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ON THE ACTION OF DEPOLARIZING DRUGS ON SYMPATHETIC GANGLION CELLS OF THE FROG

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Drugs which depress synaptic transmission in mammalian sympathetic ganglia may be divided into two groups. One group, which includes nicotine, acetylcholine, carbamylcholine (carbachol) and tetramethylammonium, also depolarizes the ganglia and produces the discharge of action potentials along the post-ganglionic axons (Langley & Dickinson, 1889; Feldberg & Vartiainen, 1935; Brink, Bronk & Larrabee, 1946; Paton & Perry, 1953; Eccles, 1956); the other group, which includes tubocurarine, hexamethonium and decamethonium, does not (Paton & Zaimis, 1951; Paton & Perry, 1953; Eccles, 1963). There is less information on the action of drugs on sympathetic ganglion cells of the frog. It is however known that acetylcholine depolarizes these cells (Blackman, Ginsborg & Ray, 1963*a*), that nicotine produces ganglionic blockade preceded by stimulation (Dixon, 1902) and that tubocurarine and hexamethonium depress synaptic transmission without depolarizing the cells (Blackman *et al.* 1963*a*). The results of the experiments to be described in the first section of this paper show that the other drugs mentioned above also have similar effects on sympathetic ganglion cells in the frog to those that they have in the mammal.

It is generally assumed that the action of the depolarizing drugs results from their combination with the same variety of receptors as react with the transmitter released from the presynaptic nerve terminals. However, it has recently been suggested, on the basis of data obtained from the superior cervical ganglion of the cat, that it is the presynaptic nerve terminals which are especially sensitive to carbachol and tetramethylammonium and that the primary action of these substances is to cause the release of acetylcholine from the presynaptic nerve (Koelle, 1961; Volle & Koelle, 1961). In the frog it seems possible to detect the release of individual quanta of acetylcholine by virtue of the fact that they produce spontaneous synaptic potentials, which may be recorded from the

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ganglion cells with an intracellular electrode (Nishi & Koketsu, 1960; Blackman, Ginsborg & Ray 1963*b*). It was thought to be of interest, therefore, to examine the action of depolarizing substances on frog's sympathetic ganglion cells in order to see if any part of it was due to an action on the presynaptic nerve.

METHODS

Single cells in the lumbar sympathetic ganglia of the *Rana pipiens* were impaled with micro-electrodes in the way described by Blackman *et al.* (1963*a*) (cf. Nishi & Koketsu, 1960). In most experiments the preparation was contained in a bath of 25 ml. The bathing

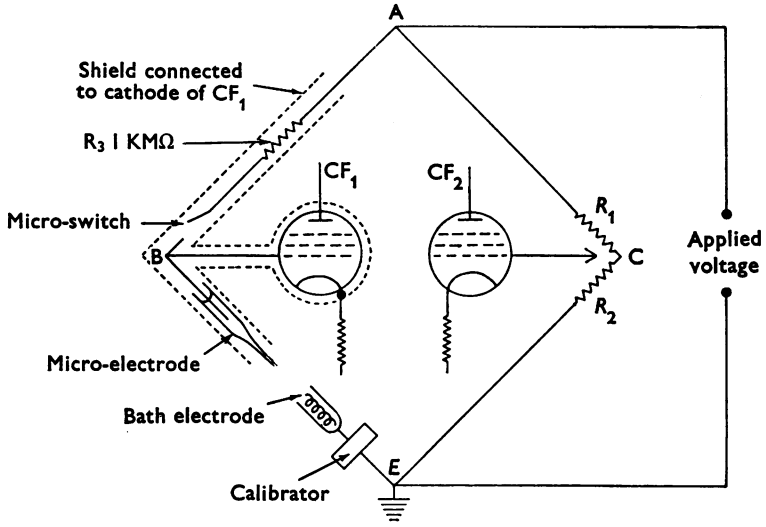

Fig. 1. Circuit used for polarizing and recording from a cell with the same micro-electrode. CF, cathode followers of equal gain. $R_1 + R_2$, 10-turn helical potentiometer of 10 KΩ. The current flowing through the micro-electrode was not measured directly, but was taken as a first approximation to be equal to the voltage across AE divided by R_3. The 'input time constant' was about 150 μsec with micro-electrodes of 30 MΩ.

solution had the same composition as that described by Blackman *et al.* (1963*a*) and the experiments were made at room temperature (17–23° C). Drugs were added to the bath from syringes, in a volume of usually not more than 1 ml., with as much force as possible to obtain rapid stirring and mixing. As might be expected, this procedure occasionally dislodged the micro-electrode from the cell. In some experiments the bath was freed from a drug, without dislodgement of the electrode, by the addition of a large volume of fresh bathing fluid from a wash bottle, the fluid level being kept constant by suction. Unless otherwise stated in the results, at least 45 min was allowed to elapse between washing the preparation and adding a second drug (or a second dose of the same drug) to the bath.

In a number of experiments the membrane potential of a ganglion cell was changed by passing current through the same micro-electrode that was used for recording. We are indebted to Professor C. C. Hunt and Dr P. G. Nelson for advice in this connexion. A circuit similar to that described by Araki & Otani (1955) (cf. Nishi & Koketsu, 1960; Frank & Fuortes, 1956; Martin & Pilar, 1963) was used and is illustrated in Fig. 1. After

a cell had been impaled a steady voltage was applied between A and E such that the amplitude of the antidromic action potential was unaltered when the switch at B was opened and closed. Since the resistance of the cell at the peak of the action potential is negligible with respect to 1000 MΩ the effect of this procedure is to restore the loss in resting potential caused by current drawn from the ganglion cell in the circuit BAE. The p.d. across the micro-electrode due to the current flowing through it was balanced out by adjusting the position of C, i.e. of the value of R_1/R_2, during the passage of a current pulse, until the 'artifact' caused by the pulse at its beginning and end was reduced to a minimum. In several experiments the balance point was checked by observing the difference between an action potential evoked by an outward current pulse during and after the passage of the pulse (Fig. 2). On the assumption that the resistance of the cell at the peak of the

