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EFFECT OF SOME BLOCKING DRUGS ON THE PRESSOR RESPONSE TO PHYSOSTIGMINE IN THE RAT

BY

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Bretylium and guanethidine blocked the pressor effect of physostigmine and potentiated the responses to adrenaline and noradrenaline on the blood pressure of the rat. Morphine and atropine in small doses blocked the pressor effect of physostigmine without interfering with the actions of adrenaline and noradrenaline. Chlorpromazine in small doses (0.5 to 2.5 mg/kg) blocked the pressor effect of physostigmine and potentiated the responses to noradrenaline whilst those to adrenaline remained unaltered. 3,6-Di(3-diethylaminopropoxy)pyridazine di(methiodide) (Win 4981) blocked the pressor effect of physostigmine and, in its early stages, this block was partially reversed by choline chloride. *N*-Diethylaminoethyl-*N*-isopentyl-*N'N'*-diisopropylurea (P-286), in a dose that reduced the effect of dimethylphenylpiperazinium, had no effect on the pressor response to physostigmine or on the responses to adrenaline and noradrenaline. Hexamethonium, even in large doses (100 mg/kg), only blocked partially the effect of physostigmine while mecamylamine produced a complete block ; the responses to adrenaline and noradrenaline were potentiated in both instances.

The pressor response to physostigmine in the rat probably arises from an activation of central nervous sympathetic mechanisms (Varagić, 1955 ; Dirnhuber & Cullumbine, 1955 ; Medaković & Varagić, 1957 ; Lešić & Varagić, 1961 ; Varagić & Vojvodić, 1962). Reduction or abolition of the response by antiadrenaline agents, by large doses of hexamethonium and by bretylium or guanethidine suggests that the central stimulant effect of physostigmine may be mediated by way of the established peripheral sympathetic pathways. The pressor response to physostigmine in the rat is thus in many ways similar to the effects of sympathetic nerve stimulation and has been used by Cass & Spriggs (1961) to assess sympathetic function.

The discovery of drugs like reserpine, bretylium and guanethidine which specifically block the function of postganglionic sympathetic nerves and the hypothesis of Burn & Rand (1960) concerning the wider involvement of cholinergic mechanisms in the autonomic nervous system has focused much attention on sympathetic mechanisms. The results of investigations in this field are often contradictory and many issues remain the subject of controversy (Chang & Rand, 1960 ; Day & Rand, 1961 ; Gillespie & MacKenna, 1961 ; Bentley, 1962).

The present paper describes the effects of some drugs on the pressor responses to physostigmine, adrenaline and noradrenaline in the rat.

METHODS

Albino rats of either sex weighing between 150 and 300 g were used and were anaesthetized with urethane (1.5 g/kg, intraperitoneally, 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 electromanometer (Model 121 C) and a Sanborn twin-viso recorder (Model 60-1300). Injections were made through a polyethylene cannula in a 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 by means of a miniature Ideal respiration pump (Palmer).

The drugs used were: physostigmine salicylate; bretylium tosylate; guanethidine sulphate; morphine sulphate; chlorpromazine hydrochloride; hexamethonium chloride; mecamylamine hydrochloride; dimethylphenylpiperazinium iodide; 3,6-di(3-diethylaminopropoxy)pyridazine di(methiodide) (Win 4981); *N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-diisopropylurea (P-286); choline chloride; atropine sulphate; and (±)-noradrenaline hydrochloride. The doses are of the salts. (−)-Adrenaline base was dissolved in 0.9% saline and the doses are of the base. Heparin (1,000 U/kg) was administered intravenously as the anticoagulant.

RESULTS

A fixed dose (60 μg/kg) of physostigmine, injected intravenously at 30 to 40 min intervals, produced a rise of blood pressure the duration of which varied from 18 to 35 min in different experiments. The magnitude of the pressor effect, usually 50 to 75 mm Hg, was remarkably constant for a period of 5 to 6 hr during individual control experiments (n=5) although the value varied from rat to rat.

After two equipressor responses to physostigmine had been obtained, the drug under study was injected intravenously at a specified period before the next injection of physostigmine.

