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THE SYMPATHETIC POSTGANGLIONIC FIBRE AND THE BLOCK BY BRETYLIUM; THE BLOCK PREVENTED BY HEXAMETHONIUM AND IMITATED BY MECAMYLAMINE

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Acetylcholine, in the presence of atropine, has an action like that of sympathetic stimulation. When injected into the splenic artery it causes contraction of the spleen, but this action is blocked by hexamethonium; stimulation of the splenic nerves, however, is still effective. Thus hexamethonium distinguishes between sympathetic nerve stimulation and the action of acetylcholine. If bretylium is used instead of hexamethonium, there is no such distinction, for bretylium blocks the response to nerve stimulation as well as that to acetylcholine. It appeared that hexamethonium might block the action of acetylcholine by preventing its entry into the sympathetic fibre. Acetylcholine has some structural similarity to bretylium, since acetylcholine is a derivative of trimethylammonium and bretylium is a derivative of dimethylethylammonium. It has been found that hexamethonium, pentolinium and hemicholinium (HC-3), which are all *bis*-quaternary compounds, block the action of bretylium, presumably by preventing its entry into the fibre. Consistent with the view that ability to enter the fibre is important is the observation that mecamylamine and pempidine, which are ganglion-blocking agents, but not either *mono-* or *bis*-quaternary compounds, often abolish the response to stimulation of the sympathetic postganglionic fibre.

Burn & Rand (1959) suggested that the impulse passing down the sympathetic postganglionic fibre released acetylcholine, and this in turn released noradrenaline. One of the observations pointing to this concept was that acetylcholine, in the presence of atropine, acted like sympathetic stimulation in many organs. Ferry (1963) has recently found that the injection of acetylcholine into an artery leading to the spleen caused a centripetal discharge in the C fibres of the splenic nerves, and that this discharge was abolished by hexamethonium. He concluded that acetylcholine excited the sympathetic postganglionic nerves to the spleen somewhere near their endings. Since Daly & Scott (1961) had found that hexamethonium did not abolish the contraction of the spleen caused by stimulating the splenic nerves, Ferry (1963) concluded that his observation explained why acetylcholine acted like sympathetic stimulation. He inferred that this action did not imply the existence of a cholinergic link between the nerve impulse and the release of noradrenaline.

To examine this conclusion we have carried out experiments with bretylium, which was found by Boura & Green (1959) to abolish the effect of stimulating sympathetic postganglionic fibres. Exley (1960) showed that its action was exerted at the endings

of these fibres. We chose bretylium because in structure it differs from hexamethonium in being a *mono*-quaternary compound, and not *bis*-quaternary. We wished to discover in the first place whether bretylium would block the action of acetylcholine and of nicotine as well as that of sympathetic stimulation. We have further determined the effect of hexamethonium on the action of bretylium, to see if bretylium blocked the response to sympathetic stimulation in the presence of hexamethonium. We have examined pentolinium and hemicholinium (HC-3) in the same way. Finally we have tested mecamylamine and pempidine to see if they had a bretylium-like action. All experiments were carried out on the preparation described by Finkleman (1930).

METHODS

Small loops of ileum between 3 and 4 cm long were removed from freshly killed rabbits together with the mesentery containing the vessels supplying each loop. The contents of the loop were washed out with Locke or Tyrode solution. Loops not used at once were kept in the solution gassed with 5% carbon dioxide in oxygen. A silk thread was tied round the main artery supplying the loop, and was threaded through the hole in a pair of Saxby electrodes (Burn & Rand, 1960a). The end of the artery with surrounding fat was gently pulled through the hole where it was firmly held. The loop was set up in an organ-bath containing 50 ml. of Locke or Tyrode solution at 33° C. The electrodes were immersed in the bath, the leads being connected to a rectangular wave stimulator from which supramaximal stimuli of 1 msec duration could be supplied at known frequencies.