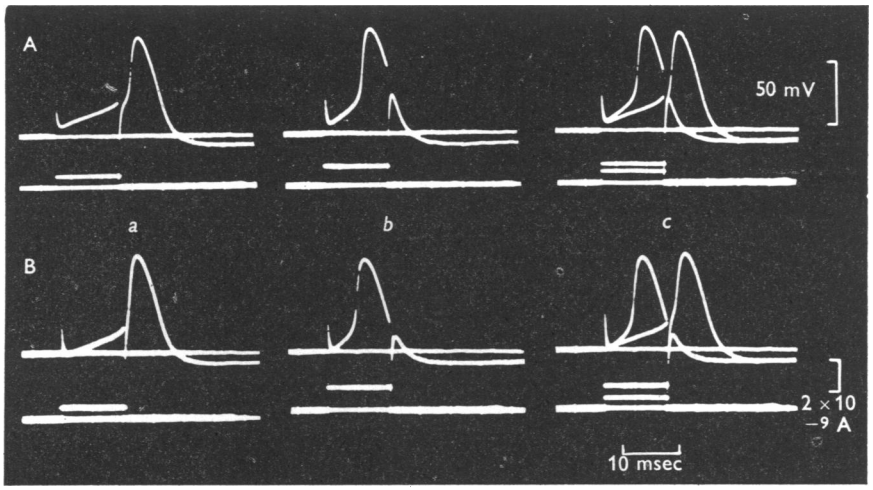

Fig. 2. Effect of bridge balance on action potentials (upper traces) of ganglion cell (cell 177) produced by passing current pulses (lower traces) outward through micro-electrode; *a*, action potential elicited after end of current pulse; *b*, during current pulse; *c*, *a* and *b* superimposed. Voltage change due to current flowing through micro-electrode has probably not been completely annulled in A, and in B it has been overcompensated.

action potential is too small for the amplitude of the action potential to be affected by the current which is flowing through the micro-electrode, the correct balance point may be obtained by adjusting C so that the membrane potential is the same at the peak of each of the two action potentials. It was usually found that the two methods of balancing the bridge gave reasonably concordant results: when it was balanced so that the 'on-off artifacts' due to a current pulse were almost minimal, the membrane potential at the peak of an action potential, however generated, was apparently affected to only a small extent by current passing through the micro-electrode. An additional check was that the threshold of the 'orthodromic action potential' also appeared relatively independent of the current passing through the micro-electrode. Nevertheless, the method in most of our experiments was unlikely to have allowed the determination of membrane potentials during current flow to an accuracy of greater than ±5 mV and it was clearly unsuitable for refined measurements such as that of the resistance of the cell at the peak of an action potential.

In agreement with results of Nishi & Koketsu (1960), when the membrane was hyperpolarized the antidromic action potential was blocked and, with a greater degree of hyperpolarization, the orthodromic action potential was converted into a synaptic potential. It was usually necessary to hyperpolarize the cell by 40 mV (corresponding to a resting potential of between about −90 and −100 mV) or more to abolish the antidromic action potential; however, in one cell (out of a total of about 150 cells) the response to an antidromic stimulus was unable to invade the cell body at the normal resting potential. After several minutes, when the resting potential had fallen by a few mV, a normal variety of antidromic action potential could be evoked.

RESULTS

Depolarization and blockade

The changes in membrane potential and in the repsonses to orthodromic and antidromic stimulation of sympathetic ganglion cells caused by the addition of acetylcholine, carbachol, nicotine or tetramethylammonium chloride to the bath have been studied in about a hundred cells. Before considering the experiments concerned with the details of the way in which

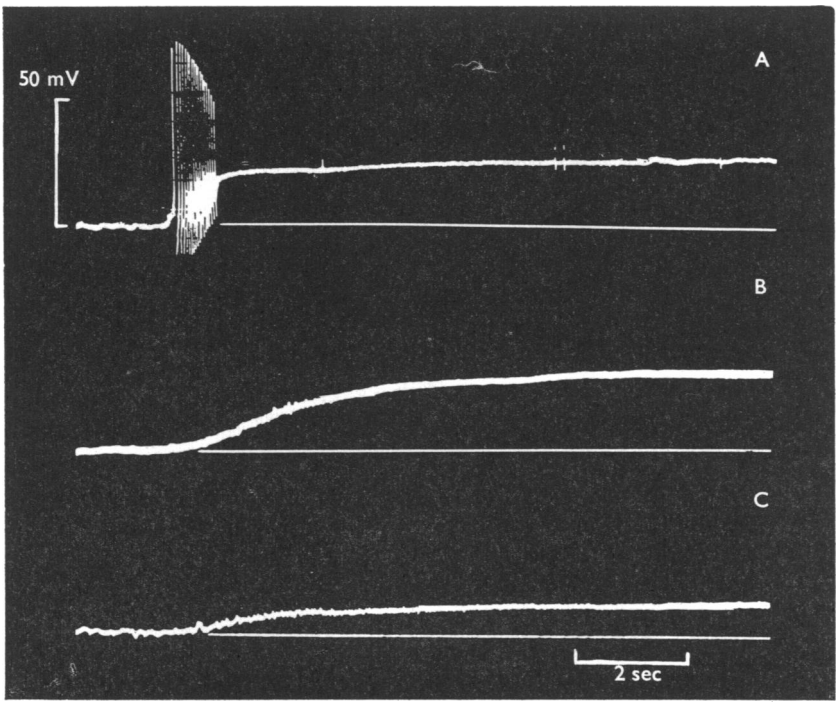

Fig. 3. Depolarization of ganglion cells produced by the addition of carbamylcholine chloride to the bath. A, Cell 116, final concentration of carbachol 5mM; B, Cell 118, final concentration 1mM; C, Cell 117, final concentration 10^{-4}M. The action potentials in A were due to the presence of the carbachol, no stimuli having been applied to either pre- or post-ganglion nerve (see text p. 201).

these substances stimulate a ganglion cell it is convenient to describe their general effects. They were similar to those observed by Paton & Perry (1953) in experiments made by recording with external electrodes from the superior cervical ganglion of the cat.

