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# THE CARDIOVASCULAR EFFECTS OF INTRAVENTRICULAR CLONIDINE AND BAY 1470 IN CONSCIOUS HYPERTENSIVE CATS

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1 Intraventricular administration of clonidine (5-30 μg) and an analogue, BAY 1470 (15-30 μg) to conscious renal hypertensive cats produced a fall in mean blood pressure lasting for approximately 3 hours. This fall in blood pressure was accompanied by a marked bradycardia.

2 Pretreatment with intraventricular phentolamine (100-200 μg), piperoxan (40-200 μg) or tolazoline (75-200 μg) abolished the cardiovascular effects of intraventricular clonidine (20 μg).

3 The cardiovascular effects of intraventricular clonidine (20 μg) were not modified by the pretreatment with either haloperidol (1 mg/kg i.p.) or desmethylimipramine (1 mg/kg i.p.).

4 Emesis was observed 1-2 min after the administration of either clonidine (5-20 μg) or BAY 1470 (30 μg). This preceded the cardiovascular actions and was still seen after pretreatment with haloperidol, desmethylimipramine, phentolamine, piperoxan or tolazoline.

5 It is concluded that the centrally mediated cardiovascular responses observed after intraventricular administration of small doses of clonidine are due to stimulation of central α-adrenoceptors and are independent of central catecholamine uptake mechanisms and dopamine receptors.

## Introduction

Clonidine is a hypotensive agent which is known to act on central cardiovascular sites and this mediates a decrease in peripheral sympathetic tone (Kobinger & Walland, 1967; Schmitt, Schmitt & Fénard, 1973a; Haeusler, 1973). The action of clonidine at these centres is thought to be due to stimulation of central α-adrenoceptors since it has been shown that phentolamine, yohimbine, and piperoxan antagonize its hypotensive action (Schmitt, Schmitt & Fénard, 1971, 1973b; Bucher, Buckingham, Finch & Moore, 1973; Haeusler & Finch, 1973).

Studies with clonidine are complicated by the observation that the drug also exerts a peripheral α-sympathomimetic effect (Dollery & Reid, 1973) which masks its initial central action and some investigators even suggest that part of its hypotensive action may be due to its peripheral effects (Zaimis & Hanington, 1969). There are also reports of drug interactions with lysergic acid diethylamide and desmethylimipramine which do not fully support the proposed central α-receptor stimulation mechanism for clonidine (Holman, Shillito & Vogt, 1971; Reid, Briant & Dollery, 1973; Van Spanning & Van Zwieten, 1973). In other studies it has been shown that clonidine interferes with centrally evoked pressor responses involved in baroreceptor and forebrain pathways which suggests that clonidine exerts its action on several intermediate neurones which may not be noradrenergic (Struker Boudier & Van Rossum, 1972; Haeusler, 1973; Klevans, Kepner & Kovacs, 1973).

In this study interactions of clonidine, administered centrally, with various agents that block α-adrenoceptors, dopamine receptors and also uptake blocking agents have been investigated in conscious renal hypertensive cats.

## Methods

### *Experimental hypertension and cannulation*

Cats were anaesthetized with pentobarbitone (35 mg/kg i.p.), the left kidney was wrapped with cellophane and contralateral nephrectomy was also performed at the same operation. Hypertension developed over a period of 3-6 weeks following these surgical procedures. At a separate operation a cannula (P.V.C. SH90) was passed down the right common carotid artery and the tip located in

the thoracic aorta. A one-way valve, attached to the catheter, was tied into the skin at the back of the neck using the method described by Day & Whiting (1972). Some cats were also prepared with an indwelling venous cannula. The cat was then placed in a Kopf stereotaxic frame and a trephine hole drilled 7 mm posterior and 5 mm lateral to the bregma. After tapping the skull, four retaining screws were placed in position before the ventricular cannula (method of Hayden, Johnson & Maickel, 1966) was cemented into position using dental acrylic. Before death methylene blue was injected via the cannula in order to check its correct placement.

