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The central action of clonidine and its antagonism

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Summary

1. We have examined the central actions of clonidine (2-(2-6-dichlorphenylamine)-2-imidazoline hydrochloride). It has been confirmed that when infused into the vertebral artery at 2 μg/min, it caused a decrease in blood pressure and a slight increase in heart rate. The same dose given intravenously or into the carotid artery had no effect.

2. Intravertebral clonidine also greatly reduced the reflex response to carotid occlusion and the effects of an intravertebral infusion of angiotensin (1 ng/kg)/min.

3. This central action of clonidine was antagonized by the adrenergic neurone blocking drug bethanidine (4–5 mg/kg intravenously) even after the cervical cord had been transected at C_4–C_6 suggesting that bethanidine also has central actions.

4. Other drugs which also antagonized the central effects of clonidine were guanethidine (4–5 mg/kg intravenously), bretylium (10 mg/kg intravenously) and phentolamine (0·2 mg/kg intravenously).

5. It is suggested that there are central adrenergic neurones which inhibit cardiovascular autonomic reflexes and that the central autonomic effects of clonidine are due to stimulation of inhibitory adrenoceptors. The antagonism by adrenergic neurone blocking drugs of the effect of clonidine could therefore be due to blockade of these inhibitory pathways.

6. The central action of clonidine could only be demonstrated when a high concentration was infused into the vertebral artery and could not be shown with oral doses of (20 μg/kg)/day for seven days. It is concluded that the hypotensive action of therapeutic doses is unlikely to be due to the central action of clonidine.

Introduction

Clonidine (2-(2-6-dichlorophenylamine)-2-imidazoline hydrochloride) lowers blood pressure on intravenous or oral administration (Bock, Heimsoth, Merguet, & Scheonermark, 1966; Hoefke & Kobinger, 1966; Michel, Zimmerman, Nassehi & Seraphim, 1966). Several workers have suggested that this hypotensive effect is due to central inhibition of the sympathetic nervous system (Hoefke & Kobinger, 1966; Kobinger & Walland, 1967). Constantine & McShane (1968) injected clonidine into the vertebral artery of dogs in doses which had no effect when given intravenously or into the carotid artery, and suggested that the resulting hypotension and bradycardia were centrally mediated and that the site of action was the hind brain. Sattler & Van Zweiten (1967) observed the same

phenomenon in cats, and in addition found that the carotid occlusion reflex was considerably reduced, probably also via a central mechanism.

In this paper we present further experiments on the central actions of clonidine after infusion into the vertebral artery of the dog, demonstrating the effect of the drug, not only on the central pathways involved in the response to carotid occlusion, but also on the pathways which mediate the central effects of angiotensin. These effects of angiotensin can be demonstrated by infusion of low doses (1 ng/kg)/min into the vertebral artery (Lowe & Scroop, 1969) and appear to make a significant contribution to the overall effects of the hormone when infused intravenously (Scroop, Katic, Joy & Lowe, 1971).

Methods

Greyhounds, weighing between 20 and 30 kg and of either sex, were anaesthetized with α-chloralose (120–140 mg/kg intravenously) after premedication with morphine (2 mg/kg intravenously). The animals were artificially ventilated throughout the experiments.

Intravascular pressures were registered by Statham pressure transducers connected to polythene catheters. Heart rate was recorded from the electrocardiograph with a Grass cardiotachometer. The tracings were recorded on a Grass model 7 polygraph. Drugs were infused into one vertebral artery through a polythene catheter (external diameter 1 mm), 5 mm of which was inserted centrifugally into the artery, the opposite vessel being ligated. The catheter was held in place with a ligature tied loosely around the vessel without obstructing blood flow. Leakage from the catheter entry site was avoided by using a puncture needle with an external diameter slightly smaller than that of the catheter. Carotid artery catheters were inserted in a similar fashion. Intravenous infusions were given through a polythene catheter inserted into the femoral vein. In all experiments infusions of the drugs were preceded and followed by control infusions of physiological saline (0·9% w/v NaCl) at the same rate (1 ml/min).