The initial phase of depolarization (Fig 3). reached a peak in a period of between a few seconds and about a minute after the addition of the drug to the bath, the period depending on the final concentration of the drug and the rapidity of mixing. The depolarization thereafter slowly subsided (Fig. 4). In a few experiments, in which the electrode remained in the cell long enough, the membrane potential could be seen to return to within a few milivolts of its original value in a period of between 5 and 20 min.

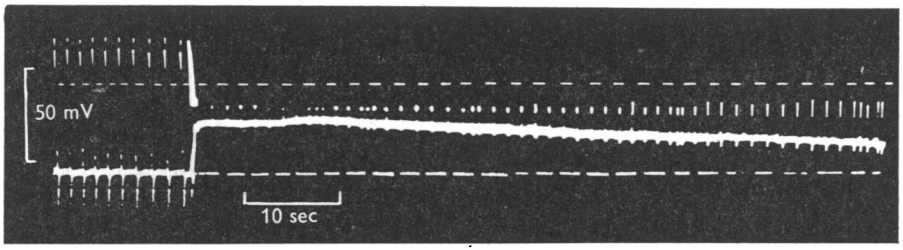

Fig. 4. Initial depolarization and partial recovery of resting potential after the addition of acetycholine choride to bath, final concentration 1.1 mM. Cell 38. Antidromic followed by orthodromic stimuli were applied at z rate of about 1/1.2 sec. The acetylcholine was added to the bath immediately after the tenth pair of stimuli. A burst of 'drug-induced'action potenrials occurred on the rising phase of the depolarization and a few such action potentials are interspersed among the responses to antidromic stimuli in the later part of the record.

During the phase of depolarization the amplitudes of both the orthodromic and antidromic action potentials were reduced (Fig. 5) and so also was the resistance of the cell (Fig. 6). The intensity of the action of the transmitter was reduced and, with a sufficiently high concentration of drug, transmission was blocked, orthodromic stimuli produced synaptic potentials only (Fig. 7; see also Fig. 5 amd 6). As the nembrane potential recovered towards its original value, so also did the membrane resistance (see Fig. 11 D) and the amplitude of the antidromic response. The reduction in intensity of the transmitter action, however, persisted indefinitely in the presence of the drug.

In one experiment advantage was taken of a preparation in which it was found possible to impale an unusually large number of cells. Carbachol was added to the bath to give a final concentration of 2×10^{-4} M, a concentration which had previously been found to produce an initial depolarization usually greater than 10 mV (see for example, Table 1). In each of the seven cells impaled in the first 15 min after the addition of the drug, the

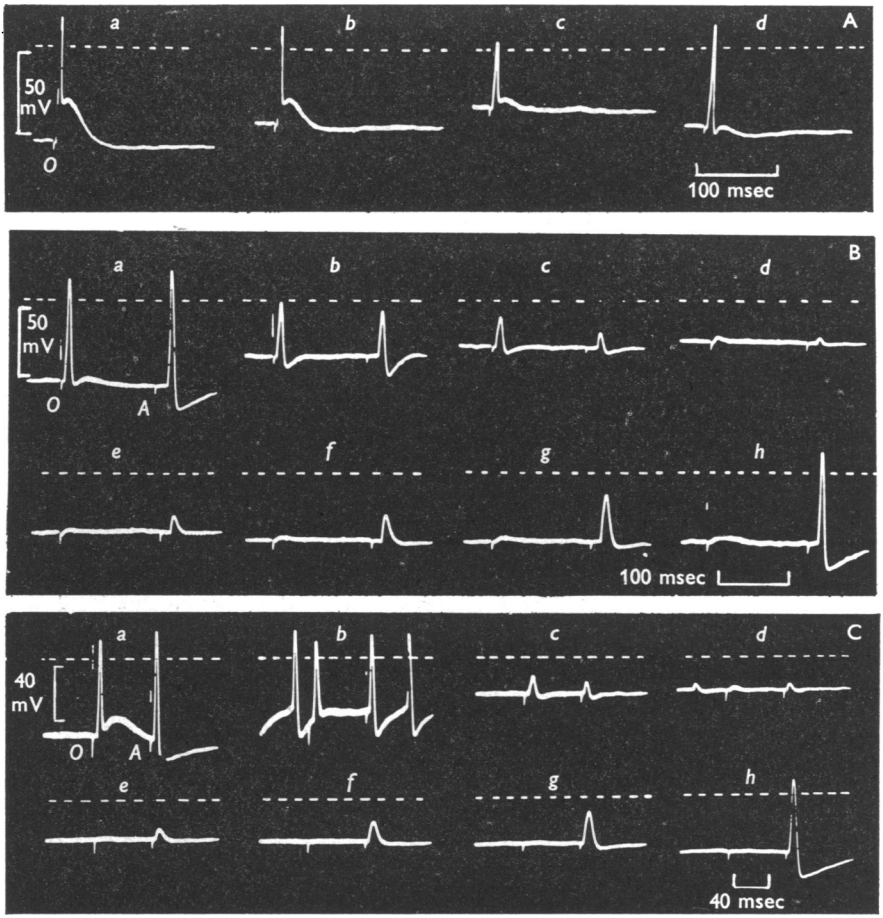

Fig. 5. Action of depolarizing substance on 'orthodromic' (*O*) and 'antidromic' (*A*) action potential of ganglion cell. A, cell 15, tetramethylammonium chloride added to bath between *a* and *b*, final concentration 2×10^{-5} M. Records taken at following times after addition of drug to bath: *b*, 8 sec; *c*, 1 min; *d*, 3 min 50 sec. Note depression in intensity of action of transmitter in *d* as indicated by reduction in amplitude of 'negative wave' and increase in amplitude of after-hyperpolarization. B, cell 13, tetramethylammonium chloride added to bath between *a* and *b*, final concentration 2×10^{-4} M. Records taken at following times after addition of drug to bath; *b*, 10 sec; *c*, 12 sec; *d*, 20 sec; *e*, 30 sec; *f*, 1 min; *g*, 2 min 30 sec; *h*, 5 min 15 sec. C, cell 43, Nicotine hydrogen tartrate added to bath between *a* and *b*, final concentration 5×10^{-4} M. Records taken at following times after addition of drug to bath: *b*, 14 sec; *c*, 18 sec; *d*, 30 sec; *e*, 1 min; *f*, 1 min 30 sec; *g*, 2 min; *h*, 6 min 30 sec. Note 'drug-induced' action potentials in *b* and *d* (see text, p. 201). Zero p.d. between micro- and bath electrode corresponds to interrupted line in each record.

