Br. J. Pharmac. Chemother. (1966), **27**, 51–63.

THE ACTION OF ACETYL CHOLINE AND OTHER DRUGS UPON THE TERMINAL PARTS OF THE POSTGANGLIONIC SYMPATHETIC FIBRE

BY

R. CABRERA*, R. W. TORRANCE† AND H. VIVEROS*

From the Institute of Physiology, National University of Chile, Santiago, Chile

(*Received November* 8, 1965)

Acetyl choline produces a sympathomimetic effect upon the isolated heart by releasing noradrenaline from stores within the postganglionic sympathetic fibres of the heart (Cabrera, Cohen, Middleton, Utano & Viveros, 1966). It could do this by exciting the postganglionic sympathetic fibres at some point so that impulses spread throughout the ramifications of the fibres and release noradrenaline in a normal way (Coon & Rothman, 1940). Alternatively it might release noradrenaline from sympathetic fibres without initiating impulses in the fibres (Burn & Rand, 1965). If the first is the true explanation of the effects of acetyl choline, then one would expect the impulses initiated peripherally to be conducted antidromically back towards the cell bodies in the sympathetic ganglion.

In one of the earliest papers on the sympathomimetic actions of acetyl choline, Coon & Rothman (1940) suggested that Ach sets up propagated impulses, in order to account for their observation that acetyl choline, injected into the skin, gave piloerection at a distance of several cm from the point of injection with a latency of a very few sec and a duration of about a min. Since that date, many workers have found that acetyl choline can excite sensory nerve fibres near to their terminations (Brown & Gray, 1948 ; Douglas & Gray, 1953) but not at more proximal parts of their course (Diamond, 1959). Ferry (1963) has shown that acetyl choline can also excite the postganglionic sympathetic fibres of the spleen. In the present work we have used the collision technique to show that acetyl choline initiates antidromic impulses in the postganglionic fibres that go to the heart and other thoracic viscera in the inferior cardiac nerve. We have also used this technique to investigate various drugs which modify the sympathomimetic effect of acetyl choline or have sympathomimetic effects themselves. Ferry (1963) used a degeneration method so that he could be sure that he was investigating sympathetic postganglionic fibres rather than sensory fibres. To achieve the same end we have used preganglionic rather than postganglionic stimulation.

* Address: Casilla 6524 Santiago, Chile.

† Present address: University Laboratory of Physiology, Oxford.

METHODS

Cats of 2–3 kg body weight of both sexes were anaesthetized with chloralose 80 mg/kg i.v. after induction with ether. The trachea was cannulated and artificial respiration was maintained with a Harvard 1063 pump. The thorax was opened in the midline and the anterior parts of the ribs were removed bilaterally. The thoracic cavity was filled with liquid paraffin at 38°.

The sympathetic chain was cut 2 cm caudal to the stellate ganglion and was put on stimulating electrodes. The inferior cardiac nerve was defined and separated from mediastinal tissue for a distance of 1 cm as near as possible to the heart and was placed on the recording electrodes. The preganglionic sympathetic fibres were stimulated supramaximally with a Grass S-4 stimulator with a stimulus isolation unit SIU-4 at a frequency of 3/sec. The postganglionic action potential was led to a Tektronix 122 preamplifier and then to a Tektronix 502 oscilloscope which was photographed with a Cossor camera.

Drugs were injected in a volume of 1 ml. through a cannula tied into the left atrial appendage or into the femoral vein. The arterial supply to the stellate ganglion was tied except for the main

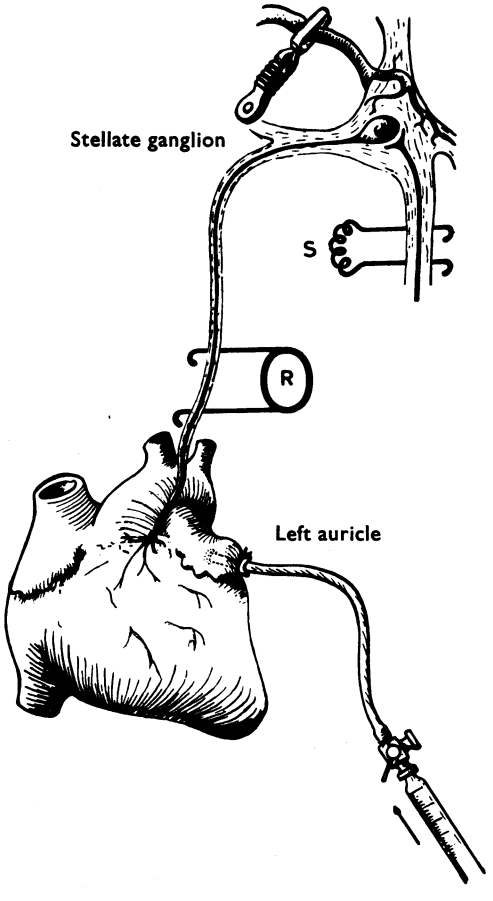

Fig. 1. Diagram of experimental assembly. The stimulus is applied to preganglionic fibres of the stellate ganglion through electrodes, S, and the postganglionic action potential is recorded from the inferior cardiac nerve with electrodes R. Drugs are injected through a cannula tied into the left atrial appendage and, during their injection, their access to the stellate ganglion is impeded by placing a bulldog clip on the subclavian artery.