Effects of bretylium and guanethidine

In eight experiments, bretylium (10 mg/kg) produced a fall (25 to 50 mm Hg) of arterial blood pressure, which in one experiment was followed by a secondary rise (10 mm Hg). After 30 min from the injection of bretylium the pressor response to physostigmine was greatly reduced but the pressor responses to adrenaline and noradrenaline were enhanced. In five experiments, guanethidine (5 mg/kg) produced a fall (10 to 20 mm Hg) of arterial blood pressure; in three others the drug produced an initial rise (20 to 30 mm Hg) which was followed by a gradual fall. After 15 min from the administration of guanethidine the pressor response to physostigmine was almost completely blocked while the pressor responses to adrenaline and noradrenaline were potentiated.

Effect of morphine

An initial intravenous injection of 2 mg/kg of morphine produced a substantial but transient fall of blood pressure (60 to 70 mm Hg) and bradycardia. Subsequent injections of the same or higher doses produced only a small fall of blood pressure (10 to 15 mm Hg) and no bradycardia. After 10 min from the administration of morphine, pressor responses to a standard dose of physostigmine were much reduced (Fig. 1, A, B). The extent of the blocking action of morphine varied in different animals. In six experiments the pressor effect of physostigmine was reduced by 40 to 90%. Pressor responses to adrenaline and noradrenaline, however, were

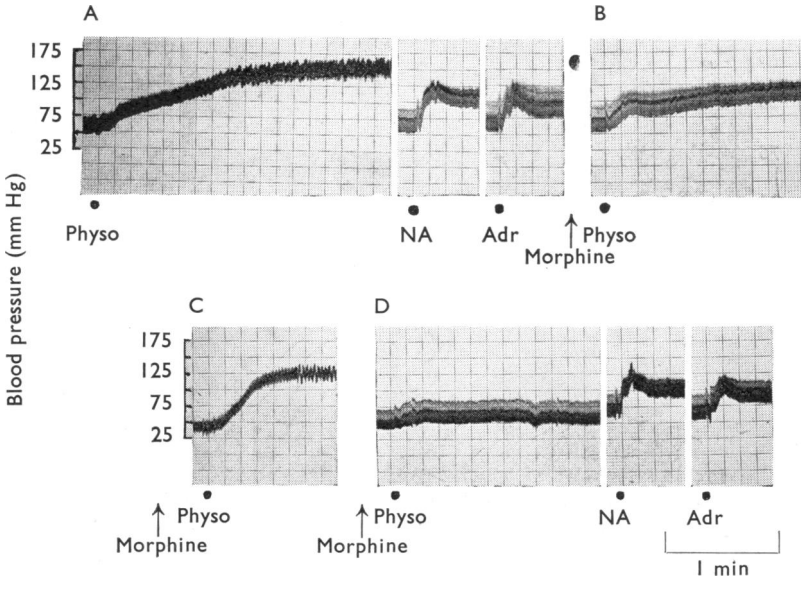

Fig. 1. Rat, 168 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA) and to adrenaline (0.2 μg at Adr). Morphine (2 mg/kg) was given between A and B and between B and C; 4 mg/kg was given between C and D. Time mark, 1 min. Injections were intravenous.

unaffected. The blocking action of morphine was relatively short-lived and full recovery of the pressor response to physostigmine was apparent within 30 to 60 min. Then a second dose of 2 mg/kg of morphine was always ineffective in blocking the pressor response to physostigmine. Indeed in a few instances actual potentiation of the response to physostigmine was observed. A higher dose (4 mg/kg) of morphine was now required to block the pressor effect of physostigmine (Fig. 1, D).

Effect of chlorpromazine

Chlorpromazine (0.5 to 2.5 mg/kg) produced a transient fall (10 to 25 mm Hg) of blood pressure which lasted for 2 to 6 min. After 10 min from the administration of chlorpromazine the pressor response to physostigmine was considerably reduced (Fig. 2, A, D). After 0.5 mg/kg of chlorpromazine a mean block of 25% (n=7) was seen. Cumulative doses of 1.0, 1.5, 2.0 and 2.5 mg/kg of chlorpromazine gave mean blocks of 30, 55, 65 and 86% respectively (n=7). In these experiments the cumulative doses of chlorpromazine were injected at 30 min intervals. At all the doses, chlorpromazine potentiated the pressor effects of noradrenaline both in size and duration, but the responses to adrenaline were usually unaffected (Fig. 2).

