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SOME ACTIONS OF CENTRALLY ACTIVE AND OTHER DRUGS ON THE TRANSMISSION OF SINGLE NERVE IMPULSES THROUGH THE ISOLATED SUPERIOR CERVICAL GANGLION PREPARATION OF THE RABBIT

BY

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The effect of some centrally-active and other drugs on the transmission of single nerve impulses through the isolated superior cervical ganglion preparation of the rabbit has been studied by recording both preganglionic and postganglionic action potentials. Block of conduction in the axon could be distinguished from block of the synaptic mechanism. The drugs did not appear to exert any one characteristic form of blocking action. A continuous spectrum of drug action linked an agent such as meprobamate which acted predominantly on the synapse to benactyzine which acted mainly by blocking axonal conduction. The drugs have been divided into three groups. Group I: hexamethonium, meprobamate, paraldehyde, amylobarbitone, methylpentynol and azacyclonal; these acted relatively selectively at the ganglion. Group II: N714C (the *cis*-isomer of chlorprothixene), prochlorperazine, methylpentynol carbamate, pipradrol, promethazine, perphenazine and procaine; the action of these drugs on the ganglion could be accounted for entirely in terms of their axonal depressant action. Group III: chlorprothixene, promazine, N720 (dihydrochlorprothixene), chlorpromazine, hydroxyzine and benactyzine; these drugs also blocked axonal conduction but in addition they appeared to exert a "facilitating" action at the ganglionic synapse. The actions of adrenaline, adrenochrome, iproniazid, ergotoxine, mescaline and lysergic acid diethylamide on transmission were also studied. The implications of the modifications of ganglionic transmission produced by these drugs is discussed.

The lack of anatomical complexity and the physical accessibility of the mammalian superior cervical ganglion and its contained synapses has led our colleagues (Quilliam, 1959; Brown & Quilliam, 1964; Matthews & Quilliam, 1964; Quilliam & Shand, 1964) and ourselves to use it as a model upon which to study the modes of action of centrally-active drugs. We were particularly interested to assess how far information about drug action at a peripheral synapse might be helpful in the understanding of the mechanisms by which centrally-active drugs exert their effects at synapses in the brain. Pre- and postganglionic action potentials were recorded from the rabbit superior cervical ganglion *in vitro* and the blocking action of the drugs on transmission across the synapse was studied. Dose/response

curves were plotted and the ED50 for ganglion block for each drug was calculated. We also sought to elucidate the mechanism by which the drugs modified ganglionic transmission.

METHODS

Lop-eared rabbits (about 2 kg) were killed rapidly by air embolism and the superior cervical ganglion was quickly excised (Elliott, 1963). The desheathed ganglion preparation was transferred to eight palladium-silver alloy electrodes (60% palladium and 40% silver) being secured under slight stretch to the end electrodes. The first two electrodes on the preganglionic trunk were stimulating electrodes, the next served as the earth electrode, and the remaining electrodes were used to record evoked potentials from various points on the preparation. The electrode system was incorporated in the lid of a moist chamber assembly (Eccles, 1952). The chamber (Fig. 1) was mounted in a thermostatically controlled water bath so adjusted that the temperature was usually 32° C. A stream of warm moist gas mixture of 95% oxygen and 5% carbon dioxide flowed continuously through the chamber. The preparation was always immersed in

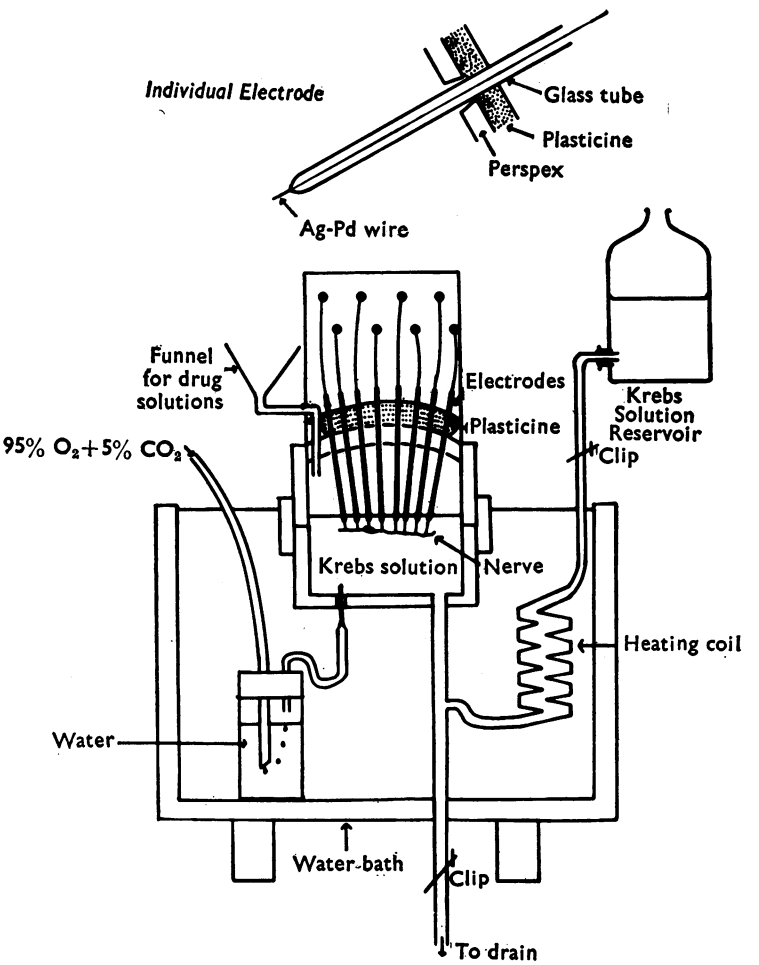

Fig. 1. Diagram of the apparatus used to record potentials from the rabbit isolated superior cervical ganglion preparation.

Krebs solution except for recording purposes. An electronic stimulator similar to that described by Bell (1957) provided single supramaximal rectangular pulses of 50 μsec duration, which were applied to the stimulating electrodes through an isolating transformer. Action potentials were recorded using a Cossor oscilloscope (Type 1049 Mark III) after amplification by a condenser-coupled preamplifier. The time constant of the overall amplification system for half decay was more than 1 sec.

The preparation was allowed to equilibrate for about 1 hr in the bath fluid. For the purposes of recording, the chamber was drained so that the preparation was suspended in the warm moist atmosphere for not more than 2 min, after which time the preparation was reimmersed in the bath fluid.

