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Clonidine-induced potentiation of reflex vagal bradycardia in anaesthetized cats

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The interactions between clonidine and the selective α_1- and α_2-adrenoceptor antagonists, prazosin and yohimbine, on the angiotensin-induced reflex vagal bradycardia have been investigated in propranolol-treated chloralose-anaesthetized cats. Clonidine (1–10 μg intracisternally) had little effect on the pressor responses to angiotensin, only slightly increased the peak but markedly increased the duration of the reflex bradycardia. Neither prazosin nor yohimbine (200 μg intracisternally of either antagonist) had any effect on the reflex bradycardia to angiotensin. Yohimbine greatly diminished the clonidine-induced potentiation of the response, but prazosin was without effect. Clonidine appears to potentiate the vagal reflex bradycardia by stimulating central α_2-adrenoceptors.

Clonidine facilitates the vagally mediated reflex bradycardia elicited by intravenous injection of angiotensin in β-adrenoceptor-blocked, anaesthetized dogs (Kobinger & Walland 1972a). The facilitation occurs centrally and can be prevented by α-adrenoceptor antagonists (Kobinger & Walland 1972b). The antagonists alone depress the reflex bradycardia to angiotensin in normal, but not in catecholamine-depleted dogs (Kobinger & Walland 1973), which suggests that endogenous catecholamines modulate, rather than mediate, the reflex. In contrast to its action in dogs, clonidine is reported to have little effect on the vagal reflex in cats (Kobinger & Pichler 1978). We have re-investigated this interaction, found that clonidine *can* modulate the vagal reflex in cats, and have characterized the receptors involved using the α_1- and α_2-adrenoceptor selective antagonists, prazosin and yohimbine.

A brief account of these findings has been presented to the British Pharmacological Society (Connor et al 1981).

MATERIALS AND METHODS

General procedure

Cats of either sex (1·6–2·95 kg) were anaesthetized with chloralose (60 mg kg^{-1} i.p.) after induction with halothane and nitrous oxide. Animals were artificially respired with room air via a tracheal cannula and arterial blood samples were taken throughout the experiments for blood gas analysis using an ABL 1 acid-base laboratory (Radiometer: Copenhagen). pH, Po_2 and Pco_2 were maintained within normal limits (i.e. 7·3–7·4; 70–100 mmHg and 24–32 mmHg respectively) by alteration of the tidal volume. Blood pressure was measured via a cannula placed in the right femoral artery and heart rate was derived electronically from the pressure pulse. Blood pressure and heart rate were displayed on a Devices M2 or MX6 chart recorder. Some drugs were administered via a cannula placed in the cephalic vein. Body temperature was maintained at 37 °C by means of a thermostatically controlled electric blanket.

After the cat had been positioned in a David Kopf stereotaxic apparatus the occipital crest was exposed. The superficial neck muscles were retracted until the atlanto-occipital membrane was clearly accessible. A steel guide cannula (o.d. 0·8 mm), held at an angle of 48° to the horizontal was then introduced into the cisterna magna via a small hole pierced in the membrane. 0·9% NaCl (saline) or drugs were later injected intracisternally via a cannula positioned inside the guide cannula and connected to an Agla micrometer syringe. The injection volume was 10–40 μl.

Experimental protocol

Shortly after anaesthesia, propranolol (1 mg kg^{-1}) was injected intravenously to produce β-adrenoceptor blockade. This dose of propranolol abolished the positive chronotropic response to isoprenaline (30 ng kg^{-1} i.v.). When blood pressure and heart rate had stabilized, incremental doses of angiotensin (0·3–30 ng kg^{-1}) were injected intravenously at intervals of 10 min. Angiotensin caused a

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dose-dependent increase in blood pressure and reduction in heart rate. A single submaximal dose of angiotensin that reduced mean heart rate by approximately 25–35 beats min^{-1} was chosen from this dose range. This dose was subsequently injected at intervals of 10 min throughout the remainder of the experiment. When three or four reproducible bradycardias had been obtained to the chosen dose of angiotensin, saline (10–40 μl) or yohimbine (200 μg in 40 μl) or prazosin (2 × 100 μg, each in 40 μl, at 30 min intervals) was injected intracisternally (i.ci.) 5 min before the next dose of angiotensin. Thirty minutes later clonidine (1 or 3 μg in 10 μl) was injected intracisternally 5 min before the following dose of angiotensin: subsequent doses of clonidine (each dissolved in a volume of 10 μl) were injected at intervals of 40 min in a cumulative dose schedule, to give a final dose of 10 or 30 μg.

