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Central noradrenergic neurones and the cardiovascular actions of clonidine in the rabbit

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Summary

1. Clonidine (1 μg/kg), given by intracisternal injection to anaesthetized rabbits, lowered mean arterial blood pressure by 33 mmHg and heart rate by 32 beats/min.

2. In animals pre-treated with 6-hydroxydopamine (6-OHD 500 μg/kg intracisternally) 7–10 days before, intracisternal clonidine (1 μg/kg) reduced mean arterial blood pressure by only 2·5 mmHg and heart rate by 4 beats/minute.

3. The hypotensive action of intravenous clonidine was reduced to 49% of control by pre-treatment with intracisternal 6-OHD. In unanaesthetized normal animals intravenous clonidine (30 μg/kg) lowered mean arterial blood pressure by 19·3 mmHg, while after 6-OHD it fell only 9·4 mmHg.

4. These studies suggest that the central hypotensive effect of clonidine is dependent on the integrity of central monoaminergic neurones.

Introduction

Clonidine (2-(2,6 dichlorophenylamino)-2-imidazoline hydrochloride) (Catapres) is a potent blood pressure lowering drug (Conolly, 1970; Parsons & Morledge, 1970). Previous studies have indicated that clonidine exerts a hypotensive action when injected into the cisterna magna or lateral cerebral ventricle of several animal species (Kobinger & Walland, 1967a); Schmitt, Schmitt, Boissier, Guidicelli & Fichelle, 1968; Sherman, Grega, Woods & Buckley, 1968), after vertebral artery infusion (Sattler & van Zweiten, 1967) and in cross circulation experiments (Sherman *et al.*, 1968). The hypotensive effect appears to be achieved by a reduction in central sympathetic tone, and a reduction in peripheral sympathetic discharges to the heart and resistance vessels (Kobinger & Walland, 1967a; Schmitt *et al.*, 1968; Klupp, Knappen, Otsuka, Streller & Teichman, 1970). The site of central action is at the level of the medulla (Schmitt & Schmitt, 1969; Klupp *et al.*, 1970; Shaw, Hunyor & Korner, 1971b).

Clonidine has a direct α-adrenoceptor stimulant action on peripheral smooth muscle (Hoefke & Kobinger, 1966; Kobinger & Walland, 1967b; Farnebo & Hamberger, 1971) and it has been proposed that the central hypotensive action results from stimulation of central noradrenaline receptors. Clonidine reduces central noradrenaline turnover (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin & Svensson, 1970) and the hypotensive effect is abolished by pre-treatment with α-adrenoceptor blocking drugs (Bolme & Fuxe, 1971).

There is increasing evidence that central noradrenergic neurones play a role in the control of peripheral sympathetic tone (Chalmers & Wurtman, 1971b; Chalmers & Reid, 1971). 6-Hydroxydopamine (6-OHD) causes a selective reversible degeneration of peripheral adrenergic neurones (Porter, Totaro & Stone, 1963; Laverty, Sharman & Vogt, 1965; Thoenen & Tranzer, 1968). Intracisternal or intraventricular 6-OHD results in a profound and long-lasting depletion of noradrenaline and, to a lesser extent, of dopamine from the central nervous system with histological evidence of neuronal degeneration, particularly of noradrenergic nerve endings (Ungerstedt, 1968; Bloom, Algeri, Groppetti, Revuetta & Costa, 1969; Uretsky & Iversen, 1970; Breese & Traylor, 1971; Chalmers & Reid, 1972). 6-OHD by the intracisternal route in rabbits does not alter noradrenaline concentrations in heart or spleen (Chalmers & Reid, 1972).

The present study was designed to investigate the role of central noradrenergic neurones in the hypotensive action of clonidine by examining the effects of ablation of central noradrenergic neurones with intracisternal 6-OHD on the cardiovascular responses to intravenous and intracisternal clonidine.