artery which arises from the subclavian artery. Central to this branch a ligature was passed round the subclavian artery so that the blood supply to the ganglion could be stopped temporarily with a bulldog clip during the injection of drugs. This procedure delayed access of drugs to the ganglion though at times it did not stop it completely.

Fig. 1 is a diagram of the experimental assembly. On stimulation at S an action potential is recorded at R. We call the C potential recorded the control orthodromic potential. Antidromic activity of C fibres is revealed as a reduction in this control potential and the reduction in this potential is proportional to the number of fibres activated and their frequency of discharge, at any given distance between S and R.

To show that any reduction in the control potential which occurs on injection of drugs into the heart is due to antidromic impulses arising in the heart and not to a block of conduction between S and R, conduction of such antidromic impulses is blocked by crushing the nerve between the heart and R. If changes in the control potential are due to impulses arising in the heart, crushing the nerve here should abolish the effect. To show that the control potential arises from the activity of postganglionic sympathetic fibres, a ganglionic blocking agent hexamethonium is injected. Then any potential of C conduction velocity that remains comes from postganglionic fibres of cells caudal to the stimulating electrodes, or from sensory C fibres.

During recording the animal was ventilated with oxygen at the minimum rate and depth that would suppress spontaneous respiratory efforts, in order to reduce recording artifacts to a minimum.

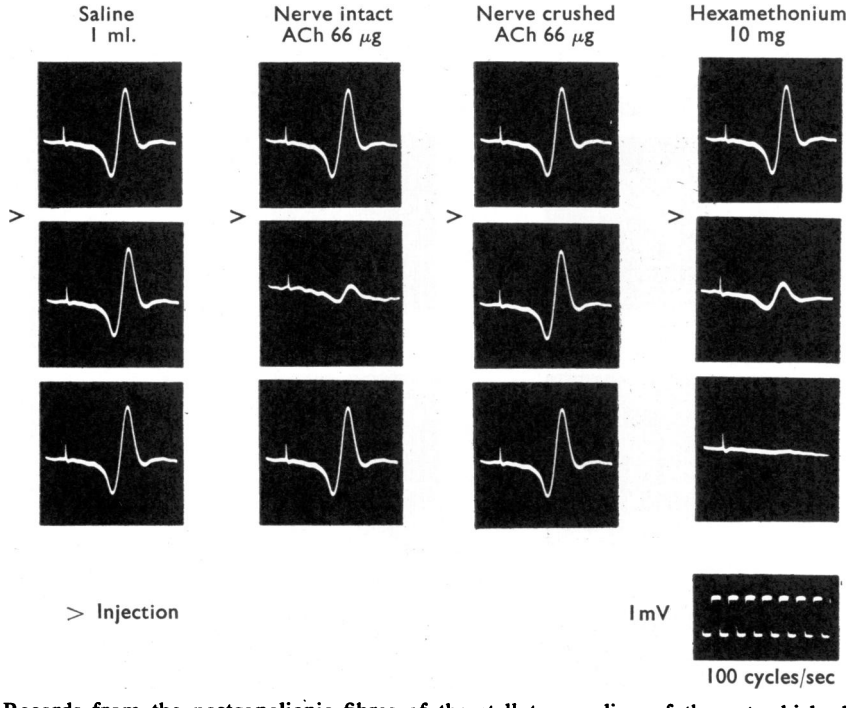

Fig. 2. Records from the postganglionic fibres of the stellate ganglion of the cat which show that acetyl choline sets up antidromic impulses in them. In each column the first record is the control potential. In the first three columns, the second record was taken 2 sec, and the third record 6 sec, after an injection into the left atrium. Saline had no effect on the potential (first column), ACh reduced it transiently (second column), but its effect was blocked by crushing the nerve distal to the recording electrodes (third column). In the fourth column, hexamethonium, given intravenously, reduced the potential at 5 sec (second record) and abolished it by 15 sec (third record).

The arterial pressure was recorded from the femoral artery with a mercury manometer and the body temperature was held at 37–38° C. Experiments were carried out on either side of the animal.

