THE MECHANISM OF ACTION OF CLONIDINE

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NUMEROUS experiments have documented the central hypotensive action of clonidine. As a matter of fact perfusion of the cerebral cavities from the lateral ventricle to the cisterna magna (BOUSQUET *et al.*, unpublished), intracisternal injections (SCHMITT *et al.*, 1973) and perfusion of clonidine through the vertebral artery (SATTLER and VAN ZWIETTEN, 1967), always produce a fall in arterial pressure together with a slight bradycardia.

(a) Nevertheless, the mechanism of the hypotensive action of clonidine is not well known; that mechanism could be of an adrenergic type (SCHMITT, 1971) although noradrenaline, when injected intraventricularly or intracisternally, has not, as a rule, produced a hypotensive effect.

The structure of the clonidine molecule has been established from an analysis of its i.r., u.v. and NMR spectra (WERMUTH *et al.*, 1973). In the protonated form which predominates *in vivo* (pK_a of clonidine is 8·2) the positive charge is delocalized on the carbon atom and the three nitrogen atoms in the guanidine function. The imidazoline ring rotates around the ArN-C bond. Clonidine has the characteristics of an α-mimetic drug as described by COUBEILS *et al.*, (1972) when the rings of the molecule lie at right angles to each other. The distance between the center of the aromatic nucleus and one of the imidazoline nitrogen atoms of clonidine or the amine function of a sympathomimetic agent is identical, i.e. 5·1Å. Similarities between clonidine and noradrenaline are evident and even more so between clonidine and dopamine.

(b) As for the mechanism of action of clonidine, its site of action also has not been well established. However, in a recent study, BOUSQUET and GUERTZENSTEIN (in press) have demonstrated that the action of clonidine is localized on the ventral surface of the brain stem of the cat; these data are consistent with the work of FELDBERG and GUERTZENSTEIN (1972) which had enabled localization of the vaso-depressive action of pentobarbitone sodium in a region caudally to the trapezoid bodies and laterally to the pyramids. In this region a topical application of clonidine produced a fall in arterial pressure.

The anatomic description of this area has been made by PETROVICKY (1968): it is a very precisely delimited region where, the glia marginalis being very thin, the neurons are found immediately under the pia mater whether cell bodies or dendrites. These neurons form a small nucleus which is 2-mm long, 1-mm wide and 1-mm deep. This nucleus is made up of two kinds of cells and its rostral part is in contact with the nucleus paragiganto-cellularis.

METHODS

The experiments were performed on cats weighing 1·7–3 kg. The cats were anaesthetized by intraperitoneal injection of pentobarbitone sodium (30 mg/kg). In a few experiments, anaesthesia was induced with ether.

For artificial respiration (Logic 03 respirator) the trachea was cannulated. The left femoral vein was catheterized for intravenous injections. The left femoral artery was also cannulated and connected to a physiological pressure transducer (Statham P23 Db). The arterial pressure was recorded by a Minipolygraph Gilson M 5 P (module CHCBPP). In some experiments arterial pressure was recorded by means of a potentimetric module (SE 21 servomodule Gilson) and the mean heart rate by the means of a cardiotachymeter (module CT 27 Gilson) connected to the module CHCBPP.

The method using the topical application of drugs on the ventral surface of the brain stem was realized in the same way as originally described by FELDBERG and GUERTZENSTEIN (1972) and GUERTZENSTIEN (1973) by means of two plastic rings. The drugs were applied in each ring in a volume of 10 μl. The rings were placed just under the pons on each side of the midline on the surface of the medulla oblongata under light pressure; thus the fluid cannot flow out of the rings. After each application of the drug, the surface was washed out with artificial CSF (MERLIS, 1940). The drugs were also dissolved in artificial CSF.

RESULTS

(1) Figure 1 shows the fall in arterial pressure which is regularly caused by the topical application of 10 μl of a solution of clonidine during 5 min on each side of the ventral surface of the brain stem.

Clonidine proves to be active in very low concentrations: 100 μg/ml (which is equivalent to the application of a dose of 2μg/kg) induces a fall in arterial pressure of