Methods

Animals

All studies were performed on male New Zealand white rabbits weighing 2·3 to 2·7 kg. Rabbits were housed in individual cages and fed food pellets (R. G. Pellets, Ware, Herts) and water *ad libitum.*

Operative procedures

Under local anaesthesia a polypropylene catheter (P.P. 50 tip cemented into P.P. 100 catheter) was introduced into the central ear artery (Chalmers & Reid, 1972) and blood pressure measured with a Statham P23D6 strain gauge transducer and a Devices M.2 recorder. Mean arterial blood pressure was calculated (diastolic pressure $+\frac{1}{3}$ pulse pressure) and the heart rate was measured by direct counting from the arterial pressure trace.

A P.P. 50 catheter was passed via the central ear vein to the right atrium for the 'intravenous' administration of drugs (Chalmers & Reid, 1972).

Intracisternal administration of drugs was performed under light pentobarbitone sodium (Abbott Laboratories) anaesthesia. Compounds were given in volumes not exceeding 100 μl from a Hamilton microlitre syringe with a 26G half inch short bevel needle introduced percutaneously into the cisterna magna (Chalmers & Wurtman, 1971a; Chalmers & Reid, 1972).

Drugs

Clonidine (Boehringer Ingelheim Limited), dissolved in 0·9% w/v NaCl solution was injected i.v. over a period of 5–7 s in doses of 3, 10, 30 μg/kg in a total volume of 1 ml. Control 0·9% w/v NaCl solution (saline) injections had no significant effect on blood pressure or heart rate. Intracisternal clonidine was given in a dose of 1 μg/kg in saline in a total volume of 50 μl.

6-Hydroxydopamine (Sigma) was dissolved in saline with 1 mg/ml of ascorbic acid as an antioxidant. It was freshly prepared at the time of injection and the dose of 500 μg/kg given in a total volume of 100 μl. Vehicle injections consisted of 100 μl of saline plus ascorbic acid 1 mg/ml.

Experimental protocol

After the insertion of the catheters, the rabbits were allowed to recover individually in boxes for 1 hour. They then received intravenous doses of clonidine 3, 10 and 30 μg/kg in random order. Animals were left for a period of not less than 1 h after recovery of blood pressure to control levels before the next intravenous dose was given. Not less than 1·5 h after the final intravenous dose, the animals were anaesthetized and the intracisternal dose of clonidine given. Blood pressure was measured before and continuously for the first two min after each dose and then at 5, 10, 15, 20 and 60 minutes.

Three days after these studies the rabbits were randomly divided into two groups. One group received 6-OHD 500 μg/kg intracisternally and the remainder were given the saline-ascorbic acid vehicle to serve as controls.

Eight to ten days following this procedure, the intravenous and intracisternal injections of clonidine were repeated as described above in both the 6-OHD-treated and the control groups.

Two days later, the animals were killed with pentobarbitone sodium given i.v., and the whole brain removed, frozen and stored at $-70°$ C until assayed. Endogenous noradrenaline concentration of whole brains was measured fluorometrically (von Euler & Lishajko, 1961) after alumina column chromatographic extraction (Anton & Sayre, 1962). The noradrenaline content of 6-OHD treated rabbits was expressed as a percentage of that endogenous noradrenaline in control animals.

Statistical methods

The mean and standard error of the mean (S.E.M.) of blood pressure, heart rate and rise or fall in these variables was determined for each group of animals at each time interval, and the significance of difference investigated by the use of Student's *t* test (Snedecor & Cochrane, 1967). The log dose-response relation of heart rate and the hypertensive and hypotensive action of intravenous clonidine was determined by regression analysis. The maximum change in heart rate and pressure response, and the greatest fall in blood pressure between 2 and 10 min after the dose, was used for these analyses.

Results

Endogenous noradrenaline concentration

The mean whole brain noradrenaline concentration in control animals was 261 ± 10 ng/g. In animals pre-treated with 6-OHD 500 μg/kg 10 days before, the noradrenaline content was reduced to $57{\cdot}5 \pm 8$ ng/g (22% of control).

