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THE RELATIVE EFFECTS OF GANGLION-BLOCKING COMPOUNDS ON THE SYMPATHETIC AND PARASYMPATHETIC GANGLIA SUPPLYING THE CAT HEART

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The use of ganglion-blocking drugs in clinical medicine is limited by the fact that they attack sympathetic and parasympathetic ganglia indiscriminately (Paton and Zaimis, 1952), and this has prompted the search for compounds which might exhibit a selective action on one or other of these autonomic pathways. One method of seeking selective blocking action lies in the study of the transmission process at the two sites to discover if any differences between them suggest a method of differential block. Both sites are, however, cholinergic (Suden, Hart, and Marrazzi, 1952; Perry and Talesnik, 1953). The latter workers showed that the ganglionic action potential of the ciliary ganglion is remarkably similar to that of the superior cervical ganglion save in its shorter duration, which may well be a characteristic not of the parasympathetic system but only of the ciliary ganglion from which neurones run to supply the pupillary muscles. Moreover, the ganglion-blocking compounds studied were similar in action at both sites; so little hope of determining a selectivity of action was offered by this method.

Another method of searching for such a selective action is the screening of large numbers of compounds for this effect on a few chosen ganglia of either system. Great difficulties in interpretation arise, however, because of the differing sensitivity not only of different animal species but of different end-organs within one particular species. Thus, to compare the effect of a drug on the nictitating membrane of the cat (sympathetic ganglion-block) with the effect on the guinea-pig ileum (parasympathetic ganglion-block) is meaningless. Even if the effects of a drug are tested in a single species, such as the cat, by recording comparatively block of nictitating membrane (sympathetic stimulation) and salivary secretion (parasympathetic stimulation), the extreme sensitivity of the latter to all blocking compounds (Paton and Perry, unpublished) makes comparisons very difficult.

We considered that the interpretation of such results might be easier if the effect of ganglion-blocking compounds was studied concurrently on the same end-organ. For this purpose we had to choose an end-organ with a dual innervation, both presynaptic nerve supplies being accessible for stimulation; this paper describes the results we have obtained in one such preparation, namely, the cat heart.

METHODS

Cats were anaesthetized with ethyl chloride and ether followed by intravenous chloralose (80 mg./kg.). Under artificial respiration the chest was opened widely on one side in order to expose the vagus and sympathetic nerves throughout their course to the heart; the pericardium was left intact. Care was taken to ensure that the blood supply to the thoracic sympathetic ganglion was not interrupted. Blood pressure was recorded through a cannula in the femoral artery. Pulse rate and pressure were recorded by transmission through a cannula in the carotid artery to a rubber diaphragm carrying a light balsa-wood lever which wrote on a kymograph.

Presynaptic parasympathetic fibres to the heart were stimulated in the cervical vagus nerve; presynaptic sympathetic fibres were stimulated in the thoracic sympathetic trunk between the 2nd and 3rd thoracic ganglia. A third stimulating electrode was placed on postsynaptic sympathetic fibres in the accelerator nerve. The nerves and vessels on the other side of the chest were left intact. Platinum-wire stimulating electrodes were used for all nerves, which were kept moist and were often covered with liquid paraffin. Square-wave pulses of 0.5 msec. duration, at a frequency of 17/sec., were used for stimulation of each of the nerves. Suitable pulses were of 5–10 volts. The sympathetic and parasympathetic presynaptic nerves were stimulated alternately at

