Brit. J. Pharmacol. (1964), **23**, 273–284.

THE SELECTIVITY OF DRUGS BLOCKING GANGLIONIC TRANSMISSION IN THE RAT

BY

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(*Received February* 21, 1964)

By comparing the effects on ganglionic transmission and on the pre- and postganglionic nerves in the isolated superior cervical ganglion preparation of the rat, the selectivity of several drugs was assessed quantitatively. Hexamethonium, tetraethylammonium, nicotine and tubocurarine blocked transmission in concentrations which did not affect nervous conduction and were considered to be highly selective in action. Atropine, amylobarbitone and paraldehyde depressed nervous conduction appreciably in ganglion-blocking doses, but not enough to account wholly for the block in transmission and they were therefore considered as being moderately selective. The ganglion blocking actions of mephenesin, procaine, methylpentynol, methylpentynol carbamate and benactyzine were nonspecific, showing general depression of neuronal activity. Ganglion block with bretylium was nonselective in its site of depression of the postganglionic neurone in concentrations which only partly depressed the preganglionic nerve.

Many drugs of varied chemical structure can block ganglionic transmission. The present investigation was carried out in an attempt to estimate quantitatively the action of some of these drugs upon the component parts of a typical peripheral ganglionic synapse by comparing their effects on ganglionic transmission and on conduction in the pre- and postganglionic nerves of the isolated superior cervical ganglion preparation of the rat.

METHODS

Wistar strain rats of either sex, weighing between 150 and 250 g, were anaesthetized with 1.5 g/kg of urethane injected intraperitoneally, which does not block ganglionic transmission in anaesthetic doses (Larrabee & Posternak, 1952). The superior cervical ganglion with its postganglionic internal carotid nerve and the preganglionic cervical sympathetic trunk was then excised, transferred to a dish of Krebs solution at room temperature and bubbled with a mixture of 95% oxygen and 5% carbon dioxide, and the connective tissue sheath around the ganglion and the postganglionic nerves was removed.

The preparation was then mounted horizontally in a bath of Krebs solution at 30° C equilibrated with 95% oxygen and 5% carbon dioxide. Stimuli, in form of rectangular pulses of 500 μsec duration and of variable voltage, could be applied through a pair of platinum stimulating electrodes (Fig. 1, S) linked by an isolating transformer to a stimulator like that described by Bell (1957). Stimuli were delivered at 6 shocks/min, a frequency at which successive stimuli showed minimal interaction. The nonpolarizable silver-silver chloride-agar saline recording electrodes (Fig. 1, R_1 to R_5) with balsa wood tips or bristle wicks were connected to a DC amplifier (Copeland, 1952), a calibrator and a cathode-ray oscilloscope. For recording, the preparation was raised above the fluid level in the bath, so providing moist