*Recording of blood pressures and drug administration*

Cats were starved overnight since it was found that both clonidine and BAY 1470 produced emesis (see results section). Trained cats of either sex (2-3.5 kg), were placed in wire mesh cages for the duration of the experiment. Blood pressures were recorded with a Statham pressure transducer connected to the one-way valve by a long length of polythene tubing (PP200) and displayed on a Grass 7 polygraph. Heart rate was counted directly by increasing the chart speed. Basal pressures were obtained for 1 h before starting any drug treatment.

Intraventricular administration was carried out in conscious animals with a Trumo microlite syringe having a needle cut to size so that it penetrated directly into the lateral ventricle. Volumes of drugs were never higher than 50 $\mu$l. In some experiments animals received the same volume of vehicle adjusted to the pH of the drug solution by the addition of dilute HCl.

*Drugs*

Drugs were made up in sterile 0.9% w/v NaCl solution (saline) immediately before administration. Drugs used: BAY 1470, 2-(2,6,dimethylphenylamino)-4H-5,6 dihydro-1,3-thiazin (Bayer AG), clonidine hydrochloride (Boehringer Ingelheim), desmethylimipramine (Pertofran, Ciba-Geigy), haloperidol (Janssen), phentolamine (Regitine, Ciba-Geigy), piperoxan 1-(2-benzodioxyanyl-methyl)piperoxan, tolazoline (Priscol, Ciba-Geigy).

## Results

*Cardiovascular effects of intraventricular clonidine and BAY 1470*

Conscious renal hypertensive cats were found to have a mean resting blood pressure of 141 ± 10 mmHg($n$ = 10) and a resting heart rate of 213 ± 14 beats/min ($n$ = 10). Clonidine (5-30 $\mu$g) when administered intraventricularly produced a dose-dependent fall in blood pressure which was accompanied by a dose-dependent bradycardia. Clonidine (20 $\mu$g) was chosen as the dose for further studies since the fall of 50-60 mmHg, onset of effect (5 min), and duration of action were reproducible and submaximal (Figure 1). The accompanying bradycardia, a fall of 80 beats/min, observed after intraventricular clonidine (20 $\mu$g) was maximal 30-60 min after administration and was of shorter duration than the fall in blood pressure (Figure 2). Intraventricular administration of the vehicle solution (20 $\mu$l) of the same pH as the clonidine solution did not produce any marked changes in the resting blood pressure or heart-rate (Figures 1, 2). Administration of BAY 1470 (10-30 $\mu$g), an analogue of clonidine (Heise, Kroneberg & Schlossmann, 1971) produced a dose-dependent fall in blood pressure and heart rate. It was found that BAY 1470 was slightly less potent than clonidine in lowering blood pressure (Figures 1, 2).

The same doses of clonidine, 20 $\mu$g equivalent to 0.01 mg/kg and BAY 1470, 30 $\mu$g equivalent to 0.015 mg/kg, were given by intravenous route to three cats, on different occasions. Both drugs produced a small rise in blood pressure (10 mmHg) which lasted for a period of approximately 5 min after which time the blood pressure and heart rate returned to their resting levels for the next 180 minutes.

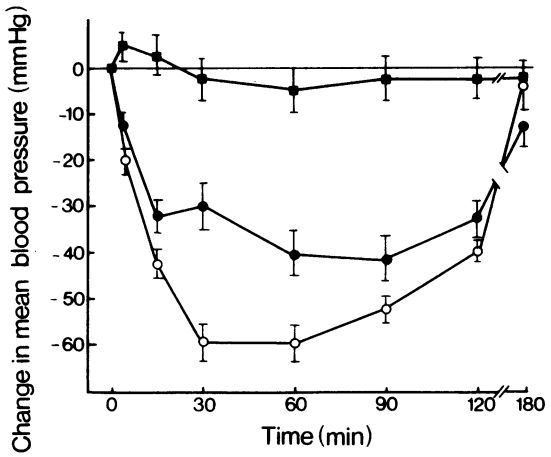


**Fig. 1** Effects of intraventricular administration of clonidine (20 $\mu$g; ○); BAY 1470 (30 $\mu$g; ●); and 20 $\mu$l vehicle (■) on the mean blood pressures of conscious renal hypertensive cats. Each point represents the mean, vertical bars indicate s.e. mean; $n$ = 10 for clonidine group, and $n$ = 4 for other groups.

