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The central hypotensive action of amphetamine, ephedrine, phentermine, chlorphentermine and fenfluramine

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The influence of amphetamine, ephedrine, phentermine, chlorphentermine and fenfluramine on blood pressure after infusion into the vertebral artery was studied in anaesthetized cats. All drugs lowered blood pressure after infusion into the vertebral artery but showed hypertensive properties upon intravenous administration in the same dosage, although chlorphentermine (i.v.) decreased pressure after a minor initial rise. The hypotensive action of amphetamine was blocked by piperoxan or yohimbine (α-adrenoceptor blocking agents) and by haloperidol which is known to block central adrenoceptors. The hypotensive action of amphetamine was abolished in reserpinized cats and usually a small increase in blood pressure was observed in these animals after infusion of amphetamine in the vertebral artery. The hypotensive action of amphetamine is probably due to the mobilization of noradrenaline in the brain. The results suggest that the drugs show central hypotensive properties probably brought about by stimulation of central α-adrenoceptors, thus causing a *decrease* in peripheral sympathetic tone. This general principle would also explain the central hypotensive properties of α-methyldopa, α-methylnoradrenaline, L-dopa and m-tyrosine.

The antihypertensive agent clonidine [2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride] decreases the activity of the peripheral sympathetic nervous system via a primarily *central* mechanism. An inhibition of the medullary vasomotor centres seems the most likely initial effect of this drug (Kobinger, 1967; Sattler & van Zwieten, 1967; Schmitt & others, 1967; Klupp, Knappen & others, 1970), Schmitt, Schmitt & Fenard (1971) postulated that this mechanism is brought about by the activation of central α-adrenoceptors. Thus the strong hypotensive action of clonidine would be the result of the drug's *central* α-adrenoceptor stimulant properties. If this is correct, other sympathomimetic agents upon central application should also decrease sympathetic tone. Classical α-adrenoceptor stimulant agents like noradrenaline hardly penetrate the blood brain barrier, so possible central properties can be demonstrated only after intraventricular injection. Noradrenaline via this route indeed evokes a decrease in blood pressure (Kaneko, McCubbin & Page, 1960; McCubbin, Kaneto & Page, 1960). The influence of sympathomimetic amines that pass through the blood brain barrier owing to the lack of phenolic hydroxyl moieties has not yet been examined in this context. We established the influence on blood pressure of amphetamine and ephedrine applied either intravenously or into the vertebral artery (central application). The appetite suppressants phentermine, chlorophentermine and fenfluramine, which hardly possess peripheral sympathomimetic properties, were included. The results suggest that the stimulation of central

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α-adrenoceptors by amphetamine and related drugs decreases peripheral sympathetic tone similarly to clonidine, although clonidine is much more potent. Part of the results have been reported previously in a preliminary communication (Hoyer & van Zwieten, 1971).

METHODS

All experiments were made on cats of either sex (2–4 kg) that had been kept on a normal diet. The animals were anaesthetized by a single intraperitoneal injection of chloralose 60 mg/kg. Throughout, the animals were artificially respired by the cannulated trachea. A catheter was inserted into a femoral vein. After surgical intervention each animal received approximately 2500 i.u. of heparin intravenously. To infuse drugs into the left vertebral artery the first three ribs were severed from the sternum and the thorax was opened.

The left subclavian artery was exposed and all its side branches (thyrocervical trunk, costocervical trunk, left mammary artery and internal thoracic artery), except the left vertebral artery, were ligated. A catheter was inserted into the distal end of the subclavian artery until its tip lay a few mm on the distal side of the entrance of the vertebral artery. Slow infusion of drugs in a small volume of saline resulted in an infusion of drug into the vertebral artery at a rate of 0·1 ml kg^{-1} min^{-1}. Details of this technique have been described (van Zwieten, Bernheimer & Hornykiewicz, 1966; Reis and van Zwieten, 1967; Henning & van Zwieten, 1968). Blood pressure in a femoral artery was recorded continuously. In some cats, heart rate was established by ecg. In some experiments the blood glucose concentration was measured at regular intervals in venous blood samples, using *o*-toluidine as reagent (cf. Hultmann, 1959); in these experiments sodium pentobarbitone (50 mg/kg, i.p.) was used for anaesthesia to avoid possible interference of anaesthetic with the assay.

