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OBSERVATIONS ON THE MODE OF ACTION OF SOME CENTRAL DEPRESSANT DRUGS ON TRANSMISSION THROUGH THE CAT SUPERIOR CERVICAL GANGLION

BY

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Methylpentynol, paraldehyde, amylobarbitone and procainamide blocked transmission through the cat superior cervical ganglion, and antagonized the ganglion-stimulating actions of acetylcholine and carbachol injected intra-arterially to the ganglion. Comparison with the effects of tetraethylammonium indicated that the impaired response to acetylcholine could not wholly account for the failure of transmission, which suggested that an impaired release of transmitter substance was a contributory factor. Methylpentynol, paraldehyde and procainamide also blocked the ganglion-stimulating action of potassium chloride. In contrast, amylobarbitone and pentobarbitone did not block the stimulating action of potassium chloride, but antagonized specifically the actions of acetylcholine and carbachol. The anti-acetylcholine activities of the two barbiturate drugs at this site accord with their relative ganglion-blocking activities. It is concluded that the ganglion-blocking action of methylpentynol, paraldehyde and procainamide arises from a nonspecific depression of both presynaptic and postsynaptic elements in the ganglion, but that barbiturate compounds act more specifically on the acetylcholine receptor.

Central depressant drugs can block transmission through the cat superior cervical ganglion (Exley, 1954; Quilliam, 1957, 1959; Marley, 1959; Brown & Quilliam, 1964). Studies on the perfused preparation (Exley, 1954; Marley & Paton, 1959; Matthews & Quilliam, 1964) have shown that these drugs not only antagonized the ganglion-stimulating action of injected acetylcholine, but could also impair the liberation of acetylcholine from the preganglionic nerve fibres.

The present investigation sought to determine how far the block of transmission produced by methylpentynol, paraldehyde and amylobarbitone could be attributed to their antagonism of acetylcholine on the postsynaptic membrane, and so, by inference, to estimate the part played by any reduction of transmitter release in the ganglion-blocking action of these drugs.

Further information on the nature of the antagonism to acetylcholine has been obtained by comparing the effects of central depressant drugs on the ganglionic responses to intra-arterially injected acetylcholine and potassium chloride. The latter is a ubiquitous stimulant of nerve cells, irrespective of the presence or absence of acetylcholine receptors (Brown & MacIntosh, 1939); its action on the cat superior

cervical ganglion is unaffected by competitive antagonists to acetylcholine, such as tubocurarine (Brown & Feldberg, 1936a), tetraethylammonium (Acheson & Pereira, 1946) or hexamethonium (Trendelenburg, 1959), but is opposed by depolarizing drugs (Trendelenburg, 1957) or by local anaesthetic agents like procaine (Harvey, 1939). Thus, with the aid of potassium chloride, we attempted to ascertain whether the effect of central depressant drugs on the ganglion cells arose from a specific affinity for acetylcholine receptors or from a more general neuronal depressant action.

METHODS

Cats, anaesthetized with chloralose (60 mg/kg), were prepared as described by Brown & Quilliam (1964). Transmission through the superior cervical ganglion was studied by recording the contracture of the nictitating membrane elicited by stimulating repetitively the preganglionic cervical sympathetic nerve trunk. Trains of rectangular stimulating pulses (0.2 msec duration, 50 shocks/sec and of supramaximal strength) were delivered to the preganglionic nerve for a duration of 15 sec at 1 min intervals. Contractures of the membrane were also elicited by injecting acetylcholine, carbachol or potassium chloride intra-arterially to the superior cervical ganglion through the lingual artery, according to the method of Morrison & Paton (1953). Before each experiment, hyoscine (1 mg/kg) was injected intravenously to oppose the peripheral parasympathomimetic effects of injected acetylcholine and carbachol. Anticholinesterases were not used. The central depressant drugs were administered intra-arterially to the ganglion in a volume of 0.5 ml. Drugs were dissolved in 0.9% saline.

Doses of the following drugs are expressed as weights of salt injected in 0.5 ml. of solution: acetylcholine chloride, carbachol chloride, potassium chloride, hyoscine hydrobromide, atropine sulphate, benactyzine hydrochloride, tetraethylammonium bromide, hexamethonium chloride, procaine hydrochloride, procainamide hydrochloride, hydroxyzine hydrochloride. sodium amylobarbitone and sodium pentobarbitone. Doses of methylpentynol, paraldehyde, mephenesin and troxidone refer to the weights of the pure compound dissolved in 0.5 ml. of 0.9% saline.

RESULTS

Antagonism to the ganglion-stimulating actions of acetylcholine and carbachol

Procainamide. Concentrations of procainamide which impaired the transmission of nerve impulses through the cat superior cervical ganglion also reduced the ganglion-stimulating actions of acetylcholine and carbachol. In Fig. 1 contractures of the cat nictitating membrane of matching amplitudes were elicited by intra-arterial injections of 50 μg of carbachol to the superior cervical ganglion and by 15 sec bursts of stimuli at 50 shocks/sec applied to the preganglionic nerve each minute. The intra-arterial injection of 2.5 mg of procainamide to the ganglion impaired the effects of both forms of stimulation by approximately equal amounts (Fig. 1, *a*). Control experiments showed that procainamide did not affect the response of the nictitating membrane to postganglionic nerve stimulation, so that the effects observed in Fig. 1, *a* were due to an action at the ganglion.