Hyoscine hydrobromide, acetylcholine bromide, bretylium tosylate, nicotine acid tartrate, hexamethonium bromide, pentolinium tartrate, mecamylamine hydrochloride and pempidine hydrochloride were used. Doses of these drugs are stated in terms of the salts except for nicotine the dose of which is given in terms of base.

RESULTS

Sympathomimetic action of nicotine blocked by bretylium. Gillespie & Mackenna (1960) observed that nicotine in low concentrations inhibited the pendular movements of isolated loops of the rabbit colon, and that even in higher concentrations it produced inhibition when atropine (10^{-4}) was present. However, they were unable to observe this action in loops of ileum, because the motor effects of nicotine were not abolished. We used hyoscine instead of atropine, and in the presence of hyoscine (10^{-7}) nicotine inhibited the ileum.

Fig. 1 shows an experiment in Tyrode solution. The left-hand panels (*a*) show in succession the inhibitory effect of stimulating the periarterial nerves at 5 shocks/sec for 40 sec, the inhibitory effect of nicotine (10^{-6}) when added to the bath for 2 min, and the inhibitory effect of stimulation at 10 shocks/sec for 20 sec. Bretylium (10^{-5}) was then added, and the observations in the middle panel (*b*) were made 25 min later. The effects of stimulation at the two frequencies were much less, and the effect of nicotine was very much less. The right-hand panel (*c*) shows that 1 hr later the effect of stimulation had gone, but nicotine still exerted a trace of inhibitory action. We observed that bretylium reduced or abolished the inhibitory effect of nicotine as well as that of sympathetic stimulation in six other experiments.

The action of acetylcholine. Because it is difficult to abolish the stimulant action of a large dose of acetylcholine by using hyoscine, we did not expect to

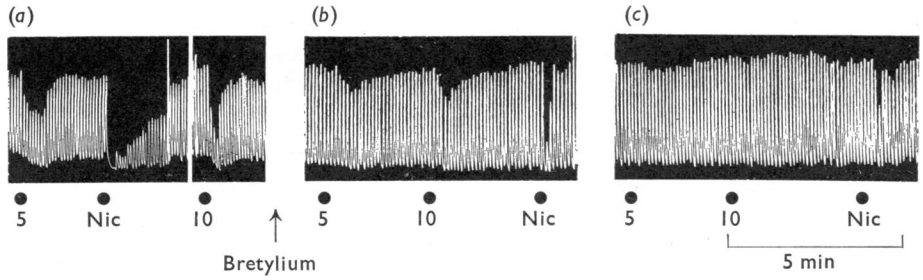

Fig. 1. Rabbit ileum preparation in Tyrode solution. The left-hand panels (*a*) show the inhibitions caused by stimulating the periarterial nerves at 5 shocks/sec for 40 sec, by adding nicotine (Nic, 10^{-6}) to the organ-bath for 2 min and, after washing out the bath, by stimulating at 10 shocks/sec for 20 sec. The middle panel (*b*) was recorded 25 min after the addition of bretylium (10^{-5}) to the bath; the responses to stimulation and to nicotine were much reduced. The left-hand panel (*c*) was recorded 1 hr after the middle panel; the responses to stimulation were abolished, and nicotine now exerted only a trace of effect. Numbers give stimulus frequencies in shocks/sec.

observe an inhibitory action of acetylcholine. However, we added acetylcholine in the course of observations with nicotine, and noticed that the stimulant action of acetylcholine was often preceded by a transient inhibition. To study this more closely, we added acetylcholine when the drum was moving at four times its usual rate. Fig. 2 shows an experiment of this kind. In the first panel, the addition of acetylcholine (40 μg/ml.) to the bath inhibited the pendular movements for about 20 sec; the inhibition was then overcome by a strong contraction. The bath fluid was changed, hyoscine was again added, and the periarterial nerves were