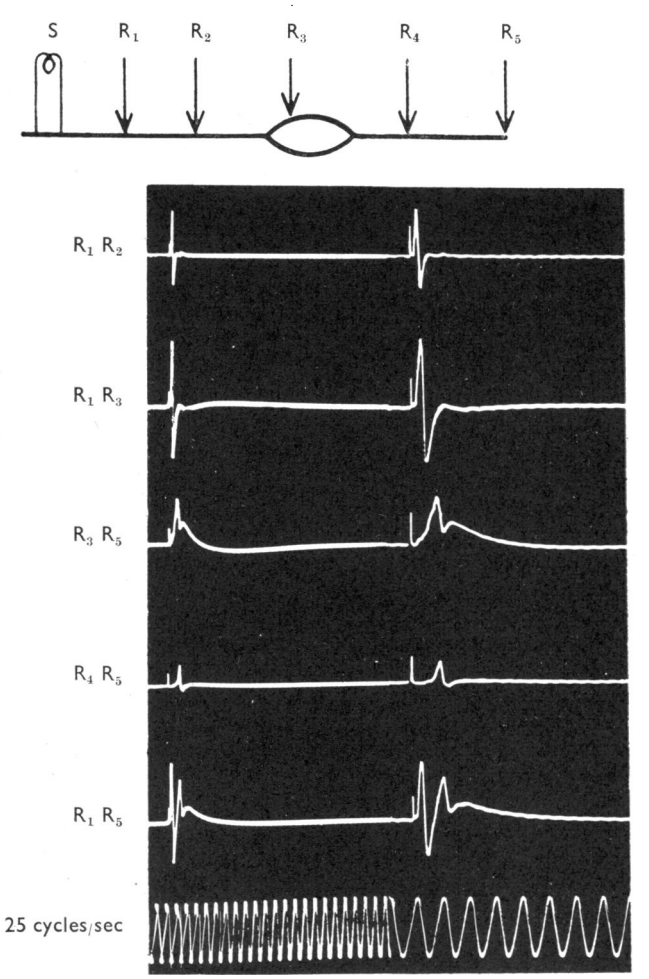

Fig. 1. Action potentials recorded from the isolated superior cervical ganglion of the rat. The positions of the recording electrodes (R) are shown together with the action potentials recorded from them following maximal preganglionic nerve stimulation through the platinum stimulating electrodes, S. Electrodes R_1 and R_2 were on the preganglionic nerve, R_3 on the ganglion, R_4 on the postganglionic nerve and R_5 on the thread tied to the cut end of the postganglionic nerve. The action potentials recorded from pairs of these electrodes are shown at two sweep speeds, the time marker at the bottom of the traces being 25 cycles/sec. The height of the time marker was adjusted at 1 mV.

R_1, R_2 : the preganglionic action potential, which was diphasic as both electrodes were on undamaged tissue.

R_1, R_3: the potential recorded between the ganglion and the preganglionic trunk was similar to that recorded from R_1, R_2, except that a small postganglionic potential could be seen following the positive phase of the preganglionic potential in the slow sweep speed recording.

R_3, R_5 : ganglionic action potential showed a spike potential followed by the N- and P-waves Both waves are visible in the slow sweep-speed recording.

R_4, R_5 : postganglionic action potential. Note that the ganglionic afterpotentials were decreased markedly with distance of these electrodes from the ganglion.

R_1, R_5 : preganglionic-postganglionic action potential complex showed easily distinguishable pre- and postganglionic components.

chamber conditions, stimulated and the action potential in response to the third stimulus photographed.

Responses to both ortho- and antidromic stimulations were recorded. The ganglionic action potential in response to stimulation of the cervical sympathetic trunk was recorded between one electrode on the ganglion and another on the thread attached to the cut end of the postganglionic nerve (Fig. 1, R_3 R_5). Electrodes on the pre- (Fig. 1, R_1 R_2) and postganglionic nerves (Fig. 1, R_4 R_5) provided facilities for recording the pre- and postganglionic action potentials. The form of these potentials recorded with a slow and a fast sweep speed are illustrated in Fig. 1. The postganglionic nerve could also be stimulated and the antidromic response of the ganglion cells recorded between the ganglion and the postganglionic nerve (Fig. 4, R_4 R_3).

RESULTS

Potency of drugs blocking ganglionic transmission

The ganglion-blocking action of the drugs used in this study was assayed relative to that of tetraethylammonium. The reduction of the orthodromic ganglionic spike potential produced by cumulative doses of the compounds was measured. Under the present experimental conditions it was found that drugs diffused rapidly to and from the preparation, giving at least 95% of their maximal effect within 5 min. Therefore, in order to facilitate a greater number of comparisons on a given preparation, cumulative dose/responses were used. Each series of doses for the test drug was alternated with a series for tetraethylammonium. Drug effects were rapidly reversed by repeated washing, usually within 15 min, and in the following experiments 30 min was allowed between successive series of cumulative doses of each drug. The potency of the test drug was calculated as the mean of the two ED50 values for tetraethylammonium divided by the ED50 of the test drug and expressed as a percentage. Molar concentrations were used throughout. Table 1 shows the potencies of the drugs examined, together with the standard errors, the numbers of determinations (n) and the ED50's in g/ml.

