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ANTAGONISM OF THE BLOCKING ACTION OF BRETYLIUM AND GUANETHIDINE ON THE PRESSOR EFFECT OF PHYSOSTIGMINE IN THE RAT

BY

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In recent years three chemically dissimilar substances, bretylium, guanethidine and reserpine have been shown to produce a specific block of the postganglionic sympathetic fibre. There is evidence to suggest that both reserpine (Burn & Rand, 1958) and guanethidine (Cass, Kuntzman & Brodie, 1960; McCubbin, Kanako & Page, 1961) may produce their blocking action by depleting the sympathetic fibres of their noradrenaline stores. Guanethidine also interferes with the release of noradrenaline from sympathetic nerves (Maxwell, Plummer, Povalski & Schneider, 1960). Bretylium, on the other hand, probably acts by selectively anaesthetizing the sympathetic postganglionic fibres (Boura, Copp, Duncombe, Green & McCoubrey, 1960).

Recently, Day (1962) found that the block produced by bretylium or guanethidine of the responses of the rabbit isolated ileum and of the cat nictitating membrane to sympathetic nerve stimulation could be reversed by certain indirectly acting sympathomimetic amines like dexamphetamine, mephentermine, hydroxyamphetamine, ephedrine and phenylethylamine and also by the monoamine oxidase inhibitors, phenelzine, pheniprazine and tranylcypromine.

Later, Day & Rand (1962) reported that the hypotension and the block of responses of the nictitating membrane to cervical sympathetic nerve stimulation following prolonged administration (12.5 mg/kg/day, for 7 days) of guanethidine could not be reversed by an intravenous infusion either of noradrenaline or of dopamine; dexamphetamine, on the other hand, significantly restored the effects of nerve stimulation and reversed the hypotensive effect of guanethidine. These authors suggested that depletion of noradrenaline stores by guanethidine is not its main action and is not responsible for its hypotensive effect.

Clinical observations also indicate that amphetamines and related drugs specifically antagonize the hypotensive action of bretylium and guanethidine (Wilson & Long, 1960; Laurence & Rosenheim, 1960). These observations are of considerable pharmacological and therapeutic interest as they provide a significant clue into the modes of action of bretylium and guanethidine and the pathogenesis of hypertension.

In the rat, the pressor response to physostigmine arises from an activation of central sympathetic mechanisms (Varagić, 1955; Medaković & Varagić, 1957; Lešić & Varagić, 1961) and is in many ways similar to the effects of sympathetic nerve stimulation; it is

blocked by bretylium, guanethidine and reserpine (Lešić & Varagić, 1961; Cass & Spriggs, 1961; Gokhale, Gulati & Joshi, 1963). The present paper describes the effects of dexamphetamine, methylamphetamine, cocaine, imipramine and methyl phenidate on the block produced by bretylium and guanethidine of the pressor effect of physostigmine in the rat. The action of reserpine was also investigated.

METHODS

Albino rats of either sex weighing between 150 and 300 g were anaesthetized with urethane (1.5 g/kg, subcutaneously in a 20% w/v solution). Arterial blood pressure was recorded from a polyethylene cannula inserted into a common carotid artery and connected to a Sanborn electro-manometer (Model 121C) and a Sanborn Twin-Viso Recorder (Model 60–1300). Injections were made through a polyethylene cannula in an external jugular vein. Drugs were injected in a constant volume of 0.1 ml. and washed in by the same volume of 0.9% saline. Artificial ventilation was given when required by means of a miniature Ideal pump (Palmer).

The following drugs were used: physostigmine salicylate; bretylium tosylate; guanethidine sulphate; imipramine hydrochloride; dexamphetamine sulphate; methylamphetamine hydrochloride; cocaine hydrochloride; methyl phenidate hydrochloride; (±)-noradrenaline hydrochloride; and reserpine (Serpasil, Ciba). Doses of all drugs are expressed in terms of the base. Heparin (1,000 U/kg) was injected intravenously as an anticoagulant.

RESULTS

Block of the pressor response to physostigmine following single intravenous injections of bretylium or guanethidine

Physostigmine (40 μg/kg) injected intravenously produced a rise of blood pressure lasting from 16 to 32 min. The magnitude of the pressor effect, though varying from rat to rat, was remarkably constant in any one experiment. In forty-seven experiments the mean basal blood pressure was 68.0±2.9 mm Hg and the mean rise of blood pressure after physostigmine was 66.3±2.4 mm Hg.