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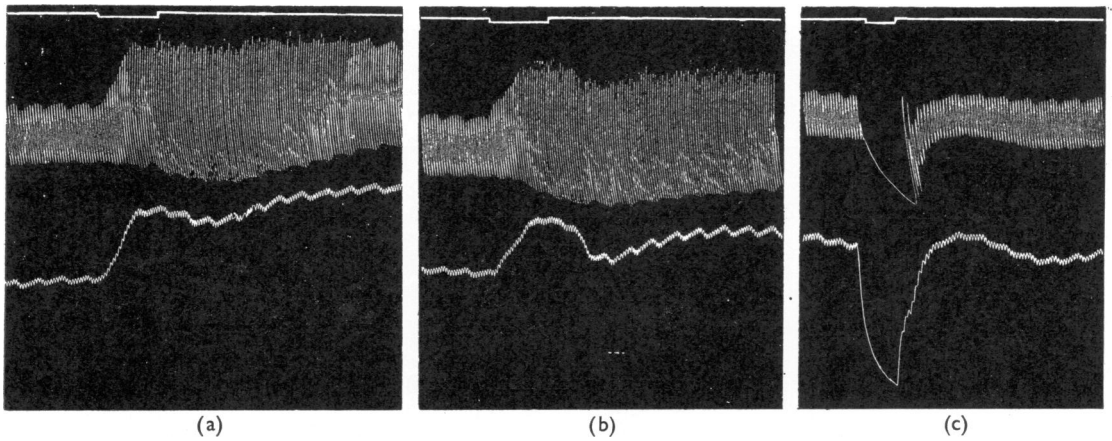

FIG. 1.—Cat, chloralose, artificial respiration. Top trace, periods of stimulation : middle trace, pulse rate and amplitude ; bottom trace, blood pressure. (a) 10 sec. stimulation of sympathetic trunk between 2nd and 3rd thoracic ganglia; (b) 10 sec. stimulation of inferior accelerator nerve; (c) 5 sec. stimulation of cervical vagus nerve.

2 min. intervals for periods of 10 and 5 sec. respectively. Occasional periods of postsynaptic sympathetic stimulation for 10 sec. were interpolated. Recovery from the effects of stimulation for these periods was nearly always complete before the end of the 2 min. cycle. Drugs were administered intravenously into the femoral vein ; hexamethonium and pentamethonium were used as the chlorides, azamethonium as the bromide (" Pendiomide "), and tetraethylammonium (TEA) and tetramethylammonium (TMA) as the bromides.

RESULTS

Effects of Stimulation

Presynaptic Sympathetic Stimulation.—Stimulation of the thoracic sympathetic chain between the 2nd and 3rd thoracic ganglia for 10 or 15 sec. produced an increase of blood pressure and acceleration of the heart rate. The onset of this effect was delayed and began some 3–4 sec. after the start of stimulation. A typical record is shown in Fig. 1(a). The blood pressure rose from 110 to 140 mm. Hg and the heart rate increased from 136 to 168 beats/min. There was also an increase in pulse amplitude. The effect lasted for about 60 sec., heart rate and blood pressure gradually returning to normal. Stimulation was repeated at 2 min. intervals for several hours and the same pattern of response obtained; the accelerator response was independent of the initial heart rate and blood pressure, but the pressor response was smaller if the initial blood pressure was high. Consequently, we used as the index of the normal response to stimulation the percentage increase in heart rate. This percentage increase was then taken as a 100% response, and responses after blocking drugs were expressed similarly. The initial heart rate was obtained by counting the heart beats during the 5 sec. just before stimulation, and the final rate by counting the beats during 5 sec. at the height of the response. Thus in Fig. 1(a) the initial heart rate was 136 and the final heart rate was 168, so that the percentage increase was $32/136 \times 100 = 24\%$. If after a dose of a ganglion-blocking drug the percentage increase in heart rate was found to be 12%, this is then taken to represent 50% inhibition of transmission.