The experimental procedures have been described in greater detail by Elliott (1963). First a control value for the ganglionic potential was obtained and then the ganglion was exposed to successively increasing concentrations of the drug, for 10 min at each concentration, so permitting the construction of a cumulative dose/response curve and the estimation of the 50% blocking dose (ED50). There was little difference between the ratio of the ED50 for tetraethylammonium to that for hexamethonium by this cumulative method and the ratio obtained by the conventional but tedious technique involving 10 min exposure to the drug concentration followed by prolonged washing. It proved impossible to wash out some drugs sufficiently well to regain the original value of the control potential. If after washing for 2 hr a constant value was obtained, this was taken as the new control for succeeding drug exposures.

The drugs used were: adrenaline acid tartrate (Samoore); adrenochrome (Light & Co.); sodium amylobarbitone (Amytal, Samoore); azacyclonal hydrochloride (Frenquel, Merrell); benactyzine hydrochloride (Cevanol, I.C.I.); chlorpromazine hydrochloride (Largactil, May & Baker); chlorprothixene (N714T, Truxal, Lundbeck, Copenhagen); ergotoxine ethanesulphonate (Burroughs Wellcome); hexamethonium chloride (Geigy); hydroxyzine hydrochloride (Atarax, Pfizer); iproniazid (Marsilid, Roche); meprobamate (Miltown, Wallis); mescaline sulphate (Light & Co.); methylpentynol (Oblivon, British Schering); methylpentynol carbamate (Oblivon C, British Schering); (−)-noradrenaline (Carnegie); dihydrochlorprothixene hydrobromide (N720, Lundbeck, Copenhagen); N714C (the *cis*-isomer of chlorprothixene) hydrochloride (Lundbeck, Copenhagen); paraldehyde (B.D.H.); perphenazine (Fentazin, British Schering); pipradrol hydrochloride (Meratran, British Schering); procaine hydrochloride (B.D.H.); prochlorperazine methanesulphonate (Stemetil, May & Baker); promazine hydrochloride (Wyeth & Co.); promethazine hydrochloride (Phenergan, May & Baker); and tetraethylammonium bromide (B.D.H.). All these drugs were made up in Krebs solution at the concentration required in the bath, except adrenaline, noradrenaline, mescaline and amylobarbitone, which were made up as concentrated solutions in distilled water on the day of the experiment. The concentrated stock solutions of the sympathomimetic amines were kept at 4° C in the dark, and a suitable small volume of the stock concentrated drug solution was added to 50 ml. of warm Krebs solution to provide the required concentration immediately before use. The drug solution after warming to chamber temperature was run into the chamber through a funnel in the lid.

The composition of the Krebs solution was (in g/100 ml.): NaCl 0.69, KCl 0.035, $CaCl_2$ 0.028, $NaHCO_3$ 0.21, KH_2PO_4 0.016, $MgSO_4$ $7H_2O$ 0.029 and glucose 0.2.

RESULTS

Transmission across the synapse was studied by stimulating the preganglionic nerve trunk supramaximally and comparing the records of the evoked preganglionic and ganglionic potentials. The ganglionic potential was recorded with one electrode on the ganglion and the other on the postganglionic trunk and was taken as the postsynaptic potential. By recording the preganglionic as well as the ganglionic

potential, it was possible to differentiate between a drug which affected mainly axonal conduction and one which acted predominantly at the ganglionic synapse.

Since many of the drugs reduced the potential from the preganglionic trunk, it was important to establish that there was no substantial number of postganglionic fibres in the preganglionic trunk. These might arise either from small accessory ganglia in the preganglionic trunk itself or they might be recurrent from the superior cervical ganglion. The latter possibility has been excluded by Elliott (1963) who

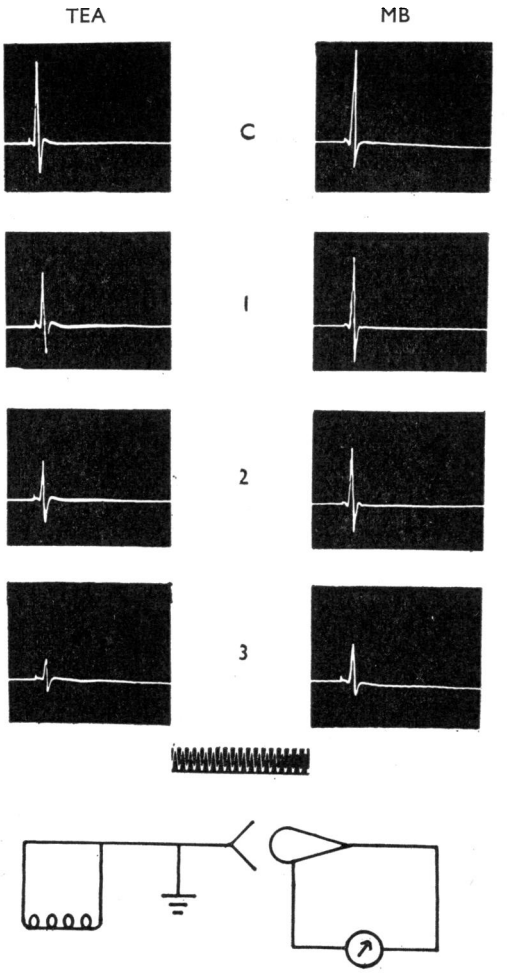

Fig. 2. Comparison of the ganglion-blocking actions of tetraethylammonium and meprobamate. Records of the ganglionic potentials ; the position of the stimulating and recording electrodes is shown on the diagram. Time mark, 50 cycles/sec. C, controls. The numerals 1, 2 and 3 denote increasing concentrations of drugs, in which the ganglion was immersed for 10 min periods, for tetraethylammonium (TEA) 100, 150 and 200 μg/ml. and for meprobamate (MB) 400, 800 and 1,600 μg/ml. were used respectively. The dose/response curves derived from these records are shown in Fig. 3.

showed that section of the preganglionic trunk near the ganglion did not alter the preganglionic potential.

When two pairs of electrodes were placed on the preganglionic trunk, one pair distant and one pair close to the ganglion, and were used alternately for stimulating and for recording, there was no substantial difference in the B fibre component of the preganglionic action potential recorded in the two positions. This finding indicated the absence of a synapse between the two pairs of electrodes on the preganglionic trunk. The accessory ganglion described by Douglas, Lywood & Straub (1960) therefore probably lay caudal to the point at which the preganglionic trunk was severed in our experiments when removing the ganglion from the rabbit.