An intravenous injection of bretylium (5.8 mg/kg) or guanethidine (3.4 mg/kg) produced a sustained fall (20 to 40 mm Hg) of arterial blood pressure. After 30 min, the pressor response to physostigmine was almost totally blocked whereas the pressor effect of noradrenaline (0.16 μg) was considerably potentiated. The blocking effects of bretylium and guanethidine persisted during the entire length (4 to 5 hr) of individual control experiments (five experiments); enhancement of the pressor effect of noradrenaline was also maintained during this period.

After bretylium or guanethidine had produced a total block of the pressor effect of physostigmine, the drugs being studied were injected intravenously and their effect on subsequent responses to physostigmine (given at 30-min intervals) was examined over a period of 2 to 4 hr.

Effect of dexamphetamine. Dexamphetamine (0.19 mg/kg) produced a rise (40 to 65 mm Hg) of blood pressure which lasted for 4 to 6 min. At 10 min after the injection the blood pressure had returned to, and was maintained at, a level corresponding to that before bretylium or guanethidine, and the pressor effect of physostigmine was completely restored. Indeed, in four out of six experiments it was potentiated and, in addition, the enhancement of the pressor response to noradrenaline was abolished (Fig. 1).

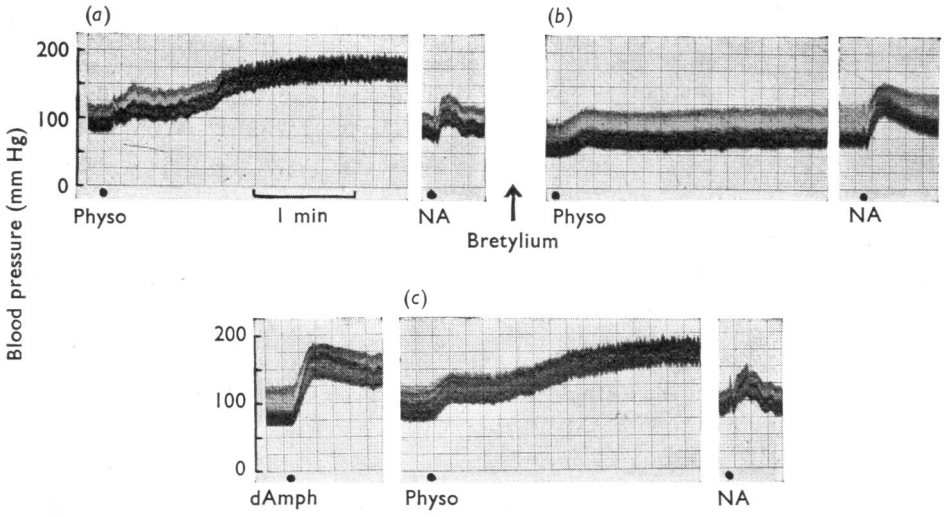

Fig. 1. Rat, 186 g. Record of carotid arterial blood pressure. Responses to physostigmine (40 μg/kg at Physo) and to noradrenaline (0.16 μg at NA). Between (*a*) and (*b*), bretylium (5.8 mg/kg) was given 30 min before (*b*); between (*b*) and (*c*) and 10 min before (*c*), dexamphetamine (0.19 mg/kg at dAmph) was injected, completely restoring the pressor effect of physostigmine. Time mark, 1 min. Injections were intravenous.

A second injection of bretylium or guanethidine given at this stage caused but a slight fall of blood pressure and only partially blocked the pressor effect of physostigmine. Thus dexamphetamine not only reversed the blocking action of bretylium or guanethidine but, if given first, also prevented its full development.

Effect of methylamphetamine. Methylamphetamine (0.4 mg/kg) produced a rise (40 to 60 mm Hg) of blood pressure which lasted for 5 to 6 min. At 10 min after its administration methylamphetamine produced effects which were indistinguishable from those described for dexamphetamine.