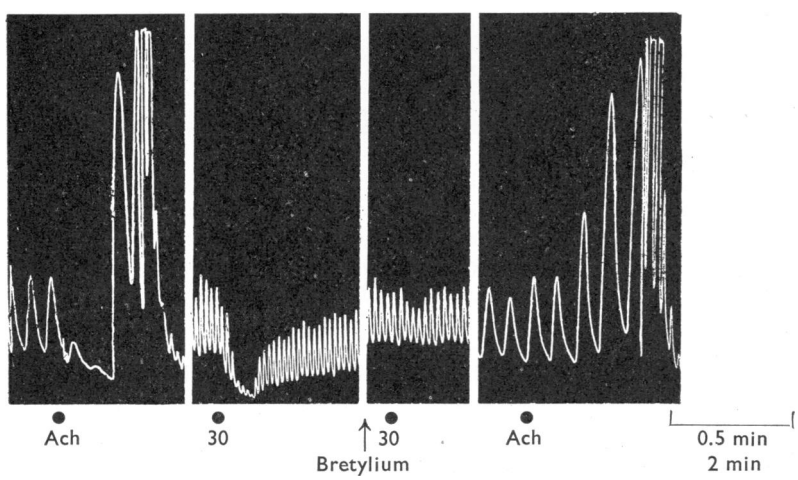

Fig. 2. Rabbit ileum preparation in Locke solution. Hyoscine (2×10^{-7}) was present in the organ-bath. Acetylcholine (Ach, 40 μg/ml.) caused inhibition which was arrested by a strong contraction. In the second panel, stimulation of periarterial nerves at 30 shocks/sec for 30 sec caused inhibition. Bretylium (40 μg/ml.) was added to the bath between the second and third panels. In the third panel 30 min later, stimulation was without effect, and, in the fourth panel, acetylcholine had lost its inhibitory action. The time calibration is 0.5 min for the outer panels, and 2 min for the inner panels.

stimulated, causing inhibition. Bretylium (40 μg/ml.) was added to the bath and, 25 min later, stimulation was without effect; addition of acetylcholine produced a contraction immediately, the inhibitory action having been abolished.

Effect of hexamethonium on the action of bretylium. At the suggestion of Dr D. B. McDougal, Jr, we carried out experiments to see if the presence of hexamethonium modified the effect of bretylium on sympathetic stimulation. We compared the effect of bretylium (4×10^{-6}) on adjacent loops of ileum, using one loop as control. Stimulation was applied 1 min after adding bretylium, and then at 3 min intervals during 30 min. The response became gradually smaller as shown in Fig 3, *a*. A second loop was then set up in the bath and the experiment was repeated

