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Localization of the central cardiovascular action of clonidine

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Summary

1. The fall in arterial blood pressure with bradycardia that occurs on injection of clonidine into the cerebral ventricles and into the cisterna magna is attributed to an action on 'chemosensitive zones' situated at the ventral surface of the brain stem. This conclusion is based on the following results obtained in cats anaesthetized with pentobarbitone sodium.

2. The fall in blood pressure no longer occurs on injection of clonidine (10 to 100 μg) into the cerebral ventricles when the passage of clonidine into the subarachnoid space is prevented by cannulation of the aqueduct. In this condition, the injections produce instead a rise in blood pressure.

3. Applied bilaterally, by means of perspex rings, to the ventral surface of the brain stem, clonidine (10 μl of a 50 to 1,000 μg/ml solution placed in each ring) produces a fall in blood pressure with bradycardia, but only when the perspex rings cover the 'chemosensitive zones' from which changes in blood pressure and heart rate are obtained with various drugs.

Introduction

In anaesthetized cats, dogs, rabbits and rats, the main cardiovascular effects of intravenous clonidine are a fall in arterial blood pressure with bradycardia. The fall is preceded by a transient rise which is a peripheral effect due to vasoconstriction. But the fall is central in origin and has been obtained with minute doses of clonidine injected into the vertebral arteries (Sattler & van Zweiten, 1967; Constantine & McShane, 1968; Katic, Lavery & Lowe, 1972) into the cisterna magna (Kobinger, 1967; Kobinger & Walland, 1967; Schmitt, Schmitt, Boissier, Giudicelli & Fichelle, 1968; Dollery & Reid, 1973), and into the lateral or third cerebral ventricle (Schmitt, 1970). The fall is not abolished by vagotomy and is due to a decrease in sympathetic vasomotor tone. A decrease in sympathetic discharge produced by clonidine was found to be particularly pronounced in the splanchnic and cardiac nerves (Schmitt, 1970). Further evidence for the role of the sympathetic nervous system in the hypotensive action of clonidine was the finding in immuno-sympathectomized rats that clonidine no longer produced its depressor effect, but only the rise in blood pressure (Zaimis, 1970).

To localize the site where clonidine acts when producing its central cardiovascular effects, Schmitt & Schmitt (1969) performed transsection experiments in cats and dogs. They found that the action must be on structures in the medulla oblongata, since clonidine still produced a fall in blood pressure, bradycardia and a reduction in the sympathetic discharge after decerebration at mid-collicular

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level as well as after brain stem transsection above the medulla oblongata; but the effects were no longer obtained after transsection of the spinal cord at the level of C_1 to C_3.

The results of the present experiments suggest that the vasodepressor and cardio-inhibiting effects of clonidine are produced by an action on 'chemosensitive zones' at the ventral surface of the rostral part of the medulla oblongata. In anaesthetized cats injections of clonidine into the cerebral ventricles no longer produced a fall in arterial blood pressure and bradycardia, when the passage of clonidine into the subarachnoid space through the foramina of Luschka was prevented by cannulation of the aqueduct. Instead, the injections caused a rise in blood pressure. However, when clonidine was applied topically to that region of the ventral surface of the medulla oblongata where previously pressor and depressor effects had been obtained with various drugs (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973) it produced a fall in blood pressure with bradycardia.

Methods

Male cats weighing between 3·6 and 4·8 kg were anaesthetized by an i.p. injection of pentobarbitone sodium (30 mg/kg) supplemented whenever required later in the experiment by an i.v. injection of 12 or 18 mg pentobarbitone sodium. For intravenous injections the left femoral vein, and for recording arterial blood pressure, the left femoral artery was cannulated. The blood pressure was recorded on a Smith's Servoscribe potentiometric recorder with a transducer connected through a Cambridge pre-amplifier (Type 72342). The trachea was cannulated, and some cats were artificially ventilated throughout the experiment with a Palmer respiratory pump. In some experiments 2 mg/kg atropine methyl nitrate, which does not pass the blood brain barrier was given intravenously.