Postsynaptic Sympathetic Stimulation.—Stimulation of either or both accelerator nerves on the

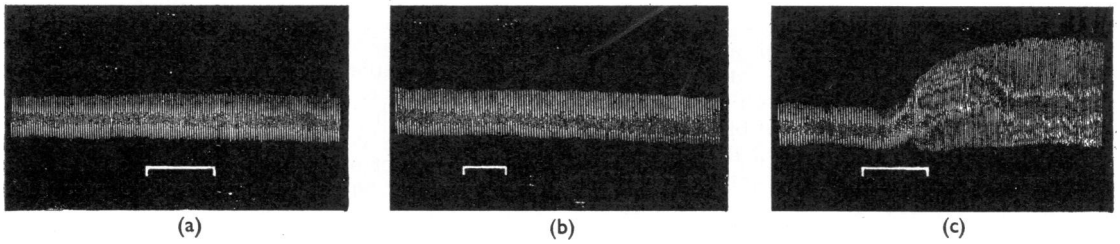

FIG. 2.—Cat, chloralose, artificial respiration. Pulse rate and amplitude after pentamethonium 400 mg. intravenously in divided doses. At signals stimulation as in Fig. 1 of (a) thoracic sympathetic trunk, (b) vagus, (c) accelerator nerve.

one side of the animal for 10 sec. led to the same typical response as that described above after stimulation of the presynaptic sympathetic nerves in the thoracic sympathetic trunk. The magnitude of the effect was similar with either the superior or inferior accelerator nerve, and concurrent stimulation of both did not materially increase the response. A typical record is shown in Fig. 1(b).

The responses were calibrated as described for the response to presynaptic stimulation. The response to postsynaptic sympathetic stimulation was unaffected by the doses of ganglion-blocking drugs used, and test periods of stimulation of this kind were interpolated in all experiments in order to ensure that the effects observed were not peripheral. Unfortunately, it was not possible to ensure that this is true of the parasympathetic innervation ; but the results of Perry and Talesnik (1953) can be used as a basis for inferring that the two systems behave in similar fashion in this respect.

Presynaptic Vagal (Parasympathetic) Stimulation.—Stimulation of the vagus for 5 sec. produced cardiac arrest or very considerable slowing of the heart rate. A typical record is shown in Fig. 1(c).

The time of stimulation used was shorter than that used for the sympathetic nerves, since 10 sec. stimulation of the vagus produced an effect which lasted longer than the interval between successive periods of stimulation (i.e., longer than 2 min.). Longer intervals between successive periods of stimulation made it very difficult to follow the course of block produced by some of the drugs studied. The complete, or almost complete, cardiac arrest lasted throughout the 5 sec. period. Thereafter the heart rate often increased to a level at which it exceeded the normal rate for a short period. The responses were calibrated in the same way as that used for responses to sympathetic stimulation.

Effects of Ganglion-blocking Drugs

"Competitive" Blocking Drugs

We have used in these experiments azamethonium, hexamethonium, pentamethonium, and tetraethylammonium (TEA). All these drugs