The action of tetraethylammonium (Fig. 1, *b*) differed from that of procainamide. Although the intra-arterial injection of tetraethylammonium greatly reduced the response to the ganglion-stimulating action of carbachol, it impaired only slightly transmission following preganglionic nerve stimulation. Thus, relative to their

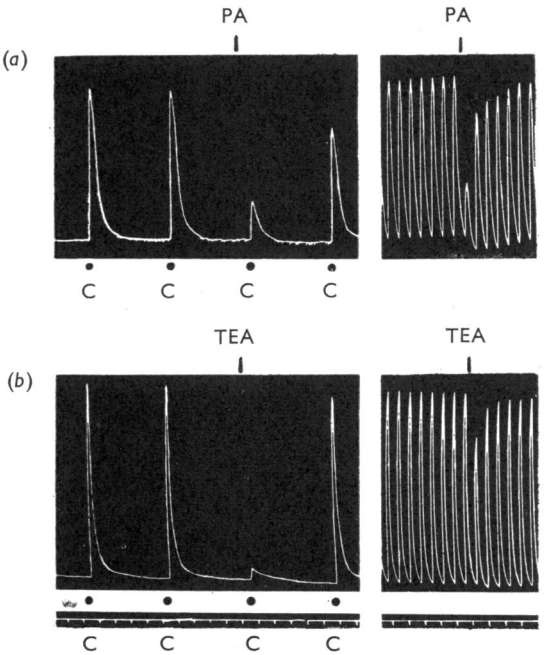

Fig. 1. The effects of (*a*) procainamide and (*b*) tetraethylammonium on the ganglion-stimulating action of carbachol (left-hand records) and on the transmission of nerve impulses (right-hand records) through the cat superior cervical ganglion preparation. Cat,3·3 kg, chloralose anaesthesia. Hyoscine (1 mg/kg) had been injected intravenously approximately 1 hr before recording. Time marks, 1 min. The contractures of the nictitating membrane were elicited either by the intra-arterial injection of 50 μg of carbachol (C) to the superior cervical ganglion (at the dots in the left-hand records) or by stimulating the preganglionic cervical sympathetic nerve trunk at 50 shocks/sec for 15 sec periods every 1 min (right-hand records). The intra-arterial injection of 2·5 mg of procainamide (at PA in *a*) reduced the responses to both forms of stimulation. Injection of 200 μg of tetraethylammonium (at TEA in record *b*) blocked the effect of carbachol more than it blocked the transmission of nerve impulses.

antagonism to carbachol, procainamide was much more active than tetraethylammonium in depressing ganglionic transmission.

To obtain a quantitative estimate of this difference between tetraethylammonium and procainamide, the percentage block of the response to each of the two forms of stimulation shown in Fig. 1 was plotted against the dose of the drug (Fig. 2). With tetraethylammonium (Fig. 2, *a*) the dose/response curve for the reduction of the response to carbachol lay to the left of the curve for the block of the responses to preganglionic nerve stimulation. The dose of tetraethylammonium which blocked nervous transmission by 50% was 295 μg while the ED50 against carbachol was 89 μg. The ratio R_{TEA} =(ED50 against nerve stimulation)/(ED50 against carbachol) was thus 3.31.

By contrast, the two curves for procainamide (Fig. 2, *b*) coincided, so that the ratio R_{PA}=(ED50 against nerve stimulation)/(ED50 against carbachol) was unity.

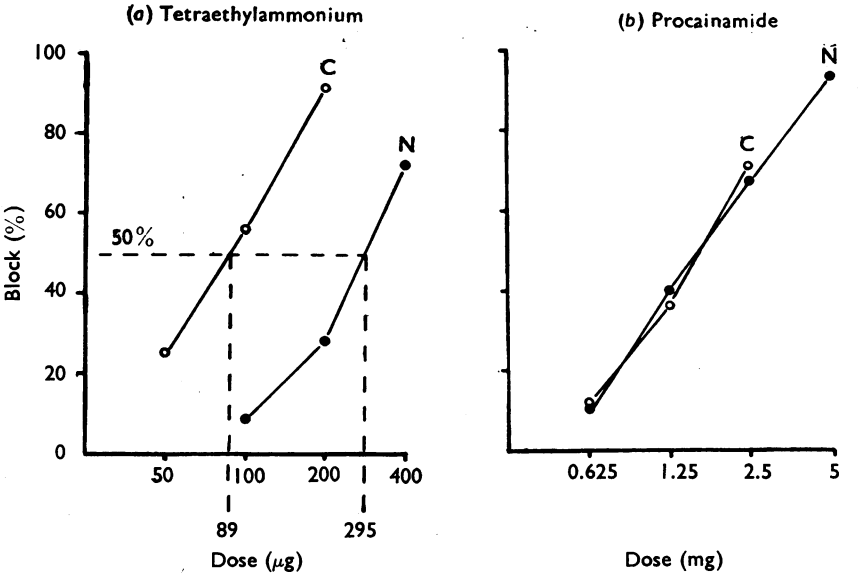

Fig. 2. The effects of a series of doses of (*a*) tetraethylammonium and (*b*) procainamide on the ganglion-stimulating action of carbachol (C, ○ —— ○) and on the transmission of nerve impulses through the superior cervical ganglion (N, ● —— ●). Points for the graphs were obtained from a single experiment, part of which is shown in Fig. 1. Ordinates : percentage block of the responses of the nictitating membrane after the intra-arterial injection of tetraethylammonium or procainamide to the superior cervical ganglion. Abscissae (log scale) : dose of tetraethylammonium (μg) or procainamide (mg) injected intra-arterially in 0·5 ml. of fluid. The dotted lines indicate how the ED50 values for the ratio $R_{TEA/PA}$ were estimated (see text).