Intravenous clonidine

The arterial blood pressure in unanaesthetized rabbits was not significantly different in the control and 6-OHD pre-treated group ($77{\cdot}7 \pm 3{\cdot}3$ and $72{\cdot}8 \pm 3{\cdot}2$ mm Hg respectively), nor were the resting heart rates different. The mean arterial pressure and heart rate response to 30 μg/kg of clonidine intravenously is shown in Figure 1. In both groups of animals intravenous clonidine caused a dose-related,

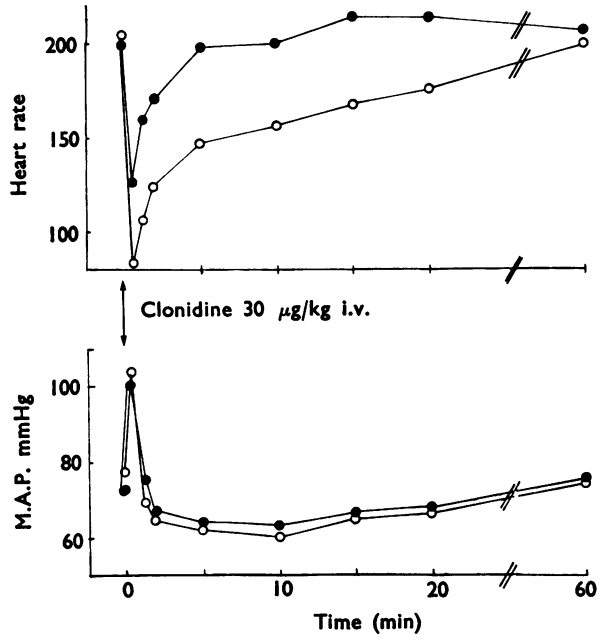

FIG. 1. Heart rate (beats/min)—top panel and mean arterial blood pressure (M.A.P.)—lower panel—after intravenous clonidine (30 μg/kg). Closed circles indicate animals pre-treated with 6-hydroxydopamine (500 μg/kg) and the open circles animals pre-treated with vehicle injection of saline and ascorbic acid. Each point represents the mean of seven animals in each group at each time interval.

acute pressure rise 10 to 30 s after injection and a later fall in pressure which was maximal at 5–10 min and lasting for 20–60 minutes. In all animals the heart rate slowed after 15 to 30 s and this lasted from 5 to 60 minutes (Fig. 1).

Acute pressor effect

The log dose-response relations for the pressor action are shown in Figure 2. There was no significant difference between the pressor action in control or 6-OHD-treated animals at any of the doses tested.

Hypotension

A fall in blood pressure evident two min after the intravenous injection of clonidine was seen particularly at the higher doses (10 and 30 μg/kg). As shown in Fig. 1 there was no significant difference between the hypotensive effect in control and 6-OHD-treated animals when the averages of the mean arterial pressure for each group at each time interval were compared. However, when the maximum fall in each individual animal between 2 and 10 min after dosing was considered, there was a reduction in the hypotensive effect in the 6-OHD-treated group. This is shown in the log dose-response curve (Fig. 3). At 30 μg/kg dose the hypotensive effect was reduced to 49% of control, and this difference was statistically significant ($P<0.025$).