The effects of drugs on the angiotensin-induced bradycadia were measured in terms of the peak and of the integrated response; in the latter case changes in the duration, as well as the peak of the response were taken into account. The integrated response was measured using a digitizing board linked to a Wang mini-computer. Using this technique the units of measurement are beats min^{-1} × min = beats.

In each experiment the mean of three or four control responses to angiotensin was obtained. The mean of the 2nd and 3rd responses obtained after intracisternal injection of saline or antagonist was also derived, and so was that of the 2nd, 3rd and 4th responses obtained after injection of each dose of clonidine. (The first angiotensin-response after each drug treatment was omitted from the calculations because it has been shown that clonidine and α-adrenoceptor blocking agents take 5–10 min to act after administration by this route (Schmitt & Schmitt 1969). Drug-induced changes in the bradycardia were expressed as a percentage change compared with the control responses.

Drugs used

The following drugs were used: angiotensin amide (Hypertensin-Ciba), atropine sulphate (BDH), clonidine hydrochloride (Boehringer-Ingelheim), prazosin hydrochloride (Pfizer), propranolol hydrochloride (ICI) and yohimbine hydrochloride (Sigma). All drugs were dissolved in saline except for prazosin and yohimbine, which were dissolved by warming in distilled water in concentrations of 2·5 and 5·0 mg ml^{-1} respectively. Doses mentioned in the text refer to the free base.

RESULTS

Resting parameters

The resting mean blood pressure (diastolic + $^1/_3$ pulse pressure) and heart rate before experimentation in the control (saline treated) cats were 117 ± 7 mmHg and 177 ± 15 beat min^{-1} (n = 5). In the groups later treated with prazosin and yohimbine these values were 101 ± 6 mmHg and 179 ± 3 beats min^{-1} (n = 4) and 116 ± 3 mmHg and 194 ± 9 beats min^{-1} (n = 5) respectively. Angiotensin (0·3 – 30 ng kg^{-1} i.v.) produced dose-dependent increases in blood pressure and reflex falls in heart rate. In individual experiments, doses of angiotensin ranging between 0·7 and 10 ng kg^{-1} were found to cause reproducible and sub-maximal responses. The pressor responses ranged between 20–40 mmHg in individual preparations, and the mean peak bradycardias produced were 30 ± 5, 27 ± 2 and 36 ± 3 beats min^{-1} before intracisternal injection of saline, prazosin or yohimbine, respectively. Individual peak and integrated heart rate responses are shown in Table 1.

Interaction between intracisternally injected α-adrenoceptor antagonists and clonidine on the reflex vagal bradycardia

Saline had little or no effect on resting blood pressure or heart rate. Yohimbine had no effect on blood pressure but increased heart rate slightly and prazosin reduced blood pressure 10–15 mmHg but did not alter heart rate. Resting blood pressure declined 7–28 mmHg following the injection of clonidine in the saline- or yohimbine-pretreated groups, but not in the prazosin-pretreated group. Resting heart rate was reduced only after 10 μg of clonidine in the saline group and after 10 and 30 μg of clonidine in the yohimbine-pretreated group. Heart rate changed little after clonidine in the prazosin-pretreated group. Results are shown in Fig. 1.