TABLE 1

POTENCIES OF GANGLION-BLOCKING DRUGS

Potencies have been calculated on a molar basis relative to that of tetraethylammonium. Values are means with standard errors; the number of determinations (n) are also given. The potency of tetraethylammonium, the reference drug, was fixed arbitrarily at 100. Drugs with a greater ganglion-blocking activity than tetraethylammonium had potencies in excess of 100 (such as nicotine, 8,504), while those with a lesser activity had potencies less than 100 (such as paraldehyde, 1·08)

Drug	Potency	n	ED50 (g/ml.)
Nicotine	8,504 ± 805	4	$2{\cdot}3 \times 10^{-6}$
Atropine	885 ± 107	4	$3{\cdot}5 \times 10^{-5}$
Hexamethonium	648 ± 117	4	$1{\cdot}6 \times 10^{-5}$
Benactyzine	410 ± 103	3	$3{\cdot}1 \times 10^{-5}$
Procaine	337 ± 69	3	$3{\cdot}0 \times 10^{-5}$
Tubocurarine	325 ± 41	4	$6{\cdot}5 \times 10^{-5}$
Bretylium	102 ± 4	3	$1{\cdot}4 \times 10^{-4}$
Tetraethylammonium	100		$8{\cdot}0 \times 10^{-5}$
Amylobarbitone	48 ± 7·5	3	$2{\cdot}1 \times 10^{-4}$
Mephenesin	15 ± 2·3	3	$3{\cdot}6 \times 10^{-4}$
Methylpentynol carbamate	6·1 ± 0·83	4	$9{\cdot}9 \times 10^{-4}$
Methylpentynol	1·49 ± 0·49	4	$2{\cdot}4 \times 10^{-3}$
Paraldehyde	1·08 ± 0·34	4	$5{\cdot}0 \times 10^{-3}$

Having shown that the drugs being studied blocked orthodromic transmission, it was necessary to determine whether an impairment of conduction in the pre- or postganglionic nerve fibres contributed to the ganglionic block.

Effects on the preganglionic nerve

By recording between the preganglionic nerve and the cut end of the postganglionic nerve, the compound action potential elicited by preganglionic stimulation exhibited easily distinguishable pre- and postganglionic components (Fig. 1, R_1 R_5; Fig. 2, 0). The other records in Fig. 2 show the effects of cumulative doses of

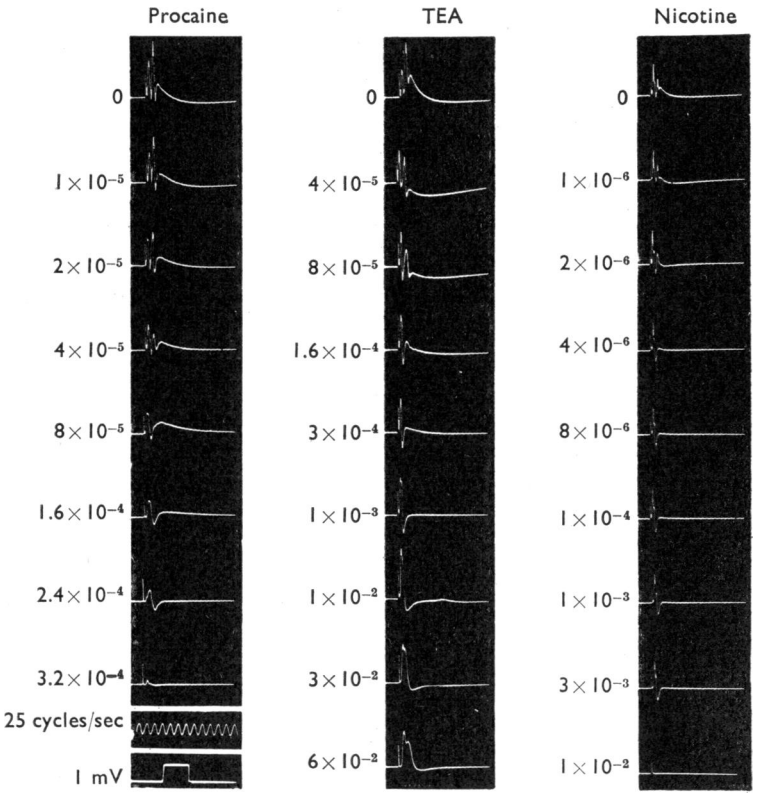

Fig. 2. Effects of procaine, tetraethylammonium (TEA) and nicotine on preganglionic and transmitted action potentials. Potentials were recorded from electrodes R_1, R_5 (Fig. 1), following maximal preganglionic nerve stimulation, and show distinct pre- and postganglionic components. Control records (0) at the top of each column are followed by the effects of increasing concentrations of procaine, tetraethylammonium and nicotine as labelled. Calibrations of 25 cycles/sec and 1 mV are given below the column labelled procaine and are the same for all the tracings. Note : in the control records following the stimulus artefact the first spike is preganglionic and the second postganglionic, being followed by N- and P-waves.