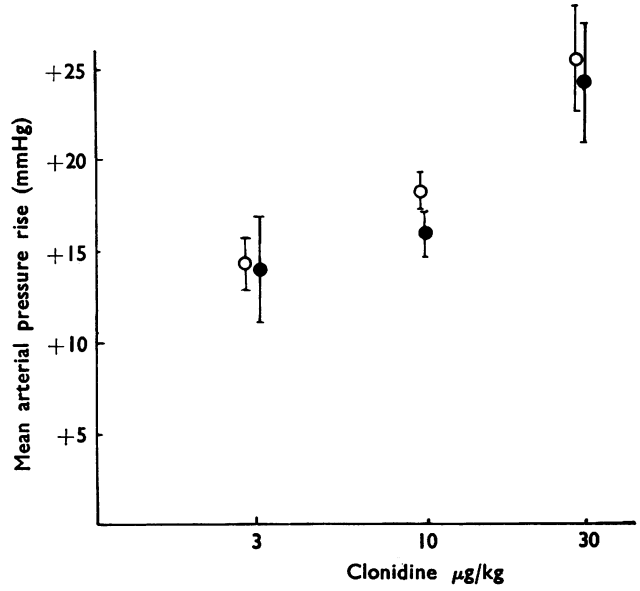

FIG. 2. The log dose-response relation of the acute rise in mean arterial pressure (mean ± standard error) following intravenous clonidine in groups of seven rabbits. The closed circles represent animals pre-treated with 6-hydroxydopamine (500 μg/kg) intracisternally and the open circles those pre-treated with vehicle injections of saline and ascorbic acid.

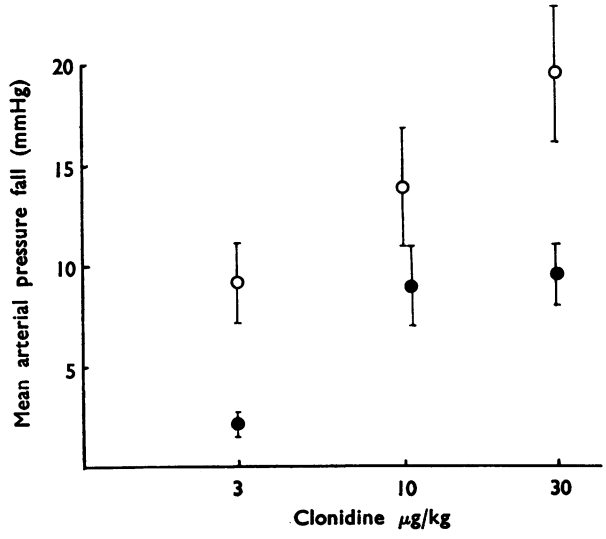

FIG. 3. The log dose-response relation of the maximum fall in mean arterial pressure (mean ± S.E., $n=7$) following intravenous clonidine in rabbits. The closed circles represent animals pre-treated with 6-hydroxydopamine (500 μg/kg) intracisternally and the open circles those pre-treated with vehicle injections of saline and ascorbic acid.

Heart rate

Clonidine, given i.v., caused a bradycardia whose duration and intensity were related to the dose (Fig. 4). In 6-OHD pre-treated animals the negative chrono-

tropic effect was less than in controls. This reduction in response was statistically significant at the 30 μg/kg dose ($P<0.025$).

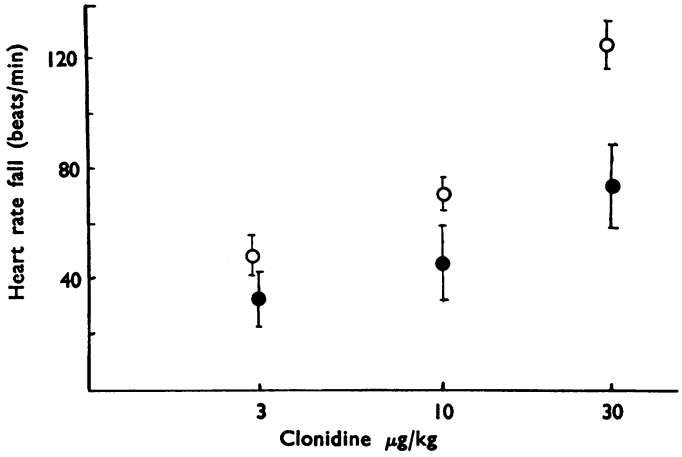

FIG. 4. The log dose-response relation of the fall in heart rate following intravenous clonidine in groups of seven rabbits (mean fall ± standard error). The closed circles indicate animals pre-treated with 6-hydroxydopamine (500 μg/kg) intracisternally, and the open circles animals pre-treated with vehicle injection of saline and ascorbic acid.

