c.v.) (open columns) and clonidine (3 μg i.c.v.) (stippled columns) before and after a 30 min pretreatment with (−)-propranolol (25–100 μg i.c.v.) and metoprolol (25–100 μg i.c.v.). 7 rats were used at each dose level and the mean maximal response is shown, the vertical bars indicating the standard error of the mean. Values of significance shown are: $*P<0{\cdot}05$, $**P<0{\cdot}01$, $***P<0{\cdot}001$.

pretreatment had any significant effect on the maximal adrenaline (10 μg i.c.v.)-induced falls in blood pressure and heart rate (Fig. 3), the maximal hypotensive effect of clonidine (3 μg i.c.v.) was significantly antagonized by these drugs. The bradycardia induced by clonidine (3 μg i.c.v.) was also significantly antagonized by metiamide (400 μg i.c.v.) and cimetidine (400 μg i.c.v., Fig. 3).

DISCUSSION

In conscious and urethane-anaesthetized SH rats we have consistently obtained falls in blood pressure and heart rate after the intracerebroventricular administration of adrenaline (Borkowski & Finch 1977, 1978). Since i.c.v. administrations of clonidine also induced hypotension and bradycardia in anaesthetized rats other similarities in the cardiovascular activities of centrally administered clonidine and adrenaline were looked for in support of the hypothesis that clonidine-induced hypotension may be due to central adrenaline receptor activation (Bolme et al 1974; Bolme & Fuxe 1975).

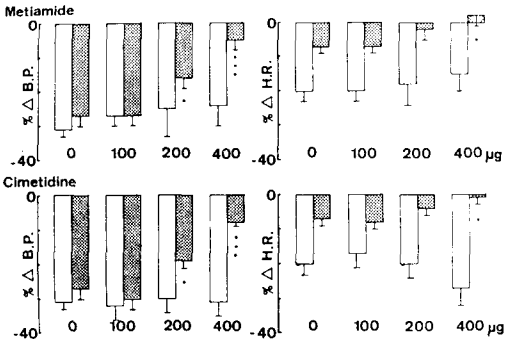

FIG. 3. The percentage changes in blood pressure and heart rate induced by adrenaline (10 μg i.c.v.) (open columns) and clonidine (3 μg i.c.v.) (stippled columns) before and after a 30 min pretreatment with metiamide (100–400 μg i.c.v.) and cimetidine (100–400 μg i.c.v.). 6 rats were used at each dose level and the mean maximal response is shown, the vertical bars indicating the standard error of the mean. Values of significance shown are: $*P<0{\cdot}05$, $***P<0{\cdot}001$.

The hypotension induced by clonidine was significantly antagonized by central pretreatment with the α-adrenoceptor blocking agents piperoxan and yohimbine. Clonidine-induced bradycardia was also effectively antagonized by central piperoxan pretreatment and these results are in accord with the work of Schmitt et al (1971, 1973) who have shown both piperoxan and yohimbine to be the most potent antagonists of the hypotensive actions of clonidine. However, neither α-adrenoceptor antagonist had any effect on the hypotension and bradycardia induced by adrenaline (10 μg i.c.v.) and it was, therefore, not possible to substantiate the proposals of Bolme & Fuxe (1975) at least in the urethane-anaesthetized SH rat that piperoxan is a preferential antagonist of adrenaline.

On the other hand, central pretreatment with (−)-propranolol or with metoprolol, two β-adrenoceptor blocking agents which significantly antagonized both the hypotension and bradycardia induced by adrenaline, was without effect on clonidine induced hypotension and bradycardia. These results suggest that the centrally induced hypotensive and bradycardic effects of adrenaline, but not those of clonidine, may be mediated by stimulation of central β-adrenoceptors in this hypertensive model.

Though the mechanisms of the interaction between histamine H_2-receptor antagonists and clonidine-induced hypotension and bradycardia are obscure, central pretreatment with metiamide and cimetidine significantly antagonized clonidine (i.c.v.)-induced hypotension. This further confirms an interaction between central clonidine and histamine H_2-receptors (Karppanen et al 1976; Finch et al 1978). The hypotension and bradycardia induced by adrenaline (i.c.v.) were unaffected by central pretreatment with either histamine H_2-antagonist, a result which indicates no histamine H_2-receptor mechanism involvement with the centrally induced cardiodepressor effects of adrenaline.

While both clonidine and adrenaline are capable of inducing hypotension and bradycardia when injected into the cerebral ventricles of urethane-anaesthetized SH rats, no evidence was found to

suggest that the hypotensive effects of clonidine may be mediated by central adrenaline receptor activation and, from the present data, it is concluded that different central mechanisms are involved in mediating the hypotension and bradycardia induced by clonidine and by adrenaline.

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