Fig. 2 shows the ganglionic potentials obtained with tetraethylammonium and meprobamate and Fig. 3 shows the dose/response curves obtained. To obtain a comparison between different drugs on different ganglia, each drug was compared on each ganglion with a standard ganglion-blocking agent, tetraethylammonium. The ratio of the ED50 percentage block with the drug to the ED50 percentage block with tetraethylammonium was expressed as a percentage, values for this ratio above 100% thus indicating a potency less than that of tetraethylammonium (see legend to Fig. 3).

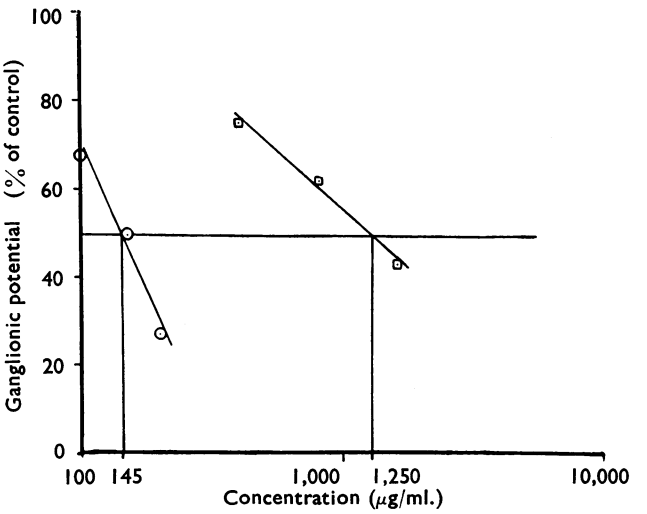

Fig. 3. Dose/response curves for tetraethylammonium (circles) and for meprobamate (squares) derived from Fig. 2. The ED50 value for tetraethylammonium was 145 μg/ml., and for meprobamate was 1,250 μg/ml., yielding a ratio R_1 (Table 1) of 860% and indicating that meprobamate was 8·6-times less potent as a blocking agent than the standard, tetraethylammonium.

Central depressant drugs

Table 1 shows the values of the ED50 percentage block for the drugs examined and their potency relative to that of tetraethylammonium. The ratios are expressed both gravimetrically (R_1) and on a molar basis (R_2). Another series of experiments was performed with phenothiazine compounds in which a direct comparison of

TABLE 1

MEAN 50% BLOCKING DOSE OF CENTRAL DEPRESSANT DRUGS AND OTHER COMPOUNDS, TOGETHER WITH THE RATIOS OF THE ED50 VALUES OF THE TEST DRUGS TO THOSE WITH TETRAETHYLAMMONIUM IN THE SAME EXPERIMENT ON THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE RABBIT

The ED50 values are expressed in gravimetric and in molar concentrations. Ratio R_1 is gravimetric [$R_1 = 100 \times$ (ED50 % drug)/(ED50 % tetraethylammonium)] and R_2 has the same derivation but uses molar concentrations

Compound	No. of experiments	ED50 % block: Gravimetric (μg/ml.)	ED50 % block: Molar (mM)	Ratio R_1 (gravimetric)	Ratio R_2 (molar)
Test compounds					
Paraldehyde	6	2,720·0	20·6	5,480·0	8,720·0
Methylpentynol	6	2,270·0	23·2	2,820·0	6,040·0
Methylpentynol carbamate	5	580·0	4·1	1,060·0	1,578·0
Meprobamate	5	1,046·0	4·8	1,054·0	1,015·0
Azacyclonal	6	202·0	$6·6 \times 10^{-1}$	136·5	94·4
Amylobarbitone	6	71·0	$3·1 \times 10^{-1}$	78·0	72·5
Hexamethonium	5	28·6	$1·1 \times 10^{-1}$	49·6	38·3
Procaine	4	50·0	$1·8 \times 10^{-1}$	33·0	25·4
Benactyzine	6	16·5	$4·5 \times 10^{-2}$	19·0	11·0
Hydroxyzine	8	19·1	$4·3 \times 10^{-2}$	21·0	9·8
Promethazine	5	6·3	$2·0 \times 10^{-2}$	11·4	7·5
Chlorpromazine	5	6·2	$1·8 \times 10^{-2}$	10·0	5·9
Prochlorperazine	5	12·5	$2·2 \times 10^{-2}$	13·1	4·9
Perphenazine	8	8·1	$2·0 \times 10^{-2}$	7·6	4·0
Promazine	10	2·8	$8·6 \times 10^{-3}$	3·6	2·4
Control compound					
Tetraethylammonium	90	96·4	$4·6 \times 10^{-1}$	100·0	100·0

TABLE 2

AN INTERCOMPARISON OF THE MEAN 50% BLOCKING DOSES OF FIVE PHENOTHIAZINE COMPOUNDS

On each of six ganglia, chlorpromazine was compared with two or more of the other phenothiazine drugs. Results are expressed as in Table 1, but relate to chlorpromazine rather than to tetraethylammonium. The ratio is [100 × (ED50 molar drug)/(ED50 molar chlorpromazine)], and the tabulated values are the means of the ratios and not the ratios of the means. Pooled phenothiazine ED50 values, excluding those with promazine, are (mean and standard error) $1·53 \times 10^{-2} \pm 0·076 \times 10^{-2}$ mM. The mean of promazine ED50 values is $0·96 \times 10^{-2} \pm 0·044 \times 10^{-2}$ mM. There was a significant difference between the ED50 values for promazine and those of the pooled phenothiazines ($P \geqslant 0·01$)

Compound	No. of experiments	ED50 % block: Gravimetric (μg/ml.)	ED50 % block: Molar (mM)	Ratio (molar × 100)
Chlorpromazine	6	5·61	$1·58 \times 10^{-2}$	100
Promethazine	3	5·50	$1·71 \times 10^{-2}$	99·5
Perphenazine	4	5·53	$1·37 \times 10^{-2}$	89·6
Prochlorperazine	3	8·13	$1·44 \times 10^{-2}$	83·4
Promazine	5	3·08	$9·6 \times 10^{-3}$	60·8

the drugs was made on the same ganglion. The results are shown in Table 2. Table 3 gives results of a direct comparison between azacyclonal and its isomer, pipradrol.