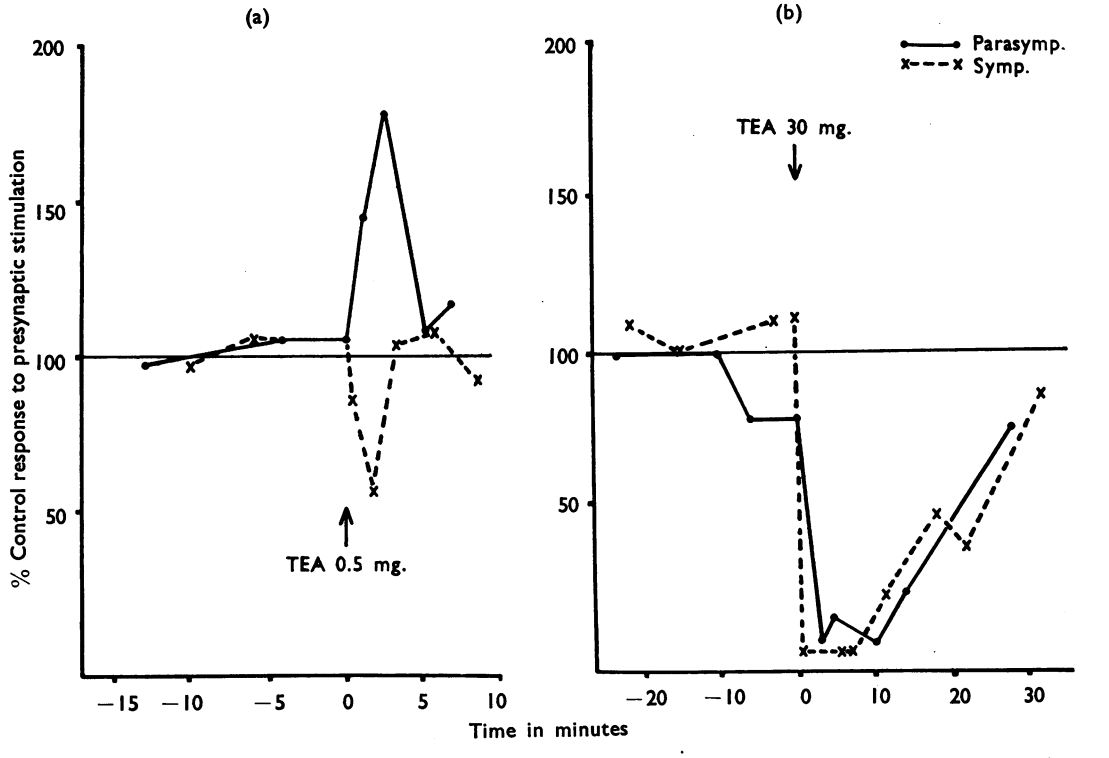

FIG. 3.—Graphs of time-course of block of presynaptic stimulation produced by TEA 0.5 mg. i.v. (a) and 30 mg. i.v. (b). Abscissa, time in min. before and after injection of drug. Ordinate, response of heart as % of control response. Solid line, response to parasympathetic stimulation. Dotted line, response to sympathetic stimulation.

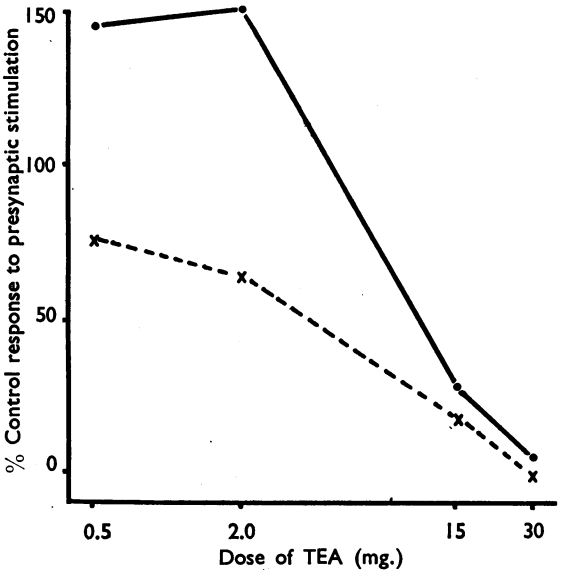

FIG. 4.—Graph of log dose-response lines of effect of TEA. Abscissa, dose of TEA in mg. Ordinate, response of heart as % of control response. Closed circles, response to parasympathetic stimulation. Crosses, response to sympathetic stimulation.

block the response to presynaptic sympathetic stimulation while leaving the effect of postsynaptic (accelerator nerve) sympathetic stimulation intact. Fig. 2 shows, for example, a record obtained using pentamethonium. The block of presynaptic sympathetic responses is always accompanied by a block of the vagal stimulation.

Tetraethylammonium.—The effects of TEA on sympathetic and parasympathetic ganglia appeared to be almost identical, although there was a slight apparent selectivity of action on the sympathetic cells. Fig. 3 shows the effects of graded doses of TEA on each system. Fig. 3(a) illustrates how 0.5 mg. TEA produced a transient block of sympathetic function of about 40%; there appears to have been no such block of vagal function and, in fact, the responses to vagal stimulation were increased, presumably due to the depression of sympathetic tone on the intact non-experimental side, since the two effects ran very parallel. When the dose of TEA was increased to 30 mg. (in the same experiment) there was almost complete block of both systems (Fig. 3(b)); the onset of sympathetic block was, however, rather more rapid, although the duration of block and rate of recovery were similar for both systems. Doses of TEA intermediate between these two doses gave responses intermediate in both magnitude and duration. It will be seen that the block produced by a dose of 30 mg. TEA lasted for some 30 min.