The intracisternal injection of saline, prazosin or yohimbine had little or no effect on the peak bradycardia produced by angiotensin injection, or upon its overall magnitude (Table 1, Fig. 2). In the saline-treated group the subsequent injection of clonidine (1, 3 and 10 μg i.ci.) increased the peak bradycardia by a mean of only 34, 33 and 48%, respectively but the duration of the response was prolonged (see Fig. 2). The latter effect was even more marked in two of the five cats to which a higher dose of clonidine (30 μg i.ci.) was administered. The effect of clonidine on the integrated response, taking the changes in both peak and duration into account, are shown in Table 1. It can be seen that the

Table 1. The interaction between prazosin or yohimbine and clonidine on the angiotensin-induced reflex vagal bradycardia in chloralose-anaesthetized cats.

Experiment	Dose of angiotensin (ng kg^{-1} i.v.)	Pretreatment heart rate response: Peak (beats min^{-1})	Pretreatment heart rate response: Integrated (beats)	% Change of integrated response after intracisternal injection of: Saline (10–40 µl)	Clonidine (µg): 1	3	10	30
1	3	16	8	+10	+192	+376	+658	
2	10	29	15	+ 8	+149	+ 49	+376	
3	10	33	44	−10	+ 9	+ 27	+112	
4	6	45	8	−16	+ 41	+ 90	+194	+445
5	0·7	27	25	− 9	+ 76	+154	+319	+682
Mean		30	23	− 3	+ 93	+139	+332	+564
				Prazosin (200 µg)				
6	10	23	18	+24		+200	+546	+701
7	6	32	15	− 7		+ 48	+122	+367
8	10	23	11	+37		+170	+423	+691
9	1	29	25	− 5		+ 67	+128	+229
Mean		27	17	+12		+121	+305	+497
				Yohimbine (200 µg)				
10	3	44	24	+11		+137	+ 64	+146
11	3	33	33	−17		+ 45	+108	+129
12	10	28	28	+30		+ 48	− 23	− 13
13	4	34	16	−45		− 12	+ 12	+ 61
14	3	39	35	− 2		− 2	− 13	+ 27
Mean		36	27	− 5		+ 43	+ 30	+ 70

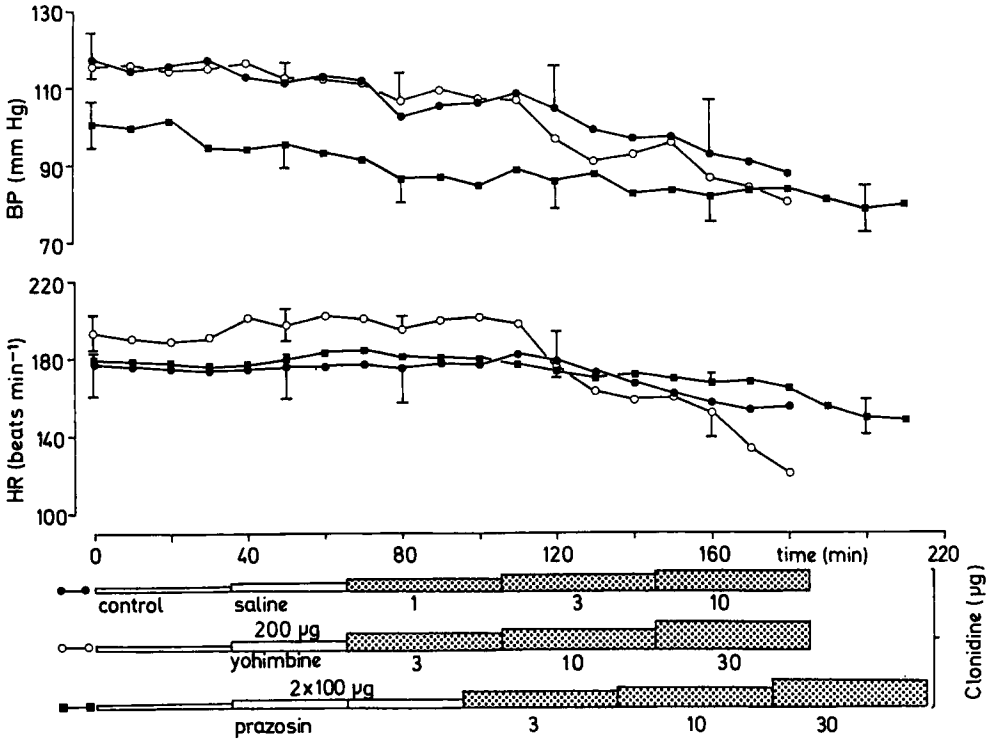

FIG. 1. The effects of intracisternal injection of clonidine alone, or after pretreatment with prazosin or yohimbine, on the resting blood pressure and heart rate of chloralose-anaesthetized cats.