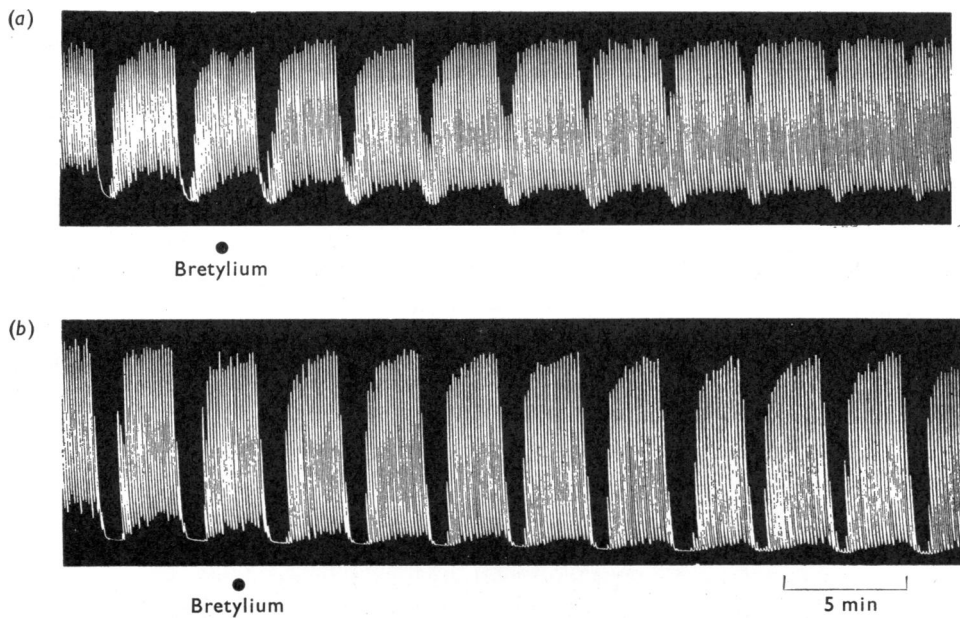

Fig. 3. Rabbit ileum preparation in Tyrode solution. The periarterial nerves were stimulated at 30 shocks/sec for 15 sec at intervals of 3 min. The top panel (*a*) shows control observations with bretylium (4×10^{-6}) added at the mark. The lower panel (*b*) shows observations made when hexamethonium (0.2 mg/ml.) was added to the bath 9 min before the mark when bretylium (4×10^{-6}) was added. Note that in the presence of hexamethonium bretylium had no effect

in the presence of hexamethonium (2×10^{-4}). Bretylium was added 9 min after the hexamethonium. The effect of stimulation remained unchanged throughout the period of 30 min, as shown in Fig. 3, *b*. Thus in the presence of hexamethonium bretylium was unable to exert a blocking action. A third loop was prepared, and the experiment was repeated in the presence of a lower concentration of hexamethonium (0.66×10^{-4}). Bretylium exerted some blocking action, but this was much less than in the control, and was incomplete after 30 min. The results are illustrated in Fig. 4.

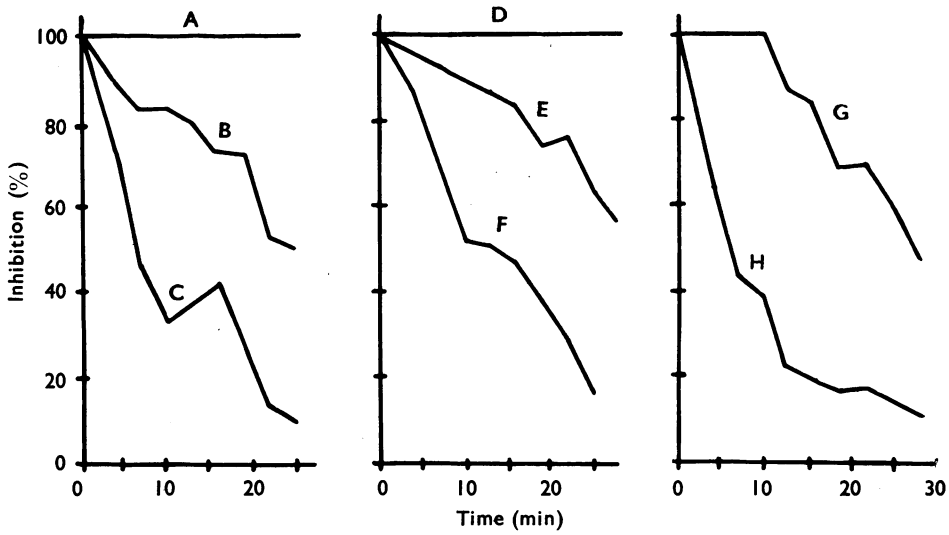

Fig. 4. Diagram showing effect of bretylium (4×10^{-6}) in presence of *bis*-quaternary blocking agents. Ordinates show inhibition (%) produced by stimulation and abscissae show time in minutes. C, F and H are control observations in the presence of bretylium only. A shows that bretylium had no effect in the presence of hemicholinium (2×10^{-4}); B shows that bretylium had a reduced effect in presence of hemicholinium (0.66×10^{-4}); D shows that bretylium had no effect in presence of hexamethonium (2×10^{-4}); E shows a reduced effect in the presence of hexamethonium (0.66×10^{-4}); and G shows reduced effect of bretylium in the presence of pentolinium (2×10^{-4}).