Effect of methyl phenidate. In five experiments methyl phenidate (4.3 mg/kg) produced a rise (40 to 60 mm Hg) of blood pressure which lasted for 8 to 10 min. At 20 min after injection of methyl phenidate the hypotensive action of bretylium was annulled, the pressor response to physostigmine was partially restored and the potentiation of the pressor effects of noradrenaline had disappeared.

In five other experiments methyl phenidate was injected 10 min before bretylium. The blocking action of bretylium was prevented; the pressor response to physostigmine was not blocked and the pressor effects of noradrenaline were not potentiated (Fig. 2).

In five experiments methyl phenidate did not reverse the blocking action of guanethidine. However, if methyl phenidate was given before guanethidine (five experiments) it partially antagonized the blocking action of guanethidine. In these experiments guanethidine did not totally block the pressor effect of physostigmine but reduced it by about half.

Effects of cocaine and imipramine. Neither cocaine (1.8 mg/kg, six experiments) nor imipramine (3.5 mg/kg, six experiments) reversed the sympathetic nerve blocking action of bretylium or guanethidine. However, if the drugs were given before bretylium, they

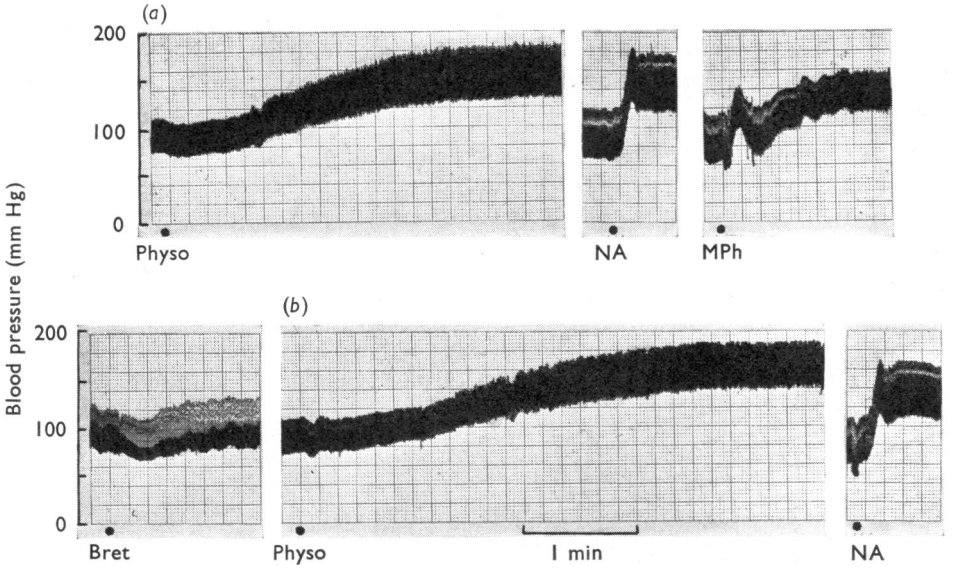

Fig. 2. Rat, 240 g. Record of carotid arterial blood pressure. Responses to physostigmine (40 μg/k at Physo) and to noradrenaline (0.16 μg at NA). Between (*a*) and (*b*), methyl phenidate (4.3 mg/k at MPh) was given followed 10 min later by bretylium (5.8 mg/kg at Bret) given 30 min before (*b*) Time mark, 1 min. Injections were intravenous.

completely prevented the development of its blocking action (six experiments). Given before guanethidine they had no such effect (six experiments).

These results are in general agreement with the findings of Day (1962) who reported that cocaine was less effective in reversing sympathetic blockade than in preventing it and that it was more effective in preventing blockade produced by bretylium than that by guanethidine.

Blockade of the pressor effect of physostigmine following prolonged administration of guanethidine

Rats were given daily subcutaneous injections of guanethidine (3.4 mg/kg) for 8 days, the animals being used for experiments 24 hr after the last injection. In these animals the basal blood pressure was 70.0$\pm$4.5 mm Hg, not significantly different from that of untreated rats (68.0$\pm$2.9 mm Hg). However, the pressor response to physostigmine was considerably reduced though not completely abolished. In twelve experiments the mean rise of blood pressure following the standard dose of physostigmine was 16.0$\pm$3.4 mm Hg. Noradrenaline was not used in these experiments as it would have modified tissue amine content.