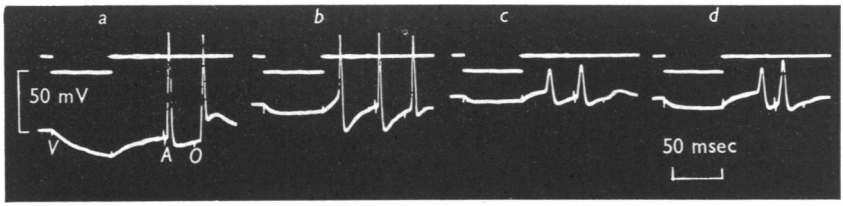

Fig. 6. Reduction in resistance of ganglion cell caused by addition of carbachol (final concentration $1{\cdot}5 \times 10^{-4}$ M) to bath. Cell 159. Upper trace in each record shows voltage step applied to 1 KMΩ resistor in bridge circuit (see Fig. 1). Lower trace, membrane potential. *V*, change in membrane potential, due to current flowing through micro-electrode; *A*, response to antidromic stimulus; *O*, response to orthodromic stimulus. Note additional action potentials due to 'anode-break' stimulation of cell in *b* and *c*. The drug was added to the bath between *a* and *b*. Records taken at following times after addition of drug: *b*, 10 sec; *c*, 30 sec; *d*, 2 min 10 sec. The initial resistance of the cell was about 70 MΩ and the minimum value was about 5 MΩ at the maximum of the depolarization caused by the carbachol.

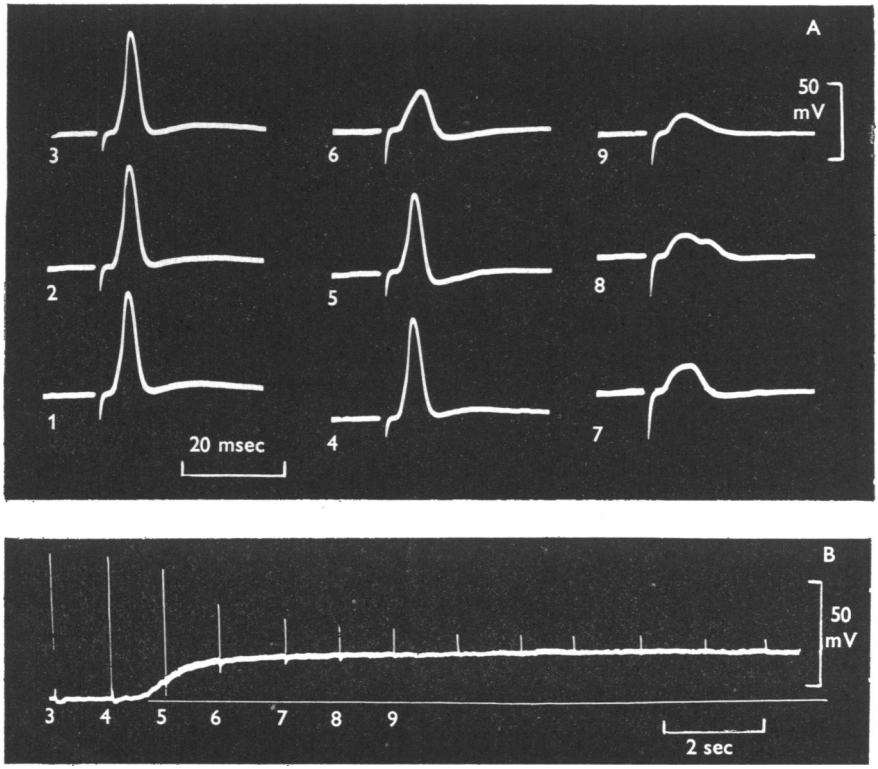

Fig. 7. Depression of synaptic transmission by carbachol. Cell 125. A, nine successive responses of the cell to orthodromic stimuli at a rate of about 1/sec. Carbachol (final concentration 2×10^{-4} M) added to bath between 3 and 4. The depolarization is shown in the continuous record, B, corresponding responses being marked with the same numbers as in the upper trace.

antidromic action potential was less than 65 mV. Twelve impalements were made in the period between 15 min and 1 hr after the addition of the drug and in four of the cells antidromic action potentials greater than 70 mV were evoked. No responses to orthodromic stimuli were obtained from any of the nineteen cells, nor were miniature synaptic potentials seen.

The depolarization could be cut short by the addition of tubocurarine to the bath (Fig. 8) and the blockade of synaptic transmission was sometimes reversed by repetitive orthodromic stimulation (Fig. 9) (cf. Lundberg & Thesleff, 1953).