The drugs used were clonidine ('Catapres', Boehringer-Ingelheim); angiotensin II (val^5-hypertensin II-asp-β-amide, Hypertensin, CIBA); α-chloralose ($C_8H_{11}O_6Cl_3$, BDH); bethanidine sulphate (Esbatal, B.W.); bretylium tosylate (Darenthin, B.W.); guanethidine (Ismelin, CIBA); phentolamine (Rogitine, CIBA); phenoxybenzamine (Dibenyline); tolazoline (Priscol, CIBA); (±)-propranolol (Inderal, I.C.I.); hyoscine (Scopolamine); imipramine (Tofranil); methamphetamine (Methedrine) and morphine sulphate.

Results

The central action of clonidine

Effect of vertebral artery infusions of clonidine

Vertebral artery infusions of 2 μg/min for ten minutes caused a reduction in arterial pressure. Figure 1 illustrates a typical response. The mean reduction for a series of 6 dogs was 16·6 mmHg ($P<0·10$). The pressure gradually returned to normal during the next hour. The same dose given intravenously or into the carotid artery had no effect on arterial pressure.

The fall in arterial pressure with vertebral artery infusion of clonidine was usually

accompanied by a small rise in heart rate. The mean increase for a series of six dogs was 11·3 beats/min ($P<0·10$). The heart rate returned to normal with the blood pressure. A slight bradycardia usually occurred when the same dose was given intravenously or into the carotid artery.

Effect of clonidine on the central action of angiotensin and on the response to carotid occlusion

To study the effect of clonidine on the central effects of angiotensin, the latter was infused into the vertebral artery at 32 ng/min for five min, before and after an intravertebral infusion of clonidine (2 μg/min for 10–20 minutes). The effect of angiotensin by this route is to cause an increase of heart rate and arterial pressure, which is due predominantly to inhibition of parasympathetic vagal tone to the heart (Scroop & Lowe, 1969). After clonidine, the heart rate response was abolished and the pressor response reduced as shown in Fig. 2, an effect which is very similar to that seen in the vagotomized animal in which the parasympathetic effects are abolished and the small residual pressor response is mediated by the sympathetic nerves.

The effects of carotid occlusion were also inhibited by clonidine leaving only a small residual pressor and heart rate response (see Fig. 2). Both this inhibitory effect and the reduction of the response to angiotensin lasted for only an hour, but a sustained inhibition could be produced by a continuous infusion of clonidine (1 μg/min) for as long as the experiment lasted.

Clonidine also altered the response to intravenous infusion of angiotensin. Figure 3 shows the effect of intravertebral clonidine (2 μg/min for 10–20 min) on the blood pressure and heart rate responses to intravenous infusions of angio-