To inject clonidine into the cerebral ventricles a Collison cannula was implanted into the left lateral ventricle near the foramen of Monro. The clonidine was injected intraventricularly in a volume of 0·2 ml and washed in by 0·05 ml artificial CSF. In those experiments in which it was intended to prevent the injected clonidine from passing into the subarachnoid space through the foramina of Luschka, the aqueduct was cannulated by a fine polyethylene tube, filled with artificial CSF, inserted through the opened cisterna magna and passed under the cerebellum up to the middle of the aqueduct, as described by Bhattacharya & Feldberg (1958). The open outside end of the tube was bent upwards and raised a little above the top of the skull, so that when filled with artificial CSF, some fluid remained in the cerebral ventricles. If the polyethylene tube was correctly positioned and snugly fitted the lumen of the aqueduct, no leakage occurred. This was checked by slowly infusing 0·1 ml artificial CSF through the ventricular cannula, resulting immediately in the same volume of outflow from the free end of the tube.

To apply the clonidine to the ventral surface of the brain stem the head of the anaesthetized supine cat was fixed to the ear bars and mouthpiece of a Dell-Moruzzi stereotaxic instrument. The method for exposing the ventral surface of the brain stem and of applying drugs to it was that of Feldberg & Guertzenstein (1972) recently described in detail with a diagram of the perspex rings and their holder (Guertzenstein, 1973).

The clonidine used was from Boehringer Ingelheim, and the atropine methyl nitrate from Sigma Chemical Company. The composition of the artificial CSF was that of Merlis (1940).

Results

Intraventricular injections

A fall in arterial blood pressure was produced by injection of 10 to 100 μg clonidine into the cerebral ventricles. The top record of Fig. 1 illustrates the effect obtained with 100 μg. After an initial rather steep fall there was partial recovery followed by a gradual prolonged fall. It then took usually 2 to 3 hours

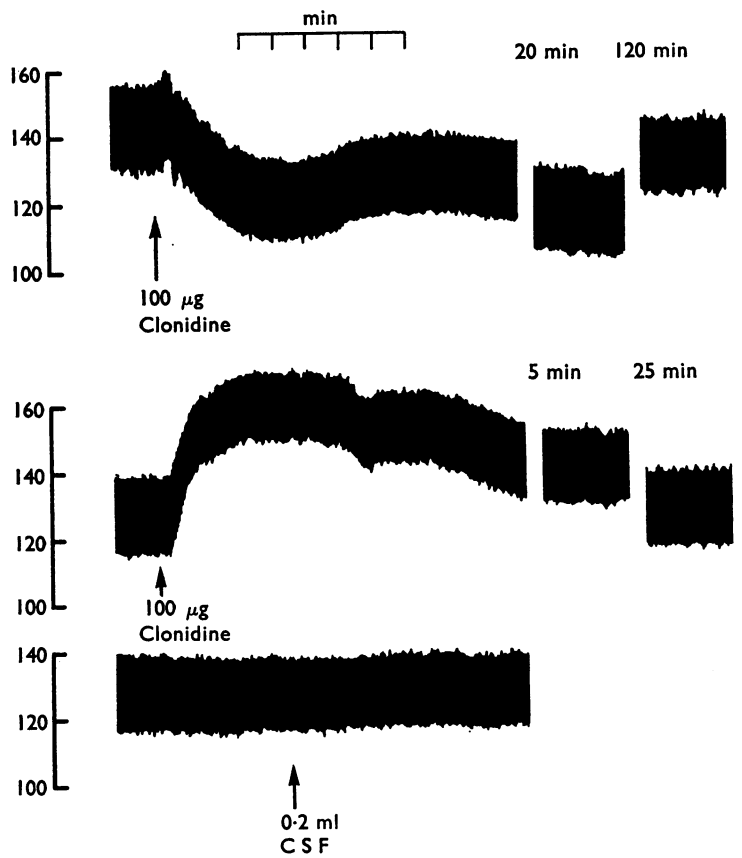

FIG. 1. Records of arterial blood pressure from a 4·5 kg cat anaesthetized with i.p. pentobarbitone sodium and artificially ventilated. The arrows in the top and middle records indicate intraventricular injections of 100 μg clonidine and the arrow in the bottom record indicates an intraventricular injection of 0·2 ml artificial CSF. Between top and middle records the aqueduct was cannulated. The breaks in the top and middle records represent intervals of 5 to 120 min as indicated. Arterial blood pressure in mmHg. Time in minutes.