clonidine-induced potentiation of the integrated response was generally dose-dependent, and tended to be inversely proportional to the magnitude of the control response.

Because of the small group sizes, and because different doses of angiotensin were selected in each experiment to produce the desired effect, no statistical analysis of the data in Table 1 has been attempted; instead, the individual results are presented. Nevertheless, it is clear that yohimbine pretreatment greatly diminished the effect of clonidine, whereas prazosin pretreatment did not.

The pressor responses to angiotensin were unchanged over the course of the experiments, except after the highest doses of clonidine in each group, when the pressor responses were reduced by 3 to 10 mmHg.

In three of the prazosin and three of the yohimbine treated cats the injection of atropine (1 mg kg^{-1}) at the end of the experiment greatly reduced or abolished the angiotensin-induced bradycardia, confirming that it was mediated via the vagus.

DISCUSSION

The intravenous injection of angiotensin in chloralose-anaesthetized cats, pretreated with propranolol, caused an increase in blood pressure and a reflex vagal bradycardia. These effects resemble those seen in dogs, but the effects of the α-adrenoceptor agonist, clonidine, and the α-adrenoceptor antagonists, prazosin and yohimbine, on these responses are somewhat different in cats. In particular, clonidine had little effect on the peak bradycardia caused by angiotensin but, instead, caused a marked, dose-dependent prolongation of the response. In further contrast to its effects in pentobarbitone-anaesthetized dogs (Kobinger & Walland 1972a) and cats (Hamilton et al 1980), clonidine had comparatively little effect on resting blood pressure or heart rate in chloralose-anaesthetized cats. This probably reflects a general characteristic of chloralose anaesthesia because Bousquet et al (1977) have shown that the cardiovascular effects of clonidine are much less marked in chloralose than in pentobarbitone-anaesthetized rats. Whatever the explanation for this finding it seems unlikely that changes in the resting parameters are responsible for the modest effect of clonidine on the peak bradycardia produced by angiotensin in the present experiments.

Neither prazosin nor yohimbine, alone, influenced the reflex bradycardia to angiotensin in chloralose-anaesthetized cats. This contrasts with the inhibitory effect of phentolamine and chlorpromazine in pentobarbitone-anaesthetized dogs (Kobinger & Walland 1973). Thus, we could find no evidence that endogenous central catecholamines modulate the vagal reflex arc in cats.

Pretreatment with yohimbine greatly reduced the effects of clonidine on the angiotensin-induced reflex