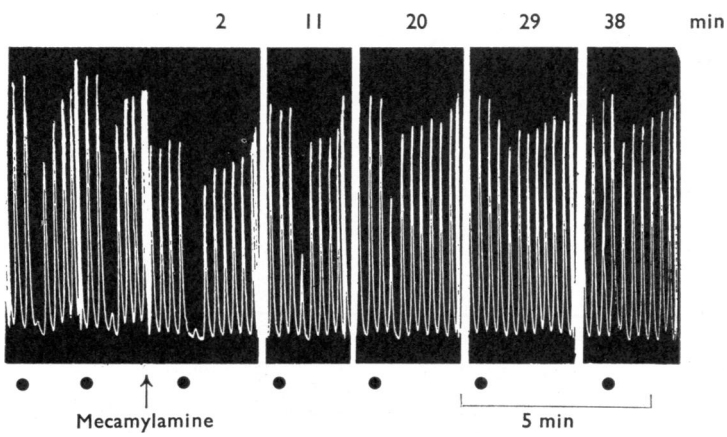

Fig. 5. Rabbit ileum preparation in Tyrode solution. At the dots, the periarterial nerves were stimulated at 60 shocks/sec for 5 sec. At the arrow mecamylamine was added in a concentration of 5×10^{-5}. The effect of stimulation steadily diminished and was very small at the end of 29 min (times in minutes at top of record).

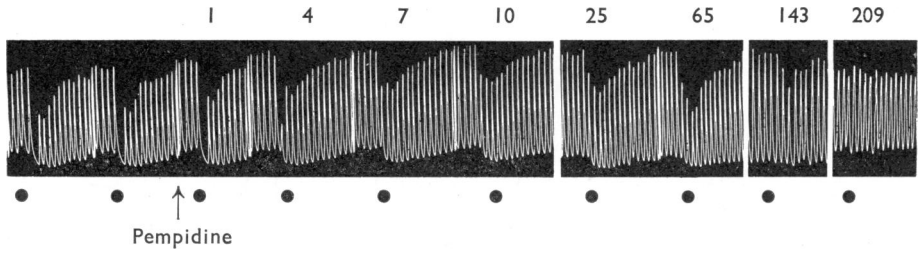

Fig. 6. Rabbit ileum preparation in Tyrode solution. At the dots the periarterial nerves were stimulated at 60 shocks/sec for 5 sec. The last two controls are shown before the addition of pempidine (5×10^{-5}) at the arrow. The effect of stimulation was much reduced in 10 min, after which it slightly increased as was evident at 65 min. Thereafter it declined and disappeared between 143 and 209 min (times in minutes at top of record).

The action of pentolinium and hemicholinium. The two compounds pentolinium and hemicholinium, which are both *bis*-quaternary compounds, were also tested in the same way as hexamethonium, and were found to have the same action. In their presence bretylium was unable to exert its effect. Hemicholinium was quantitatively equal to hexamethonium, but pentolinium was weaker. The results are illustrated in Fig. 4.