Statistical evaluation of the results was made using Student's *t*-test. With hypotensive effects, *P* values for the *minimum* on blood pressure were given compared to control values. For this purpose a statistical comparison with the pre-infusion blood pressure level was made.

Drugs used: (±)-Amphetamine hydrochloride (Nordmark GmbH., Uetersen); (±)-ephedrine hydrochloride (Merck AG., Darmstadt); phentermine hydrochloride (Tropon AG., Köln); chlorophentermine hydrochloride; (±)-fenfluramine, piperoxane hydrochloride and yohimbine hydrochloride (kindly put at our disposal by Prof. Dr. H. Schmitt, Paris). All were administered in saline as pure compounds, devoid of commercial solvents. Heparin (NOVO, Copenhagen), sodium pentobarbitone (Nembutal, Deutsche Abott GmbH., Ingelheim), Reserpine (Serpasil, CIBA AG., Basel) and haloperidol (Haloperidol Janssen, Beerse, Belgium) were administered as commercial preparations.

RESULTS

The mean blood pressure of all chloralose-anaesthetized cats about 30 min after the described surgical intervention amounted to 117 ± 4 mm Hg (mean ± s.e., n=75). Without drug this level remained virtually unchanged over at least 2 h. The effect of a control infusion of saline into the vertebral artery is shown in Fig. 1.

In each animal the mean arterial blood pressure just before drug administration was taken as 100% and was only considered to be constant if it had not changed for

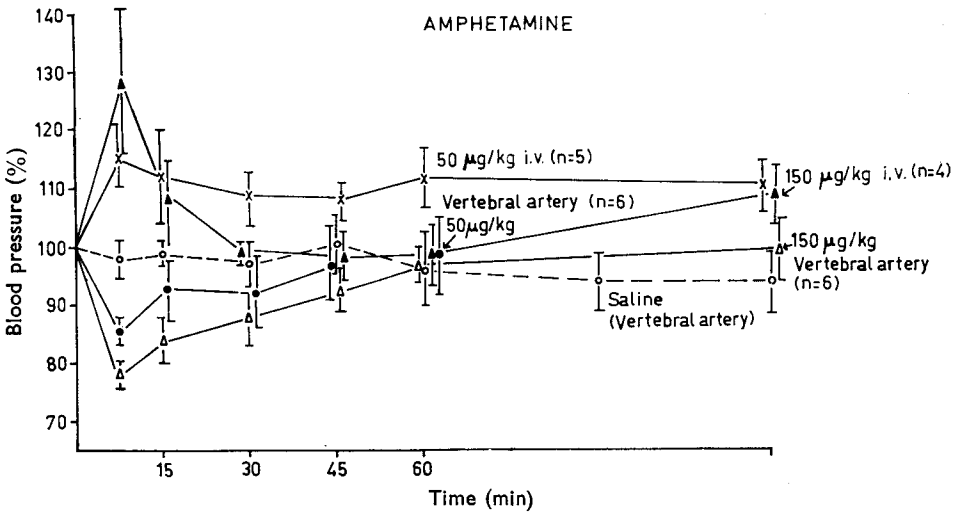

FIG. 1. Influence of ±-amphetamine given either intravenously (upper curves) or infused into the left vertebral artery on arterial blood pressure (femoral artery) in anaesthetized cats. The blood pressure before drug administration was taken as 100% and the subsequent values were expressed as percentage of the initial level and plotted against time. Each point on the curves represents the mean ± s.e. for n individual animals. The infusion of saline into the vertebral artery (left) (similar to amphetamine) has been depicted by the dotted line.

at least 20 min. All values after injection of drug were expressed as percentage of the initial level and plotted against time.

Amphetamine

Intravenously administered (±)-amphetamine (either 50 or 150 µg/kg) increased blood pressure. The initial rise was more pronounced after 150 µg/kg, but a longer hypertension was observed after the lower dose (Fig. 1). The slow infusion of amphetamine in the same doses into the left vertebral artery induced a pronounced although transient *hypotension* (Fig. 1).