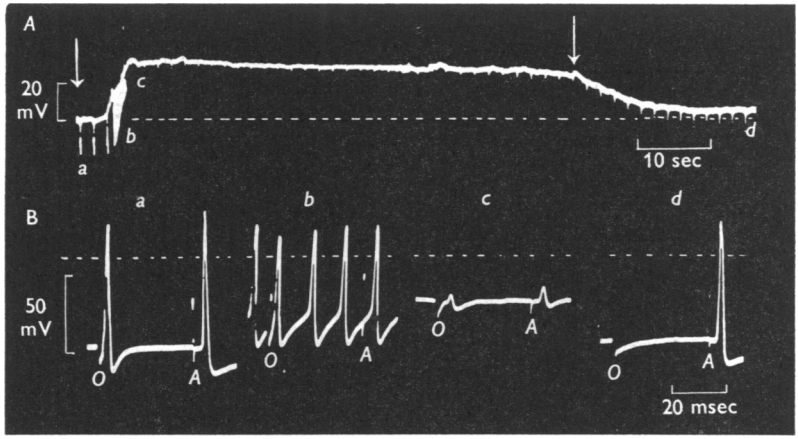

Fig. 8. Acceleration of recovery from 'carbachol-depolarization' by addition of tubocurarine to bath. Cell 136. A, carbachol added to bath to give a final concentration of 5 mM at first arrow and tubocurarine to give a final concentration of 4×10^{-4} M at second arrow. B, individual traces of responses to the orthodromic (*O*) followed by antidromic (*A*) stimuli recorded at the times indicated in *A* by the letters a, b, c, d. The interrupted line in B indicates zero p.d. between the micro- and bath electrode.

In several experiments two successive additions of the same drug were made to the bath, the second being made after the depolarization due to the first had largely subsided. It was invariably found that the sensitivity of the cell had been reduced by the initial application of the drug. For example, in one cell (cell 130, Table 1) in the presence of 2×10^{-5} M carbachol, which had initially produced a depolarization of 7 mV, no depolarization was observed when the concentration was raised to 2×10^{-4} M.

That the sensitivity of the cell to the transmitter was also diminished by the action of these drugs was suggested by the fact that after their addition to the bath the amplitude of miniature synaptic potentials was reduced even after the depolarization had subsided and the resistance of the cell had presumably recovered. In one experiment (cell 15, cf. Fig.

5 A), in the period 48 sec before the application of tetramethylammonium to a final concentration of 2×10^{-5} M, 56 spontaneous synaptic potentials occurred with a mean amplitude of 4·8 mV (range 1·2–13 mV). In a period of 48 sec, 4 min after the application of the drug when the resting potential was within 5 mV of its original value, 21 spontaneous synaptic potentials could be detected with a mean amplitude of 2·6 mV (range 1 mV–5 mV).

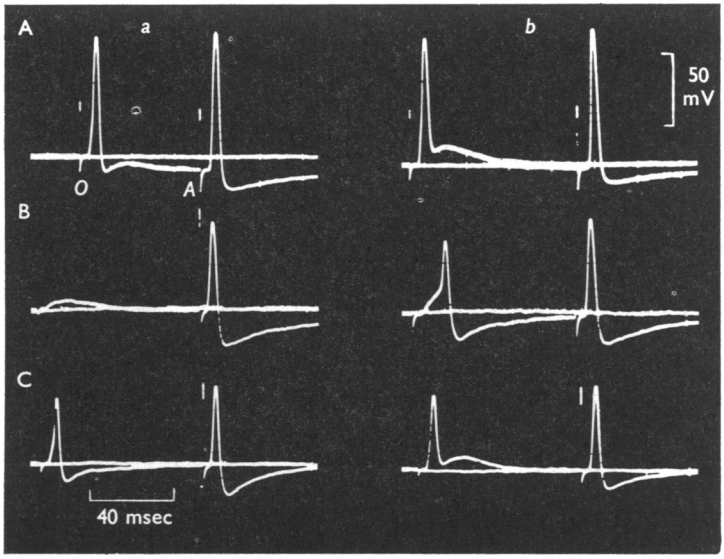

Fig. 9. Reversal by repetitive orthodromic stimulation of depression of synaptic transmission caused by carbachol. Cell 27. A, before addition of carbachol; *a*, response to isolated orthodromic (*O*) and antidromic (*A*) stimulus; *b*, responses 2 sec after stimulation at about 4/sec for 10 sec. B, 4 min after addition of carbachol to a final concentration of 8×10^{-5} M. C, after replacing bath with carbachol-free solution. The electrode was partly dislodged during the washing the resting and potential was reduced.

Since deflexions smaller than 1 mV could not be distinguished from base-line noise the apparent reduction in frequency cannot be regarded as significant, but the reduction in amplitude was presumably due, at least in part, to a reduction in chemosensitivity. In the experiment illustrated in Fig. 10, there happened to be a very high recurrence frequency of spontaneous synaptic potentials; 5 min after the addition of carbachol (to a final concentration of 2×10^{-4} M), when the resting potential was within a few mV of its initial value, the amplitudes of the spontaneous synaptic potentials had apparently been reduced to a level below that of the base-line noise.

It seems reasonable to suppose that the prolonged blocking action of the

substances tested is at least in part due to a reduction in the chemosensitivity of the cell membrane. The possibility, however, that it also involves a reduction in the evoked output of acetylcholine from the presynaptic nerve is not excluded and requires investigation.

The sensitivities of different cells varied rather widely. In one cell, for example, a depolarization of 15 mV was produced by the application of 6×10^{-5} M acetylcholine although in a number of other cells concentrations of up to 10^{-4} M produced no effect on the membrane potential. Concentrations of carbachol greater than 4×10^{-6} M, and of tetramethylammonium greater than 3×10^{-5} M usually produced a detectable depolarization.

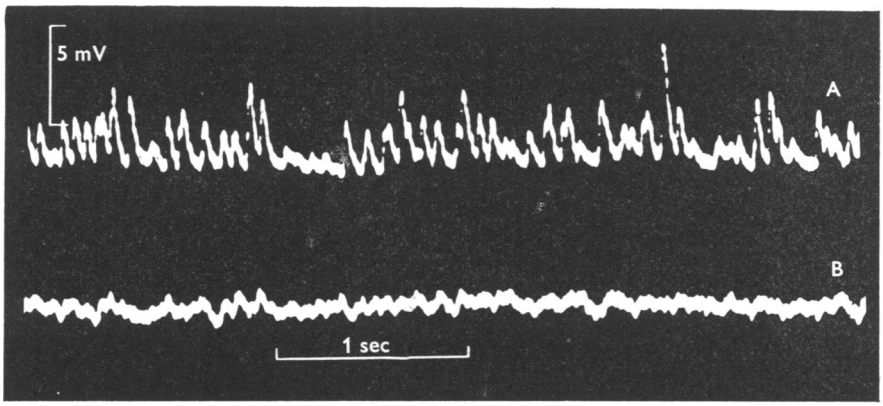

Fig. 10. Reduction in amplitude of spontaneous synaptic potential in presence of carbachol (2×10^{-4} M). A, before, and B, 5 min after the addition of the drug (see text).