Intravenous injections of 10 mg/kg of chlorpromazine produced a sustained fall (30 to 40 mm Hg) of blood pressure ; after 10 min pressor responses to physostigmine and to adrenaline were absent but the pressor effect of noradrenaline was unaffected (n=4).

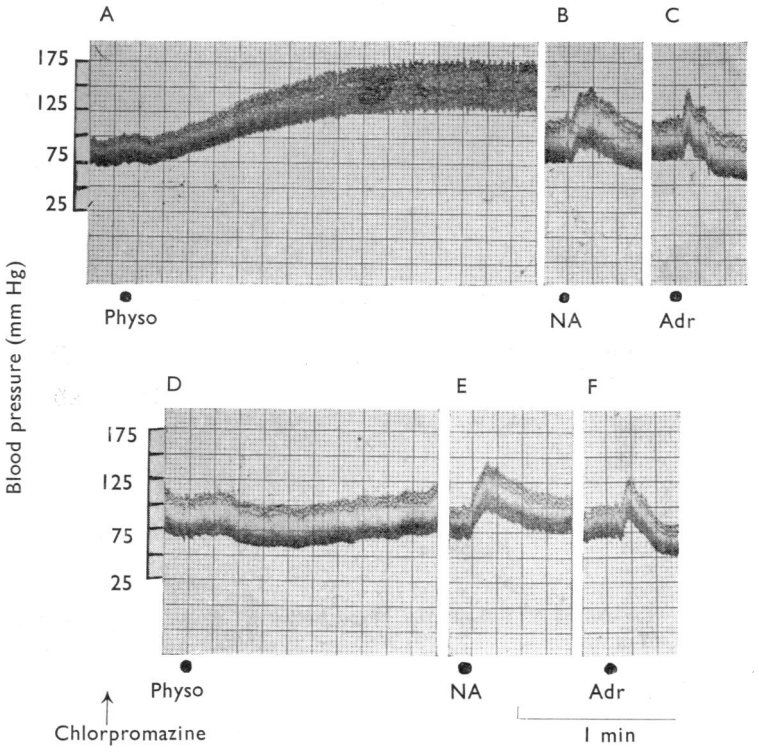

Fig. 2. Rat, 250 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA) and to adrenaline (0.2 μg at Adr) before (in A, B and C) and after (in D, E and F) cumulative intravenous administration of 2.5 mg/kg of chlorpromazine. Time mark, 1 min. Injections were intravenous.

Effect of Win 4981

3,6-Di(3-diethylaminopropoxy)pyridazine di(methiodide) (Win 4981) is claimed to have an action closely resembling that of hemicholinium (Gesler, Lasher, Hoppe & Steck, 1959; Gesler & Hoppe, 1961). The compound was administered by slow intravenous injection over a period of 5 to 15 min. Artificial ventilation was routinely employed shortly after the injection of Win 4981 and was continued throughout the duration of the experiment. A dose of 2 to 6 mg/kg had no immediate effect on blood pressure or heart rate and, during the first 20 min after the administration, the pressor responses to physostigmine were largely unaltered. After 30 min, the pressor response to a standard dose of physostigmine was almost totally abolished and the pressor responses to adrenaline and noradrenaline were potentiated (n=8). Then an intravenous injection of 10 to 20 mg/kg of choline chloride could produce a brief rise of blood pressure and thereafter partially restored the pressor effect of physostigmine (Fig. 3). The restoration of the pressor response to physostigmine by choline was only temporary, the next injection of physostigmine being again ineffective.

In the absence of choline, 1 to 1.5 hr after the administration of Win 4981 the blood pressure had usually fallen by 30 to 40 mm Hg, the pressor response to physostigmine was abolished and the responses to adrenaline and noradrenaline were much reduced (Fig. 3, D). At this phase, administration of choline chloride did not produce a rise of blood pressure and did not reverse the blocking action of Win 4981 on the responses to physostigmine, adrenaline and noradrenaline.