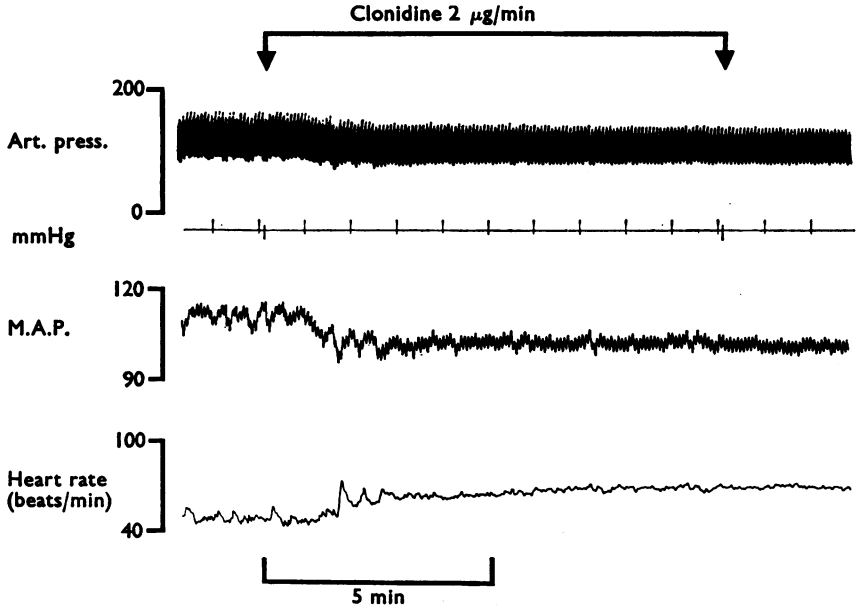

FIG. 1. Effects of vertebral artery infusion of clonidine at 2 μg/min in the anaesthetized dog. Pulsatile arterial pressure (Art. press.) mean arterial pressure (M.A.P.) and heart rate are illustrated. One division on the time scale represents one minute.

tensin at three different doses. The pressor response to each dose was reduced and there was then a reproducible bradycardia whereas before clonidine there was no consistent change of heart rate.

Antagonism of the central action of clonidine

By analogy with previous experiments (Scroop & Lowe, 1969) in the vagotomized animal, it seemed likely that after treatment with clonidine the small residual