for the blood pressure to return to the pre-injection level. In the experiment of Fig. 1, blood pressure fell nearly 30 mm but the sensitivity varied from cat to cat. In some cats, the injection of 100 μg produced a fall of 80 mm and the injection of 10 μg a fall of 25 mmHg. However, in the same cat the depressor

effects of clonidine did not vary much when the same dose of clonidine was injected two or three times at intervals of 2 to 3 hours.

The fall was associated with bradycardia. In the experiment shown in the top record of Fig. 1, heart rate fell from 230 to 210/minute. In other experiments, bradycardia was more pronounced. For instance, in one cat it fell from 260 to 200/min, in another from 250 to 180/minute.

When the aqueduct was cannulated so as to prevent the clonidine from entering the subarachnoid space through the lateral recesses an intraventricular injection no longer produced a fall in blood pressure. Instead, it produced a rise. In Fig. 1, the rise shown in the middle record, which is from the same cat as the top record, was produced by 100 μg clonidine injected intraventricularly after the aqueduct had been cannulated. The rise was not due to any mechanical effect of the injection itself, because a control injection of artificial CSF had no effect on the arterial blood pressure. This is shown in the bottom record. The pressor effect of clonidine after cannulation of the aqueduct could be obtained repeatedly. In the experiment of Fig. 1, it was again produced when clonidine was injected after the control injection of artificial CSF had been given.

Topical application to the ventral surface of the brain stem

A fall in arterial blood pressure, associated with bradycardia, was produced when clonidine was applied bilaterally by means of perspex rings to the area of the ventral surface of the brain stem from where depressor and pressor effects were previously elicited with various drugs (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973). The area is indicated in the diagram of Fig. 2 by the circles numbered 2. The effects obtained with clonidine applied bilaterally to this area in concentrations of 0·05 to 1 mg/ml were not prevented by an intravenous injection of 2 mg/kg atropine methyl nitrate. In experiments without artificial ventilation the clonidine produced in addition slight bradypnoea followed by tachypnoea after washing out the drug from the rings. Applied either more rostrally or more caudally to the ventral surface of the brain stem, i.e. when the perspex rings covered the areas indicated in the diagram by the circles numbered 1 or 3, the clonidine was ineffective as illustrated in the experiment shown in the top record of Figure 2.

In this experiment the perspex rings through which the clonidine was applied covered first the areas 1 (at A), then the areas 2 (at B), and finally the areas 3 (at C). Only when the rings covered the areas 2 did the clonidine produce a fall in blood pressure although its concentration was the same when the rings covered the areas 1 and twice as strong when they covered the areas 3. In this experiment heart rate fell (at B) only from 210 to 200/min, although no atropine had previously been injected intravenously into the cat.

The effects of topical application of clonidine to the areas 2, were not due to its absorption into the blood stream. This is evident from the results shown in the bottom record of Fig. 2 which is from another cat. The clonidine produced a depressor effect when applied in a concentration of 0·2 mg/ml at D, and a little stronger effect when applied in a concentration of 0·5 mg/ml at F. The volume in which the clonidine was introduced into each ring was 10 μl so that the total amount applied was 4 and 10 μg respectively. If all the clonidine