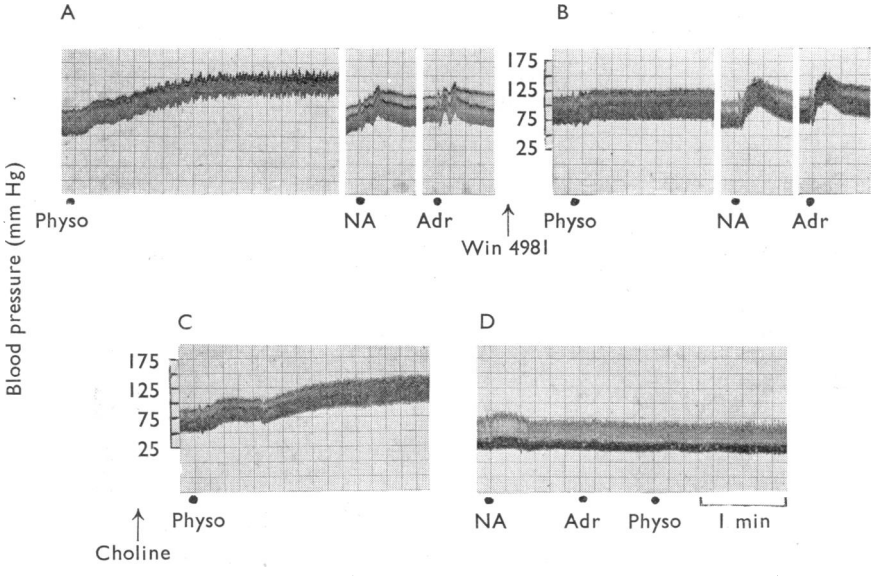

Fig. 3. Rat, 240 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA) and to adrenaline (0.2 μg at Adr). Between A and B, 4 mg/kg of Win 4981 was injected 30 min before B; between B and C, 15 mg/kg of choline chloride was injected, partially restoring the pressor effect of physostigmine. In D are shown the responses to noradrenaline, adrenaline and physostigmine, 1.5 hr after the administration of Win 4981. Artificial ventilation was given after administration of Win 4981. Time mark, 1 min. Injections were intravenous.

In two rats, however, pressor responses to physostigmine as well as to adrenaline and noradrenaline were reduced 30 min after the administration of Win 4981, and choline chloride did not restore the pressor effect of physostigmine.

Effect of P-286

N-Diethylaminoethyl-*N*-isopentyl-*N'N'*-diisopropylurea (P-286), the most active member of a series of aminoalkylureas studied by Gardier, Abreu, Richards & Herrlich (1960), appears to possess the property of blocking specifically the adrenal medulla without producing a concomitant sympathetic ganglionic blockade. De Schaepdryver (1959) has also reported that P-286 reduces the release of adrenaline and noradrenaline from the adrenal medulla caused by acetylcholine, nicotine, insulin and carotid arterial occlusion. In the present experiments, P-286 was used

to test whether release of amines from the adrenal medulla plays any part in the pressor effect of physostigmine in the rat.

P-286 (2 mg/kg) produced no appreciable effect on blood pressure or heart rate. In three experiments, 15 min after the injection of P-286 pressor responses to physostigmine, adrenaline and noradrenaline were unaltered while the pressor response to a small dose (20 to 40 μg/kg) of dimethylphenylpiperazinium was reduced (Fig. 4).

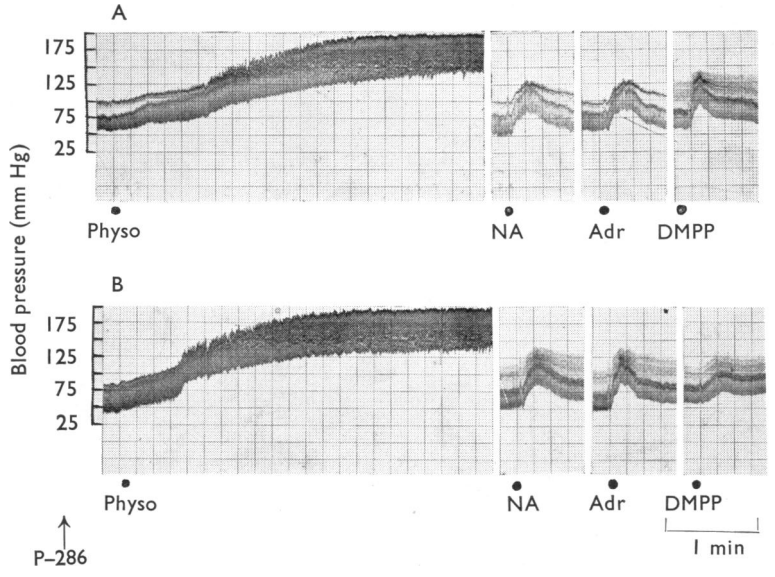

Fig. 4. Rat, 240 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA), to adrenaline (0.2 μg at Adr) and to dimethylphenylpiperazinium (20 μg/kg at DMPP). P-286 (2 mg/kg) was given after A and 15 min before B. Time mark, 1 min. Injections were intravenous.