Except where stated animals were given 1 mg/kg atropine at the beginning of the experiment and the dose was repeated later if necessary. Drugs used were: Acetyl choline hydrochloride, atropine sulphate, pentobarbitone sodium as salt, tubocurarine chloride, hexamethonium bitartrate, dimecaine hydrochloride, procaine hydrochloride, nicotine, propylene glycol, ethyl ether, α-chloralose, guanethidine sulphate, bretylium tosylate, reserpine as pure hydrochloride dissolved immediately before use in concentrations of 1–6 mg/ml. in propylene glycol or 50% propylene glycol 50% distilled water.

All drugs were injected at 37° C with 5 min between injections, except where stated otherwise.

The photographic records were printed at an enlargement of about 25 times and areas were measured with a planimeter. Results are presented as a percentage reduction from the area of the control potential, produced by injection of the drug being studied.

RESULTS

Excitation of postganglionic cardiac sympathetic fibres by acetyl choline

Fig. 2 shows the potential recorded from the inferior cardiac nerve in response to a maximal stimulus applied to the sympathetic chain caudal to the stellate ganglion. The potential appears at a latency of 30 msec measured from the stimulus artifact to the peak of the potential. The distance between the stimulating and the recording electrodes

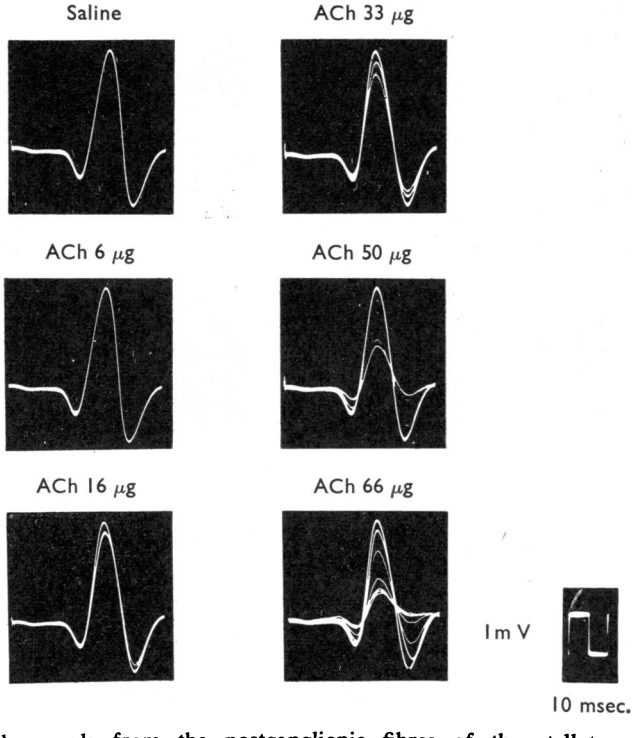

Fig. 3. Superimposed records from the postganglionic fibres of the stellate ganglion made at intervals of ½ sec. The reduction of the potential produced by the injection of acetyl choline into the left atrium is greater, the greater the dose.

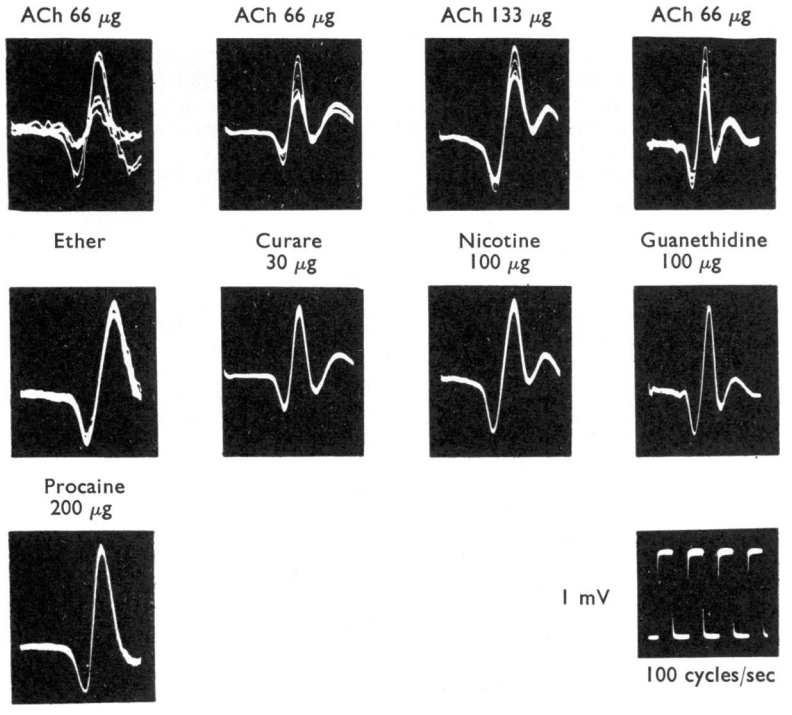

Fig. 4. The effect of ether, procaine, D-tubocurarine, nicotine and guanethidine in blocking the effect of acetyl choline on postganglionic sympathetic fibres. Each column of superimposed records is from a different experiment. The top row of superimposed records shows that an injection of acetyl choline into the left atrium reduces the size of the postganglionic action potential. The records beneath these show that after the administration of a second drug the effect of acetyl choline is reduced or abolished. The testing doses of acetyl choline were given at the following intervals after the conditioning drug. Ether: 2 min after the start of inhalation. Procaine: 2 min ; Curare: 2 min ; Nicotine: 5 min ; Guanethidine: 30 min.