Then, the ratio $R_{TEA/PA}$ (or R_{TEA}/R_{PA}) becomes 3.3. Thus, for equal anticarbachol activity, procainamide was 3.3 times more active than tetraethylammonium in blocking the transmission of nerve impulses through the superior cervical ganglion. Three further experiments with procainamide gave values for $R_{TEA/PA}$ of 3.6, 1.3 and 3.3, with a mean of 2.9 (standard error, ± 0.5 ; $n=4$).

When acetylcholine replaced carbachol as the stimulant drug, the value (mean and standard error) for $R_{TEA/PA}$ was 2.7 ± 0.4 ($n=3$). This accords with the view that both acetylcholine and carbachol act on the same receptors on the ganglion cells as they appear to do at the motor endplate (del Castillo & Katz, 1957 ; Jenkinson, 1960).

Central depressant drugs. Experiments analogous to those illustrated in Figs. 1 and 2 were undertaken to compare, in turn, the actions of methylpentynol, paraldehyde and amylobarbitone with those of tetraethylammonium. As the ratio $R_{TEA/PA}$ with acetylcholine fell so close to that with carbachol, carbachol was used as the stimulant drug in subsequent experiments ; it also produced more consistent contractures of the nictitating membrane and had less tendency to produce side-effects such as stimulation of the adjacent neck muscles. The results of these experiments, expressed in terms of $R_{TEA/X}$ where X refers to the drug being

tested, and TEA refers to tetraethylammonium, are given in Table 1. Six control experiments, in which the effects of tetraethylammonium were assessed twice in the same cat to give values designated $R_{TEA/TEA}$, were carried out to obtain an indication of the repeatability of the experimental technique and of experimental error.

Ideally, the control value, $R_{TEA/TEA}$, should be unity. In practice the value (mean and standard error) for $R_{TEA/TEA}$ was 0.9 ± 0.1 (n=6), which does not differ significantly from unity at the 5% probability level. The standard error and range of the control results were rather less than those of the other results in Table 1. This may mean that the different values of $R_{TEA/X}$ obtained in different experiments are not entirely reflections of experimental errors, but also of some variation of the action of the drugs tested on different cats.

TABLE 1

COMPARISON OF THE EFFECTS OF SOME DRUGS ON GANGLIONIC TRANSMISSION AFTER REPETITIVE PREGANGLIONIC NERVE STIMULATION AND ON THE GANGLION-STIMULATING ACTIONS OF ACETYLCHOLINE OR OF CARBACHOL, EXPRESSED IN TERMS OF THE EFFECT OF TETRAETHYLAMMONIUM

The ratio $R_{TEA/X}$ (individual values, means and standard errors are given) was obtained as follows :

$$R_{TEA} = \frac{\text{ED50 of tetraethylammonium (TEA) as an agent blocking action of preganglionic nerve stimulation}}{\text{ED50 of tetraethylammonium as an antagonist to carbachol or acetylcholine}}$$

R_X = corresponding ratio for the blocking agent (X)

$R_{TEA/X} = R_{TEA}/R_X$

Blocking agent (X)	Stimulant drug	$R_{TEA/X}$	
		Individual	Mean ± s.e.
Tetraethylammonium (TEA)	Carbachol	0·8 0·5 0·9 0·9 0·7 1·3	0·9 ± 0·1
Procainamide (PA)	Acetylcholine	3·3 2·9 2·0	2·7 ± 0·4
	Carbachol	3·3 3·6 1·3 3·3	2·9 ± 0·5
Amylobarbitone (AB)	Carbachol	2·0 2·6 1·7	2·1 ± 0·3
Paraldehyde (P)	Carbachol	2·0 0·7 2·2	1·6 ± 0·5
Methylpentynol (MP)	Carbachol	1·0 1·5 0·9 1·3 2·1	1·4 ± 0·2

With amylobarbitone (AB), all of the values for $R_{TEA/AB}$ exceeded the highest control value, and the mean value for $R_{TEA/AB}$ (2.1 ± 0.3, $n=3$) was significantly greater than the mean control value at the 5% probability level. Amylobarbitone was thus more than twice as active as tetraethylammonium in depressing the transmission of nerve impulses through the superior cervical ganglion when administered in doses producing equivalent reductions of the response to carbachol. In this respect, amylobarbitone showed some resemblance to procainamide.

Paraldehyde (P) and methylpentynol (MP) showed a similar tendency to depress ganglionic transmission to a greater extent than that predicted by their antagonism to carbachol. The value for $R_{TEA/P}$ clearly exceeded the control values in two out of three cats, and that of $R_{TEA/MP}$ in one out of five cats tested. However, there was considerable variation from cat to cat with these drugs, and the mean values for $R_{TEA/P}$ and $R_{TEA/MP}$ did not differ significantly ($P>0.05$) from the mean control value.

The effect of drugs on the ganglion-stimulating action of potassium chloride

To study the effect of drugs on the ganglion-stimulating action of potassium chloride, the responses of the nictitating membrane to alternate intra-arterial injections of acetylcholine, or more usually carbachol, and of potassium chloride to the superior cervical ganglion were recorded. Doses of each stimulant drug were chosen to produce approximately equal contractures of the membrane. To check that potassium chloride acted solely on the ganglion, and not directly on the nictitating membrane, the ganglion was excised at the end of each experiment and the injection of potassium chloride repeated. In no instance was a response of the nictitating membrane elicited under these conditions.