Effect of atropine

Large doses (2 to 7 mg/kg) of atropine have been reported to block the pressor action of physostigmine (Medaković & Varagić, 1957). In our experiments very small doses (10 to 20 μg/kg) of atropine given intravenously produced a transient rise of blood pressure (15 to 40 mm Hg) and had no effect on the pressor response to physostigmine. A dose of 50 μg/kg reduced the response by about 35%. Cumulative doses of 150 to 200 μg/kg (in six experiments) almost totally blocked the pressor effect of physostigmine while responses to adrenaline and noradrenaline were unaltered (Fig. 5).

Effect of hexamethonium

Hexamethonium (10 to 20 mg/kg) produced a small (10 to 20 mm Hg) but sustained fall of blood pressure. After 10 min from the administration, the pressor response to physostigmine was reduced by about half (n=8). Simultaneously the

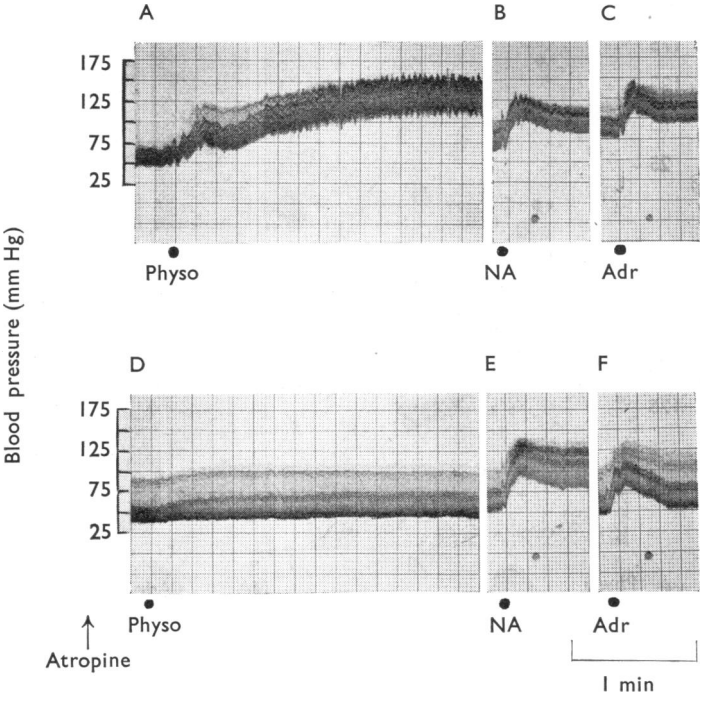

Fig. 5. Rat, 176 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA) and to adrenaline (0.2 μg at Adr) before (in A, B and C) and after (in D, E and F) an intravenous cumulative dose of 200 μg/kg of atropine. Time mark, 1 min. Injections were intravenous.

pressor responses to adrenaline and noradrenaline were increased. Cumulative administration of up to 100 mg/kg of hexamethonium did not decrease the pressor effect of physostigmine further while the responses to adrenaline and to noradrenaline were still enhanced.

Effect of mecamylamine

In view of the possible differences between the mechanisms of ganglionic blockade by hexamethonium and mecamylamine (Bennett, Tyler & Zaimis, 1957) it was considered worthwhile to study the effect of mecamylamine on the pressor response to physostigmine in the rat. We found that intravenous doses of mecamylamine up to 0.5 mg/kg had no effect on the pressor response to physostigmine. In five rats, 1 mg/kg produced an initial transient rise of blood pressure (10 to 25 mm Hg) which was followed by a gradual fall (10 to 20 mm Hg). After 10 min the pressor effect of physostigmine was considerably reduced. After cumulative administration of 1.75 to 2.5 mg/kg of mecamylamine the pressor effect of physostigmine was almost completely blocked ; simultaneously the pressor effects of adrenaline and noradrenaline were potentiated (Fig. 6).