was 30 mm so the conduction velocity of about 1 msec is appropriate for C fibres. Injection of 1 ml. normal saline into the left auricle with the circulation to the ganglion temporarily blocked did not alter the size of the control orthodromic potential (A), but the injection of 66 μg acetyl choline in an equal volume of saline reduced it by 90% (B). Crushing the nerve between the recording electrodes and the heart abolished the reduction of the control potential by acetyl choline (C). Hexamethonium in a dose which blocks ganglionic transmission abolished the postganglionic potential (D).

The reduction of the control orthodromic potential by acetyl choline can be observed repeatedly and is very constant in any animal, lasting for 3 to 6 sec, depending on the dose injected. The effect was seen regularly in 31 cats, three of which had not been given atropine.

In five experiments the nerve was crushed distal to the recording electrodes and this, without exception, abolished the effect of acetyl choline. In 11 experiments hexamethonium given intravenously abolished the orthodromic potential completely.

In eleven of the 31 cats the effects of increasing doses of acetyl choline were studied.

TABLE 1

THE EFFECT OF VARIOUS DRUGS UPON THE ACTION OF ACETYL CHOLINE IN EXCITING POSTGANGLIONIC SYMPATHETIC FIBRES

The dose of acetyl choline that would reduce the postganglionic potential by about one-half was first determined and then the effect of the same dose of acetyl choline was tested at various times after the injection of another drug. In the column headed " block," the symbols 0, +, ++, +++ indicate that the effect of this dose of Ach was reduced by the second drug by less than 25%, between 25% and 50%, between 50% and 75% and by more than 75% respectively. The figures refer to the maximum block produced. Only in the case of pentobarbitone, guanethidine and bretylium did the block develop slowly over several minutes so that its development could be followed. Each observation with any drug was made in a different experiment. In each experiment a second drug was only used when the effect of a first one had completely passed off

	Dose (mg/kg)	Route	Block	Duration
Ether	—	inhal.	+++	10
	—	inhal.	+++	15
	—	inhal.	+++	15
Pentobarbitone	0·5	i.a.	+++	2
	0·5	i.a.	+++	15
	2·5	i.a.	+++	15
	17·0	i.a.	+++	>30
	17·0	i.a.	+++	>30
	35·0	i.v.	+++	>90
	35·0	i.v.	+++	>90
Chloralose	5	i.a.	+++	15
	10	i.a.	+++	>10
	80	i.v.	+	10
	80	i.v.	+	20
	80	i.v.	++	60
	80	i.v.	+++	>5
	80	i.v.	+++	40
	80	i.v.	+++	95
Dimecaine	1	i.a.	+++	15
	2·5	i.a.	+++	10
	2·5	i.a.	+++	25
	10	i.a.	+++	100
Procaine	1	i.a.	+++	5
	1	i.a.	+++	5
	1	i.a.	+++	15
	2·5	i.a.	+++	15
D-Tubocurarine	0·015	i.a.	++	10
	0·015	i.a.	++	10
	0·015	i.a.	+++	5
Nicotine	0·05	i.a.	+++	10
	0·10	i.a.	0	—
	0·10	i.a.	+++	25
	0·10	i.a.	+++	40
	1·0	i.a.	+++	>5
Guanethidine	0·005	i.a.	+++	>30
	0·05	i.a.	+++	>300
	5	i.a.	+++	>120
Bretylium	0·003	i.a.	0	0
	0·003	i.a.	0	0
	0·005	i.a.	+	60
	0·01	i.a.	0	0
	0·025	i.a.	+++	>60
	0·03	i.a.	0	0
	0·07	i.a.	+	10
	0·3	i.a.	+++	>30
	0·3	i.a.	+++	>90
	0·7	i.a.	+++	>60
Tyramine	0·01– 1·0	i.a.	0	0
Reserpine	0·5 –21·0	i.a.	0	0

In all of these, the size of the effect depended on the dose. The threshold was between 15 and 30 μg injected into the left auricle, and maximal effects were obtained with doses of 66 to 133 μg. This effect of the size of dose is shown in Fig. 3.