Fig. 3 illustrates the effects of atropine, procainamide and benactyzine on the ganglion-stimulating actions of carbachol and potassium chloride. The intra-arterial injection of 0.5 mg of atropine (Fig. 3, *a*) slightly reduced the response to potassium chloride, but abolished that to carbachol. Increasing the dose of atropine to 2.5 mg (not shown) did not further reduce the effect of potassium chloride.

On the other hand, doses of procainamide (Fig. 3, *b*) or benactyzine (Fig. 3, *c*) which antagonized the action of carbachol produced a more pronounced depression of the response to potassium chloride than did atropine. Slight increases of the doses of procainamide and benactyzine over those in Fig. 3 completely abolished the action of potassium chloride.

The competitive blocking agents tetraethylammonium and hexamethonium also slightly reduced the effects of potassium chloride, even when injected in quite low doses (see, for example, Fig. 4, *a*). This may have been because the abolition of the response to carbachol produced by these agents reduced the sensitivity to the succeeding dose of potassium chloride. However, since tetraethylammonium also reduced the response to potassium chloride injected when no carbachol had been given, the true explanation may reside in the observation of Brown & Feldberg (1936b) that, apart from directly stimulating the ganglion cells, potassium ions also provoke a discharge of acetylcholine from the preganglionic fibres. The acetyl-

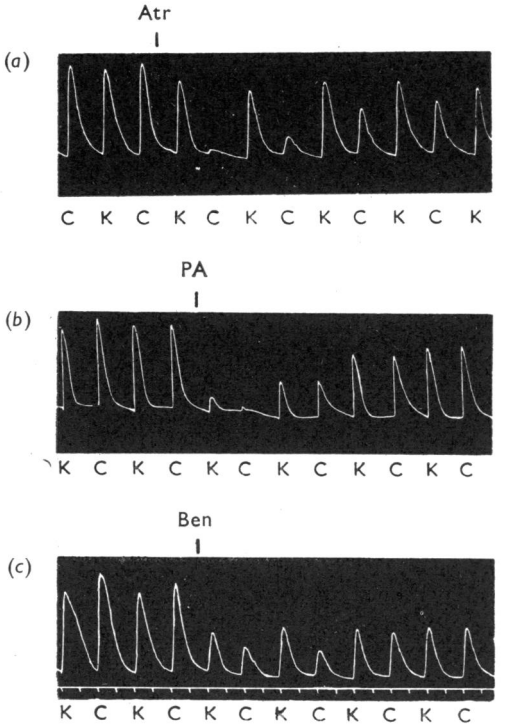

Fig. 3. The effects of (*a*) atropine, (*b*) procainamide and (*c*) benactyzine on the ganglion-stimulating actions of carbachol and potassium chloride. Cat, 2·4 kg, chloralose anaesthesia. Hyoscine (1 mg/kg, intravenously) was administered approximately 1 hr before recording. Time marks, 1 min. Records of the contractures of the cat nictitating membrane elicited by intra-arterial injection to the superior cervical ganglion of 50 μg of carbachol (C) or 5 mg of potassium chloride (K). The intra-arterial injection of 0·5 mg of atropine (at Atr in *a*) specifically antagonized the acion of carbachol. By contrast, the intra-arterial injection of 5 mg of procainamide (at PA in *b*) or of 0·5 mg of benactyzine (at Ben in *c*) decreased the responses both to carbachol and to potassium chloride.

choline so released might then stimulate the ganglion cells, but would be ineffective in the presence of drugs competing with acetylcholine. Thus, small reductions of the ganglion-stimulating action of potassium chloride, such as that produced by atropine in Fig. 3, *a*, are compatible with a competitive ganglion-blocking action, provided that the response to potassium chloride was not further blocked to any great extent by small increases in the dose of blocking drug (see Fig. 4, *a*).

Amylobarbitone and pentobarbitone resembled tetraethylammonium, hexamethonium and atropine in that a dose of drug which completely blocked the action of acetylcholine or of carbachol on the superior cervical ganglion produced only a slight reduction of the response to potassium chloride injections.

Methylpentynol, paraldehyde, troxidone, mephenesin, hydroxyzine and procaine, like procainamide and benactyzine (Fig. 3, *b* and *c*), showed no selectivity in their