Compared with the pre-infusion blood pressure, the effects of both doses were statistically significant: 50 µg/kg, $P < 0{\cdot}01$ (n=6), and 150 µg/kg, $P < 0{\cdot}001$ (n=6). The relative decrease in blood pressure was dose-dependent and the effect of the lower dose was shorter-lasting (Fig. 1). Amphetamine (10 µg/kg) by infusion did not influence arterial pressure significantly ($P > 0{\cdot}1$).

Shortly before administration of amphetamine, the mean cardiac frequency of four open-chest cats amounted to 234 ± 9 beats/min. Infusion of amphetamine (150µg/kg) into the left vertebral artery caused a small increase in frequency by about 5–10% of the initial value. The onset of the effect was approximately 10 min after the end of the infusion; the maximum increase was about 1 h later.

The hypotensive effect of amphetamine (150 µg/kg) could be blocked completely upon pretreatment with the α-adrenoceptor blocking agents piperoxan or yohimbine. Piperoxan (1 mg/kg, i.v.) given approximately 10 min before amphetamine by slow infusion caused a decrease in blood pressure by 8 ± 1·5% (mean ± s.e., n=4) of the initial level. This effect was only transient; return to normal pressure occurred within 5 min. Immediately after normalization of blood pressure, amphetamine 150 µg/kg, was infused. The pressure was not significantly different ($P > 1{\cdot}0$) from the control, indicating a blockade by piperoxan of the amphetamine effect.

A similar blockade of the hypotensive action of amphetamine (150 µg/kg) was

effected by yohimbine (0·6 mg/kg, i.v.), given 10–15 min before amphetamine. Yohimbine decreased blood pressure maximally by 25 ± 3·4% (n=4). Normal pressure returned within 15 min and amphetamine (150 μg/kg) was then infused into the left vertebral artery. As after piperoxan, the blood pressure decrease [4·0 ± 1·2% (n=4] was not significant. Thus yohimbine, too, blocks the hypotensive effect of amphetamine.

Haloperidol blocks central adrenoceptors although at high doses (Andén, Corrodi & others, 1970). To test its influence, we administered 1 mg/kg intravenously, approximately 30 min before the amphetamine infusion. Arterial pressure just before amphetamine in the haloperidol-treated cats was 106 ± 8 mm Hg (mean ± s.e., n=4), which was not significantly different from that of anaesthetized control animals at 117 ± 4 mm Hg (mean ± s.e., n=75) ($P > 1·0$). In four haloperidol-pretreated cats, blood pressure of 105 ± 3% (mean ± s.e., n=4) after infusion of amphetamine 150 μg/kg was not significantly different ($P > 1·0$) from the pre-amphetamine pressure level, but the same dose of amphetamine caused a significant hypotensive effect in cats that had not received haloperidol (Fig. 1). Thus the hypotensive action of amphetamine is blocked also by haloperidol.

To study the action of amphetamine in *reserpinized* animals, cats were treated with reserpine 1 mg/kg, i.p.) 16-24 h before the experiment and showed sedation, ptosis, diarrhoea. They were anaesthetized with chloralose (40 mg/kg). The blood pressure in the anaesthetized animals was 68 ± 13 mm Hg (n=3) and was significantly lower than that of untreated control animals ($P < 0·001$). In the reserpinized cats, amphetamine (150 μg/kg) by slow infusion caused a non-significant increase [8 ± 1·5 mm Hg (n=3)] of the pre-infusion blood pressure level persisting for at least 30 min. Thus, after reserpine, the pronounced central hypotensive effect of amphetamine is negated or at most converted into a small increase in blood pressure.

Ephedrine, phentermine, chlorphentermine and fenfluramine

(±)-Ephedrine (50 μg/kg), which, like amphetamine, acts indirectly, caused an increase in arterial pressure after intravenous injection. The maximum increase was 35 ± 3·8% (n=4) of the initial level. Slow infusion of the same dose caused a slowly developing effect that lasted longer than that evoked by amphetamine. Maximal effect was about 15 min after ephedrine infusion; the relative decrease in pressure amounted to 19·5 ± 0·8% (n=4) of the pre-infusion level and was statistically significant ($P < 0·01$).