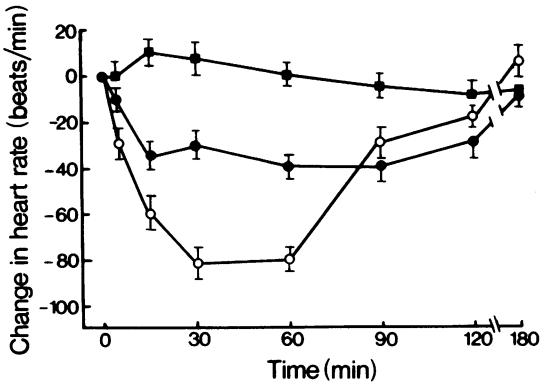


**Fig. 2** Effects of intraventricular administration of clonidine (20 μg; ○); BAY 1470 (30 μg; ●); and 20 μl vehicle (■) on the resting heart rate of conscious renal hypertensive cats. Each point represents the mean, vertical bars indicate s.e. mean; $n$ = 10 for clonidine group, and $n$ = 4 for other groups.

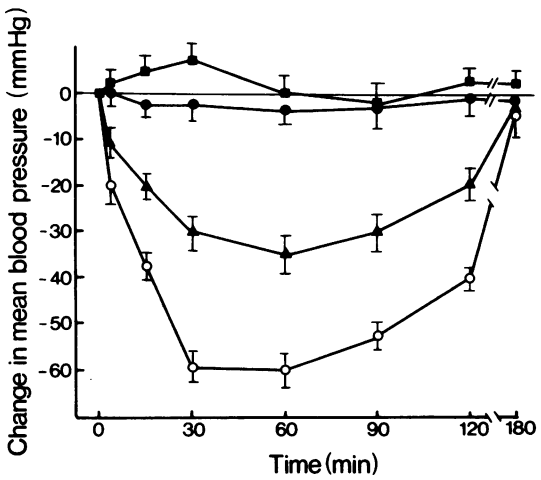


**Fig. 3** Effects of intraventricular administration of clonidine, 20 μg (○); phentolamine (200 μg; ■); phentolamine (100 μg) given 30 min before clonidine (20 μg; ▲); phentolamine (200 μg) given 30 min before clonidine (20 μg; ●) on the mean blood pressure of conscious renal hypertensive cats. Each point represents the mean, vertical bars indicate s.e. mean; $n$ = 10 for clonidine alone group and $n$ = 4 for other groups.

One to two minutes after the administration, by intraventricular route of clonidine (5-30 μg) and BAY 1470 (15-30 μg), violent emesis was always observed. This usually preceded any of the observed cardiovascular changes. Administration of the vehicle (20 μl) at the same pH as the clonidine solution did not induce emesis.

*Interaction of clonidine with α-adrenoceptor blocking agents*

Pretreatment with phentolamine (100 μg-200 μg), intraventricularly, showed a dose-dependent antagonism of the cardiovascular effects of intraventricularly administered clonidine (20 μg) and at the highest dose of phentolamine, clonidine produced no significant fall in resting blood pressure or heart rate (Figure 3). Intraventricular phentolamine (100 μg-200 μg) when given alone did not produce any significant changes in the resting blood pressure or heart rate. Very similar results were observed with intraventricular administration of piperoxan (40-200 μg) and tolazoline (75-200 μg) which at the highest doses completely prevented the cardiovascular effects produced by intraventricular clonidine (20 μg).