We have attempted to relate the potencies of TEA on the two systems by comparing the dose-response lines for each. The results of one such attempt is shown in Fig. 4. It is obvious that no direct parallelism exists, since the two effects are so closely interrelated that partial block of the sympathetic fibres is accompanied by a potentiation of the effect of vagal stimulation. Nevertheless, at doses which are sufficient to produce block of both, there appears to be no selective action on either system—at high doses the potency ratio being less than 1.5.

In view of the differences in rate of onset, duration and recovery of block, we used another method of illustrating these effects—similar to that used by Paton and Perry (1953). This is shown for another experiment with TEA in Fig. 5. The same type of reaction is seen, namely, a relatively rapid onset of sympathetic block, a slower development of vagal block—the later phases of which are accompanied by partial sympathetic recovery—and, finally, a steady recovery from both effects, reaching normal concurrently.

We have described the effects of TEA in detail, since they provide the basis for describing the action of the other blocking drugs.

Azamethonium.—The results which we obtained with azamethonium are almost identical with those already described for TEA. The onset of paralysis is rapid on the sympathetic ganglion, but the para-

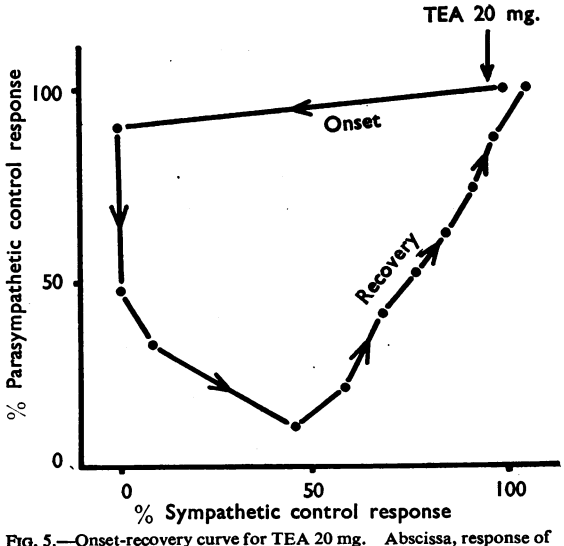

FIG. 5.—Onset-recovery curve for TEA 20 mg. Abscissa, response of heart to parasympathetic stimulation as % of control response. Ordinate, response of heart to sympathetic stimulation as % of control response. Arrows indicate course of block.

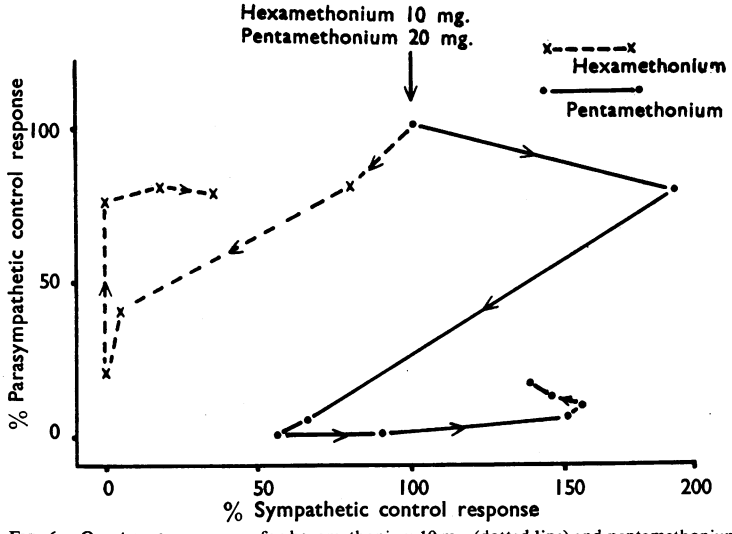

FIG. 6.—Onset-recovery curves for hexamethonium 10 mg. (dotted line) and pentamethonium 20 mg. (solid line). Conventions as in Fig. 5. Arrows indicate course of block.

lysis lasts slightly longer in the parasympathetic ganglia. With 5 mg. the block lasts some 15 min.; with 15 mg. the block is slightly greater, lasting 45 min.