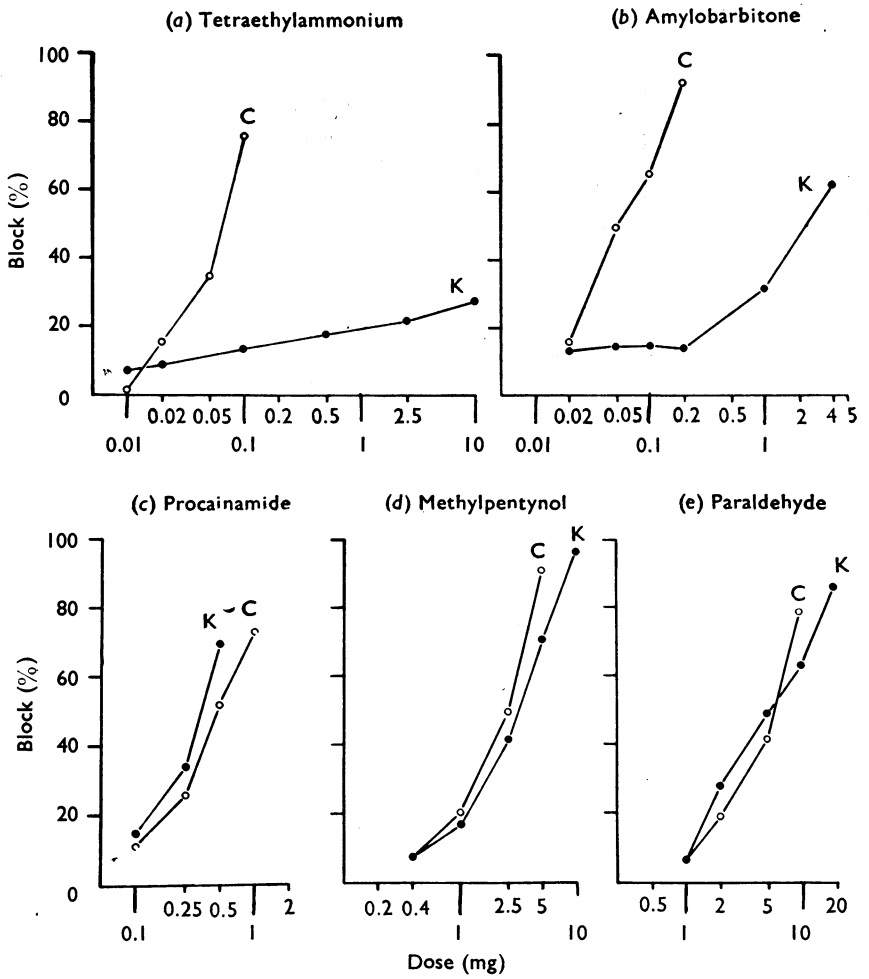

Fig. 4. The effects of (*a*) tetraethylammonium, (*b*) amylobarbitone, (*c*) procainamide, (*d*) methylpentynol and (*e*) paraldehyde on the ganglion-stimulating actions of carbachol (C, ○ —— ○) and potassium chloride (K, ● —— ●). Matched contractures of the cat nictitating membrane were elicited by the intra-arterial injection of carbachol and potassium chloride to the superior cervical ganglion. Ordinates : percentage block of the response of the nictitating membrane after the intra-arterial injection of depressant drugs to the ganglion. Abscissae (log scales) : dose of depressant drug (mg) injected in 0·5 ml. of fluid. The results in (*a*) and (*b*) were obtained from a single experiment. Graphs (*c*) to (*e*) were derived from three further separate experiments.

actions on the ganglion, blocking to the same extent the responses to acetylcholine, to carbachol and to potassium chloride.

Measurement of specificity. To obtain quantitative information regarding the specificity of the antagonism to choline esters, dose/response curves were constructed relating the dose of depressant drug injected intra-arterially to the percentage block of the ganglion-stimulating actions of carbachol and potassium chloride

TABLE 2

COMPARISON OF THE EFFECTS OF SOME DRUGS ON THE GANGLION-STIMULATING ACTIONS OF CARBACHOL, ACETYLCHOLINE AND POTASSIUM CHLORIDE

The index of specificity gives the values of the ratios (ED50 against potassium chloride)/(ED50 against carbachol) and (ED50 against potassium chloride)/(ED50 against acetylcholine). Each value was obtained from a single experiment

Drug	Index of specificity: Potassium choride / Carbachol	Index of specificity: Potassium chloride / Acetylcholine
Specific		
Hexamethonium	>100	—
Tetraethylammonium	>70	—
	>80	—
Atropine	10	—
Amylobarbitone	37	40
	5·3	45
	34	
Pentobarbitone	—	10
	—	10
Nonspecific		
Paraldehyde	3·4	—
	1·0	
Methylpentynol	2·6	0·7
	1·4	
	0·9	
Hydroxyzine	1·1	—
Procainamide	0·6	—
	1·0	

(Fig. 4). These results confirmed our previous findings that tetraethylammonium and amylobarbitone were highly specific antagonists to carbachol. Tetraethylammonium (up to 10 mg intra-arterially) only reduced the response to potassium chloride by 30% or less, whereas 100 μg sufficed to antagonize strongly the action or carbachol (Fig. 4, *a*). Amylobarbitone was more effective than tetraethylammonium in opposing the ganglion-stimulating action of potassium ions, but the dose required was also well in excess of that which antagonized carbachol (Fig. 4, *b*), or of that which blocked ganglionic transmission (see Fig. 5, *a*). On the other hand, procainamide (Fig. 4, *c*), methylpentynol (Fig. 4, *d*) or paraldehyde (Fig. 4, *e*) opposed stimulation of the superior cervical ganglion by carbachol or by potassium chloride with equal facility.

To provide an index of specificity, the ED50 as an antagonist to potassium chloride was compared with the ED50 as an antagonist to carbachol. The indices of specificity are given in Table 2. Each value was obtained from a single experiment. In most experiments, carbachol was used as the specific activator of acetylcholine receptors. In a few experiments acetylcholine was used and the results did not differ materially from those obtained using carbachol.

Drugs possessing an index of specificity of 5 or more could antagonize completely the action of carbachol without depressing the effect of potassium chloride by more than 30%, a degree of depression of the response to potassium chloride which was

the maximum ever observed after the injection of tetraethylammonium or hexamethonium. Hexamethonium, tetraethylammonium, atropine, amylobarbitone and pentobarbitone were specific antagonists to carbachol, whereas paraldehyde, methylpentynol, hydroxyzine and procainamide reduced the responses of the ganglion in a nonspecific manner.

Comparison of amylobarbitone and pentobarbitone

The ganglion-blocking activities of members of the barbiturate group of drugs are closely related neither to their general anaesthetic activities nor to their physical properties (Exley, 1954). For instance, pentobarbitone, which is an isomer of amylobarbitone, is a more potent depressant of the central nervous system than is amylobarbitone (Goldbaum, 1948), yet possesses less than half of the ganglion-blocking activity of amylobarbitone (Exley, 1954; Brown & Quilliam, 1964).