The bretylium-like action of mecamylamine and pempidine. We have also tested mecamylamine and pempidine, which are non-quaternary ganglion-blocking agents, to see if they possessed a bretylium-like action. We found that they had such an action, as is shown in Figs. 5 and 6. In these experiments the drugs were present in a concentration of 5×10^{-5}. Experiments with pempidine at lower

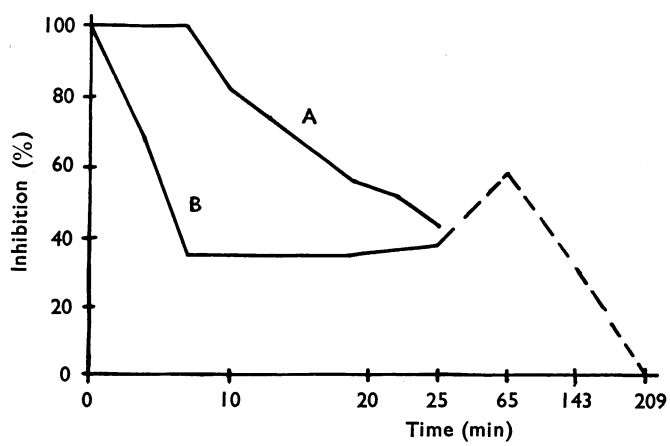

Fig. 7. To show the onset of block in the postganglionic fibre when pempidine was added to the organ-bath. The ordinate gives the inhibition (%), taking inhibition before pempidine was added as 100. The abscissa is the time in minutes (alinear scale). A shows the onset of block in the presence of pempidine (10^{-5}). B shows the onset of block in the presence of 5×10^{-5} of pempidine; note the initial rapid onset of effect followed by a period in which the block did not increase, and at 25 min the block appeared to decrease. Block was complete between 143 and 209 min.

concentrations showed that 2×10^{-5} and 10^{-5} also blocked the effect of sympathetic stimulation. A graph showing the course of the inhibition is shown in Fig. 7. The time required to reduce the inhibition to one-half was 7 min when pempidine (5×10^{-5}) was used. This may be compared with a time of about 7 min when bretylium (4×10^{-6}) was used. Thus pempidine was found to be less active than bretylium ; it appears to have 5 to 10% of the activity of bretylium. Figs. 6 and 7 show that there were two phases of block with the higher concentration of pempidine ; an early onset which was rapid (see curve B, Fig. 7), followed by a period in which there was no further advance, and indeed at the end of 1 hr the block was less. However the block increased at the end of 2 hr and became complete at the end of 3 hr. It is possible that pempidine, like bretylium, has some action in increasing the effect of noradrenaline as well as in blocking its release by the sympathetic fibre ; such an action might neutralize the later stage of block production before the block was complete.

DISCUSSION

The conception that acetylcholine exerts an action like that of sympathetic stimulation by stimulating the presynaptic terminals of the postganglionic fibres takes no account of the evidence that acetylcholine is released when the splenic nerves are stimulated in cats treated with reserpine (Burn & Rand, 1960b ; Brandon & Rand, 1961). Nor does it take acount of the amount of acetylcholine shown to be present in the terminations of the splenic nerves ; Brandon & Rand (1961) found that it was as much as 40% of the amount of noradrenaline.

The evidence that hexamethonium blocks the action of acetylcholine in releasing noradrenaline from the spleen, but does not change the effect of stimulating the splenic nerves, can now be considered in the light of the fact that bretylium blocks both the action of acetylcholine, and also that of sympathetic stimulation. Huković (1960) showed that this was true in the isolated atria of the rabbit heart, and also in the vessels of the perfused rabbit ear. Boura & Green (1959) showed that bretylium blocked the response to sympathetic stimulation in the spleen, and Brandon & Rand (1961) showed that it blocked the action of acetylcholine in causing contraction of isolated strips of spleen when atropine was present. The experiments described in this paper show that bretylium blocks sympathetic stimulation and also acetylcholine and nicotine in the ileum. Thus when bretylium is used rather than hexamethonium, there is no difference between the effect on sympathetic stimulation and that on acetylcholine.

What is the relation between bretylium and hexamethonium ? Hexamethonium is a *bis*-quaternary compound, and would have more difficulty in entering a postganglionic sympathetic fibre than *mono*-quaternary compounds like bretylium or acetylcholine. From the work of Boura, Copp, Duncombe, Green & McCoubrey (1960) it is known that bretylium enters the postganglionic sympathetic fibre in cats, and observations by Stewart, Hutchinson & Szaz (unpublished) have shown that it enters the sympathetic fibres in man in the course of a few minutes.