procaine, tetraethylammonium and nicotine, each dose being in contact with the preparation for 5 min. Doses were increased eventually above those necessary to block ganglionic transmission in order to block preganglionic conduction (lower records in Fig. 2) and so to obtain a quantitative measure of the selectivity of action of each drug. By plotting dose/response curves (Fig. 3) for block of preganglionic (open symbols) and transmitted postganglionic action potentials (solid symbols), it was possible to compare the effects of the drugs on conduction in the preganglionic nerve and on synaptic transmission. From such graphs, the

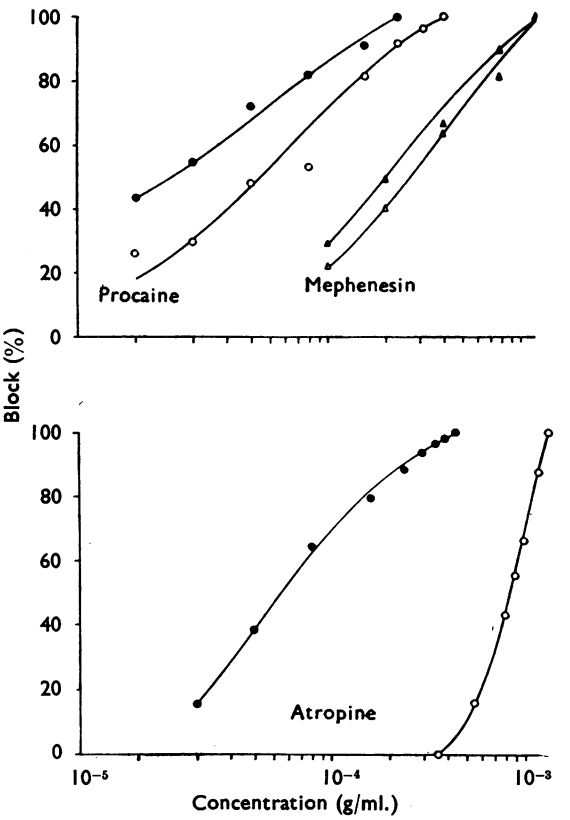

Fig. 3. Preganglionic selectivity of procaine, mephenesin and atropine. Graphs constructed from measurements of action potentials like those shown in Fig. 2. Ordinates : percentage block. Solid symbols, postganglionic block ; open symbols, preganglionic block. Abscissae : concentrations of drug in g/ml. as log scale. Upper graph : circles, procaine ; triangles, mephenesin. Lower graph : circles, atropine. Note : the preganglionic selectivity ratio is the concentration giving 50% preganglionic block divided by the concentration giving 50% postganglionic block. From these graphs, the preganglionic selectivity ratios were procaine, 2·65; mephenesin, 1·30 ; and atropine, 11·30.

preganglionic selectivity ratio, that is the ratio of the dose required to give 50% block of conduction in the preganglionic nerve and the dose giving 50% block in transmission, was calculated together with the percentage preganglionic block when transmission had been abolished. The results are summarized in Table 2.

Effects on the postganglionic nerve

The selectivity of the drugs for blocking synaptic transmission as opposed to blocking conduction in the postganglionic nerve was investigated by comparing drug effects on the orthodromically induced ganglionic action potential with those on the antidromic ganglionic action potential. The technique and the results of a typical experiment are illustrated in Fig. 4,A. As the postganglionic nerve is short in the rat (3 to 4 mm), these experiments proved technically difficult, and

TABLE 2

PREGANGLIONIC SELECTIVITY RATIOS OF THE GANGLION-BLOCKING DRUGS

The preganglionic selectivity ratios are given for each of the drugs, together with the number of determinations (n) and the height of the preganglionic action potential at full block of ganglionic transmission expressed as a percentage of the control height