Many of the drugs exerted a marked depressant action on conduction in the preganglionic trunk. In order to allocate a numerical value for this depressant action the percentage depression of the preganglionic potential when the ganglionic potential was reduced to 50% of its control value by the drug was measured.

TABLE 3

DIRECT COMPARISON OF THE 50% BLOCKING DOSES OF AZACYCLONAL AND PIPRADROL IN FIVE GANGLIA

The last row gives means and standard errors. There was a significant difference between the mean ED50 values for azacyclonal and pipradol ($P > 0.01$)

ED50 % block		
Azacyclonal (μg/ml.)	Pipradrol (μg/ml.)	Ratio (azacyclonal/pipradrol)
72	30	2·4
72	21	3·4
200	19	10·5
115	23	5·0
160	14·5	11·0
Mean 123·8 ± 25·1	21·5 ± 2·6	6·46 ± 1·81

The results are shown in Table 4 in which the drugs have been divided into three arbitary groups according to their preganglionic depressant action:

(I) those having little or no preganglionic depressant action;
(II) those having a moderate preganglionic depressant action;
(III) those drugs exerting a strong preganglionic depressant action.

Next, the antidromically evoked ganglionic potential was examined in order to assess the contribution of a postganglionic axonal depressant action to the reduction

TABLE 4

THE PERCENTAGE DEPRESSION OF THE PREGANGLIONIC POTENTIAL WHEN THE GANGLIONIC POTENTIAL WAS REDUCED TO 50% OF ITS CONTROL VALUE BY DRUGS

Fig. 9. illustrates the method of deriving the value for the preganglionic potential. An asterisk (*) indicates that in at least one experiment the preganglionic potential was 100%, namely unaffected by that drug

Category	Compound	Mean height of preganglionic potential (% of control)	No. of experiments
Group I			
Agents with little or no preganglionic depressant action	Hexamethonium	100	4*
	Meprobamate	86	3*
	Paraldehyde	86	1
	Amylobarbitone	83	4*
	Methylpentynol	82	1
	Azacyclonal	82	9*
Group II			
Agents with moderate preganglionic depressant action	N714C	81	1
	Prochlorperazine	61	4
	Methylpentynol carbamate	60	4
	Pipradrol	50	3
	Promethazine	46	4
	Perphenazine	43	7
	Procaine	43	4
Group III			
Agents with a strong preganglionic depressant action	Chlorprothixene	40	1
	Promazine	37	5
	N720	36	1
	Chlorpromazine	31	9
	Hydroxyzine	29	5
	Benactyzine	24	8

of the orthodromically evoked ganglionic potential produced by some drugs. Owing to technical difficulties only a few experiments could be carried out.

The action of drugs on the amplitude of the orthodromic and antidromic potentials was compared. Hexamethonium and tubocurarine reduced the orthodromically evoked ganglion potential by a highly selective block of transmission which left the antidromically evoked potential unaffected. Amylobarbitone was moderately selective reducing the orthodromic ganglionic potential to 14% and the antidromic potential to 92% of its control value (Fig. 4). Methylpentynol and paraldehyde

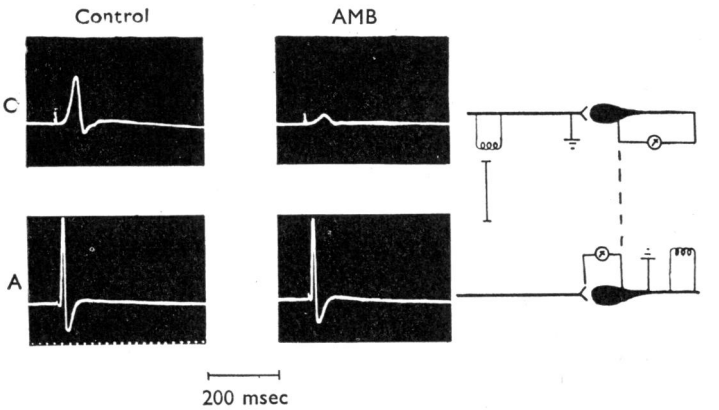

Fig. 4. Ganglionic action potentials with the electrode positions illustrated. The effects of amylobarbitone on the orthodromically (O) and antidromically (A) evoked ganglion potentials of the rabbit superior cervical ganglion. The input to the amplifier was reversed when recording the antidromic response so that both potentials are shown in the same direction. The concentration of sodium amylobarbitone (AMB) was 0·2 mg/ml. The orthodromic ganglionic potential was reduced to 14% of its control value and the antidromic potential to 92%. A chamber temperature of 20° C was used to slow potentials and thus reduce interference from the stimulus artefact when recording the antidromic potential. Vertical calibration, 1 mV.

were less selective whilst methylpentynol carbamate produced considerable depression of the antidromic potential (to 43% of the control) and of the orthodromic potential (to 23%, Fig. 5). Amylobarbitone and methylpentynol carbamate never reduced the antidromic potential more than the orthodromic potential.

With chlorpromazine the antidromic response was invariably reduced more than the orthodromic response. Thus, in one experiment (Fig. 6), after 4 μg/ml. of chlorpromazine the orthodromic ganglionic response was 84% of the control value, the antidromic potential was 69% of its control value, whilst the orthodromically evoked preganglionic action potential was only 50% of its control. These findings strongly suggested some facilitatory action of chlorpromazine at the ganglionic synapse.

In some experiments in which the preganglionic and ganglionic potentials obtained by maximal orthodromic stimulation were compared, drugs produced an increase in amplitude of the ganglionic potential without affecting the preganglionic potential. A " facilitatory action " of this type was seen with the phenothiazine

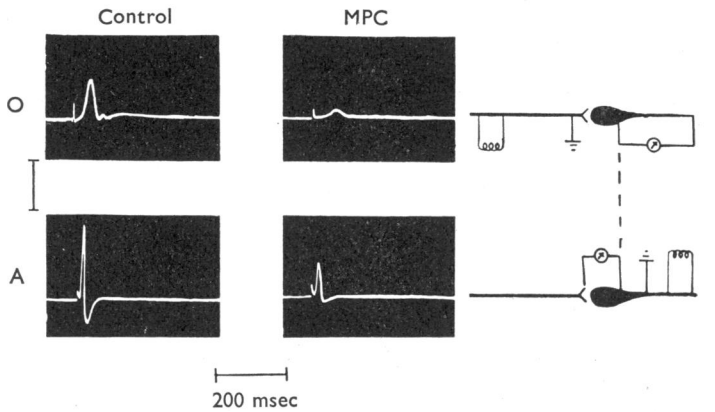

Fig. 5. Ganglionic potentials recorded from the positions illustrated. The effect of methylpentynol carbamate on the orthodromically (O) and antidromically (A) evoked ganglionic potentials of the rabbit superior cervical ganglion. The input to the amplifier was reversed when recording the antidromic potentials so that ganglionic and antidromic potentials are shown in the same direction. A concentration of 1 mg/ml. of methylpentynol carbamate (MPC) reduced the orthodromic ganglion potential to 23% and the antidromic potential to 43% of the control values. Temperature 20° C. Vertical calibration, 1 mV.