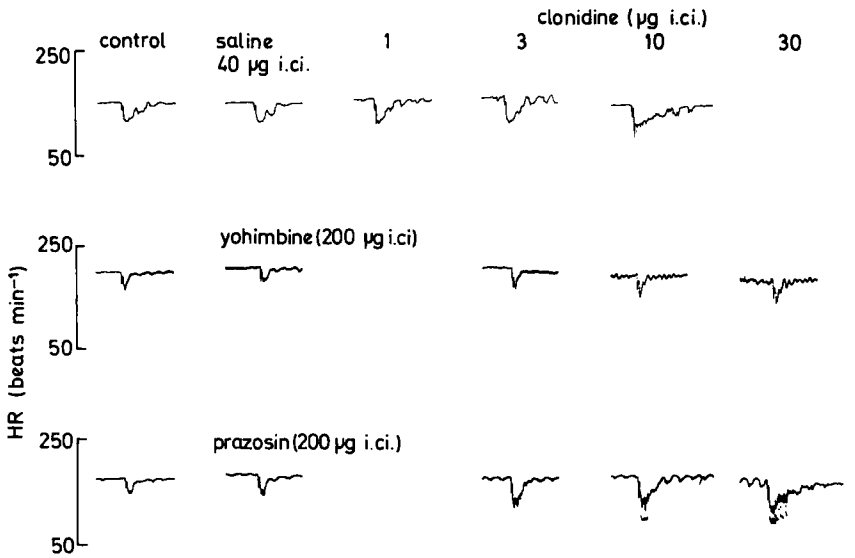

FIG. 2. The effects of intracisternal injection of clonidine alone, or after pretreatment with prazosin or yohimbine, on the reflex vagal bradycardia produced by intravenous injection of angiotensin in chloralose-anaesthetized cats.

bradycardia, but prazosin pretreatment did not. These differences cannot be ascribed to the different effects of the antagonists on the basal parameters, because prazosin, rather than yohimbine, caused a small reduction in resting blood pressure and neither antagonist reduced resting heart rate. The most obvious explanation for these findings is that yohimbine selectively antagonized clonidine; similar doses of yohimbine and other α-adrenoceptor antagonists have been reported to antagonize the central hypotensive effects of clonidine in anaesthetized cats and dogs (Schmitt et al 1973) although prazosin is ineffective in cats (Hamilton et al 1980).

Experiments carried out in isolated tissues have shown that yohimbine is approximately 100 times more potent than prazosin at blocking α_2-adrenoceptors, whereas the reverse is true at α_1-adrenoceptors (Doxey et al 1977; Drew 1979). Thus, the failure of prazosin to prevent the effect of clonidine on the angiotensin-induced reflex bradycardia suggests that clonidine acted at central α_2-adrenoceptors. Although further experiments with other selective agonists and antagonists would be needed to confirm this view, it is supported by the findings of Timmermans & van Zwieten (1980) who showed that central α_1-adrenoceptors in cats could be blocked by prazosin administered via the vertebral artery in a dose as low as 3 μg kg^{-1}.

One other feature of the present experiments deserves comment. Although, as previously stated, clonidine, alone, had much less effect on resting blood pressure and heart rate in chloralose- than in pentobarbitone-anaesthetized cats, the interactions between clonidine and the α-adrenoceptor antagonists on these parameters were the very opposite of those reported in pentobarbitone-anaesthetized cats (Hamilton et al 1980). They also differed from the way in which these drugs interacted with the angiotensin-induced reflex bradycardia *in the same animals*. Thus, relatively high doses of clonidine (10 and 30 μg intracisternally) reduced resting blood pressure in yohimbine, but not prazosin, pretreated cats. This could, at least in part, be due to the fact that resting blood pressure was lower at the beginning of the experiments in the prazosin pretreated group (although it was still quite high in both groups). However, a difference in the basal levels cannot account for why clonidine caused a much greater fall in resting heart rate in the yohimbine than in the prazosin-pretreated group.

Thus it appears that the effect of clonidine on basal blood pressure and heart rate in chloralose-anaesthetized, β-adrenoceptor-blocked cats, though muted, is mediated via central receptors more closely resembling α_1- than α_2-adrenoceptors. The effects of clonidine on resting heart rate, at least, must be due to elevated vagal output, whereas it is probably due to withdrawal of efferent sympathetic tone in pentobarbitone-anaesthetized cats in which vagal tone is almost absent (Olmsted & Page 1966). Indeed, the effect of clonidine on resting blood pressure may simply be the result of a heart rate-induced decrease in cardiac output. Whatever the explanation, these findings suggest that the choice of anaesthetic might have some bearing on whether the central cardiovascular effects of clonidine are mediated via central α_1- or α_2-adrenoceptors. Thus, pentobarbitone anaesthesia may reveal the central α_2-adrenoceptor stimulant effects of clonidine, whereas its actions at α_1-adrenoceptors are seen more clearly under chloralose anaesthesia.

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