The observations described in the preceding section indicate that, unlike other central depressant drugs, the action of amylobarbitone and pentobarbitone on the ganglion cells is rather like that of competitive ganglion-blocking agents, that is they block the acetylcholine receptors of the ganglion cell. If this antiacetylcholine action of amylobarbitone were greater than that of pentobarbitone, it might account for the more powerful ganglion-blocking action of amylobarbitone. To test this hypothesis, the activities of the two barbiturates as antagonists to the stimulating

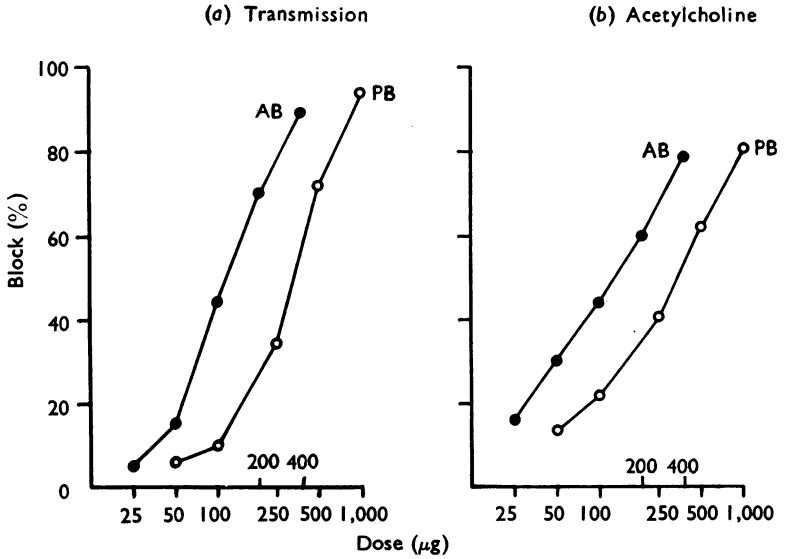

Fig. 5. The effects of amylobarbitone (● —— ● AB) and pentobarbitone (○—— ○, PB) on (*a*) ganglionic transmission and (*b*) the ganglion-stimulating action of acetylcholine. Matched contractures of the cat nictitating membrane were elicited in (*a*) by 15 sec bursts of 50 shocks/sec to the preganglionic cervical sympathetic nerve, and in (*b*) by the intra-arterial injection of 200 μg of acetylcholine to the superior cervical ganglion. Ordinates : percentage block of the response of the nictitating membrane after the intra-arterial injection of barbiturate to the ganglion. Abscissae (log scale) : dose of barbiturate (μg) injected in 0·5 ml. of fluid. These results were obtained in a single experiment.

action of injected acetylcholine on the cat superior cervical ganglion were measured and compared with their relative blocking activities on the transmission of nerve impulses through the ganglion. The results of one experiment are plotted graphically in Fig. 5. Here, pentobarbitone possessed 44% of the blocking activity of amylobarbitone on nervous transmission through the ganglion (Fig. 5, *a*), and 41%

TABLE 3

COMPARISON OF THE ACTIONS OF AMYLOBARBITONE AND PENTOBARBITONE ON THE TRANSMISSION OF PREGANGLIONIC NERVE IMPULSES THROUGH THE CAT SUPERIOR CERVICAL GANGLION AND ON THE GANGLION-STIMULATING ACTION OF ACETYLCHOLINE

The values in the second and third columns give the activity of pentobarbitone as a percentage of the activity of amylobarbitone, determined from the doses required to block by 50% the responses to preganglionic nerve stimulation or to intra-arterially injected acetylcholine. The last column gives the ratios of the values in the second to those in the third column

No. of expt.	Ganglion-block (%)	Antagonism to acetylcholine (%)	Ratio (block/antagonism)
1	47	39	1·2
2	44	41	1·1
3	68	44	1·5
Mean ± s.e.	53 ± 8	42 ± 2	1·3 ± 0·1

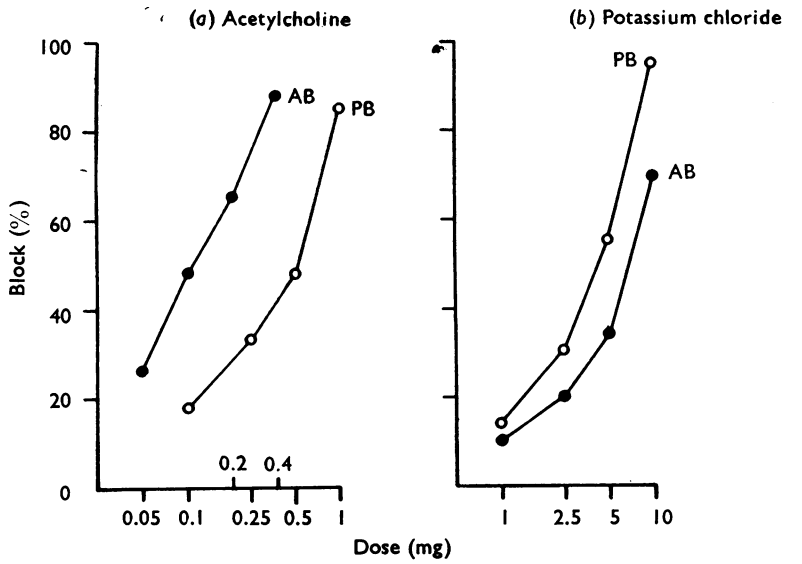

Fig. 6. The effects of amylobarbitone (●—— ●, AB) and pentobarbitone (○—— ○, PB) on the ganglion-stimulating actions of (*a*) acetylcholine and (*b*) potassium chloride. Matched contractures of the cat nictitating membrane were elicited by the intra-arterial injection of 300 μg of acetylcholine in (*a*) and of 4 mg of potassium chloride in (*b*) to the superior cervical ganglion. Ordinates : percentage block of the response of the nictitating membrane after the intra-arterial injection of barbiturate to the ganglion. Abscissae (log scale) : dose of barbiturate (mg) injected in 0·5 ml. of fluid. These results were obtained from a single experiment. Note that much larger doses of both barbiturates were necessary to antagonize the ganglion-stimulating action of potassium chloride (graph *b*) than those to block the responses to acetylcholine (graph *a*).