drugs and with hydroxyzine, azacyclonal and pipradrol, but was only observed with low concentrations of the drugs. This facilitatory action was observed in the following proportion of experiments: phenothiazine compounds, 22 out of 61 experiments; hydroxyzine, 2 out of 7; azacyclonal, 3 out of 6; pipradrol, 1 out of 4. The effect was small, the maximum facilitation for phenothiazine drugs being about 30%, and it was usually 10% or less.

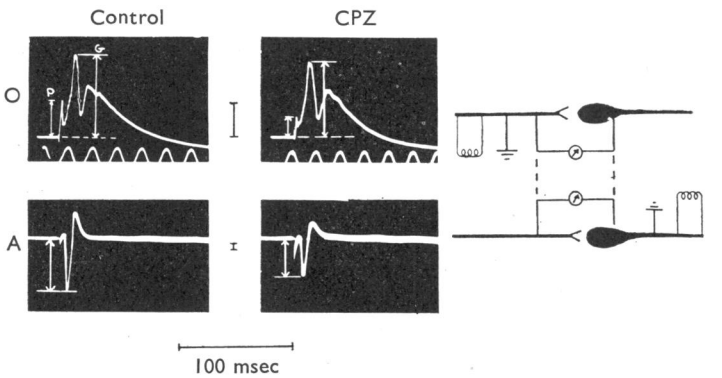

Fig. 6. Potentials recorded with electrodes in the positions indicated show the apparent facilitatory action of chlorpromazine hydrochloride. The potentials in the upper row are recorded across the ganglion and thus include the preganglionic potentials (P) and the corresponding ganglionic potentials (G). The antidromically evoked potentials (recorded without reversal of the amplifier input leads) show the effect of chlorpromazine (4 μg/ml., CPZ) on the postganglionic neurone. The preganglionic potential was reduced to a greater extent than the ganglionic potential. Actual values were preganglionic potential 50% of its control value, orthodromically evoked ganglionic potential 84% of control, and the antidromically evoked potential 69% of control. Vertical calibration, 1 mV.

An alternative explanation to a facilitatory action at the ganglion to account for the preganglionic action potential and the antidromically evoked postganglionic potential being depressed proportionately more than the orthodromically evoked ganglionic potential was that reduction of the potentials in the pre- and post-ganglionic axons was more apparent than real. This could arise because some of these compounds caused an increased dispersion of the action potentials by widening the conduction velocity spectrum. The spike recorded, which is an integrated record of all the action potentials in the individual fibres, would then fall in amplitude due to further asynchronization of the individual constituent action potentials.

An attempt was made to allow for this asynchrony by measuring the area under the spike potential as well as its amplitude. Fig. 7 illustrates some of the records from an experiment with procaine, a drug which produces increased asynchrony of the preganglionic potential, and Fig. 8 gives the derived dose/response curves

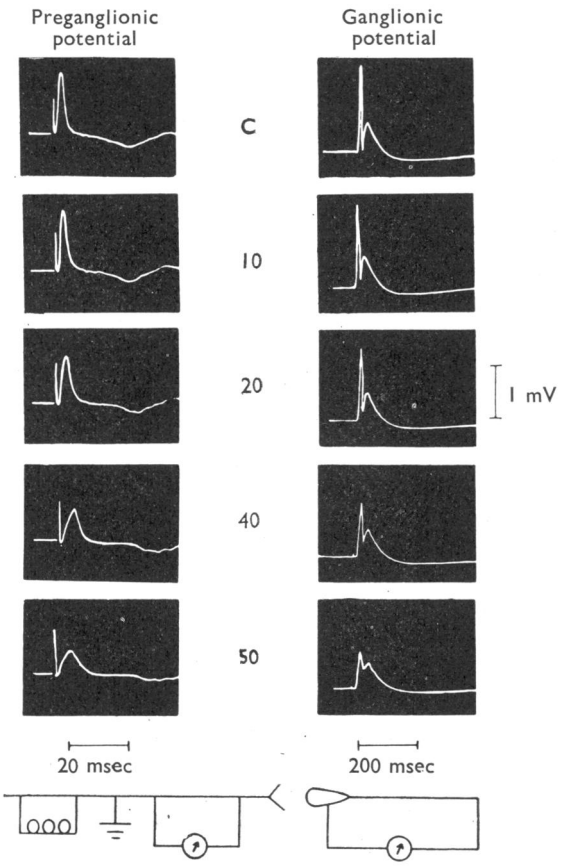

Fig. 7. Preganglionic and ganglionic potentials showing the effects of procaine on conduction and transmission in the superior cervical ganglion. C = control potentials. Increasing concentrations of procaine in μg/ml. are indicated by the numerals between the records. Dose/response curves derived from these records are shown in Fig. 8.

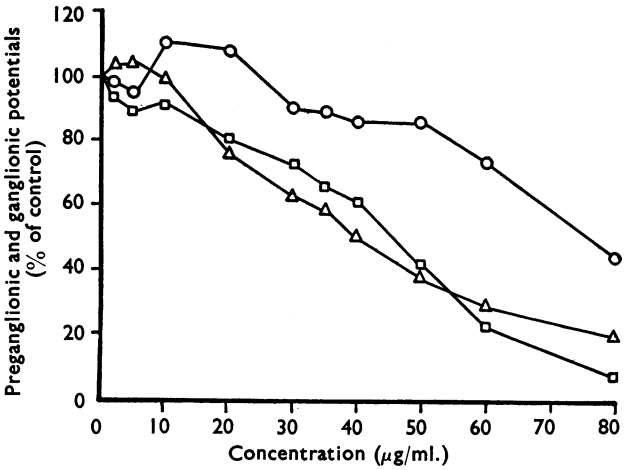

Fig. 8. The action of procaine on the preganglionic and ganglionic potentials. Some of the records from which these graphs were constructed are shown in Fig. 7. If the area of the preganglionic potential (circles) is plotted instead of its amplitude (triangles) the disproportionately greater depression of the preganglionic potential than of the ganglionic potential amplitude (squares) seen in the concentration range 15 to 55 μg/ml. is reversed.