Drug	Preganglionic selectivity ratio	n	Height of preganglionic action at 100% ganglionic block (%)
Highly selective drugs			
Nicotine	100	2	86
Hexamethonium	1,000	1	91
Tetraethylammonium	1,500	2	128
Tubocurarine	50	2	75
Moderately selective drugs			
Atropine	14·50	4	87·5
Amylobarbitone	33·00	3	73
Paraldehyde	4·55	4	64
Bretylium	17·40	3	50
Nonselective drugs			
Procaine	2·66	5	17
Methylpentynol	2·05	4	24·5
Methylpentynol carbamate	1·61	3	27
Benactyzine	1·34	2	10
Mephenesin	1·49	4	10

usually only one experiment was performed on each drug, except on tetraethylammonium, mephenesin, bretylium and procaine. The dose/response curves for block of antidromic conduction (Fig. 4,B, open symbols) and for ganglionic block (Fig. 4,B, solid symbols) were constructed. The antidromic selectivity ratio was derived by dividing the dose producing 50% block of the antidromic action potential by that giving 50% block of synaptic (orthodromic) transmission. These ratios, together with the percentage block of the antidromic spike at full ganglionic block, are given in Table 3.

TABLE 3

ANTIDROMIC SELECTIVITY RATIOS OF THE GANGLION-BLOCKING DRUGS

The antidromic selectivity ratios are given, together with the number of experiments (n) and the percentage height of the antidromic action potential at full ganglionic block

Drug	Antidromic selectivity ratio	n	Height of antidromic action potential at 100% ganglion block (%)
Highly selective drugs			
Nicotine	100	1	100
Hexamethonium	1,000	1	100
Tetraethylammonium	1,500	2	138
Tubocurarine	50	1	90
Moderately selective drugs			
Atropine	18·3	1	70
Amylobarbitone	16·5	1	80
Paraldehyde	3·0	1	50
Nonselective drugs			
Procaine	2·70	3	29
Methylpentynol	1·26	1	10
Methylpentynol carbamate	1·55	1	10
Benactyzine	1·45	1	11
Mephenesin	1·58	2	17
Bretylium	2·66	2	31

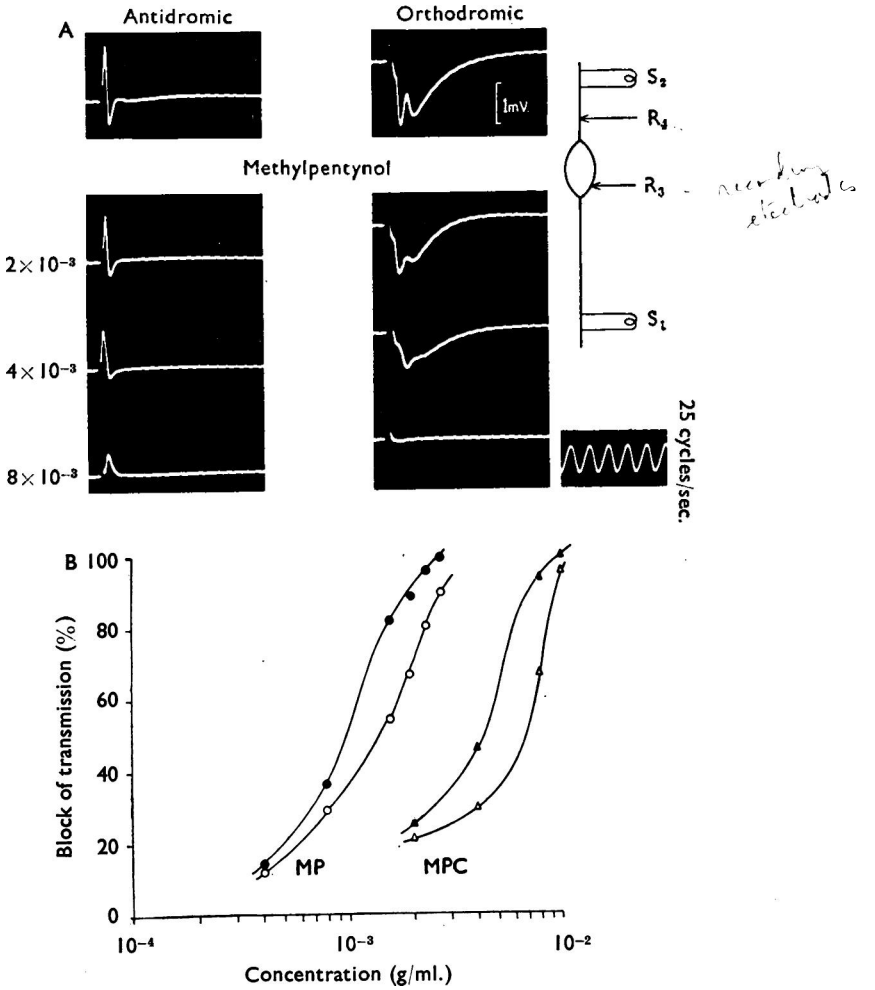

Fig. 4. Effects of methylpentynol and methylpentynol carbamate on antidromic and orthodromic postganglionic action potentials.