Phentermine (300 μg/kg, i.v.) caused a rise in blood pressure by 22 ± 2·5% (n=6). The effect persisted for at least 2 h. A transient, modest hypotension was observed upon infusion of the same dose (residual blood pressure 85 ± 3·1% (n=5) at maximal effect ($0·01 < P < 0·05$).

Chlorphentermine (300 μg/kg, i.v.) slightly, but not significantly, increased arterial pressure by 6·2 ± 1·8% (n=5) ($P > 1·0$). A pronounced and significant hypotensive action was recorded after infusion of the same dose into the vertebral artery. At maximal effect the relative decrease in blood pressure was to 32·5 ± 3·1% (n=5) of the pre-infusion level ($P < 0·01$). Blood pressure reached a new steady state approximately 30 min after drug administration, but did not return to normal values within 2 h.

Fenfluramine (100 μg/kg) infused into the left vertebral artery significantly reduced blood pressure maximally by 38·5 ± 2·4% (mean ± s.e., n=5) of its pre-infusion

control value ($P < 0{\cdot}01$). The effect had a time course similar to that after infusion of amphetamine. Pressure returned to around about 1 h after the drug. Fenfluramine given intravenously did not significantly influence mean arterial pressure.

Phenmetrazine (30–300 μg/kg) did not significantly alter arterial pressure when infused into the left vertebral artery ($P > 0{\cdot}1$).

Noradrenaline (1 μg/kg, i.v.) significantly increased arterial pressure by $36 \pm 2{\cdot}8\%$ of its initial level (n=5), $P < 0{\cdot}01$. The same dose by infusion into the vertebral artery did not significantly influence blood pressure ($P > 1{\cdot}0$) nor did a tenfold higher dose ($9{\cdot}8 \pm 1{\cdot}5\%$, $P > 1{\cdot}0$).

Blood glucose concentration

Intravenously injected amphetamine (150 μg/kg) did not influence the concentration of glucose in plasma. However, the same dose infused into the left vertebral artery caused a small but significant hyperglycaemic effect. The glucose concentration rose by $22 \pm 1{\cdot}5\%$ of its initial value (n=19, maximum effect, achieved 1 h after drug administration) ($P < 0{\cdot}01$). None of the other drugs significantly influenced the glucose concentration of the blood by either route of administration.

DISCUSSION

The results have demonstrated that amphetamine and the other amines investigated possess central hypotensive properties. Compared with clonidine, studied under identical experimental circumstances (Sattler & van Zwieten, 1967), the amines are less active.

The drugs examined (amphetamine, ephedrine, phentermine, chlorphentermine and fenfluramine) are vasoconstrictors and therefore may constrict the cerebral branches of the vertebral artery or other cerebral blood vessels. This might evoke changes in the nutritive perfusion of the neurons in the regions where blood pressure is regulated. If so, the hypotensive changes observed would be unspecific. This hypothesis was tested using noradrenaline, which does not pass the blood brain barrier but constricts cerebral blood vessels. While infusion into the vertebral artery did not influence blood pressure, the same dose given peripherally caused vasoconstriction. These results suggest that unspecific changes in blood pressure, effected by cerebral vasoconstrictor effects, do not explain the central hypotensive action of amphetamine and related compounds.

Clonidine gives rise to pronounced bradycardia, probably of central origin, and increases vagal reflex bradycardia by a central mechanism (Kobinger & Walland, 1971). Amphetamine, on the other hand, did not depress, but slightly increased, cardiac frequency when infused into a vertebral artery, possibly as a result of a peripheral effect. As observed with clonidine, pretreatment of the animals with the α-adrenoceptor blocking agents piperoxan or yohimbine abolished the hypotensive action of amphetamine (cf. Schmitt, Schmitt & Fenard, 1971; Schmitt, personal communication). This observation is in line with the hypothesis of Schmitt & others (1971), according to which the activation of noradrenergic neurons in the nucleus tractus solitarii (Dahlström & Fuxe, 1964) would stimulate an inhibitory neuron in such a manner that the *peripheral* sympathetic tone decreases. Stimulation of the carotid sinus nerve (first synapse in the nucleus tractus solitarii) is also known to decrease peripheral sympathetic activity with consequent reduction in arterial pressure (Bronk, Ferguson & others, 1936; Sarnoff, Gilmore & others, 1960). Clonidine

and the drugs studied by us either mimic the aforementioned effect of noradrenaline or may liberate noradrenaline in the brain.