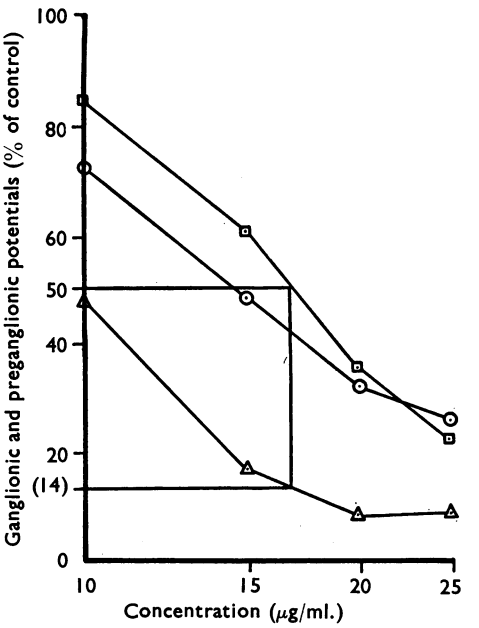

Fig. 9. The effect of benactyzine on the preganglionic and ganglionic potentials. Even when the area under the preganglionic potential (circles) instead of its amplitude (triangles) is compared with the amplitude of the ganglionic potential (squares) there is still a disproportionately greater depression of the preganglionic than of the ganglionic potential. The method of estimating the percentage reduction of the preganglionic potential corresponding to 50% reduction of the ganglionic potential (Table 4) is shown (14% normal in this experiment).

for the amplitude of the preganglionic potential, for the area under the preganglionic potential and for the amplitude of the ganglionic potential. When a comparison is made between the preganglionic and ganglionic potentials as percentages of control responses on the basis of their amplitudes, the preganglionic potential appears to be reduced more than the ganglionic potential, within the dose range 15 to 55 μg/ml. If, however, the area under the preganglionic potential, instead of its amplitude, is used as the basis for comparison this discrepancy is resolved. Increased asynchrony of the preganglionic action potential cannot however account for the findings with benactyzine (Fig. 9) and with hydroxyzine (Fig. 10) where, even when the area under the preganglionic potential is measured instead of its amplitude, the depression of the preganglionic potential exceeds that of the ganglionic potential.

Hallucinogenic agents

The concentration producing 50% block with adrenochrome was 15.92 mM (n=1), with lysergic acid diethylamide 0.31 mM (n=1) and with mescaline sulphate 3.00 mM $\pm$0.28 (standard error, n=5).

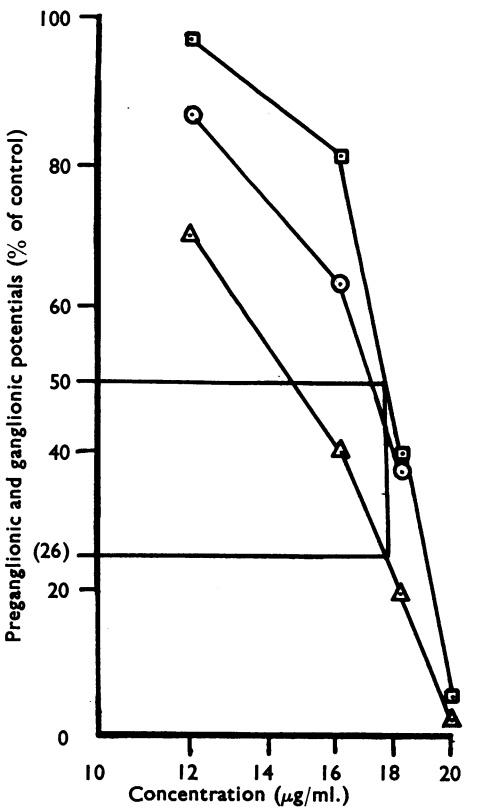

Fig. 10. The effect of hydroxyzine on the preganglionic and ganglionic potentials. Even when the area under the preganglionic potential (circles) instead of its amplitude (triangles) is compared with the amplitude of the ganglionic potential (squares) there still remains evidence of facilitation of the ganglionic response.

Mescaline sulphate produced "facilitation" at low concentrations in five out of eight experiments. The average increase was 9%, the greatest being 23% in an experiment with partial preganglionic section which permitted submaximal stimulation. This facilitation was accompanied by an apparent depression of the N wave which might suggest a depolarizing action (Paton & Perry, 1953; Eccles, 1956). This point is difficult to settle since the decreased N wave might be merely a reflection of the increased diphasic character of the spike potential due to improved postganglionic transmission. Fig. 11 illustrates this apparent "depolarization"

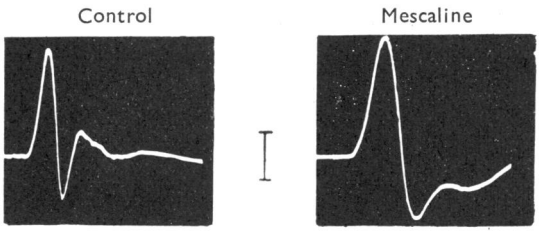

Fig. 11. The action of mescaline in increasing the amplitude and prolonging the duration of the ganglionic potential of the rabbit superior cervical ganglion. Concentration of mescaline sulphate, 1 mg/ml. Supramaximal stimulation. Recording electrodes as in Fig. 2. Vertical calibration, 1 mV.

facilitation after mescaline sulphate (1 mg/ml.) and also shows the prolongation of the ganglionic potential. This was associated with an increased dispersion of the preganglionic potential. Mescaline decreased the amplitude of the preganglionic action potential so that the mean preganglionic action potential was 71% (range 55 to 92%, n=6) of its control when the postganglionic potential was 50% of its control. In the lower concentration range, the preganglionic action potential was sometimes reduced more than the ganglionic action potential, and in two experiments potentiation of the ganglionic potential was seen in the presence of a decrease in amplitude of the preganglionic potential.

Adrenaline

As adrenaline may play some role in the transmission process in the superior cervical ganglion and elsewhere, its ganglion blocking action was studied. The adrenaline dose/response curve differed markedly from that of tetraethylammonium (Fig. 12). Reliable estimates of doses of adrenaline producing 50% block were not usually possible because the dose/response curve flattened out as it approached 50% block. The concentration producing 30% block was therefore measured. There was a large scatter in the results due to the shallowness of the dose/response curve. The ED30 value varied between 2.7 and 52 μg/ml. with a mean of 18.8 μg/ml. in eight experiments.