Intracisternal clonidine: hypotension

Following pentobarbitone sodium anaesthesia the mean arterial pressure rose in the control group by 4 mmHg. However, in the 6-OHD pre-treated group the pressure fell by 6 mmHg after anaesthesia (Table 1). The results of intracisternal

TABLE 1. *Mean arterial pressure and heart rate (mean ± S.E.M.) of rabbits before and after intracisternal injection in controls and animals pretreated with intracisternal 6-hydroxydopamine (6-OHD)*

Drug treatment (No. of animals)		Before anaesthesia	Anaesthetized	Time after injection 10 min	60 min
Clonidine 1 μg/kg Control group (7)	Mean arterial pressure	79·12 ±2·33	83·00 ±3·50	49·86 ±3·69	64·80 ±5·52
	Heart rate	211·70 ±12·37	296·10 ±7·56	264·57 ±12·76	268·00 ±19·35
Clonidine 1 μg/kg 6-OHD pretreated group (7)	Mean arterial pressure	74·50 ±3·33	68·70 ±6·25	66·20 ±3·57	72·50 ±5·60
	Heart rate	195·86 ±15·70	235·71 ±20·29	230·00 ±18·97	214·71 ±17·65
Saline control group (5)	Mean arterial pressure	78·20 ±6·89	81·40 ±4·62	76·50 ±2·40	79·70 ±4·88
	Heart rate	214·00 ±4·04	304·20 ±8·80	301·60 ±10·60	286·80 ±7·86

injection of 50 μl saline are shown in Figure 5. There was no consistent or significant change in blood pressure following the saline vehicle injection. The intracisternal injection of clonidine 1 μg/kg into normal animals caused an immediate and profound fall in blood pressure not preceded by a pressor response. Mean arterial pressure fell rapidly by 33 mmHg and had not recovered after 60 minutes. In animals pre-treated with 6-OHD, clonidine caused no significant change in pressure. There was a highly significant difference between the pressure fall in control and 6-OHD-treated rabbits at all times studied from 2 to 20 min ($P<0.001$). Thus 6-OHD pre-treatment had effectively abolished the hypotensive effect of intracisternal clonidine at this dose.

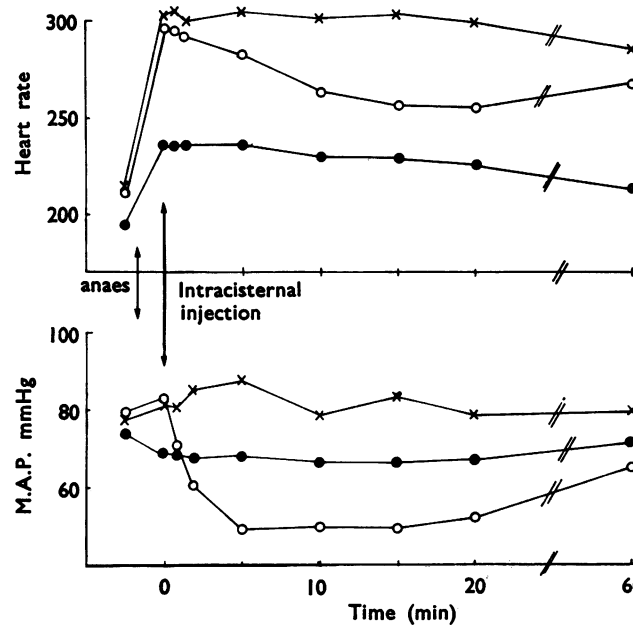

FIG. 5. Heart rate (beats/min)—top panel and mean arterial pressure (M.A.P.)—lower panel before and after pentobarbitone anaesthesia and after intracisternal clonidine 1 μg/kg in rabbits pre-treated with 6-hydroxydopamine (500 μg/kg) (closed circles) and saline/ascorbic acid vehicle (open circles). The crosses represent the effects of 50 μl saline in normal untreated animals. Each point refers to the mean of seven animals in the clonidine-treated groups and four in the saline-treated group.