The effect of drugs upon the action of Ach

Certain drugs which block the sympathomimetic action of Ach on the isolated heart or have a sympathomimetic action themselves were tested for their effects upon the action of Ach in setting up antidromic nerve impulses. In each experiment, the dose of Ach that would reduce the control orthodromic potential by approximately 50% was first determined. Then the effect of this dose of Ach was determined before and after the injection of a second drug, intravenously or into the left auricle.

Pentobarbitone, administered in subanaesthetic doses into the left auricle or in anaesthetic doses intravenously, gave a prolonged block of the effect of Ach. Ether,

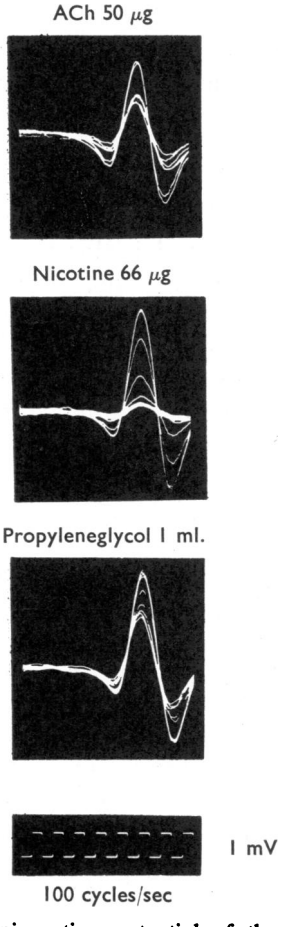

Fig. 5. Records of the postganglionic action potential of the stellate ganglion which show that nicotine and propylene glycol as well as acetyl choline set up antidromic nerve impulses in postganglionic sympathetic fibres.

administered by inhalation in oxygen for less than a min, gave a block which lasted for several min after the restoration of ventilation with pure oxygen. Chloralose in anaesthetic or subanaesthetic doses gave a shorter lasting block. Local anaesthetics, D-tubocurarine, nicotine, guanethidine and bretylium were strong blocking agents of the action of Ach. The effect of bretylium and guanethidine developed slowly over 5–30 min and the development was slower the smaller the dose used. The effect of pentobarbitone reached a peak within 5 min of injection. The effects of other drugs developed more quickly and their time courses could not be resolved. Reserpine and tyramine did not alter the effect of acetyl choline. The effect of Ach was of a typical size when reserpine (2.5 mg/kg) was administered 24 hr before the experiment. All our observations are given in Table 1, and in Fig. 4 are shown the effects of some of these drugs on the action of acetyl choline.

The effect of various drugs acting alone

Some of the drugs investigated for their antagonism to Ach have a sympathomimetic effect themselves. We therefore looked for direct effects of drugs on the control orthodromic potential. Pentobarbitone, ether, chloralose, local anaesthetics, tubocurarine, guanethidine, bretylium and tyramine had no effect. Nicotine gave a similar effect to that of Ach (Fig. 5) except that a first injection of nicotine blocked the effect of a second one for several min.

Propylene glycol (1 ml.) injected into the left auricle reduced the orthodromic potential but only for a very short time after the injection (Fig. 5) so it was difficult to pick up its effect with our method of recording. 1 ml. propylene glycol, 50% v/v in distilled water, did not reduce the control potential. To measure the effect of reserpine it was therefore necessary to compare the effect of its injection with the effect of its vehicle alone. When injected in propyleneglycol alone its effect was no greater than that of a control injection of the vehicle. When it was injected in 50% propylene glycol in water, neither the reserpine nor the control injection had any effect.

DISCUSSION

These results confirm those of Ferry (1963) on the cat spleen and those of Cabrera & Torrance (1964) on the sympathetic nerves of the skin of the same animal.

Firstly we must consider whether our results prove that acetyl choline does initiate impulses in sympathetic postganglionic fibres at a point within the heart. Crushing the nerve distal to the recording electrodes abolished the effect of acetyl choline so the effect cannot have been brought about by an action of acetyl choline itself between the stimulating and recording electrodes. The inferior cardiac nerve contains sensory fibres from the mediastinal organs and they might have been responsible for the effects of acetyl choline. But it was regularly found that the C potential we investigated was completely abolished by hexamethonium, so the effects of acetyl choline we have observed must have been upon postganglionic sympathetic fibres of cells between the stimulating and recording electrodes. The stimulus was set to be supramaximal, though not grossly so, for the C potential and it gave this by exciting preganglionic B fibres. If at the end of an experiment, after the C potential we had investigated had been completely blocked

with hexamethonium, the stimulus was increased greatly, a slight C potential might appear, presumably from sensory or postganglionic sympathetic fibres that ran without synapse between the two pairs of electrodes and, as C fibres, had a higher threshold to electrical stimulation than preganglionic sympathetic B fibres. But these fibres were not numerous and the stimuli used in the main part of the experiment did not excite them.