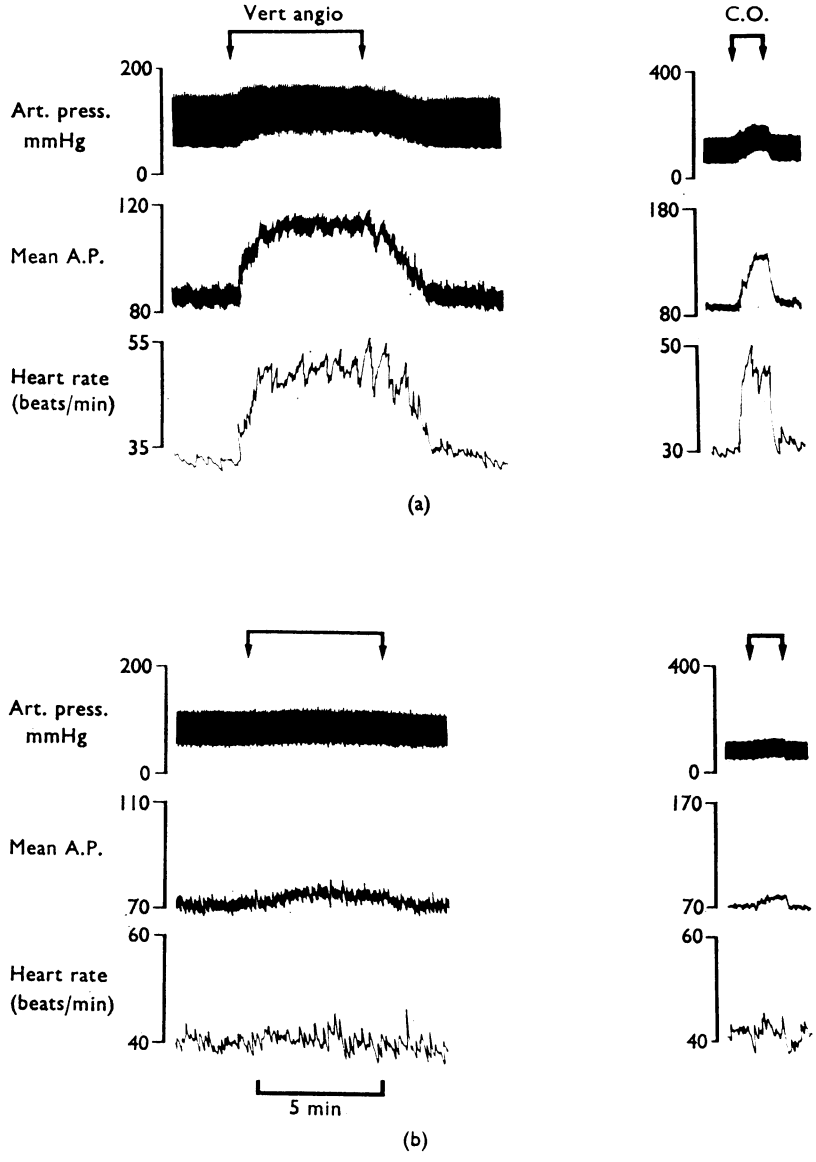

FIG. 2. Effect of clonidine on the response to infusion of angiotensin (32 ng/min for 5 min) into the vertebral artery, and to bilateral carotid occlusion (C.O.). Pulsatile arterial pressure (Art. press.), mean arterial pressure (Mean A.P.) and heart rate are illustrated. The response in the anaesthetized dog is shown before (a) and after (b) clonidine (20 μg intravertebrally).

pressor response to angiotensin was sympathetic in origin and should be abolished by bethanidine.

To test this, bethanidine (4–5 mg/kg) was infused intravenously. Contrary to our expectations, instead of abolishing the remaining pressor response, bethanidine restored both heart rate and blood pressure responses to intravertebral infusions of angiotensin. These responses could not then be reduced by further administration of clonidine. In the same way, the response to bilateral carotid occlusion returned after intravenous infusion of bethanidine. Figure 4 illustrates the mean results from six experiments. Since the response to this dose of angiotensin (32 ng/min) is mediated almost entirely via a central mechanism, this strongly suggested that bethanidine also has central actions which antagonize those of clonidine. To confirm this, in six dogs the cervical cord was transected at C_{4-6} above the level of sympathetic outflow. Both the tachycardia and the pressor response to vertebral artery infusions of angiotensin and to carotid occlusion (mediated by the vagus) were still present after cervical cord section and intravertebral clonidine still reduced or abolished this response. When bethanidine was given subsequently, the response returned. Figure 5 illustrates a typical experiment. The antagonism

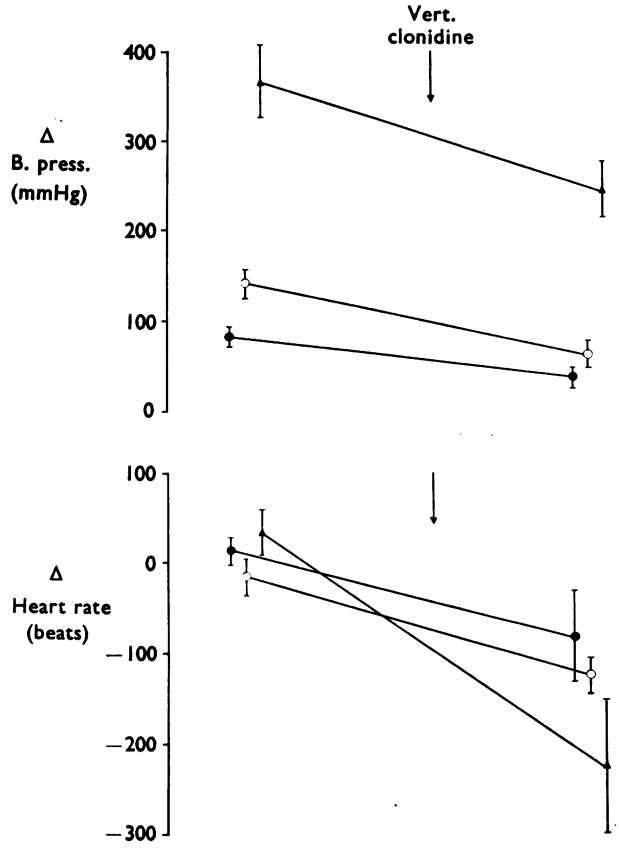

FIG. 3. Mean data from five experiments showing the effect of intravertebral clonidine (20 μg/min for 10–20 min) on the blood pressure (B. press.) and heart rate responses to intravenous infusions of angiotensin at 125 ng/min (●), 500 ng/min (○) and 2 μg/min (▲). One standard error is shown. The responses are expressed as the integral, i.e. the area of the change of heart rate or blood pressure measured by planimetry.

between bethanidine and clonidine was also demonstrated in a different way, by giving bethanidine first. Subsequent administration of clonidine failed to block the central effects of angiotensin and the response to carotid occlusion.