A. Effect of methylpentynol on antidromic and orthodromic postganglionic action potentials. In both instances, recording electrodes R_3, R_4 were used. For the antidromic action potential stimulating electrodes S_2 were employed and for the orthodromic action potential S_1. The electrical circuit was arranged so that negativity was upwards in the case of the antidromic potential. As the same recording electrodes were employed for the orthodromic potential negativity was downwards. The increasing concentrations of methylpentynol are indicated on the left. Calibrations are for 25 cycles/sec and 1 mV.

B. Graphs showing the antidromic selectivity of methylpentynol and its carbamate ester. Ordinate : percentage block. Solid symbols give block of orthodromic transmission ; open symbols, block of antidromic transmission. Abscissa : concentration of the drugs in g/ml. on a log scale. Methylpentynol (MP), circles ; methylpentynol carbamate (MPC), triangles. Note : from such graphs the antidromic selectivity ratio of the drugs was calculated as the concentration blocking antidromic action potential by 50% divided by the concentration blocking orthodromic action potential by 50%. The antidromic selectivity ratio for methylpentynol was 1·26 and for methylpentynol carbamate 1·55.

DISCUSSION

Comparison of the present results of ganglionic-blocking potency *in vitro* with those in previous studies presents difficulties. Not only has the rat ganglion itself been used only rarely for pharmacological investigation, but most other work has been performed on the superior cervical ganglion of the cat *in vivo* with various rates of stimulation of the preganglionic sympathetic nerve. The only work comparable with ours is that of Gertner (1956), who found, in the rat anaesthetized with pentobarbitone, that hexamethonium was about five-times more potent than tetraethylammonium in abolishing the contraction of the inferior eyelid of the rat following electrical stimulation of the cervical sympathetic trunk at 4 shocks/sec. This result accords with our ratio of 6.48 ± 1.17 using stimulation at 6 shocks/min. In comparisons made on the anaesthetized cat, hexamethonium is about five-times more potent than tetraethylammonium whether using a stimulus frequency of 12 shocks/sec (Wien, Mason, Edge & Langston, 1952) or 2 shocks/sec (Bainbridge & Brown, 1960). It appears, however, that the rat is less sensitive to both these compounds than the cat, for Gertner (1956) found that 5 mg/kg of hexamethonium was necessary to cause full ganglionic block in the rat, while Paton & Zaimis (1951) and Bainbridge & Brown (1960) needed only about 1 mg/kg for full ganglion block in the anaesthetized cat using stimulus frequencies of 10 and 2 shocks/sec respectively. If it is assumed that both species are equally sensitive to non-quaternary compounds, then the lower sensitivity of the rat to hexamethonium could explain why we observed that atropine and hexamethonium were nearly equipotent in the rat (Table 1) whereas Bainbridge & Brown (1960) reported that, in the cat, atropine was about five-times less active than hexamethonium.

Except with bretylium, the antidromic selectivity for each drug (Table 3) fell in the same range as the preganglionic selectivity ratios reported in Table 2. Thus it appears that these drugs affect conduction in both the pre- and postganglionic nerves quantitatively in the same manner. This finding is hardly surprising, for the fibres in both nerves of the rat are nonmyelinated (Foley & DuBois, 1940). The close agreement between the preganglionic and the antidromic selectivity ratios permits their discussion together and, on the basis of these values, the drugs were arbitrarily divided into highly selective, moderately selective and nonselective ganglion blocking agents. Bretylium has been placed in the nonselective group because it is nonselective regarding the site of its effects on the postganglionic neurone, although it showed some selectivity as regards its effects on the preganglionic nerve.