Table 1 illustrates the variation in sensitivity to carbachol, the results being taken from experiments in which the cells were initially in good condition and in which there was no reason to suspect that there was a mechanical contribution to the depolarization.

Table 2 shows the peak depolarizations produced by large concentrations of depolarizing substances. It may be seen that the value of the membrane potential at the peak depolarization was usually within or not far outside the range (-10 to -20 mV) previously reported for the 'transmitter equilibrium' potential (Nishi & Koketsu, 1960; cf. Blackman *et al.* 1963*a*). This result might be expected if the depolarizing drugs combine with the same variety of receptors as does the transmitter.

Some depression in the intensity of the action of the transmitter was observed whenever a detectable depolarization was produced. However, transmission was not usually blocked in concentrations of carbachol or tetramethylammonium of less than 5×10^{-5} M or in concentrations of acetylcholine of less than 5×10^{-4} M. In a few experiments the effects of

decamethonium iodide and succinylcholine chloride were examined. Blockade of transmission occurred when the concentration of decamethonium was raised to above 1 mM and that of succinylcholine to above 2·5 mM. There was no effect on either the resting potential or the antidromic action potential.

TABLE 1. Depolarizations caused by carbachol

Cell	Final concentration of carbachol (moles/l.)	Initial amplitude of antidromic action potential (mV)	Initial resting potential (mV)	Maximum depolarization (mV)
47	2×10^{-6}	90	−61	0
133	4×10^{-6}	85	−54	5
132	1×10^{-5}	93	−58	20
		After 7 min in continued presence of drug		
	5×10^{-3}	71	−49	24
		After 3 min in continued presence of drug		
	1×10^{-2}	71	−49	0
130	2×10^{-5}	85	−63	7
		After 4 min in continued presence of drug		
	2×10^{-4}	85	−60	0
19	4×10^{-5}	92	−61	0
58	6×10^{-5}	70	−48	23
26	1×10^{-4}	90	−58	34
30	1×10^{-4}	90	−55	26
53	2×10^{-4}	70	−41	20
117	2×10^{-4}	75	−55	13

TABLE 2. Peak depolarizations caused by large concentrations of drugs

Cell no.	Drug	Concentration (mM)	Initial amplitude of antidromic action potential (mV)	Initial resting potential (mV)	Depolarization (mV)	Resting potential at peak depolarization (mV)
69	Acetylcholine	5	80	−57	46	−11
9	Tetramethylammonium	0·7	90	−59	48	−11
68	Carbachol	4	73	−40	31	− 9
73	Carbachol	4	>75	52	45	− 7
115	Carbachol	15	80	−43	26	−17

Is the 'drug-induced' depolarization due to summation of synaptic potentials?

It has recently been suggested that exposure of sympathetic ganglia in the cat to small concentrations of carbachol and tetramethylammonium causes the release, from the presynaptic nerve terminals, of acetylcholine, which in turn causes the depolarization of the post-synaptic ganglion cells (Koelle, 1961; Volle & Koelle, 1961). If this was so in the frog, it might be expected that the drug-induced depolarization would take the form of a high-frequency discharge of spontaneous synaptic potentials. These

would be difficult to detect during the stage of the depolarization in which the chemosensitivity and the resistance of the cells are reduced; but this difficulty should not apply to the early part of the rising phase of the depolarization, which should therefore consist of a burst of spontaneous