Interaction of clonidine with other drugs

α- and β-Adrenoceptor antagonists and adrenergic neurone blocking drugs were used in place of bethanidine to determine which antagonized the effects of clonidine. Phentolamine (5 mg intravertebrally, 8 dogs), guanethidine (120 mg intravenously, 6 dogs) and bretylium (300 mg intravenously, 3 dogs) all counteracted the effects of clonidine, but the following drugs (5 mg of each intravertebrally) did not: phenoxybenzamine (6 dogs), tolazoline (4 dogs), methamphetamine (5 dogs), imipramine (4 dogs), hyoscine (4 dogs), propranolol (7 dogs).

Chronic administration of clonidine

In six dogs, clonidine was given orally (500 μg/day) for seven days. On the eighth day the dogs were given a final oral dose and then were anaesthetized with

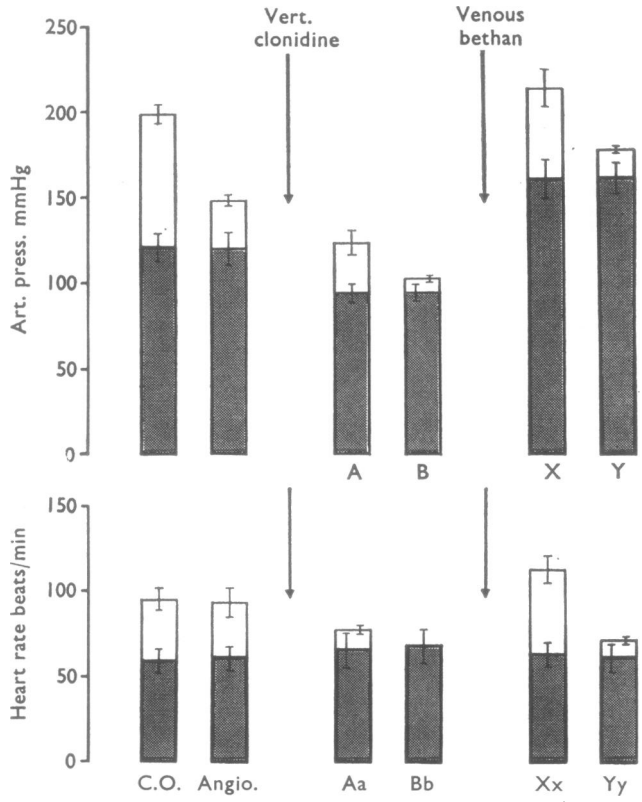

FIG. 4. Mean data from six experiments showing the antagonism of the central effects of clonidine by bethanidine. Mean arterial pressure (Art. press.) and heart rate are illustrated. The shaded areas represent the resting values and the open areas the increase in blood pressure and heart rate obtained during bilateral carotid occlusion (C.O.) and intravertebral infusion of angiotensin (Angio.) at 32 ng/min. The figure shows the effect on these responses of intravertebral infusion (20 ng) of clonidine ($P<0.02$ for each response) and the antagonism of this effect by bethanidine (Y–B, $P<0.05$; Yy–Bb, $P<0.01$; Xx–Aa, $P<0.01$; X–A, N.S.).

chloralose. The blood pressure and heart rate were not significantly different in these animals from those in normal dogs. Neither the response to vertebral artery infusion of angiotensin at 32 ng/min, nor that to bilateral carotid occlusion were significantly different from normal. Table 1 summarizes the results. Subsequent infusions of 2–4 μg/kg of clonidine intravertebrally had the effect seen in untreated animals, i.e. lowering of arterial pressure and reducing or abolishing the response to intravertebral angiotensin and to carotid occlusion, as in the acute experiments.