Heart rate

In all groups the heart rate increased after anaesthesia. However, the increase in the 6-OHD group was less than control animals (20·5% and 40·3% respectively).

Intracisternal administration of saline had no significant effect on heart rate during the first 20 min after injection. The heart rate then fell slowly over the next 2–3 h to pre-anaesthetic values as recovery from anaesthesia took place. Intracisternal clonidine in normal animals caused a slowing of heart rate which was maximal at 10–15 min with return to the same rates as vehicle-injected animals, at 1 hour. In animals treated with 6-OHD there was no significant change in heart rate up to 20 min after which it fell gradually to pre-anaesthetic values. The time course was similar to that in vehicle-injected controls.

The bradycardia induced by clonidine in the normal rabbits was significantly different from that observed after either saline or clonidine injection in 6-OHD pre-treated animals at 10, 15, and 20 min after the dose ($P<0·01$). Pre-treatment with 6-OHD thus abolished the negative chronotropic effect of intracisternal clonidine.

Discussion

The results of these studies indicate that the hypotensive action of intracisternal clonidine depends on the integrity of central monoaminergic neurones.

The considerable depletion of whole brain noradrenaline achieved after a single dose of intracisternal 6-OHD is not associated with any significant changes in

endogenous noradrenaline content of heart and spleen and peripheral sympathetic neurone function (Chalmers & Reid, 1972). The mean arterial blood pressure of resting unanaesthetized rabbits 7–10 days after 6-OHD was not significantly different from controls and this is in accord with the previous reports on rabbits (Chalmers & Reid, 1972) and rats (Haeusler, Gerald & Thoenen, 1970; Finch, Haeusler & Thoenen, 1972). The blood pressure in 6-OHD-treated animals did fall after pentobarbitone anaesthesia and this may reflect an inability of these animals to achieve the usually observed increases in sympathetic vasoconstrictor tone reported by Korner, Uther & White, 1968. These small changes in blood pressure do not, however, mask the profound difference in response to intracisternal clonidine before and after 6-OHD.

It has been proposed that clonidine acts as an α-adrenoceptor stimulant in the central nervous system (Andén *et al.*, 1970; Bolme & Fuxe, 1971) to activate mechanisms inhibiting central and peripheral sympathetic tone. As 6-OHD primarily damages catecholaminergic nerve endings and to a lesser extent cell bodies (Bloom *et al.*, 1969; Iversen & Uretsky, 1970; Breese & Traylor, 1971) it might be expected that clonidine could still act on receptors to reduce blood pressure and heart rate. Starke, Wagner & Schümann (1972) have demonstrated that clonidine reduces noradrenaline release from isolated rabbit hearts following postganglionic nerve stimulation. Although the results of the present study would appear to support such an action on adrenergic neurones, several explanations can be proposed to reconcile the findings of this study with the agonist hypothesis.

1. Although 6-OHD undoubtedly causes depletion of catecholamines from, and degeneration of, peripheral and central noradrenergic neurones (Thoenen & Tranzer, 1968; Bloom *et al.*, 1969; Uretsky & Iversen, 1970) it has been suggested that the drug may also possess a transient α-adrenoceptor blocking action on peripheral vascular muscle lasting up to 2 days (Haeusler, 1971). Such an effect has not been demonstrated in the central nervous system, but if it did occur it would have to be of longer duration than in the periphery as in the experiments reported here, clonidine was administered 8–10 days after 6-OHD.