Although no significant changes in blood pressure and heart rate were observed after pretreatment with these three α-adrenoceptor blocking agents, emesis was still observed when clonidine (20 μg) was administered, but none of the blocking agents themselves produced this effect. Tolazoline (100 μg-200 μg) produced a marked sedation lasting 3-4 hours.

*Interaction of clonidine with desmethylimipramine and haloperidol*

Pretreatment with desmethylimipramine (1 mg/kg i.p.) produced an initial rise in blood pressure, mainly systolic blood pressure, which lasted for 60 minutes. This effect was probably due to blockade of uptake of neuronal noradrenaline since rises in blood pressure produced by intravenous noradrenaline (0.5-2 μg/kg) were potentiated by this dose of desmethylimipramine. However, this pretreatment with desmethylimipramine did not affect the cardiovascular changes or emesis observed after intraventricular clonidine, 20 μg (Figure 4).

Pretreatment of cats with haloperidol (1 mg/kg i.p.) produced no change in resting blood pressure or heart rate but these cats did show marked behavioural changes including catatonia and sedation. Clonidine (20 μg) intraventricularly, still produced the normal cardiovascular changes (Fig. 5) and emesis after pretreatment with haloperidol.

## Discussion

The observation that very small doses of clonidine and BAY 1470 when administered intraventric-

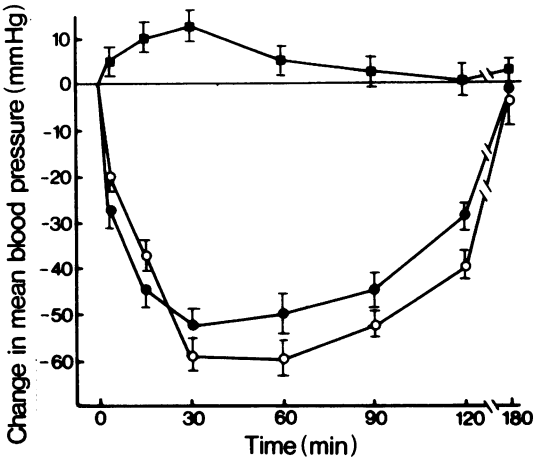


**Fig. 4** Effect of intraventricular administration of clonidine (20 μg; ○); desmethylimipramine (1 mg/kg i.p.; ■); and desmethylimipramine (1 mg/kg i.p.) given 60 min before clonidine (20 μg; ●) on the mean blood pressure of conscious renal hypertensive cats. Each point represents the mean, vertical bars indicate s.e. mean; $n = 10$ for clonidine along group and $n = 4$ for other groups.

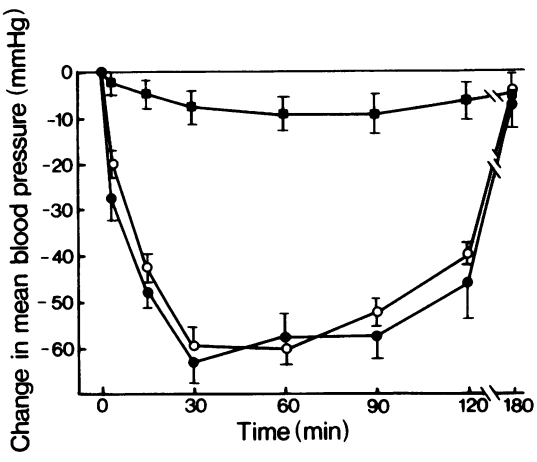


**Fig. 5** Effect of intraventricular administration of clonidine (20 μg; ○); haloperidol (1 mg/kg i.p.; ■); and haloperidol (1 mg/kg i.p.) given 60 min before clonidine (20 μg; ●) on the mean blood pressure of conscious renal hypertensive cats. Each point represents the mean, vertical bars indicate s.e. mean; $n = 10$ for clonidine alone group and $n = 4$ for other groups.

ularly to conscious cats with renal hypertension produced a marked fall in blood pressure and bradycardia confirms the hypothesis that these agents act via a central mechanism (Kobinger & Walland, 1967; Heise *et al.*, 1971; Schmitt *et al.*, 1971, 1973a). Furthermore, the same doses of clonidine given by the intravenous route did not produce any significant change in the resting blood pressure or heart rate and therefore the results in this study are independent of its peripheral sympathomimetic action (Dollery & Reid, 1973).