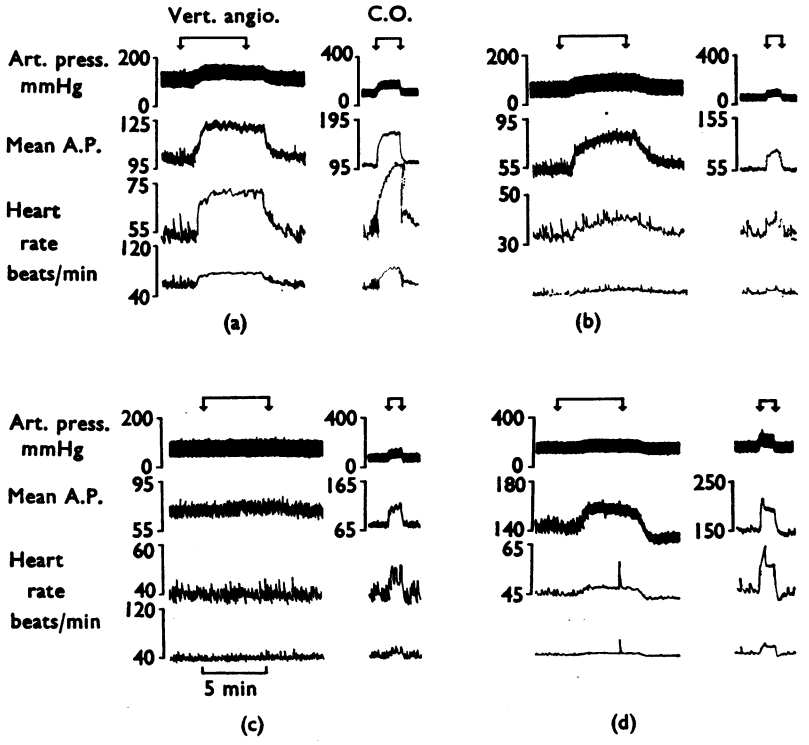

FIG. 5. Effect of infusion of angiotensin (32 ng/min for 5 min) into the vertebral artery, and of bilateral carotid occlusion (C.O.). Pulsatile arterial pressure (Art. press.), mean arterial pressure (Mean A.P.) and heart rate are shown. The responses in the intact dog are shown (a) and then the sequential effects of (b) cervical cord section, (c) clonidine (20 μg intravertebrally) and (d) bethanidine (120 mg intravenously).

TABLE 1. *Effect of oral clonidine (500 μg/day for seven days) on the increase in blood pressure and heart rate during bilateral carotid occlusion (C.O.) and vertebral artery infusion of angiotensin*

	Normal	After oral clonidine	After vertebral clonidine in the above animals
Blood pressure (mmHg)			
Mean±S.E.	108·2±5·6	108·9± 5·3	91·1 ±11·8
Response to C.O. mean±S.E.	58·0±3·5	62·5±14·4	11·5 ± 5·9
Response to angiotensin mean±S.E. (32 ng/min I-vert.)	30·0±1·3	27·6± 1·7	13·5 ± 2·6
Heart rate (beats/min)			
Mean±S.E.	47·5±3·8	54·8± 8·9	78·7 ± 6·5
Response to C.O. mean±S.E.	25·8±5·2	37·5± 8·9	15·1 ± 6·4
Response to angiotensin mean±S.E. (32 ng/min I-vert,)	32·8±9·43	23·6± 3·9	0·33± 5·5

Discussion

These experiments confirm the results of Constantine & McShane which show that intravertebral infusions of clonidine lower blood pressure by a central action. A minor difference between our results and theirs is that we observed a tachycardia on intravertebral infusion, whereas they observed a bradycardia. This difference probably reflects the different baseline heart rates which result from different anaesthesia ; we used chloralose which leaves a low resting heart rate and a high degree of resting vagal tone, whereas they used pentobarbitone which almost abolishes vagal tone and leaves a high resting heart rate. The tachycardia in our experiments is probably reflex in origin resulting from the fall of arterial pressure during clonidine infusion.