Adrenaline did not reduce the preganglionic potential unless given in extremely high concentrations (1 to 10 mg/ml.) and only then were ganglionic potentials below 50% of the control value recorded. Facilitation of the ganglionic potential by adrenaline was seen in five out of twelve experiments at concentrations between

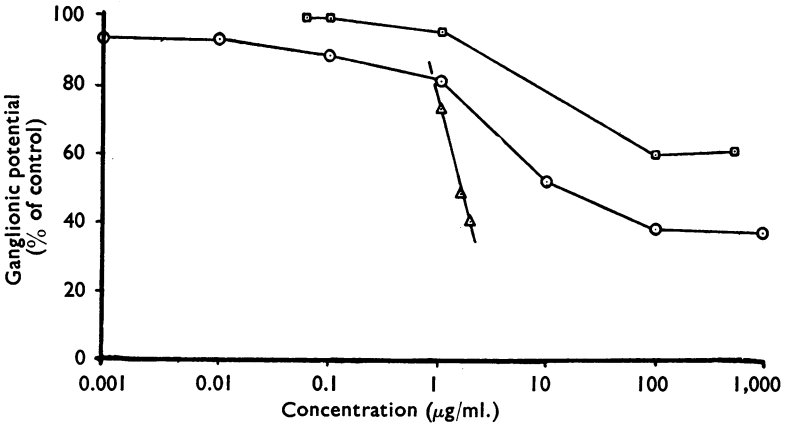

Fig. 12. The effect of adrenaline on the ganglionic potential. The upper graph (squares) shows a typical adrenaline dose/response curve. In only one experiment (circles) was more than 50% block achieved. A typical regression line with tetraethylammonium is included for comparison (triangles).

0.005 and 1 μg/ml. However, in four of the five experiments fast repetitive stimulation had been employed as well as single stimuli and it seemed possible that the facilitation could have been due to residual effects from the previous burst of repetitive stimulation, especially as these effects are known to be long lasting (Elliott, 1964). Out of seven experiments in which only single stimuli were used, only one example of facilitation (of 6%) was observed. This group included one preparation in which the preganglionic trunk had been partially sectioned to favour the display of facilitation, but none was seen.

Interactions between central depressant drugs and other compounds at the superior cervical ganglion

Attempts were made to prevent the block produced by mescaline sulphate by previous treatment with an ataractic agent. Three successive dose/response curves for mescaline were obtained, the second one being in the presence of a low concentration of an ataractic drug in the bath fluid. Chlorpromazine (0.75 and 1 μg/ml.), azacyclonal (20 μg/ml.) and meprobamate (100 μg/ml.), rather than antagonizing, tended to increase the block produced by mescaline. The reverse experiment in which mescaline sulphate (100 μg/ml.) was used in an attempt to reduce the block produced by chlorpromazine was equally ineffective. Neither chlorpromazine nor azacyclonal influenced the block produced by adrenaline.

Ergotoxine ethanesulphonate (2 to 20 μg/ml.) reduced or abolished the inhibition of transmission produced by adrenaline, suggesting that the latter action is dependent on an effect on the α-receptors of Alhquist (1948). Large doses of ergotoxine alone abolished transmission irreversibly.

After immersion of the preparation for periods up to 3 hr in concentrations of 10^{-4} to 10^{-3} g/ml. of the amine oxidase inhibitor iproniazid, considerable reduction of the ganglionic potential occurred. This was not due to a synaptic action of the

drug since the preganglionic potential was also reduced. Neither ergotoxine nor iproniazid produced facilitation. In two experiments in which distinct S_a and S_b spikes (Eccles, 1952) could be measured, iproniazid exerted a selective action on the S_a spike, reducing both the pre- and postganglionic potentials, leaving the S_b spike relatively unaffected. Adrenaline alone did not show this marked selective action. Adrenaline facilitated transmission during repetitive stimulation (Elliott, 1964) whereas iproniazid did not. These experiments suggested that the effects of iproniazid, at these concentrations and for these periods of immersion, were unrelated to its antiamine oxidase properties.

DISCUSSION

The action of central depressant drugs on the ganglion

All the central depressant drugs used blocked transmission through the rabbit superior cervical ganglion when applied in sufficient concentration. The question arises as to how far this action was synaptic as opposed to one interfering with conduction of the nerve impulse in the pre- and postganglionic axons. Depression of conduction in the preganglionic axon, which was seen with most of these central depressant agents, might reduce the orthodromically elicited ganglionic potential without involving any specific action on transmission. To investigate this point it was necessary first to establish the relation between the amplitude of the preganglionic action potential and the amplitude of the ganglionic potential. When the interval between successive stimuli of increasing strength was long enough to avoid temporal facilitation this relation was approximately linear (Elliott, 1963).

Assuming that this linear relationship applied to all the ganglia examined here, predictions as to the effect of an axonal depressant drug on synaptic transmission can be made. Thus if drug X depressed conduction in the preganglionic axon by 50% it might depress the postganglionic response by 50%. Since the postganglionic axon was also exposed to the drug in the present experiments, one might expect *a priori* the ganglionic response to be reduced by 75% (=50% + 50% of 50%) if the sensitivity of the postganglionic axon was similar. A drug having an axonal depressant action would therefore depress the ganglionic potential to an extent equal to, or greater than, its preganglionic depressant action. Table 4 shows the depression of the preganglionic action potential corresponding to a 50% block of the ganglionic action potential. There was a continuous spectrum of axonal depressant activity varying from nil with hexamethonium to 76% reduction with benactyzine. The compounds have been arbitarily divided into three groups on the basis of their axonal depressant action at the ED50 ganglion blocking concentration.

Group I : hexamethonium, meprobamate, paraldehyde, amylobarbitone, methylpentynol and azacyclonal. These acted selectively at the ganglion ; the preganglionic depression which sometimes occurred was not sufficient to account for the reduction of the ganglionic potential observed. Antidromic stimulation indicated that such axonal depression that these drugs might show was no greater on conduction in the postganglionic axon than it was on the preganglionic axon.

Group II : cis-*isomer of chlorprothixene, prochlorperazine, methylpentynol carbamate, pipradrol, promethazine, perphenazine and procaine.* The action of these drugs on the ganglion could be accounted for entirely in terms of their axonal depressant activity.