Effect of methylamphetamine. At 15 min after injection of methylamphetamine (0.4 mg/kg) the pressor response to physostigmine was completely restored. In four experiments physostigmine now caused a mean rise of 68.0$\pm$4.9 mm Hg. This figure is not significantly different from the control value of 66.3$\pm$2.4 mm Hg. In two experiments an intravenous injection of guanethidine (3.4 mg/kg) given at this stage did not cause a fall of blood pressure and only partially blocked the pressor effect of physostigmine.

Effect of dexamphetamine. Dexamphetamine (0.19 mg/kg) almost completely restored the pressor effect of physostigmine. In four experiments, 15 min after dexamphetamine administration, a standard dose of physostigmine caused a mean pressor effect of 66.0±2.4 mm Hg.

Effect of noradrenaline infusion. In five experiments, 8.2 to 16.4 μg of noradrenaline, injected intravenously over a period of 15 min, caused a considerable rise of blood pressure. At 20 min after the infusion, the blood pressure had returned to normal. In these animals physostigmine now caused a mean pressor effect of 21.0±4.0 mm Hg. This value is not significantly different from the mean pressor effect of physostigmine in animals who had not received a noradrenaline infusion (16.0±3.4 mm Hg). However, intravenous injection of dexamphetamine (0.19 mg/kg) or methylamphetamine (0.4 mg/kg) was still able to cause considerable restoration of the pressor effect of physostigmine (Fig. 3).

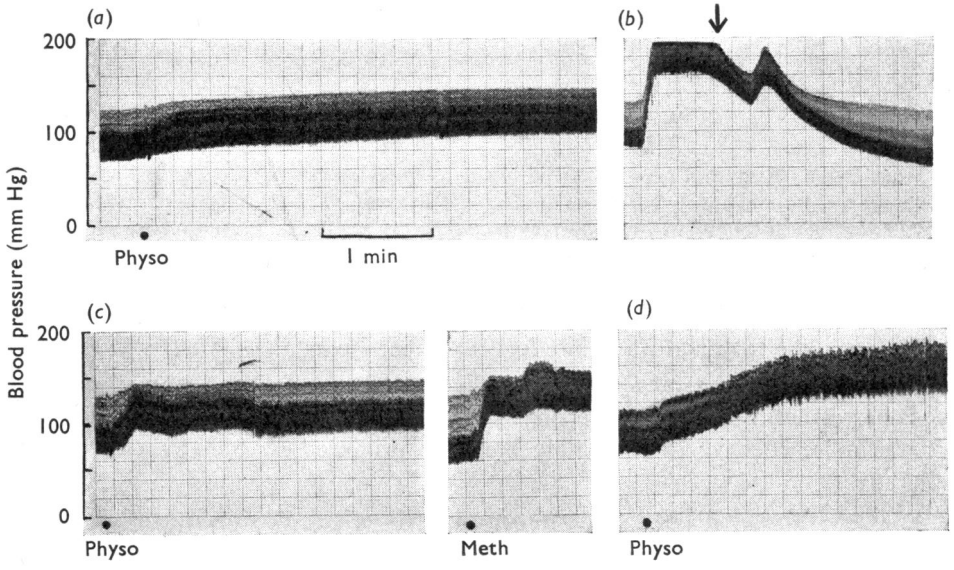

Fig. 3. Rat, 210 g, chronically treated with guanethidine (3.4 mg/kg, subcutaneously, daily for 8 days). Record of carotid arterial blood pressure. Responses to physostigmine (40 μg/kg at Physo). Between (*a*) and (*c*), an intravenous infusion of noradrenaline (10 μg, over 15 min, effect shown in (*b*), paper stopped at arrow) was given. The response in (*c*) was recorded 20 min after (*b*). Between (*c*) and (*d*), methylamphetamine (0.4 mg/kg at Meth) was injected 15 min before (*d*). Time mark, 1 min. Injections were intravenous.