We have also confirmed that intravertebral (but not intracarotid) infusion of clonidine greatly reduces at least two central autonomic effects—the reflex response to carotid occlusion and the effects of an intravertebral infusion of angiotensin (1 ng/kg)/min. The inhibition of both these effects is probably due to a central action of clonidine because an intravenous infusion of the same dose had no effect.

Intravertebral infusion of clonidine also altered the response to intravenous infusions of angiotensin. This was anticipated because at the doses of angiotensin used in these experiments (125–2,000 ng/min) the cardiovascular changes are the result of combined central and peripheral effects. The peripheral vasoconstrictor action of angiotensin causes a rise of blood pressure and a reflex bradycardia. The central action produces a tachycardia and the balance between the two is responsible for the fact that intravenous angiotensin causes little change of heart rate. When the central effects are abolished by clonidine, the reflex bradycardia is unopposed.

An unexpected finding was that the central action of clonidine was antagonized by bethanidine. To demonstrate this antagonism it was necessary to give a large dose which caused sympathetic blockade throughout the body but it can nevertheless be concluded that bethanidine was not acting on the peripheral sympathetic nervous system because the antagonism between bethanidine and the central effects of clonidine was seen even in the dogs whose cervical cord had been cut at C_4–C_6 ; the effects of carotid occlusion and of intravertebral infusion of angiotensin in these animals were due entirely to changes of vagal tone with no contribution from sympathetic nerves. Bethanidine does not sensitize the heart to changes of vagal tone (unpublished observations), and we therefore conclude that its effect in restoring the response to angiotensin and to carotid occlusion is due to a central action. A further central action of bethanidine has been described elsewhere (Lavery, Lowe & Scroop, 1971) ; it reduces the central effect of prostaglandin $F_{2\alpha}$ by some mechanism other than by interfering with transmission in post ganglionic sympathetic neurones.

We also found that several other drugs could antagonize the central effects of clonidine, namely the other adrenergic neurone blocking drugs bretylium and guanethidine and the α-adrenoceptor antagonist phentolamine, although phenoxybenzamine and tolazoline did not, nor did the other drugs tested, propranolol, methamphetamine, hyoscine and imipramine.

It is difficult to select one hypothesis to explain the effects of clonidine and its

antagonism by some adrenoceptor antagonists because some such antagonists do not affect the action of clonidine. However, the following hypothesis seems the most plausible. We suggest that the inhibition of central autonomic effects by clonidine is due to its initial stimulant action on adrenotropic receptors; we make this suggestion both because the first action of clonidine is usually to activate such receptors (Nayler, Price, Swann, McInnes, Race & Lowe, 1968; Kobinger & Walland, 1967; Constantine & McShane, 1968) and because the inhibition is short-lived. This interpretation implies that there are central adrenergic neurones which inhibit cardiovascular autonomic reflexes by preventing changes of sympathetic and parasympathetic tone. The antagonism of this inhibition by adrenergic neurone blocking drugs would then be due to blockade of these inhibitory pathways. An alternative explanation for the antagonism is that the various drugs compete with clonidine for receptors in a non-specific way and displace clonidine from its site of action.

Neither hypothesis explains why phentolamine counteracts the effect of clonidine whereas phenoxybenzamine and tolazoline do not; the different effects of the two latter drugs might reflect a difference in penetration or access to the receptors.

We were unable to demonstrate a central action of clonidine with oral doses of up to (20 μg/kg)/day. This action has only been demonstrated when a high concentration of the drug reaches the hind brain, and we conclude that the central action of clonidine probably does not contribute to the hypotensive action of therapeutic doses in man.

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