It is likely that acetylcholine also enters the fibre and that, when bretylium blocks the action of acetylcholine, the block takes place within the fibre. That acetylcholine

enters the fibre is supported by the evidence that in higher concentration it blocks the sympathetic impulse, as several workers have shown. Burn & Froede (1963) pointed out that there are at least seven *mono*-quaternary compounds which can block the sympathetic impulse. It is likely that all these do so after entering the fibre.

Hexamethonium, being a *bis*-quaternary compound, finds entry into the fibre more difficult, and therefore does not as a rule block the sympathetic impulse. It might however block the action of acetylcholine by preventing its entry into the fibre. Our colleague, Dr D. B. McDougal, Jr., suggested that we should test this view by seeing if hexamethonium would block the action of bretylium; for if hexamethonium prevented the entry of acetylcholine, it should prevent the entry of bretylium. We therefore made the experiment, and found that, in the presence of hexamethonium, bretylium did not block the sympathetic impulse. We found that two other *bis*-quaternary compounds, pentolinium and hemicholinium, had the same action as hexamethonium. We conclude from these results that hexamethonium fails to block the sympathetic impulse because it cannot enter the postganglionic fibre, and that it blocks the action of acetylcholine because it prevents acetylcholine from entering the fibre.

Burn & Froede (1963) called attention to the fact that bretylium blocks transmission in the sympathetic ganglion (Boura & Green, 1959) and that it also blocks the neuromuscular junction in the phrenic nerve-diaphragm preparation (Dixit, Gulati & Gokhale, 1961). Recently Gokhale, Gulati & Joshi (1963) have studied the rise of blood pressure caused by physostigmine when injected into the rat. This rise was described by Dirnhuber & Cullumbine (1955) and independently by Varagić in 1955. Gokhale *et al.* (1963) have found that the rise is blocked completely by bretylium, but only partly by hexamethonium. However, they observed that mecamylamine blocked the rise completely. The rise caused by physostigmine obviously involves the sympathetic nerves, but it appeared strange that the two ganglion-blocking agents, hexamethonium and mecamylamine, should act differently. Why was this ?

Mecamylamine differs from hexamethonium in structure, since it is a secondary amine and not a *bis*-quaternary compound. It occurred to us that mecamylamine might have not only the power to block ganglia, but also some power to block the postganglionic fibre as well. On the view put forward by Burn & Froede (1963) and repeated in this paper, mecamylamine should block the postganglionic fibre although hexamethonium cannot. We found that not only mecamylamine, but also pempidine, which is a tertiary amine, have a bretylium-like action, and that pempidine appears to have about 5 to 10% of the action of bretylium. This finding helps to account for differences between the action of mecamylamine and that of hexamethonium observed earlier (Bennett, Tyler & Zaimis, 1957).

For our purpose the point of interest is that substances classed as ganglion-blocking agents, which are not *bis*-quaternary in structure, have been found to have an action in blocking the sympathetic postganglionic fibre. Since they are ganglion-blocking agents they block the action of acetylcholine. Since they are secondary and tertiary amines, respectively, they can enter the postganglionic fibre, and their action in that

fibre is explained if the hypothesis is accepted that acetylcholine is an intermediary in the release of noradrenaline. The action of mecamylamine and of pempidine, like that of nicotine, was not seen in all preparations.

NOTE ADDED IN PROOF

Dr Kevin K. F. Ng (Department of Pharmacology, University of Singapore) has observed in three experiments that pempidine (30 μg/ml.) blocked sympathetic stimulation in the Finkleman preparation at a high frequency before it blocked at low frequency. This result indicates that the blocking action is not due to anaesthesia of the nerve.

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