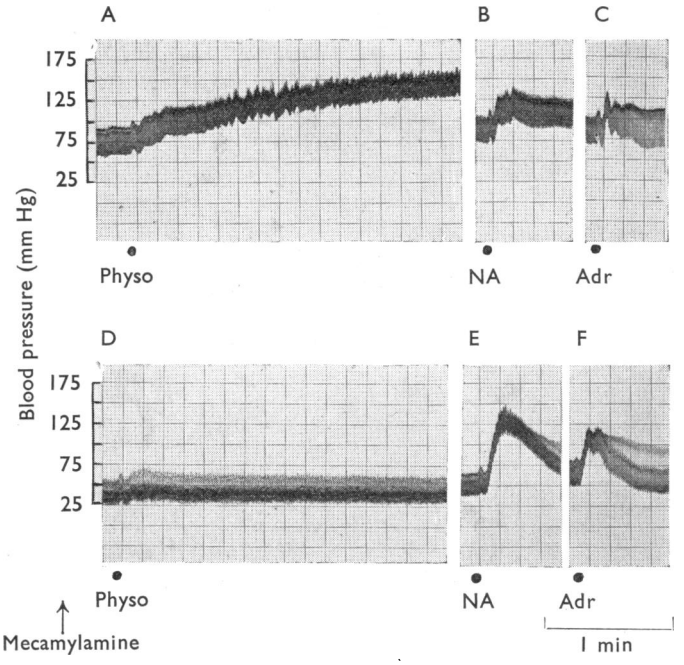

Fig. 6. Rat, 190 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (0.2 μg at NA) and to adrenaline (0.2 μg at Adr) before (in A, B and C) and after (in D, E and F) intravenous cumulative administration of 2 mg/kg of mecamylamine. Time mark, 1 min. Injections were intravenous.

DISCUSSION

Bretylium and guanethidine blocked the pressor effect of physostigmine in the rat. This finding confirms the observations of Lešić & Varagić (1961) and Cass & Spriggs (1961).

The action of morphine, blocking the pressor effect of physostigmine in the rat but not affecting the pressor effects of injected adrenaline or noradrenaline, is reminiscent of its effect on responses to sympathetic nerve stimulation. Morphine reduces the responses of the cat nictitating membrane and of the guinea-pig isolated jejunum to sympathetic nerve stimulation, without interfering with the actions of adrenaline and noradrenaline. Moreover, morphine does not block the transmission of nerve impulses through the superior cervical ganglion. On the basis of these findings it has been suggested that morphine interferes with the release of noradrenaline from sympathetic postganglionic nerve endings (Trendelenburg, 1957; Szerb, 1961; Carnie, Kosterlitz & Taylor, 1961a, b). However, there is no direct evidence of diminution of noradrenaline release to support this suggestion. The only site at which morphine has been found to reduce the amount of transmitter released is the cholinergically innervated smooth muscle of the guinea-pig ileum (Paton, 1957; Schaumann, 1957). The inhibitory effect of morphine on the pressor response to physostigmine and on the effects of sympathetic nerve stimulation could be explained

if acetylcholine were involved in the release of noradrenaline at sympathetic nerve endings.

We observed a diminution in the inhibitory action of morphine with repeated doses, rather like the findings of Paton (1957) on the guinea-pig coaxially stimulated ileum.

Small doses of chlorpromazine blocked the pressor responses to physostigmine without interfering with the pressor effects of injected adrenaline or noradrenaline. The most likely explanation of the blocking action of chlorpromazine appears to be a depression of central vasomotor tone and reactivity by the drug. Further support for this concept may be cited. First, chlorpromazine has little if any blocking action on the pressor effects of injected noradrenaline (Courvoisier, Fournel, Ducrot, Kolsky & Koetschet, 1953 ; Huidobro, 1954 ; Moran & Butler, 1956). Martin & Riehl (1956) and Martin, Riehl & Unna (1960) have shown a potentiation of the pressor effects of noradrenaline by chlorpromazine, an observation which has been confirmed in the present study. Furthermore, chlorpromazine does not block transmission through sympathetic ganglia (Huidobro, 1954 ; Holzbauer & Vogt, 1954) and, even in large doses, only partially blocks the pressor effects of splanchnic nerve stimulation (Vanlerenberghe, Robelet & Milbled, 1954). Thus chlorpromazine blocks the responses neither to sympathetic nerve stimulation nor to circulating noradrenaline, the presumed transmitter of postganglionic sympathetic fibres. Small doses (50 to 100 μg/kg) of chlorpromazine, which are without peripheral effects, were found by Dasgupta & Werner (1954) to depress central vasomotor tone and activity. Jourdan, Duchene-Marullaz & Boissier (1955) found that chlorpromazine was inactive in the spinal dog at a dose which caused hypotension in the intact animal.