Apart from its central α-adrenoceptor stimulant properties, clonidine transiently stimulates peripheral α-adrenoceptors (Hoefke & Kobinger, 1966). The question arises whether amphetamine owes its hypotensive properties to a direct stimulation of central α-adrenoceptors (like clonidine) or to the liberation of noradrenaline. The depletion of noradrenaline in the brain requires elevated doses (1–5 mg/kg) of systemically applied amphetamine (Moore & Lariviere, 1963; Carlsson, Lindqvist & others, 1965). However, the route we used does not allow a direct comparison of the doses. The "topical" application of amphetamine to brain regions as in our studies may well have caused the mobilization of noradrenaline. Our observation that the central hypotensive action of amphetamine was abolished by pretreatment with reserpine would suggest that the liberation of noradrenaline by amphetamine is probably responsible for amphetamine's hypotensive effect.

Haloperidol has been reported to block central adrenoceptors (Andén & others, 1970), although high doses were required. The central hypotensive effect of clonidine could be blocked partially by haloperidol (Bock & van Zwieten, 1971) at the dose used in the present study. We found the effect of amphetamine could be blocked completely, which would also suggest that stimulation of central α-adrenoceptors by either clonidine or amphetamine is reduced or blocked by haloperidol, clonidine being the more potent drug.

Ephedrine possesses both direct and indirect sympathomimetic properties and, as expected from its structure, it also shows central activity (Chen & Schmidt, 1926). We found it also caused a modest centrally induced hypotensive action, the duration of which suggested that not only the liberation of central noradrenaline but also ephedrine itself can stimulate central α-adrenoceptors, since ephedrine is inactivated more slowly than noradrenaline (Winder, Anderson & Parke, 1948).

Phentermine showed persistent peripheral sympathomimetic activity. However, its central hypotensive action was transient and less pronounced than that of amphetamine, probably due to its modest lipid solubility. The central hypotensive action of chlorphentermine, which is more hydrophobic than phentermine (Dubnick, Leeson & others, 1963), was more pronounced than that of phentermine. The peripheral vasopressor action of fenfluramine is much weaker than that of amphetamine(Beregi, Hugon & others, 1970); we found it to give rise to a considerable central hypotensive effect. The central effect on blood glucose concentration of the amines we used was modest (amphetamine) or negligible while clonidine shows significant central hyperglycaemic activity (Bock & van Zwieten, 1971). This potency of clonidine might at least in part be caused by its high lipid solubility. The peripheral administration of amphetamine and chlorphentermine has been reported to bring about a significant hypoglycaemia (Herold, Kemper & Opitz, 1965).

Thus the central hypotensive actions of clonidine and of sympathomimetic agents that can penetrate into the brain are probably caused by the same mechanism, possibly the stimulation of central α-adrenoceptors which might be located in the nucleus tractus solitarii.

There is evidence that these receptors differ from peripheral ones (Schmitt & others, 1971; Boakes, Bradley & others, 1971). α-Methyldopa (Henning & van Zwieten, 1968) Henning, 1969; Ingenito, Barrett & Procita, 1970), L-dopa (Rubenson, 1971a) and *m*-tyrosine (Rubenson, 1971a, b) possess central hypotensive properties which fit

this concept. In the brain, these compounds are converted into sympathomimetic agents like α-methylnoradrenaline (from α-methyldopa), which has been demonstrated to possess central hypotensive activity itself (Henning & Rubenson, 1971; Heise & Kronenberg, 1972). Thus central hypotension as a result of stimulation of central α-adrenoceptors seems to be a general principle.

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