Blockade of the pressor effect of physostigmine following treatment with reserpine

Reserpine (2 mg/kg) was administered subcutaneously on two successive days and the animals were used for experiment 24 hr after the last injection. In these animals the resting blood pressure (72.5±2.4 mm Hg) was not significantly different from that of untreated rats (68.0±2.9 mm Hg). Nevertheless the pressor response to physostigmine was almost totally abolished, the mean value in five experiments being 5.4±0.75 mm Hg. An intravenous infusion of noradrenaline (8.2 to 16.4 μg given over 15 min) produced a considerable rise of blood pressure, but did not even partially restore the pressor effect of physostigmine.

This result agrees with the findings of Lešić & Varagić (1961). Dexamphetamine (0.19 mg/kg) and methylamphetamine (0.4 mg/kg) were also ineffective in restoring the pressor effect of physostigmine in these animals.

DISCUSSION

The present experiments have shown that dexamphetamine, methylamphetamine and to some extent methyl phenidate can both prevent and reverse the blocking action of bretylium and guanethidine on sympathetic nerves.

Day & Rand (1963) suggested that the antagonism of the effects of guanethidine by amphetamine can be explained if both drugs had a similar affinity for the same receptor sites but were acting with different degrees of intrinsic activity. Our results can be explained on the same hypothesis. The inability of methyl phenidate to reverse the blocking action of guanethidine may be due to a relatively low affinity.

Neither cocaine nor imipramine could reverse the adrenergic neurone blocking action of bretylium or guanethidine. However, if given first the drugs were able to prevent the blocking action of bretylium.

Tissues innervated by the sympathetic nervous system can rapidly remove noradrenaline and inactivate it by fixation in storage sites (Hertting & Axelrod, 1961; Whitby, Axelrod & Weil-Malherbe, 1961). Furchgott, Kirpekar, Rieker & Schwab (1963) and Kirpekar & Furchgott (1964) have proposed that there are sites of a specific type on the exterior of the catechol amine store with which noradrenaline, tyramine and bretylium molecules must first combine before being transferred into the interior. These authors attribute the antagonism by cocaine of the sympathomimetic effects of tyramine and bretylium to a competitive occupation of these " transfer sites " by cocaine. It is likely that cocaine and imipramine prevent the adrenergic neurone blocking action of bretylium through a similar mechanism.

It has been suggested that the depletion in catechol amines caused by guanethidine is responsible for its adrenergic nerve blocking action (Cass *et al.*, 1960). In our experiments with rats chronically treated with guanethidine an intravenous infusion of noradrenaline did not reverse the blocking action of guanethidine; injection of dexamphetamine or methylamphetamine, however, resulted in a complete restoration of the pressor effect of physostigmine. This result suggests that the block of the pressor effect of physostigmine following chronic administration of guanethidine is due not to a depletion of tissue amine stores but rather to a failure of release of transmitter.

In rats treated with reserpine the pressor effect of physostigmine was completely blocked and neither infusion of noradrenaline nor injection of dexamphetamine or methylamphetamine could restore it. Treatment of animals with reserpine severely impairs the uptake of subsequently administered noradrenaline by storage sites (Axelrod, Whitby & Hertting, 1961; Bhagat & Shideman, 1964; Samorajski, Marks & Webster, 1964) and this might explain the inability of noradrenaline infusion to reverse the blocking action of reserpine. The inability of dexamphetamine and methylamphetamine to reverse the blocking action of reserpine suggests a mode of action quite distinct from that of bretylium and guanethidine. Thus it appears that, whereas the blocking actions of bretylium and guanethidine are closely similar, that of reserpine is quite different.

SUMMARY

1. The adrenergic nerve blocking action of bretylium, guanethidine and reserpine was investigated in rats using the pressor effect of physostigmine to assess sympathetic function.

2. Dexamphetamine, methylamphetamine and to some extent methyl phenidate each prevented and reversed the blocking effect of single intravenous doses of bretylium or guanethidine. Cocaine and imipramine were unable to reverse the blocking action of the drugs but, given first, prevented the blocking action of bretylium but not of guanethidine.

3. Block of the pressor effect of physostigmine following chronic administration of guanethidine was reversed by dexamphetamine or methylamphetamine. Intravenous infusion of noradrenaline, however, was ineffective.

4. Neither noradrenaline infusion nor dexamphetamine could reverse the blocking effect of treatment with reserpine.

5. It is concluded that the adrenergic nerve blocking actions of bretylium and guanethidine are closely similar whereas that of reserpine is quite different.

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