Hexamethonium.—We obtained differing results with hexamethonium. In some experiments the parasympathetic block was slightly more severe; in others the reverse was true. However, the difference was never so great as to suggest a really selective action. The duration of action was also similar in both sympathetic and parasympathetic ganglia. One onset-recovery curve is shown in Fig. 6, where it is compared to a similar curve for pentamethonium. In this particular experiment the parasympathetic block was both less intense and less prolonged than the sympathetic block, although the rates of onset of block were similar.

Pentamethonium. — This compound was the only one with which we observed a consistent selectivity of effect on the cat heart.

Fig. 7(a) shows the dose-response lines obtained in one experiment, and shows that pentamethonium was some 8 times as active in blocking the parasympathetic ganglia as it was in blocking the sympathetic ganglia. In a similar experiment another figure of about 12 was obtained. For comparison Fig. 7(b) shows similar dose-response lines for hexamethonium; in this case there appeared to be a slight selective action (say $\times 3$) on the sympathetic ganglia.

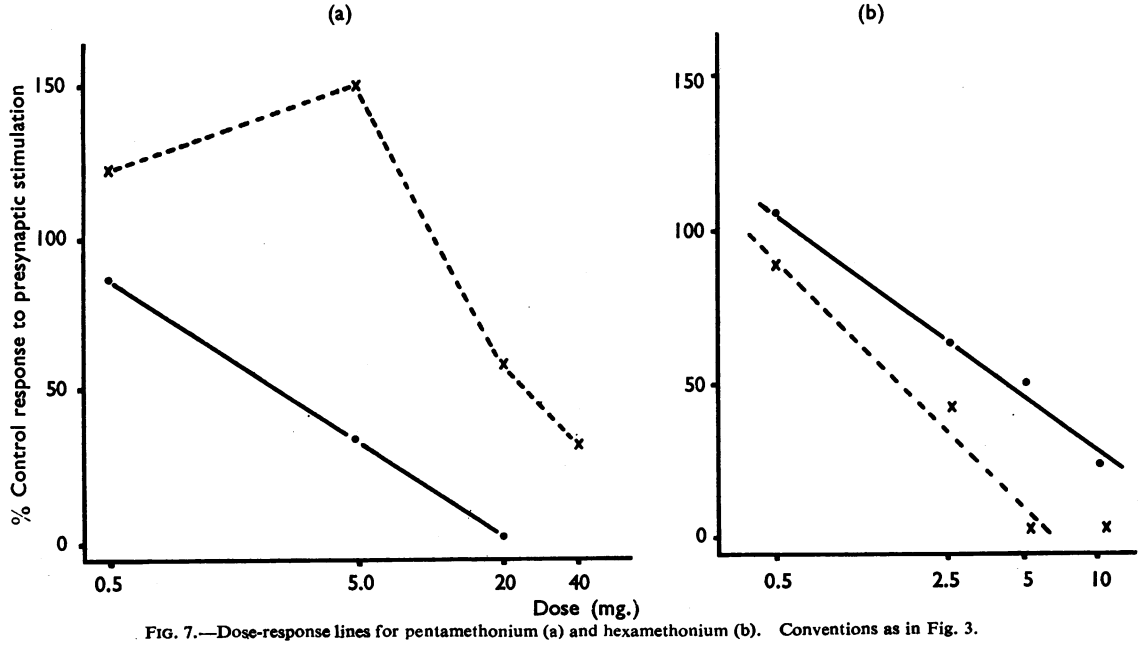

FIG. 7.—Dose-response lines for pentamethonium (a) and hexamethonium (b). Conventions as in Fig. 3.