From observations with the collision technique, one can calculate the minimum frequency at which the fibres, activated by the orthodromic stimulus, must have discharged in an antidromic direction to produce the reduction observed in the orthodromic potential. To do this one needs to know the length of the period for which an orthodromic impulse can be extinguished by an antidromic one, so producing a reduction in the control potential. According to Douglas & Ritchie (1957), this extinction time is equal to $2t+r$ where t is the conduction time from the point of stimulation to the recording electrodes and r is the refractory period at the point of stimulation.

In our experiments, t is the conduction time from the cells of the ganglion to the recording electrodes since the antidromic impulses cannot cross the ganglionic synapse, and r is the refractory period of the ganglion after the arrival of an antidromic impulse until an orthodromic impulse can again pass the synapse.

We found in one experiment that the refractory period of the ganglion after an antidromic impulse was 13 msec, a figure which agrees reasonably well with the figure of 15 msec obtained by Brown (1934) for the superior cervical ganglion. In this same experiment the distance from the ganglion to the recording electrodes was 27 mm and the velocity of conduction of the C fibres was 0.8 msec. So the extinction time was 80 msec. Thus if all fibres were to conduct antidromic impulses every 80 msec, the orthodromic potential would be completely extinguished and if the potential was completely extinguished then all fibres must have been excited at a frequency of at least 12.5/sec. In this experiment 66 μg acetyl choline abolished the control potential completely and so gave a discharge of at least this rate.

If we suppose that we can only pick up, with confidence, a reduction of the orthodromic potential of 20%, then we can detect a frequency of antidromic impulses of 3/sec. At threshold doses of Ach, the effect lasts only for 3 sec, so the method will detect a volley of less than 10 impulses in each fibre.

The threshold dose of Ach into the left auricle in these experiments was 15–30 μg and if the coronary circulation is taken to be even as much as 10% of the heart output, then the dose administered to the heart was 1.5–3.0 μg, a dose which is only about one tenth of that needed to produce a recognizable sympathomimetic effect upon the isolated heart. This may be because the electrophysiological method will detect a total of less than 10 impulses in each fibre, a number of impulses which will have little effect upon the beating of the heart.

Our results then establish that in the heart as well as the spleen and the skin, acetyl choline sets up impulses in postganglionic sympathetic fibres and that these must at least contribute to producing the sympathomimetic effects of that substance. We do not know whether these impulses are alone responsible for the full sympathomimetic effect of Ach. Burn & Rand's (1965) theory of a cholinergic link at sympathetic nerve

endings predicts that the sympathomimetic effects of Ach can only be reproduced by an orthodromic discharge that is greater than the antidromic discharge set up by Ach.

Our experiments with drugs other than Ach will now be considered. The interpretation of those in which only one drug was used is the least complex. Nicotine, like Ach, set up antidromic impulses and these must be significant in producing its sympathomimetic effects. The failure of tyramine, guanthidine, bretylium and reserpine to do this indicates that an all or nothing depolarization of the nerve membrane is not essential for them to exert their effects on transmitter release.

The interactions of other drugs with Ach are more difficult to explain. According to Ferry (1963), Ach acts upon the membrane of the nerve fibre at or near to its termination to depolarize it in a graded fashion. If this graded depolarization reaches a threshold level, an all or nothing nerve impulse is set up and propagates both peripherally and centrally throughout the nerve fibre. As the nerve impulse invades the terminal branches of the nerve fibre it releases transmitter. This sequence of events could be affected at many stages by a second drug and some of the possibilities will now be stated.

1. The reaction of Ach with the nerve membrane is blocked. Those drugs which block the action of Ach in junctional transmission probably act on the nerve fibre in this way. Such a drug should abolish in parallel both the generation of antidromic nerve impulses by Ach and the sympathomimetic effects that they produce.

2. Ach reacts with the membrane to give a graded local depolarization, but the drug stops the process by which a local depolarization gives rise to propagated all or nothing nerve impulses. If this effect on conduction were produced uniformly along the whole length of the nerve fibre, both the antidromic and the sympathomimetic effects of Ach in a normally effective dose would be blocked, but a larger dose of Ach might produce an intense enough local depolarization of the nerve terminals to cause transmitter release without setting up all or nothing impulses. So Ach would give a sympathomimetic effect without antidromic impulses. A dose of such a drug that was not large enough to block nerve conduction would raise the threshold local depolarization needed to set up propagated nerve impulses. The dose of Ach needed to elicit any given intensity of antidromic discharge would be greater and the antidromic discharge elicited by any given dose of Ach would be less.