2. Intracisternal 6-OHD results in a variable degree of depletion of endogenous noradrenaline in the brain and spinal cord of the rat (Bloom *et al.*, 1969; Iversen & Uretsky, 1970) and the rabbit (Chalmers & Reid, 1972). The pattern of depletion appears to be determined by the route of administration, and the relative predominance of nerve endings and cell bodies (Chalmers & Reid, 1972). The depletion is unselective in that 6-OHD reduces the concentration of amine in all noradrenergic and dopaminergic neurones to which it has access. Fluorescence histochemical studies have revealed that in addition to large numbers of noradrenergic neurone and nerve endings in the medulla (Fuxe, 1965) there are also descending bulbospinal noradrenergic neurones which terminate in close proximity to the sympathetic outflow from the spinal cord (Dahlstrom & Fuxe, 1965). It is likely that both the medullary and bulbospinal noradrenergic neurones in addition to suprapontine factors are involved in the maintenance and control of central autonomic tone.

Transcollicular section and high spinal cord section localize the hypotensive action of clonidine to the medulla (Schmitt & Schmitt, 1969). However, it is unlikely that clonidine directly influences the baroreflex arc (Klupp *et al.*, 1970) as the drug does not influence the diving reflex of duck (Kobinger & Oda, 1969) or the response to Valsalva's manoeuvre in man (Muir, Burton & Lawrie, 1969). If clonidine had a

selective action activating inhibitory modulating neurones in the medulla, then following destruction of both medullary and bulbospinal tracts by 6-OHD the actions of clonidine would be abolished.

The hypotension following single intravenous injection of clonidine in rabbits is of the same order as that reported by Shaw, Hunyor & Korner (1971a). In the present study, although maximum fall in blood pressure was reduced in 6-OHD pre-treated animals, the overall pattern of the intravenous hypotensive effect was very similar. These findings may be further evidence for a quantitatively important peripheral component to the hypotensive action of clonidine which has been previously proposed (Zaimis & Hanington, 1969; Shaw, Hunyor & Korner, 1971a). However, it may only reflect the effects of anaesthesia and differences between the receptor sites in the central nervous system which can be reached by intravenous and intracisternal administration. The residual central noradrenergic neurones may lie in sites inaccessible to intracisternal 6-OHD or clonidine but attainable from the intravascular compartment. The physical properties of clonidine suggest that it would readily penetrate the brain tissue (Kobinger & Walland, 1967a), but it is possible that at the low dose used in these studies, clonidine was unable to reach by the intracisternal route residual receptor sites available to the intravenously administered drug.

The bradycardia induced by clonidine intracisternally was abolished and that following intravenous dosing was reduced by 6-OHD. This supports other findings that the negative chronotropic effect of clonidine has a central basis (Robson & Kaplan, 1969; Kaplan, La Sala, Simon & Robson, 1969; Kobinger & Walland, 1971). There is considerable evidence that central noradrenergic neurones influence heart rate not only by changes in peripheral sympathetic tone, but also by modifying vagal efferent activity. Intraventricular and intracisternal administration of noradrenaline results in cardiac slowing (McCubbin, Kaneko & Page, 1960; Toda, Matsuda & Shimamoto, 1969) which is greatly reduced by vagotomy (Share & Melville, 1963). However, intracisternal 6-OHD in rabbits causes a bradycardia which is completely abolished by atropine and which may persist for up to two weeks (Chalmers & Reid, 1972).

It has been proposed that the acute transient pressor response to intravenous clonidine is caused by a direct stimulant action of the drugs on peripheral α-adrenoceptors (Hoefke & Kobinger, 1966; Kobinger & Walland, 1967a). This is supported by the observation that this effect is not influenced by central noradrenergic ablation with intracisternal 6-OHD.

In conclusion, these studies support the hypothesis that the hypotension and bradycardia observed after intracisternal clonidine is dependent upon the integrity of central noradrenergic neurones.

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