Moreover, the onset-recovery curves (Fig. 6) make the difference in the effects of these two closely related compounds very obvious indeed. Hexamethonium showed little selectivity either in degree or duration of the block, whereas pentamethonium produced a rapid onset of parasympathetic block accompanied at an early stage by potentiation of the effect of sympathetic stimulation, due presumably to release from parasympathetic tone on the other side of the animal. This was soon followed by partial block of the response to sympathetic stimulation which, however, was of lesser degree than the parasympathetic block. Furthermore, the partial sympathetic block disappeared completely, while the parasympathetic block remained complete. Thereafter there was a further potentiation of the sympathetic response, presumably due, once again, to the temporary absence of parasympathetic tone. Recovery from parasympathetic block was very slow and only the early phase is shown in Fig. 6.

Since in all the experiments quoted only one drug was used, we considered it necessary to confirm that the difference was not one caused by variation in the susceptibility of different cats; consequently, we compared in one experiment the effects of two drugs, and the same differences were observed.

"Depolarizing" Blocking Drugs

Tetramethylammonium.—TMA produced first a marked slowing of the pulse rate and a fall in blood pressure, followed by a prolonged rise of blood pressure and increase of pulse rate above the initial resting levels. The latter effect might be attributed to a stimulation of the sympathetic supply which outlasted the parasympathetic stimulation; or to an effect of adrenaline released by TMA from the adrenals. We repeated this experiment after ligating the blood supply of both adrenal glands, and the TMA then produced only a slowing of the pulse rate, indicating that the later phase of increased pulse rate was in fact due to adrenaline release. Thus it appears that TMA is a more effective stimulant of the parasympathetic than of the sympathetic ganglion cells.

After its initial stimulant effect, TMA blocked the effects of stimulation of the sympathetic and parasympathetic nerves. The degree and time-course of the block of parasympathetic and of sympathetic stimulation were almost identical. This block was relatively short-lived as compared with the block produced by the methonium drugs and closely resembled that produced by TEA.

After a dose of TMA it was necessary to test for block during the phase of increased pulse rate due to release of adrenaline. At this time there appeared to be block of both pre- and post-synaptic sympathetic stimulation. The apparent postsynaptic block was, however, less marked than the presynaptic block, because the pulse rate was already so fast that stimulation failed to produce the usual acceleration. After ligation of the blood supply to both adrenal glands, postsynaptic stimulation was fully effective after large doses of TMA which completely blocked presynaptic stimulation. Furthermore, if adrenaline was administered at this stage, the acceleration produced would again mask the effect of postsynaptic stimulation.

Relative Potencies of Blocking Drugs

We have calculated roughly by graphical methods the relative potencies of the different drugs on parasympathetic and sympathetic ganglia respectively, and these figures are summarized in Table I. Such estimates were only possible from experiments in which a number of different doses were tested.

TABLE I

MEAN RATIOS OF SYMPATHETIC/PARASYMPATHETIC BLOCK, CALCULATED FROM DOSE-RESPONSE LINES AS IN FIGS. 4 AND 7

Drug	No. of Expts.	Ratio
Tetraethylammonium ..	2	0·75
Tetramethylammonium ..	1	1·0
Azamethonium	2	0·75
Hexamethonium	3	0·6
Pentamethonium	2	10·0

DISCUSSION

The method of studying ganglion-blocking drugs which we have described represents, we believe, the first attempt to compare concurrently the effects of such compounds on the ganglion cells of the sympathetic and parasympathetic supply to a single end-organ. Such studies should provide a more useful method of determining a selectivity of action on one or other part of the autonomic system than do the more commonly used methods of testing on more convenient but different end-organs in different species of animals. The relative effects of a drug on the ganglion cells of each system may, of course, depend not only upon inherent differences in sensitivity but also on the accessibility of the cells which are morphologically very differently arranged. Thus administration of the drug by arterial injection close to the end-organ might be expected to produce a selective action on the parasympathetic ganglion cells which are located in the end-organ itself, unlike the

sympathetic ganglion cells which are collected in distinct ganglia and would be proximal to such an arterial injection. We considered that intravenous injection of the drug at a distance from the end-organ provided the best available means of minimizing such possible differences in accessibility, and we have consequently used this technique throughout.