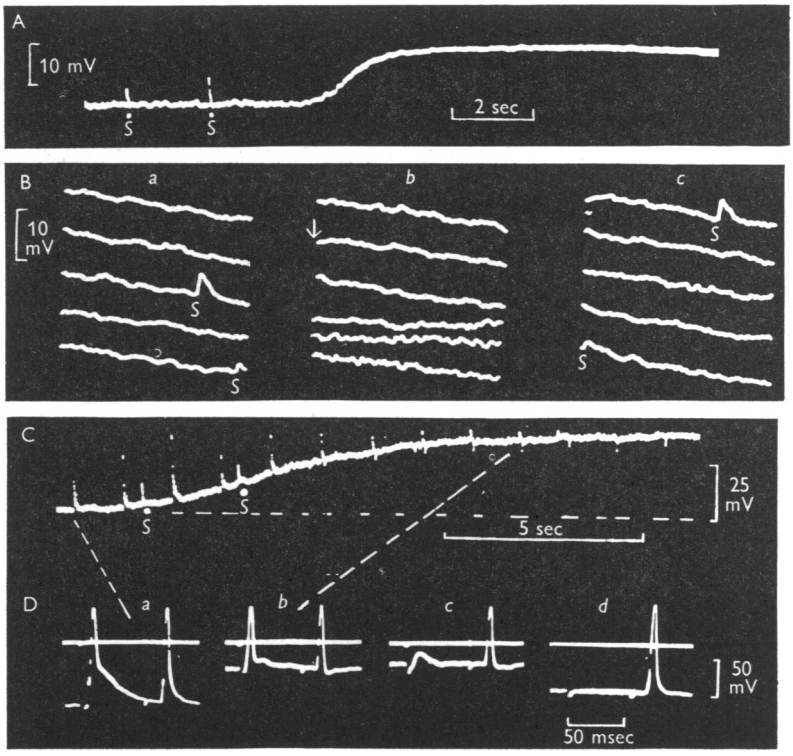

Fig. 11. Rising phase of drug-induced depolarization and spontaneous synaptic potentials. A, cell 39. Depolarization produced by 4×10^{-5} M carbachol. B, cell 40. Spontaneous synaptic potentials (*S*), before *a*, and after washing out *c*, 4×10^{-5} M carbachol. Drug added at arrow in *b*, and depolarization begins about 1 sec later. Film moving perpendicularly to free-running sweep. Records read from left to right and from above downwards. Depolarization indicated by upward movement of an individual trace. Trace length, 0·57 sec. C, D, cell 140. Depolarization produced by carbachol, 2×10^{-4} M. In order to increase the amplitude of any spontaneous synaptic potentials which occurred, the membrane was hyperpolarized by about 50 mV, by passing a current through the cell: the current was maintained constant throughout the experiment. Orthodromic followed by antidromic stimuli were applied at the rate of about 1/sec, and individual records are shown in D. Two spontaneous synaptic potentials (*S*) may be seen on the rising phase of the depolarization in C. Twenty-three such potentials occurred in the 3 min immediately before adding carbachol to the bath. D, *c* and *d* were recorded 36 sec and 5 min after the addition of the drug. The reference lines in D correspond to a p.d. between micro-electrode and bath electrode of about -25 mV.

synaptic potentials. As is shown in Fig. 11, this was not the case. It is true that in many cells oscillatory changes in membrane potential do occur, but it will be shown below that these are the consequence and not the cause of the depolarization.

Oscillations and action potentials

Oscillations and trains of action potentials (Figs. 12 and 13) were often seen when the depolarization was greater than about 10–20 mV. They

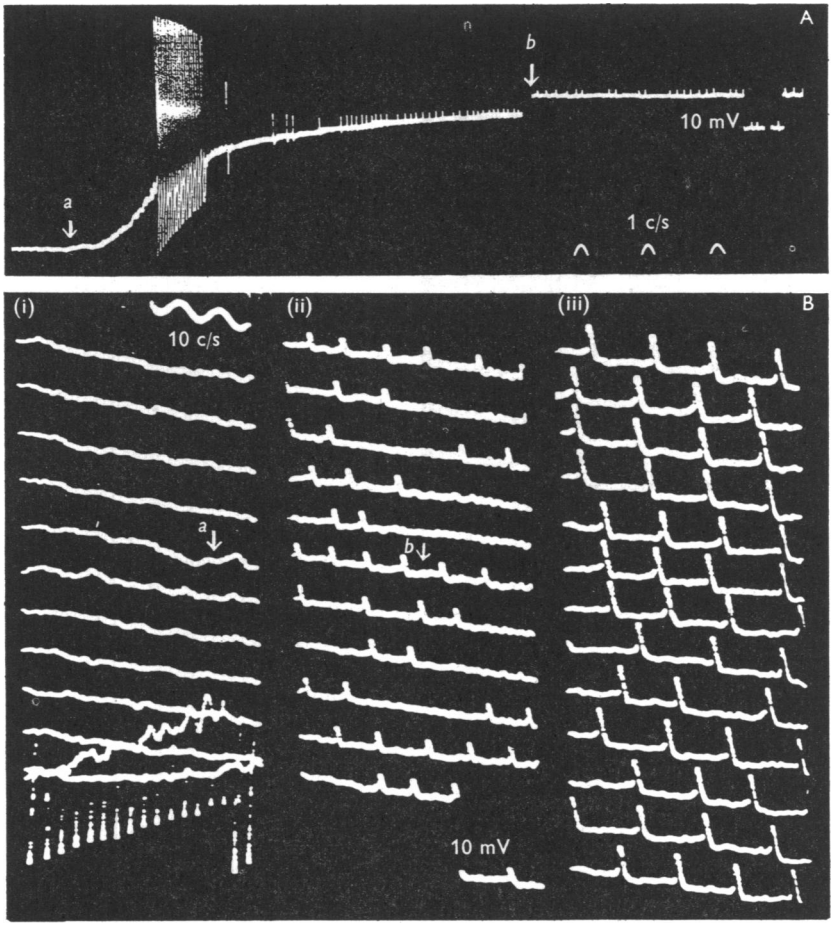

Fig. 12. Depolarization, oscillations and action potentials caused by the addition of carbachol to bath (final concentration 4 mM). Cell 73. A, drug added to bath at beginning of record; gap at *b* corresponds to period of 44 sec. B, film moving perpendicularly to free-running sweep of second oscilloscope; time increases from left to right and from above downwards; upward movement of an individual trace indicates depolarization. In (i) and (ii) letters *a* and *b* correspond to times indicated by same letters in A. B (iii) begins 3 min after addition of drug to the bath.

were probably similar in origin to those seen in other types of cell (see Hodgkin, 1951 and Huxley, 1959 for references and theory). The drug-induced action potentials were of the same amplitude as those evoked by antidromic test-stimuli and different in amplitude from orthodromic responses (Fig. 14, see also Figs. 5C and 8); even when small (as e.g. Fig. 12) they occluded responses to antidromic stimuli which happened to have been timed appropriately and were thus propagated along the post-synaptic axon (presumably with normal amplitude).