of the activity of amylobarbitone as an antagonist of the ganglion-stimulating action of acetylcholine (Fig. 5, *b*), as estimated from the ED50 values.

The results of this, and of two similar experiments, are given in Table 3. There was a significant positive correlation at the 5% probability level between the relative depressant activities of amylobarbitone and pentobarbitone on ganglionic transmission and their antagonism to the action of acetylcholine on the ganglion.

On the other hand, pentobarbitone was more effective than amylobarbitone in blocking responses induced by potassium chloride, by factors of 2.1 and 1.6 in two experiments. Fig. 6 illustrates the contrasting orders of activity of the two barbiturate drugs as antagonists to the ganglion-stimulating action of acetylcholine (Fig. 6, *a*) and potassium chloride (Fig. 6, *b*). The greater activity of pentobarbitone as an antagonist to potassium chloride accords with its greater central depressant activity, but not with its weaker ganglion-blocking activity when assessed by responses to stimulating the preganglionic cervical sympathetic nerve.

DISCUSSION

Comparison of the mean value of $R_{TEA/PA}$ (2.9 ± 0.5) with the mean control value $R_{TEA/TEA}$ (0.9 ± 0.1) shows that doses of procainamide which were as effective as tetraethylammonium in antagonizing acetylcholine or carbachol on the post-synaptic membrane of the ganglion cells were over three times more active than tetraethylammonium in blocking the transmission of nerve impulses through the ganglion. Paton & Thompson (1964) have recently reported a similar discrepancy between the effects of procainamide and hexamethonium. This might be explained by postulating the presence of a "diffusion barrier" around the synaptic areas and enclosing the receptors activated by "intrinsic" acetylcholine released by the preganglionic nerve fibres, so as to hinder the access of tetraethylammonium or of hexamethonium to the receptors but not that of procainamide. Tetraethylammonium has a smaller molecular size than procainamide, so it would be expected to diffuse through an aqueous medium more rapidly than procainamide. Hence, to account for the above results, the postulated diffusion barrier must discriminate against tetraethylammonium on the basis of some property other than molecular size, such as charge density or lipoid solubility. However, this type of barrier would then be incompatible with the observed differences between methylpentylnol and procainamide (see Table 1), for methylpentynol is un-ionized and highly lipoid-soluble, yet, like tetraethylammonium, is less effective than procainamide in blocking the transmission of nerve impulses through the ganglion when compared on a basis of equally active anticarbachol concentrations.

It seems more probable that the high value of $R_{TEA/PA}$ when compared with $R_{TEA/TEA}$ is a reflection of the interference by procainamide with the release of transmitter substance from the preganglionic fibres (Paton & Thompson, 1953, 1964), an action not shared by tetraethylammonium (Matthews & Quilliam, 1964). This implies that the reduced release of transmitter must make an important contribution to the block of transmission following procainamide.

If this interpretation of the ratios $R_{TEA/X}$ is accepted, then, as the mean of $R_{TEA/AB}$ (2.1 ± 0.3) also greatly exceeded the mean control value $R_{TEA/TEA}$ (0.9 ± 0.1), the ganglion-blocking action of amylobarbitone must likewise involve some presynaptic failure, though of lesser magnitude than that produced by procainamide. This may also be true for paraldehyde, because $R_{TEA/PA}$ exceeded by a factor of two the control value $R_{TEA/TEA}$ in two out of three cats. On the other hand, the value of $R_{TEA/MP}$ was clearly greater than the control value in only one out of five cats, suggesting that, in most animals, any reduction of acetylcholine release contributed only marginally to the block of ganglionic transmission produced by methylpentynol.

These inferences accord with the results obtained when acetylcholine output has been measured directly. Amylobarbitone, methylpentynol and paraldehyde have been observed to reduce the output of acetylcholine from the perfused cat superior cervical ganglion preparation (Exley, 1954; Marley & Paton, 1959; Matthews & Quilliam, 1964). The decrease in acetylcholine output produced by these agents was generally less marked than that with procainamide or procaine and was not readily correlated with the degree to which transmission through the ganglion was blocked. Methylpentynol and paraldehyde also impair the release of acetylcholine from motor nerves (Nicholls & Quilliam, 1956; Matthews & Quilliam, 1964).

To measure acetylcholine release from the superior cervical ganglion, the ganglion is perfused with Locke solution containing physostigmine. The effect of adding physostigmine and the substitution of Locke solution for blood can introduce complicating factors. For instance, methylpentynol carbamate appears to exert a stimulant action on the perfused ganglion (Marley & Paton, 1959; Matthews & Quilliam, 1964) whereas no evidence of stimulation has been detected using the blood-bathed ganglion (Marley, 1959; Brown & Quilliam, 1964). By avoiding perfusion techniques, our results form an essential complement to those derived from direct measurements of acetylcholine output, and provide some further evidence concerning the contribution made by presynaptic failure to the block of transmission. They suggest that, though amylobarbitone, methylpentynol and paraldehyde can impair the release of acetylcholine, their effect at this stage of the transmission process is somewhat less pronounced than that of procainamide, and that a decrease of postsynaptic excitability must also play a substantial role in the ganglion-block observed with these three compounds.