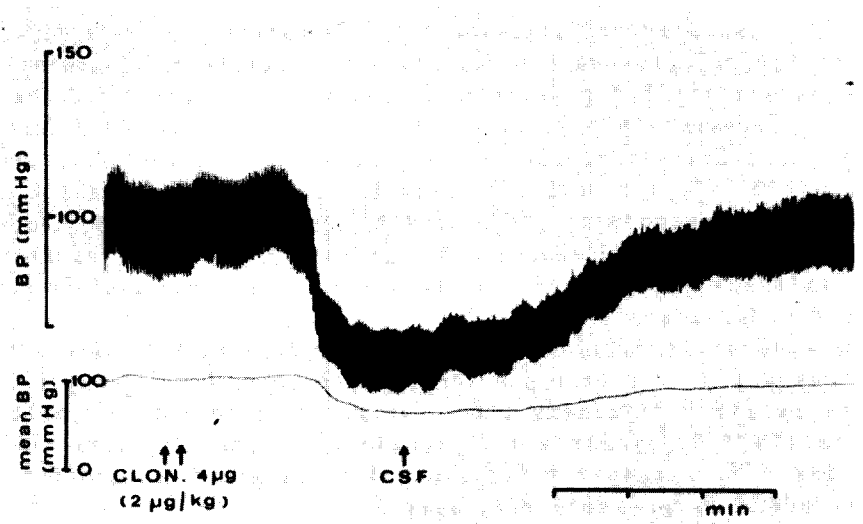


FIG. 1.—Effect of clonidine on arterial blood pressure when topically applied on the ventral surface of the cat's medulla.
↑↑ left then right application of clonidine
↑ washout with artificial CSF.

30–40 mm Hg. The application on one side only is not as effective even when the concentrations are higher, but, as a rule, hypotension appears as soon as clonidine comes into contact with the second side. The duration of the hypotensive effect is variable; the arterial pressure generally returns to the initial level after 20–60 min, sometimes even after several hours, and this delay is all the more longer that the hypotensive effect is greater. In many experiments hypotension is accompanied by a slight bradycardia.

It is impossible to state that this area of the ventral surface of the brain stem where the application of low doses of clonidine causes a fall in arterial pressure is its only site of hypotensive action; nevertheless it is a hypothesis which may be assumed. In fact, the intraventricular perfusion of clonidine does not cause any fall in arterial pressure when the drug is not in contact with the ventral face of the brain stem. Furthermore BOUSQUET and GUERTZENSTEIN (in press) have demonstrated that clonidine injected into the lateral ventricle of the cat proves to be hypertensive when the drug flows through a cannula placed in the aqueduct of Sylvius. Consequently the site of hypotensive action of clonidine can only be localized in the brain stem. Some current experiments enable us to assert that this action does not take place in the floor of the fourth ventricule, that is to say, on the dorsal surface of the brain stem.

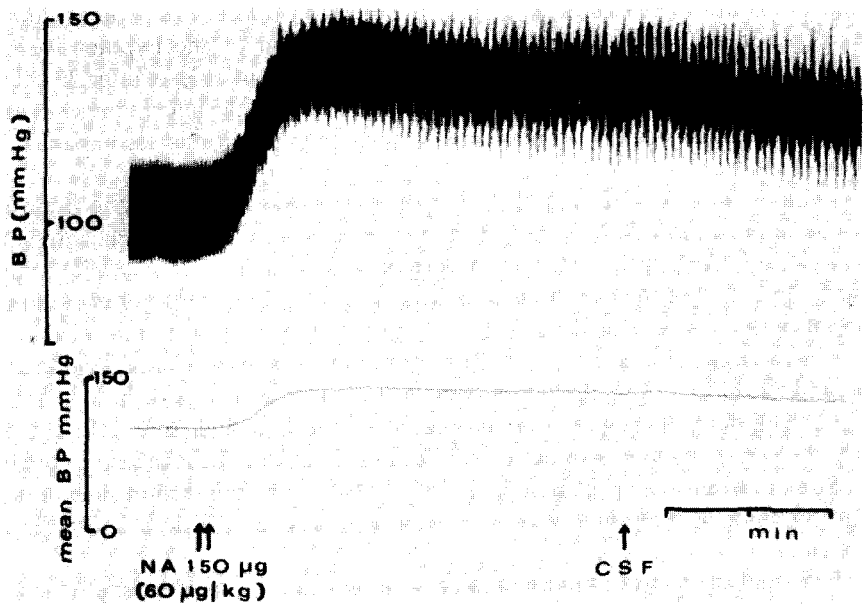
(2) The analysis of the clonidine molecule shows that it has the same structure as noradrenaline and dopamine.

(a) Therefore we applied noradrenaline in concentration of 0·5–10 mg/ml to the site where clonidine is active. At low doses, less than 2 mg/ml, noradrenaline does not modify arterial pressure; at higher concentrations of 5–10 mg/ml. a hypertension is produced which lasts for several minutes before recovery takes place; there is never any hypotensive phase (Fig. 2). Besides, the hypertensive effect which occurs when high concentrations of noradrenaline are applied may be due to a fraction of the product passing into the blood stream (the total dose applied under these circumstances corresponds to amounts of 100–200 μg).