That doses of chlorpromazine which had little or no peripheral antiadrenaline action blocked the pressor effect of physostigmine supports the view that this response is mediated through central sympathetic activation.

Chlorpromazine potentiated the pressor effects of noradrenaline whereas the responses to adrenaline were unaffected. Of the two amines, tissue binding plays a more significant part in the inactivation of noradrenaline and this could account for the selective potentiation of responses to noradrenaline by chlorpromazine.

As the blocking actions of both Win 4981 and of hemicholinium (Gesler *et al.*, 1959) depend on the rate of stimulation, are selectively reversed by choline and are characterized by a slow onset and long duration, the former drug may also block synthesis of acetylcholine in cholinergic neurones, as MacIntosh, Birks & Sastry (1956, 1958) have shown for hemicholinium which is believed to act by competitive inhibition of the transport of choline to its intracellular site of acetylation. Some support for this suggestion arises from the work of Farah (1959), who found that, in dog renal slices, both Win 4981 and hemicholinium blocked the transport of *N*-methylnicotinamide and tetraethylammonium, compounds which are transported by the same mechanism which transports choline.

In the present experiments Win 4981 blocked the pressor response to physostigmine with a slow onset and long duration. During its early stages the block

could be partially reversed by choline chloride and this might indicate interference with the synthesis of acetylcholine by the drug. It is unlikely that Win 4981, a bis-quaternary compound, would act on the central nervous system. The most obvious possibility is that the drug acts at autonomic ganglia. As hexamethonium, a highly selective ganglion blocking agent, even in very large doses, only partially blocked the pressor effect of physostigmine, Win 4981 might be assumed to act at the postganglionic sympathetic fibres or their terminals, perhaps near the " cholinergic trigger " which has recently been postulated (Burn & Rand, 1960).

As the later stages of the blocking action of Win 4981 could not be reversed by choline chloride, it probably acts by direct depression of vascular reactivity.

A small dose (2 mg/kg) of P-286, which did not reduce vascular reactivity to injected adrenaline or noradrenaline but reduced the pressor response to dimethylphenylpiperazinium (20 to 40 μg/kg), had no effect on the pressor response to physostigmine in the rat. As the pressor effects of such small doses of dimethylphenylpiperazinium are mainly mediated through a release of amines from the adrenal medulla, the above finding suggests that discharge of the adrenal medullary hormone(s) plays no part in the pressor response to physostigmine, a conclusion drawn by Lešić & Varagić (1961) and by Cass & Spriggs (1961), who demonstrated that the pressor effect of physostigmine was unaltered by adrenalectomy.

Relatively small doses of atropine (50 to 200 μg) could block the pressor effect of physostigmine. To our knowledge this blocking action of such small doses of atropine has not been reported previously. The blocking action of atropine could be due to a central nervous anticholinergic action of the drug, though other possibilities cannot be excluded. Bainbridge & Brown (1960) have demonstrated a ganglion-blocking action of atropine, but the doses used were large (1 to 4 mg/kg) and the blocking effect was relatively transient. Szerb (1961) showed that small concentrations of atropine decreased the responses of the guinea-pig jejunum to sympathetic nerve stimulation without reducing the inhibitory effect of noradrenaline. How far this action of atropine is involved in its block of the pressor response to physostigmine is not clear from our experiments.

Hexamethonium, even in very large doses (100 mg/kg), only partially blocked the pressor effect of physostigmine. Mecamylamine, on the other hand, produced an almost complete block. If the blocking action of mecamylamine in this instance is exerted at the ganglionic synapse then our findings would suggest that the action of mecamylamine is rather different from that of hexamethonium, a suggestion already made by Bennett *et al.* (1957).

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