Group III : chlorprothixene, promazine, dihydro-chlorprothixene, chlorpromazine, hydroxyzine and benactyzine. These compounds appeared to exert a dual action depressing axonal conduction and yet having a " stimulating " or " facilitating " action at the synapse itself. At lower concentrations a small facilitatory action was occasionally seen with all the phenothiazines and with azacyclonal, hydroxyzine and pipradrol.

The " stimulant " or " facilitatory " action of Group III drugs is of interest in the light of the findings of Nishi, Koketsu, Cerf & Abood (1959) with hydroxyzine. These authors showed that over a certain dose range of hydroxyzine at the neuromuscular junction, the depression of conduction in the nerve exceeded that in the muscle, a finding which they attributed to facilitation at the neuromuscular junction. Their intracellular recordings from cells of the dorsal root ganglia in the isolated spinal cord of the frog indicated that hydroxyzine exerted a depolarizing action at this site. It is not possible to follow changes in polarization with the condenser-coupled amplifier system we used but depolarization can be recognized by depression of the N wave component of the ganglionic potential. Group III drugs did not exhibit this sign of depolarization.

Dempsher, Tokumaru & Zabara (1959) have presented evidence which they interpret as suggesting the presence of preganglionic fibres inhibiting transmission through the superior cervical ganglion. It is possible that the Group III drugs might have a greater affinity for these postulated inhibitory fibres than for the excitatory fibres.

The depression of the amplitude of the preganglionic potential seen with most of the drugs examined was accompanied by a slowing of the conduction velocity and temporal dispersion. It is conceivable that some of the effects observed may be related to the acetylcholine release being slower than normal in the ganglion treated with drugs.

Central depressant drugs do not appear to exert any one specific action by which they block ganglionic transmission. With Group I compounds, synaptic block predominates, whilst with Group II and III it is the axonal depressant action which is the most important factor ; all these drugs possess the two properties but in different degrees, and a continuous spectrum of drugs links meprobamate, paraldehyde and amylobarbitone, which act selectively at the synapse, to axonal depressant agents, such as chlorpromazine, hydroxyzine and benactyzine.

The action of compounds other than central depressant agents on the ganglion

Adrenaline. No dose/response curve for the effects of adrenaline on transmission in the superior cervical ganglion appears to have been reported previously. That in Fig. 12 supports the contention of Matthews (1956) that there is a group of cells in the ganglion which are insensitive to block by adrenaline. Whilst the possibility

of facilitation by low concentrations of adrenaline was not excluded by the present experiments, it seems unlikely to be an important effect *in vitro*. It is possible that facilitation might have been apparent had shorter immersion periods been used since it has been suggested by Bülbring (1944) that facilitation precedes inhibition. It was hoped that the exposure to very dilute solutions (1×10^{-3} μg/ml.) for 10 min would mimic the effects of more concentrated solutions over a shorter period. Whilst every precaution was taken to minimize oxidation of the adrenaline solutions before the experiment, some oxidation must occur during the 10 min immersion of the ganglion in the adrenaline solution when it was bubbled with the gas mixture. Adrenochrome was relatively very inactive on the ganglion, so that inactivation of adrenaline would be expected to lead to lower ganglion-blocking potencies. It is possible therefore that the shallowness of the dose/response curve might be related to oxidation of adrenaline. Visual observation indicated that very little adrenochrome was produced, but this does not exclude the possibility of the production of colourless intermediates.

Recently Eccles & Libet (1961) have extended the acetylcholine hypothesis of synaptic transmission at ganglia to account for the late negative (LN wave) and late positive wave (P wave). On stimulation, preganglionic fibres liberate acetylcholine at the ganglion, and this reacts with nicotinic receptors on the postsynaptic membrane leading to a depolarizing potential, the N potential, which initiates the spike potential in the uncurarized ganglion. They suggested that some acetylcholine diffuses away from the N sites and binds with other sites producing the slowly rising and long-lasting LN wave. In addition, preganglionic terminals in the vicinity of chromaffin cells liberate acetylcholine which binds with muscarinic receptors on the chromaffin cells resulting in the liberation of adrenaline. The adrenaline then combines with P receptors on the ganglion cells in the vicinity leading to a hyperpolarizing potential, the P potential. The authors are careful to restrict their interpretations to the curarized ganglion, but the implication arises that adrenaline exerts a tonic inhibitory action on the uncurarized ganglion.

In our experiments ergotoxine ethanesulphonate blocked the inhibitory action of added adrenaline, and a similar result was obtained by Lundberg (1952) with dihydroergotamine. Since ergotoxine in adrenaline-blocking concentrations had no effect on the ganglion in the absence of added adrenaline it seems to follow that material quantities of adrenaline are not liberated normally at the rabbit superior cervical ganglion. On the other hand, Costa, Revzin, Kuntzman, Spector & Brodie (1961) obtained facilitation with ergotamine alone. Should the concept of Eccles & Libet (1961) prove correct, then it would furnish a good explanation for the action of the Group III drugs which all exert antiadrenaline actions, except benactyzine which has atropine-like effects and therefore might block the muscarinic sites on the chromaffin cells.

Paton & Thompson (1953) observed that adrenaline reduced uniformly the various components of the ganglionic potential, a finding confirmed here. They suggested that the action of adrenaline was twofold causing a reduction in the output of acetylcholine and a diminished sensitivity of the ganglion cells to injected acetylcholine. In the present experiments, adrenaline still exerted its characteristic

actions in the presence of an anticholinesterase, a situation in which acetylcholine was presumably present in excess.

Mescaline. This compound produced ganglion-block by virtue of its axonal depressant action. It resembled the Group III compounds in exerting a concealed facilitatory action at low concentrations. There was some indirect evidence to suggest that it produced depolarization. A characteristic feature of its action was the prolongation of the duration of the preganglionic and of the ganglionic action potentials.

The negative results in the experiments in which an attempt was made to reverse the blocking action of mescaline with tranquillizers need not necessarily imply that such an interaction never occurs, for only a few experiments were possible and the selection of suitable doses to demonstrate such interactions is notoriously difficult.

The results from these electrical studies on transmission through the isolated superior cervical ganglion of the rabbit have yielded quantitative data which can be expressed and interpreted in pharmacological terms. They suggest that the neuropharmacology of the central nervous system and its synapses should be critically examined for evidence of synaptic and/or neuronal block which could form the basis of the action of many compounds upon the function of the brain.

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