The selection of the heart as the end-organ for study was based on the relative ease of isolation of the presynaptic fibres of both systems supplying it. It is indeed probable that the clinical effects of ganglion-blocking drugs are attributable only to a small extent to their direct action on the autonomic supply to the heart. Further work on other end-organs is most desirable, and the present results merely indicate the possibilities inherent in techniques of this kind.

The results which we have obtained on the rate of onset, duration, and recovery from block of the two systems underline the complexity of the autonomic control. Block of one system may result in potentiation of the effects of the other, possibly due to release of tone ; and this potentiation may well complicate the pattern of any partial block of this second system which may be co-existent with the potentiation. In analysing these effects we have found that the onset-recovery curves, such as those illustrated in Figs. 5 and 6, have been most helpful, and we consider that they go far towards elucidating the pattern of interaction. It is worth pointing out that the potentiation of the sympathetic response seen during parasympathetic block was also observed when the sympathetic stimulation was postsynaptic, indicating that this effect was a peripheral and not a ganglionic one. As might have been expected from previous studies of the transmission process at both sites, most drugs studied showed little or no selectivity of action on the ganglion cells of one or other system. The single striking exception was pentamethonium, which did, apparently, exhibit a marked preferential block of the parasympathetic ganglion cells, being some 10 times more potent in blocking them than in blocking the sympathetic ganglion cells. This was, at first sight, a surprising finding in view of the fact that early work (Paton and Zaimis, 1951) showed pentamethonium to be much more active on the superior cervical ganglion than on the guinea-pig ileum and stressed the possible importance of this apparently selective action. Nevertheless, as Paton and Zaimis (1952) later pointed out, this relatively simple picture failed to survive when it became apparent that different end-organs varied enormously in their sensitivity—as, for example, when Paton and Perry (unpublished) showed that the salivary secretion was blocked first by all the ganglion-blocking drugs tried. The present results indicate that, on the innervation of the heart at least, pentamethonium attacks the parasympathetic cells preferentially and not, as had at first appeared probable, the sympathetic cells. The 10-fold difference in sensitivity between the two systems, which we found with pentamethonium and not with hexamethonium, is not clearly reflected in the clinical activity of the two drugs. The main use of the drugs in clinical practice has been in the treatment of hypertension. What is required in such cases is a selective block of sympathetic ganglia ; and pentamethonium is less effective than hexamethonium. It could be argued that this difference is due to the greater degree of block of parasympathetic ganglia produced by pentamethonium. The situation is obviously complex, and our results suggest that further direct comparisons of the kind we have described will be necessary to clarify it.

SUMMARY

1. A method is described for studying the action of drugs concurrently on the effect of presynaptic stimulation of the sympathetic and parasympathetic ganglia supplying the cat heart. By this method the effects of postsynaptic sympathetic stimulation can also be investigated.

2. A number of ganglion-blocking drugs has been studied using this method. TEA, TMA, hexamethonium, and azamethonium showed no selective blocking action on either the sympathetic or parasympathetic ganglia, in doses which leave the effects of postsynaptic sympathetic stimulation intact.

3. Pentamethonium is 10 times more effective in blocking the parasympathetic ganglia than in blocking the sympathetic ganglia.

4. The time-course of the block produced by these drugs on both systems is described.

5. The use of this and of other similar methods in examining compounds for selective blocking activity on one part of the autonomic system is discussed in relation to the clinical need for such differentiation.

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