Highly selective blocking drugs. These had selectivity ratios of 50 or more and, in doses abolishing ganglionic transmission, caused a slight reduction in the preganglionic action potential (usually no more than 10 to 15% but at the most 25%) which remained constant until very large doses were added to the bath fluid. Reduction of amplitude of the action potential with doses just blocking ganglionic transmission was seen in the preganglionic nerve and not the postganglionic nerve except with tubocurarine, which caused a 10% reduction (Table 3), and it is interesting to speculate whether this could be accounted for in terms of the drugs blocking accessory ganglia in the cervical sympathetic trunk between

the point of application of the stimulus and the site of the preganglionic recording electrode. Such accessory ganglia were often observed as macroscopic swellings on the preganglionic trunk which became depolarized by acetylcholine, an effect demonstrable with the moving fluid electrode technique of Pascoe (1956). As the preganglionic fibres in the rat are nonmyelinated, conduction velocities are slower and occupy a wider spectrum than in species with myelinated preganglionic nerves, and it is difficult to be certain what proportion of the preganglionic action potential was due to activation of postganglionic fibres from accessory ganglia. The presence of such accessory ganglia would only have been detected in the present experiments when the potentials of synaptically activated nerve fibres in the preganglionic trunk had been abolished by a highly selective ganglion-blocking drug. This supposition is supported by the findings that the drugs did not affect the postganglionic nerve action potentials in the postganglionic trunk which is also nonmyelinated, and that such reduction of the preganglionic action potential as did occur remained constant until the drug concentration was raised considerably above that blocking transmission through the ganglion. It is also possible that some cells in the superior cervical ganglion in the rat send their axons caudally down the preganglionic trunk, as has been demonstrated in the rabbit by Douglas, Lywood & Straub (1960). These fibres could only be selectively affected by ganglion-blocking drugs if they chanced to pass back to the preganglionic recording electrode and then passed out of the sympathetic trunk before the point of contact with the stimulating electrode. Such a possibility seems remote.

It can be seen from Tables 2 and 3 that tetraethylammonium is unique for, in ganglion-blocking doses, the preganglionic and antidromic nerve action potentials were potentiated. This finding is of considerable interest in connection with the observation of Matthews & Quilliam (1964) that tetraethylammonium can augment acetylcholine output initially from the perfused superior cervical ganglion preparation of the cat stimulated repetitively via the preganglionic nerve. Collier & Exley (1963) have reported that tetraethylammonium can cause increased release of acetylcholine from the rat isolated diaphragm repetitively stimulated via the phrenic nerve.

Nicotine, tetraethylammonium, hexamethonium and tubocurarine block ganglionic transmission in the rat by a specific effect on the transmission process and do not materially affect nervous conduction except in concentrations greatly in excess of those blocking transmission.

Moderately selective blocking drugs. Atropine, amylobarbitone and paraldehyde had selectivity ratios intermediate between those of the highly selective and the nonselective groups, ranging from 3.0 to 33.0. They were included in a separate group because they caused an appreciable block of nervous conduction in concentrations abolishing transmission. This block of conduction amounted to 12 to 50% but could account only in part for the block of transmission which must be attributed to a more specific effect. The preganglionic blocking activity of amylobarbitone accords with the findings of Exley (1954) and those of Matthews & Quilliam (1964) that this drug can cause some decreased output of acetylcholine from the cat perfused ganglion. The latter authors also found by direct measure-

ment that paraldehyde could reduce acetylcholine output, an action which had been postulated for the drug by Nicholls & Quilliam (1956) at the skeletal neuromuscular junction and by Quilliam (1959) at the ganglionic synapse.

Nonselective ganglion blocking drugs. Procaine, mephenesin, benactyzine, methylpentynol and methylpentynol carbamate had low selectivity ratios of between 1.26 and 2.70. The ratio of 2.70 for procaine accords with the finding of Harvey (1939) that this drug abolished the effects of acetylcholine (injected intra-arterially to the ganglion) a little more readily than those of potassium by the same route. As the transmission in the preganglionic nerve was 83% blocked with doses of procaine completely blocking ganglionic transmission and, as the other drugs in this group caused between 75 and 90% block of nervous conduction in full ganglionic blocking concentrations, the main effect of these agents cannot be attributed to selective synaptic block, but to a general depression of neuronal activity.

Marley & Paton (1959) showed that block of transmission through the perfused superior cervical ganglion of the cat by methylpentynol and its carbamate was associated with a marked reduction of acetylcholine release by repetitive preganglionic nerve stimulation, a finding confirmed by Matthews & Quilliam (1964), who also found that procaine caused a profound fall in acetylcholine output from the perfused superior cervical ganglion of the cat. Brown & Quilliam (unpublished observations) have observed that benactyzine, methylpentynol, procainamide and mephenesin abolished the stimulant action of both potassium and acetylcholine (intra-arterially) on the superior cervical ganglion-nictitating membrane preparation of the cat. Such effects appear due to the general neuronal blocking activity demonstrated above.