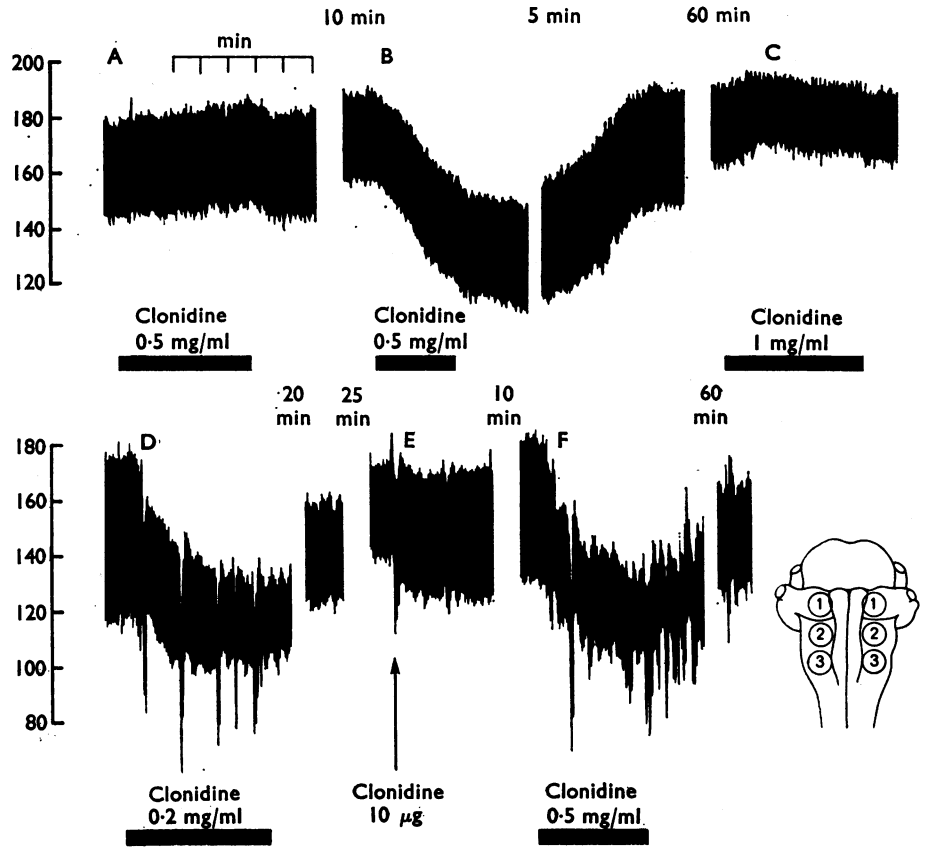

FIG. 2. Records of arterial blood pressure from a 4·8 kg (top record) and a 3·9 kg (bottom record) cat anaesthetized with i.p. pentobarbitone sodium and artificially ventilated. The cat of the bottom record had been given an intravenous injection of atropine methyl nitrate (2 mg/kg). The horizontal black bars under the records represent periods of topical application of clonidine, by means of perspex rings, to the ventral surface of the brain stem. The concentrations of the clonidine solutions are given on top of the bars. Inset, a diagram of the ventral surface of the brain stem; the numbered circles indicate the areas to be covered by the perspex rings. In the top record the rings covered the areas numbered 1 at A, numbered 2 at B, and numbered 3 at C. In the bottom record they covered the areas numbered 2. The arrow indicates an intravenous injection of 10 μg clonidine. The breaks in the records represent intervals of 5–60 min as indicated. Arterial blood pressure in mmHg. Time in minutes.

were absorbed it would not have been sufficient to produce these depressor effects, because, as shown at E, an intravenous injection of 10 μg clonidine did not lower blood pressure. In other experiments of this kind the intravenous injection of 10 μg clonidine was sufficient to lower blood pressure, but the fall was always much smaller than that which followed the introduction of 10 μl of 0·5 mg/ml clonidine into each of the two perspex rings covering the areas 2.

In the experiment of the bottom record of Fig. 2, the fall in arterial blood pressure produced during the two applications of clonidine was associated with bradycardia. During the first application heart rate fell from 260 to 230/min, and during the second application, from 250 to 216/minute. These reductions in heart rate were greater than those in the experiment of the top record of Fig. 1 although the clonidine was applied after the cat had received an intravenous injection of atropine methyl nitrate.