3. A drug acts as under (2) but it does not affect the conduction of nerve impulses uniformly along the whole length of the fibre. Suppose, for example, that it first blocked conduction at points of branching. The threshold dose of Ach for a sympathomimetic effect would not be dramatically raised because impulses would still be set up in the most peripheral parts of the nerve fibre but antidromic nerve impulses would not be conducted into the main nerve trunk. Such a drug would block the peripheral effects of centrifugal nerve impulses whilst leaving the sympathomimetic effects of Ach undisturbed, provided there were no directional element in the block of conduction at a point of branching.

4. A drug blocks conduction in nerve fibres by holding them depolarized. As such an action develops the drug itself sets up antidromic nerve impulses and so has a sympathomimetic effect. The depolarized nerve fibres cannot conduct nerve impulses and so Ach has no effect, unless the drug does not act uniformly along the nerve fibre. In this case the same sort of problem as was discussed under (3) will arise.

5. Finally, the release of transmitter by an action potential is disturbed, either by breaking the link with replete stores or else by depleting the stores. In this case Ach still elicits antidromic impulses but its sympathomimetic effects are abolished.

Now that we have enumerated five types of ideal drug which would modify a sympathomimetic effect of Ach that is mediated by antidromic impulses, we will consider whether the real drugs that we have used can be fitted neatly into these categories or whether rather the real drugs have more than a single type of action. We have found a good example of type (1) action in tubocurarine and of type (5) action in reserpine. Some of the drugs we have used certainly block nerve conduction at higher concentrations than we have used: the local anaesthetics are such drugs and they probably reduce the effect of Ach in setting up antidromic nerve impulses by raising the level of depolarization needed to set up propagated nerve impulses (type (2) or (3)). The work of Quilliam (1955) and of Thesleff (1955a and b) on muscle suggests that pentobarbitone and chloralose also act in this way. But pentobarbitone may in addition have a curariform action on striated muscle (Thesleff, 1956), as also may procaine (del Castillo & Katz, 1957). So it is possible that pentobarbitone and procaine also have a type (1) effect upon the action of Ach at the sympathetic terminal. Guanethidine and bretylium probably have a type (2) or (3) effect against the action of Ach in setting up antidromic nerve impulses. The slow development of the effects of small doses of bretylium is not surprising in view of the observation that bretylium is slowly concentrated in nerve fibres (Boura, Copp, Duncombe, Green & McCoubrey, 1960). Guanethidine and bretylium did not set up antidromic nerve impulses even when given in large doses which quickly blocked the action of Ach. This finding supports the view that they block the sympathetic nerve terminal by holding its nerve membrane polarized at, or even above, the resting level, as local anaesthetics do (Bishop, 1932; Bennett & Chinberg, 1946; Toman, Woodbury & Woodbury, 1947) rather than by depolarizing it (Brodie, Chang & Costa, 1965). Nicotine probably has first a type (4) depolarizing action but the transience of its excitatory effects and work on muscle suggest that this proceeds to a type (1) action with depolarization passing off but with the block persisting (Thesleff, 1955a).

The observation that tyramine, guanethidine and bretylium do not set up antidromic impulses indicates that they do not produce their sympathomimetic effects by setting up all or nothing nerve impulses which release transmitter. This view is consistent with the finding of Gillis & Nash (1961) that the transient initial sympathomimetic effect of guanethidine and bretylium is seen in rats in the presence of pentolinium in a dose which blocks sympathetic ganglia and so should also block a nicotinic action in setting up nerve impulses.

In general it appears to us that the use of a second drug in these experiments has served to elucidate somewhat the way in which that drug achieves its effects, rather than to test either Burn & Rand's (1965) or Ferry's (1963) theories of the mechanism of the sympathomimetic actions of Ach. That the mechanism originally proposed by Coon & Rothman (1940) and more recently re-emphasized by Ferry (1963) is important cannot be doubted, but whether it alone is involved can only be established by comparing the sympathomimetic effect of Ach with the effect of a number of efferent impulses equal to the number of antidromic impulses set up by that same dose of Ach. To do this experiment satisfactorily, one would need to work with single nerve fibres and the

effector cells they innervate. It would be impossible to interpret observations on a nerve such as the inferior cardiac nerve which innervates several organs and several different tissues within each organ, because the number of antidromic nerve impulses set up in any nerve fibre may differ greatly from the average number set up in the whole nerve, and it is only this average number that is given by the technique we have used.

SUMMARY

1. The collision technique has been used to test whether drugs set up antidromic nerve impulses in thoracic postganglionic sympathetic fibres.

2. Acetyl choline sets up antidromic impulses in postganglionic sympathetic fibres and these must at least contribute to the sympathomimetic effects of acetyl choline on the heart.

3. Nicotine and propylene glycol set up antidromic impulses but ether, pentobarbitone, chloralose, local anaesthetics, D-tubocurarine, tyramine, guanethidine and bretylium do not.