(b) On the other hand, dopamine in concentrations of 12·5–100 mg/ml (that is to say application of 250 μg–2 mg) shows a hypotensive effect as a rule, and there is a certain linear relationship between the dose and the hypotensive effect. Like clonidine, dopamine induces an important, immediate and long lasting hypotension (providing that this amine is applied on both sides) (Fig. 2); it is accompanied by a slowing of the heart. At high doses the effect may sometimes be diphasic: an initial hypertension which lasts for a few minutes followed by hypotension which remains for more than an hour even though the product is applied for not more than 5 min.

We have been able also to show in the dog that arterial hypotension can be caused by infusion of dopamine into the lateral ventricle with an outflow cannula in the cisterna magna, in doses of 10–50 μg/kg, in a total volume of 1 ml during 5 min. Same results are obtained with clonidine in doses of 1–10 μg/kg. On the contrary, under these circumstances, noradrenaline never causes hypotension and shows hypertensive effects at higher doses.

Besides, when injected intravenously, amounts of dopamine similar to these applied in cats or infused in dogs, always induce an important rise in arterial pressure.



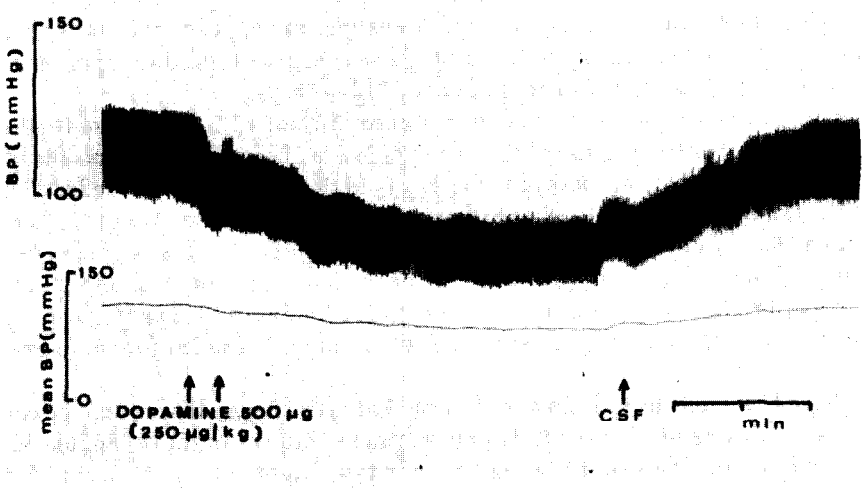


FIG. 2.—Effect of noradrenaline (above) and dopamine (below) on arterial blood pressure when applied on the ventral surface of the cat's medulla.
↑↑ left then right topical application of the drug solutions
↑ washout with artificial CSF

DISCUSSION

Many authors have studied the structure of the ventral surface of the medulla oblongata and earlier works took into account a chemosensitive area at this level. It has indeed been demonstrated that pledgets of filter paper soaked in artificial cerebrospinal fluid with high pCO_2 or H^+ concentrations as well as when containing nicotine or acetylcholine produced a hyperpnea, (LOESCHCKE *et al.*, 1958; MITCHELL *et al.*, 1963) when applied to a zone corresponding closely to that described by PETROVICKY (1968). On the contrary procaine induced a depression of respiration and a fall in arterial pressure (LOESCHCKE and KOEPCHEN, 1958a, b). Localized cooling in this area of the ventral surface of the medulla resulted in a reduction of the tidal volume and a drop in arterial pressure (SCHLAEFKE and LOESCHCKE, 1967).

Subsequently, FELDBERG and GUERTZENSTEIN (1972) have shown that pentobarbitone sodium has a vasodepressive effect in this area. Moreover, GUERTZENSTEIN (1973) demonstrated that in the same area cholinomimetic substances (physostigmine and carbachol) as well as glycine and GABA induce a fall in arterial pressure antagonized by atropine. With strychnine, leptazol and tubocurarine, an increase in arterial pressure can be observed. It is in that same area that BOUSQUET and GUERTZENSTEIN (in press) have localized the hypotensive action of clonidine. And in that area also experiments we report here demonstrate a specific vasodepressive activity of dopamine, structurally related to clonidine, while noradrenaline, also analogous to clonidine, proves to be vasopressive. Nevertheless clonidine is obviously more potent (a total dose of 4–10 μg of clonidine causes an evident hypotension which requires a dose of 250 μg of dopamine). Our experiments show that clonidine acts in an analogous fashion to dopamine but not to noradrenaline.

At the level of the structures we studied, dopamine may have a direct mediatory action, or may play the role of a modulator in underlying cholinergic mechanisms (BERTLER and ROSENGREN, 1966). Based on the results of experiments, which demonstrate hypotensive actions of both cholinomimetic agents and dopamine on the ventral surface of the brain stem, the second of these hypothesis seems more plausible.

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