The central administration of α-adrenoceptor blocking agents (phentolamine, piperoxan and tolazoline) blocked the cardiovascular effects of intraventricular clonidine in a dose-dependent manner. This confirms the previous observations obtained with anaesthetized cats in which splanchnic nerve discharges were also recorded (Schmitt *et al.*, 1973b). However, in this study α-adrenoceptor blocking agents themselves reduced splanchnic discharges when given by the intracerebral route. No real explanation can be given for the difference in these results except that it might be due to an interaction with the anaesthetic agents. However, the overall observation that the hypotensive effect of clonidine is antagonized by agents such as phentolamine and piperoxan is further evidence for a central α-adrenoceptor stimulating action of clonidine.

The cardiovascular effects of intraventricular clonidine were not modified by pretreatment with desmethylimipramine. These results with desmethylimipramine must be taken with caution since there was no experimental evidence for blockade of central catecholamine uptake mechanisms. However, the dose (1 mg/kg i.p.) was similar to that used in other studies both in conscious rabbits and anaesthetized cats in which the hypotensive response to clonidine was markedly reduced by desmethylimipramine (Reid *et al.*, 1973; Van Spanning & Van Zwieten, 1973). It is possible that the partial antagonism of the hypotensive effects of clonidine observed by these investigators was due to a prejunctional action of α-receptors (Starke & Altmann, 1973). However this possibility is not supported by results in both cats and rats depleted of brain catecholamines by pretreatment with reserpine, α-methyl-*p*-tyrosine and 6-hydroxydopamine which failed to modify the hypotensive action of clonidine (Bucher *et al.*, 1973; Haeusler, 1974). The doses used in the present study (1-10 μg/kg) were very small as compared with those used by other workers even though comparable systemic changes were observed suggesting that anaesthetic agents may depress the actions of clonidine (Haeusler, 1973; Dollery & Reid, 1973; Poyser, Shorter & Whiting, 1974). The failure of haloperidol to antagonize the cardiovascular effects of intraventricular clonidine

was not surprising although it has been shown that dopaminergic pathways may be important in the central regulation of blood pressure (Bolme & Fuxe, 1971).

The emetic action of clonidine and its structurally related analogue BAY 1470 would appear to be by a mechanism different from those involved in their cardiovascular actions since α-adrenoceptor blocking agents did not prevent the vomiting. Furthermore, emesis was also observed after pretreatment with haloperidol which is known to antagonize apomorphine-induced vomiting (Janssen, Niemegeers & Schellekens, 1965). It also does not seem to be due to an irritant action of the drugs since emesis was not observed after injection of equivalent volumes of vehicle at the same pH and a close structural analogue, tolazoline, did not exert any emetic action even when administered in much larger doses. However, this emetic action of clonidine and BAY 1470 would not appear to be of any clinical significance since peripheral administration of the drugs to cats does not induce vomiting (Finch, unpublished observations).

In the present experiments with conscious renal hypertensive cats it has been demonstrated that clonidine in small doses exerts a centrally mediated hypotensive effect and an accompanying bradycardia. These cardiovascular effects were not modified by peripheral administration of haloperidol or desmethylimipramine whilst central administration of α-adrenoceptor blocking agents produced a dose-dependent reduction. Therefore, these results strongly suggest that clonidine exerts its effects independent of dopamine receptors, does not require an intact catecholamine uptake mechanism but acts via stimulation of central α-adrenoceptors.

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