4. Ether, pentobarbitone, chloralose, local anaesthetics, D-tubocurarine, guanethidine and bretylium reduce the effects of Ach in setting up antidromic impulses. Reserpine and tyramine do not.

5. These results are discussed in relation to the sympathomimetic and/or sympathetic blocking actions of the drugs used.

This research was supported by the Faculty of Medicine of the University of Chile (Grant 60–27) and by the Rockefeller Foundation (Grant 63015) under a joint programme. R. W. T. wishes to thank the Royal Society, the Osler Memorial Fund and the Lockey Bequest for travel grants, and Professor Samuel Middleton for the hospitality of his laboratory.

REFERENCES

BENNETT, A. L. & CHINBERG, K. G. (1946). The effects of several local anaesthetics on the resting potential of isolated frog nerve. *J. Pharmac. exp. Ther.*, **88**, 72–81.

BISHOP, G. H. (1932). Action of nerve depressants on potential. *J. cell. comp. Physiol.*, **1**, 177–194.

BOURA, A. L. A., COPP, F. C., DUNCOMBE, W. G., GREEN, A. F. & MCCOUBREY, A. (1960). The selective accumulation of bretylium in sympathetic ganglia and their postganglionic nerves. *Br. J. Pharmac. Chemother.*, **15**, 265–270.

BRODIE, B. B., CHANG, C. C. & COSTA, E. (1965). On the mechanism of action of bretylium and guanethidine. *Br. J. Pharmac. Chemother.*, **25**, 171–178.

BROWN, G. L. (1934). Conduction in the cervical sympathetic. *J. Physiol. (Lond.)*, **81**, 228–242.

BROWN, G. L. & GRAY, J. A. B. (1948). Some effects of nicotine-like substances and their relation to sensory nerve endings. *J. Physiol. (Lond.)*, **107**, 306–317.

BURN, J. H. & RAND, M. J. (1965). Acetyl choline in adrenergic transmission. *A. Rev. Pharmac.*, **5**, 163–182.

CABRERA, R., COHEN, A., MIDDLETON, S., UTANO, L. & VIVEROS, H. (1966). The immediate source of noradrenaline released in the heart by acetyl choline. *Br. J. Pharmac. Chemother.* In the press.

CABRERA, R. & TORRANCE, R. W. (1964). Excitatión antidrómica de fibras postganglionares simpáticas por dosis relativamente altas de acetilcolina. *Arch. Biol. Med. Exp. Santiago*, **1**, 169.

COON, J. M. & ROTHMAN, S. (1940). The nature of the pilomotor response to acetyl choline: some observations on the pharmacodynamics of the skin. *J. Pharmac. exp. Ther.*, **68**, 301–311.

CASTILLO, J. DEL & KATZ, B. (1957). A study of curare action with an electrical micro-method. *Proc. R. Soc. B.*, **146**, 339–356.

DIAMOND, J. (1959). The effects of injecting acetyl choline into normal and regenerating nerves. *J. Physiol. (Lond.)*, **145**, 611–629.

DOUGLAS, W. W. & GRAY, J. A. B. (1953). The excitant action of acetyl choline and other substances on cutaneous sensory pathways and its prevention by hexamethonium and D-tubocurarine. *J. Physiol. (Lond.)*, **119**, 118–128.

DOUGLAS, W. W. & RITCHIE, J. M. (1957). A technique for recording functional activity in specific groups of medullated and non-medullated fibres in whole nerve trunks. *J. Physiol. (Lond.)*, **138**, 19–30.

FERRY, C. B. (1963). The sympathomimetic effect of acetyl choline on the spleen of the cat. *J. Physiol. (Lond.)*, **167**, 487-504.

GILLIS, C. N. & NASH, C. W. (1961). The initial pressor actions of bretylium tosylate and guanethidine sulphate and their relation to the release of catecholamines. *J. Pharmac. exp. Ther.*, **134**, 1–7.

QUILLIAM, J. P. (1955). The action of hypnotic drugs on frog skeletal muscle. *Br. J. Pharmac. Chemother.*, **10**, 133–140.

THESLEFF, S. (1955a). The mode of neuromuscular block caused by acetyl choline nicotine, decamethonium and succinylcholine. *Acta physiol. scand.*, **34**, 218–231.

THESLEFF, S. (1955b). The effects of acetyl choline, decamethonium and succinylcholine on neuromuscular transmission in the rat. *Acta physiol. scand.*, **34**, 386–392.

THESLEFF, S. (1956). The effect of anaesthetic agents on skeletal muscle membrane. *Acta physiol. scand.*, **37**, 335–349.

TOMAN, J. E. P., WOODBURY, J. W. & WOODBURY, L. A. (1947). Mechanism of nerve conduction block produced by anticholinesterases. *J. Neurophysiol.*, **10**, 429–441.