The postsynaptic depressant action of methylpentynol and paraldehyde affected not only the responses to acetylcholine and carbachol but also those to potassium chloride. Procainamide, mephenesin, troxidone, hydroxyzine and benactyzine likewise antagonized the ganglion-stimulating action of potassium ions. Depolarizing agents such as nicotine and tetramethylammonium oppose the action of potassium chloride on the ganglion (Trendelenburg, 1957, 1959), but the drugs used in the present study neither stimulated nor depolarized the ganglion (Quilliam, 1959; Brown & Quilliam, 1964; Quilliam & Shand, 1964). Their action resembles more closely the nonspecific effect of procaine (Harvey, 1939; Paton, 1954). The impaired response to acetylcholine after the injection of these local anaesthetic

and central depressant drugs can therefore be ascribed to a general depression of the excitability of the ganglionic neurones, rather than to a specific block of acetylcholine receptors.

In contrast, low concentrations of atropine, amylobarbitone and pentobarbitone did not oppose the ganglion-stimulating action of potassium chloride, but specifically antagonized the actions of acetylcholine or carbachol, in a manner comparable with that of competitive ganglion-blocking agents. Comparison of amylobarbitone with pentobarbitone confirmed that the block of ganglionic transmission produced by these barbiturate compounds was related to this specific antiacetylcholine action (Fig. 5), but not to nonspecific effects on neuronal excitability (Fig. 6).

Possession of a high affinity for acetylcholine receptors could account for the exceptionally strong ganglion-blocking activity of the two barbiturate compounds (Brown & Quilliam, 1964). It would also provide an explanation for the observation of Larrabee & Posternak (1952) that pentobarbitone blocked synaptic transmission through the cat stellate ganglion at one-tenth of the concentration required to impair conduction along " through-fibres," a degree of " synaptic selectivity " well in excess of that possessed by ether, chloroform, alcohols or local anaesthetic drugs. This measure of synaptic selectivity is analogous to the measurement of acetylcholine/potassium chloride specificity made in the present study (Table 2).

The specific action of amylobarbitone on postsynaptic acetylcholine receptors raises the question of whether the effect of this drug on the release of acetylcholine might be related to an equally specific action on the acetylcholine synthesis or release mechanism, rather than to a " a local anaesthetic " effect on the conduction of the preganglionic nerve impulse. In strong contrast to procaine, concentrations of amylobarbitone which block transmission through the isolated superior cervical ganglia both of rat (Quilliam & Shand, 1964) and of rabbit (Elliott & Quilliam, 1964) have no material effect on the size of the preganglionic action potential. These observations and those of Larrabee & Posternak (1952) make it unlikely that the profound depression of acetylcholine output from the cat preganglionic cervical sympathetic nerves produced by such low concentrations as 50 to 100 μg/ml. of amylobarbitone (Matthews & Quilliam, 1964) can arise from an effect on the conduction of the preganglionic nerve impulse. Barbiturate drugs do not appear to exert a direct effect on acetylcholine synthesis *in vitro* (Elliott, Page & Quastel, 1955), but the partial antagonism by choline on the effect of amylobarbitone on acetylcholine release (Matthews & Quilliam, 1964) might be held to imply some restriction of acetylcholine synthesis *in vivo*.

Barbiturate drugs also appear to exhibit a high affinity for acetylcholine receptors at other cholinergic junctions. Pentobarbitone, in concentrations as low as 50 μg/ml., depresses strongly the response of frog skeletal muscle to acetylcholine without reducing the electrical excitability of the muscle membrane (Quilliam, 1955; Thesleff, 1956). Krnjevic & Phillis (1963), testing the response of cholinoceptive neurones in the cat cerebral cortex to acetylcholine and to the nonspecific stimulating agent, glutamic acid, found that pentobarbitone and allobarbitone depressed selectively the response to acetylcholine, whereas procaine depressed equally the responses both to acetylcholine and to glutamic acid.

On the other hand, pentobarbitone appears to block transmission at spinal motor neurones, which are insensitive to acetylcholine, not by antagonizing the depolarizing action of the transmitter substance, but by raising the threshold level of depolarization required to initiate action potentials (Eccles, 1946). This action might be considered equivalent to the nonspecific depressant action of high doses of this drug on the excitation of sympathetic ganglion cells by potassium ions. This observation of Eccles (1946) also implies that pentobarbitone did not reduce the amount of transmitter released by afferent impulses, which accords with our view that the action of barbiturate drugs on transmitter output from cholinergic nerves is linked closely to the nature of the transmitter substance and is not due to an effect on nerve conduction *per se*.

The present observations, together with the findings of others, lead to the general conclusion that local anaesthetic drugs such as procaine and procainamide, and the central depressant drugs methylpentynol and paraldehyde, produce a nonspecific depression of junctional transmission, affecting to variable extents both the release of and the response to the transmitter substance, and that their action probably does not depend critically on the chemical nature of the transmitter. Barbiturate drugs appear to produce a similar nonspecific depression of transmission at non-cholinergic synapses in the spinal cord, but there is substantial evidence that their effect on cholinergic junctional transmission is dominated by a specific block of acetylcholine receptors.

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