Discussion

Cats have no foramen of Magendie. Therefore, the previous findings that clonidine lowers arterial blood pressure and slows the heart on injection into the cerebral ventricles as well as on injection into the cisterna magna, could be explained by an action on 'chemosensitive zones' situated at the ventral surface of the brain stem. These zones are reached by drugs injected either way, though more readily on intraventricular than on intracisternal injection. For instance, pentobarbitone sodium which lowers blood pressure by an action on these zones was shown to be more effective on intraventricular than on intracisternal injection (Feldberg & Guertzenstein, 1972). These zones are reached also from the blood stream when the clonidine is injected into the vertebral arteries. So the site of action may be the same whether clonidine acts from the liquor space or from the blood stream. The results obtained by Schmitt & Schmitt (1969) in their transsection experiments are in accord with the suggestion that clonidine acts on chemosensitive zones at the ventral surface of the brain stem as they are situated at the rostral end of the medulla oblongata.

The present observations have provided experimental evidence for this suggestion. On the one hand, blood pressure fell and the heart slowed when the clonidine was applied to these zones in doses too small to be effective on intravenous injection. On the other hand, these effects no longer occurred when injections were made into the cerebral ventricles after cannulation of the aqueduct, i.e. when the passage of clonidine into the subarachnoid space was prevented. After the aqueductal cannulation the intraventricular injections of clonidine produced a central pressor effect which may explain an observation made by Shaw, Hunyor & Korner (1971). These authors found that in rabbits the depressor effect of intravenous clonidine was accentuated in 'pontine' rabbits, i.e. after transsection of the brain stem rostral to the pons. They therefore postulated a supra-bulbar action of clonidine. The pressor response unmasked after cannulation of the aqueduct on intraventricular injection may represent this action, and its elimination could account for the accentuation of the depressor response in 'pontine' rabbits.

According to results obtained by Schmitt & Schmitt (1969), clonidine would appear to act also on structures at the dorsal surface of the brain stem. In two cats they obtained a fall in blood pressure and slowing of the heart rate when pieces of blotting paper soaked in a 0·1% solution of clonidine were applied to the floor of the fourth ventricle. Their results, however, do not exclude the possibility that the action was on structures at the ventral surface of the brain stem. This possibility was not envisaged. Therefore, the necessary precautions were probably not taken to prevent an escape of small amounts through the foramina of Luschka at the lateral recesses. If only a few microlitres of the fluid had escaped through these channels and had reached the ventral surface, even in a diluted form, this would have been sufficient because the clonidine was effective in 20 times weaker concentration when applied to the ventral surface. This appears to be more likely than an action on structures at the floor of the fourth ventricle, which would imply that clonidine produces the same cardiovascular effects when acting on structures at the dorsal and ventral surface of the rostral part of the medulla oblongata. However, at present this possibility cannot be excluded.

The slowing of the heart produced by clonidine both on intraventricular injection and on topical application is mainly or wholly due to inhibition of sympathetic

tone and not to increase in vagal tone because it was not prevented by intravenous injection of atropine methyl nitrate. The cardio-inhibitory effect of intravenous clonidine too is not abolished by atropine though it is reduced (Boissier, Guidicelli, Fichelle, Schmitt & Schmitt, 1968). In the present experiments the methyl nitrate of atropine was used for the intravenous administration because it does not pass the blood brain barrier. Therefore, a possible interaction was excluded with clonidine on the chemosensitive zones at the ventral surface of the brain stem on which atropine itself has an action (Guertzenstein, 1973). Both the depressor and cardio-inhibitory effects of clonidine thus appear to result mainly or wholly from the same mechanism of action, a decrease in sympathetic discharge, which was found by Schmitt (1970) to be particularly pronounced in the cardiac nerves.

Clonidine is only one of many drugs which produce vasodepression due to decrease in sympathetic vasomotor tone when applied to the ventral surface of the brain stem. Vasodepression was obtained with pentobarbitone sodium, carbachol, physostigmine, glycine and γ-aminobutyric acid (Feldberg & Guertzenstein, 1972; Guertzenstein, 1973). Although these substances all produce this effect when acting on the chemosensitive zones at the ventral surface of the brain stem it does not follow that they act on the same synapses.

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