All the nonselective drugs had selectivity ratios greater than unity, and so it was thought necessary to compare the effects of such a block with the effects of varying the stimulus strength. The relationship between the preganglionic and transmitted postganglionic action potentials produced by varying the stimulus voltage and by the action of two nonspecific blocking drugs is shown in Fig. 5. It can be seen that, for a given reduction in the preganglionic action potential, the drugs caused a greater reduction of the transmitted action potential (open symbols) than did a reduction of the stimulating voltage (solid symbols). This disparity can be attributed to an added depressant action of the drugs on the postganglionic cells, which does not arise when reducing the stimulus strength to the preganglionic nerve. Thus for the same reduction in preganglionic action potential produced by a nonspecific drug and a reduced stimulus, the reduced amount of transmitter will have a smaller effect on ganglion cells depressed by the drug. It is concluded that the selectivity ratio of slightly greater than unity, rather than unity itself, is but a reflection of the anatomical discontinuity at the ganglionic synapse.

Bretylium deserves special mention, as it was the only drug which yielded divergent preganglionic and antidromic selectivity ratios, 17.40 and 2.66 respectively. The greater susceptibility of the postganglionic nerves accords with the observations of Green (1961). The antidromic selectivity of bretylium was within the range of the nonselective drugs, although at full ganglion-blocking concentra-

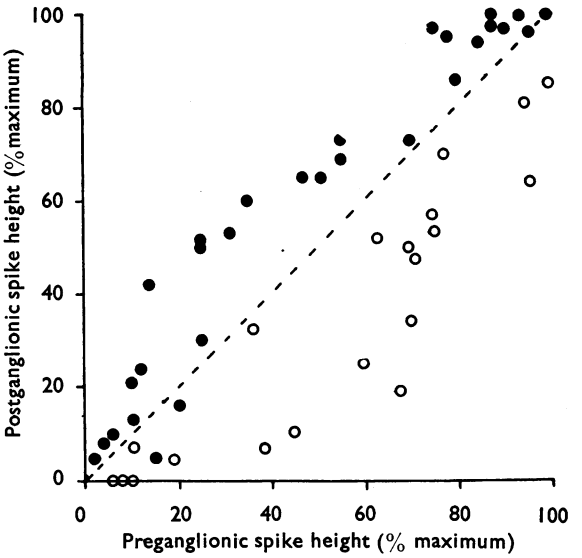

Fig. 5. Comparison of the effect of nonselective drugs and of reducing the stimulus voltage on pre- and postganglionic action potentials. Action potentials were recorded from electrodes R_1, R_5 (Fig. 1) following preganglionic nerve stimulation. Ordinate : height of postganglionic component of action potential, expressed as percentage of maximum. Abscissa : height of preganglionic component of action potential, expressed as percentage of maximum. The dotted line is drawn through the origin with a slope of 45°. Filled circles : results from variation of stimulus strength in two experiments. Empty circles : results from the effect of variation of concentrations of mephenesin or of procaine on the action potential following maximal preganglionic nerve stimulation in four experiments (two experiments with each drug). Note : all the points obtained by changing the stimulus voltage fall above the dotted line, while those for the drugs fall below the line. Therefore, for a given reduction in the preganglionic action potential, mephenesin and procaine caused a greater reduction in transmission than did reducing the stimulus strength.

tions the preganglionic nerve was still conducting. Our findings with bretylium accord with the view that the postganglionic neurone is its primary site of action and thus provide further support for its classification as an adrenergic neurone-blocking agent.

Finally, because of the good reproducibility of results and the ease with which drugs act and may be washed out, the isolated superior cervical ganglion of the rat forms a convenient preparation on which to investigate quantitatively the action of ganglion-blocking drugs.

This work was undertaken by D. G. Shand in partial fulfilment of the requirements for the degree of Ph.D. in the University of London. It is a great pleasure to acknowledge the help and advice received from Mr. P. M. G. Bell and Mr. K. A. H. Didcock. This work was supported by a grant [No. AF 61(052)-25] from the United States Air Force Office of Scientific Research (OAR).

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