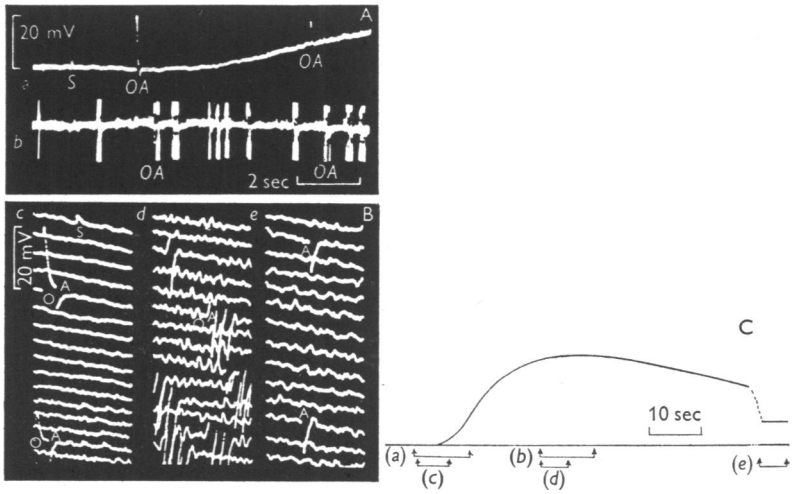

Fig. 13. Oscillations and action potentials accompanying depolarization due to carbachol (final concentration 6×10^{-5} M). Cell 58. The time course of the experiment is shown in C which indicates periods corresponding to records shown in A and B. In B film moving perpendicularly to oscilloscope sweep, time increasing from left to right and from above downwards; depolarization corresponds to decreased separation between successive traces. Trace length 575 msec. *S*, spontaneous synaptic potential, *O* and *A* indicate orthodromic and antidromic stimuli. The full amplitude of the action potentials cannot be seen in A *b*, and only the negative wave of the orthodromic, and the after-hyperpolarization of the antidromic and drug-induced action potentials can be seen in A and B.

That the 'drug-induced' oscillations and action potentials were the direct consequence of the depolarization was indicated by the effects observed when the membrane potential was altered by currents passed across the cell membrane. Thus in a number of experiments the oscillations were reduced in amplitude and the action potentials suppressed when large inward currents were used to reduce the 'drug-induced' depolarization. Furthermore, as in Fig. 15, the effects of a drug-induced depolarization were very similar to those produced by an outward current passed across the membrane.

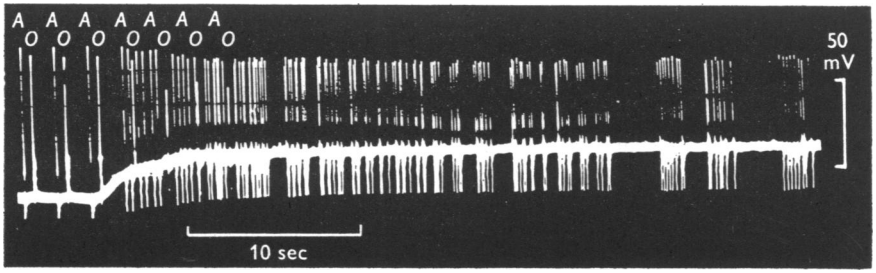

Fig. 14. Distinction between 'orthodromic' and 'drug-induced' action potentials. Carbachol added to bath to give final concentration of 4×10^{-5} M. *A* and *O* indicate responses to antidromic and orthodromic stimuli. The remaining action potentials were 'drug-induced'. Note the very small amplitude of the last three orthodromic action potentials, possibly due to a slow rate of rise and long duration of synaptic step and a consequently enhanced 'Na-inactivation' (see Blackman *et al.* 1963*a*, p. 369).

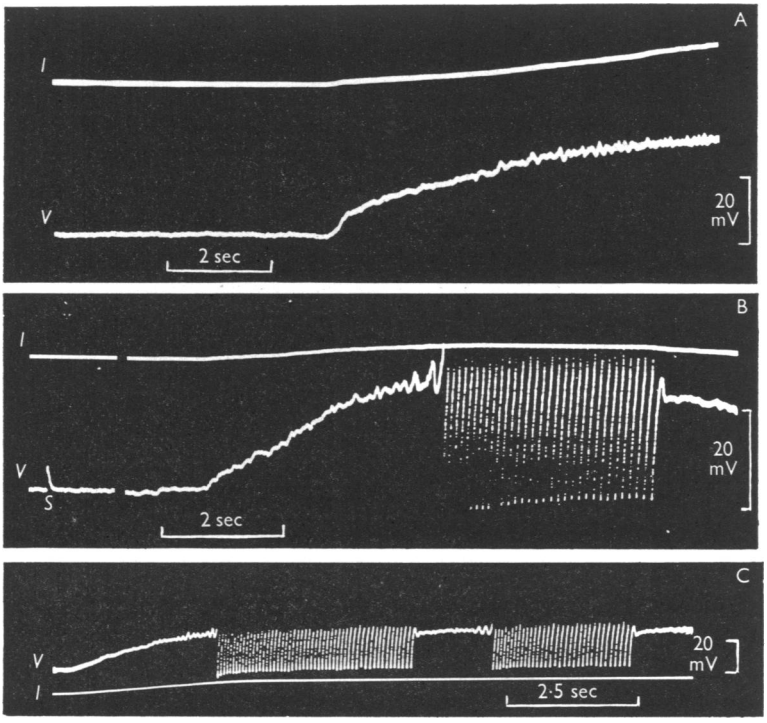

Fig. 15. Oscillations and action potentials (*V*) produced by depolarizing currents (*I*) in three different cells. Only the after-hyperpolarization of the action potentials can be seen. In C, tubocurarine, 10^{-4} M, was present in the bathing fluid. In each record the 20 mV calibration in *V* corresponds to about 2×10^{-9} A in *I*. The letter *S* in B indicates a spontaneous synaptic potential.

However, one type of 'drug-induced' response which was not mimicked by direct depolarization was the train of action potentials of small amplitude seen for example in Fig. 12. This may be related to the fact that the resistance of a cell when depolarized by a drug was very much smaller than when depolarized to the same degree by a current. If, as seems probable, the action potentials are initiated in the post-synaptic axon adjacent to the cell body, they should be attentuated to a greater extent in the cells depolarized by the action of a drug than in those depolarized by current.

DISCUSSION

The pharmacological reactions of ganglion cells of the frog appear similar to those of mammalian sympathetic ganglion cells, as described by Paton & Perry (1953). There are, however, two minor differences between the results reported in this paper and those reported by Paton & Perry. From the present experiments it appears that the resting potential of sympathetic ganglion cells of the frog cannot be entirely annulled even by very high concentrations of depolarizing substances, whereas in records made with external electrodes from the superior cervical ganglion of the cat (Paton & Perry, 1953), the amplitude of the depolarization appeared greater than that of the action potential. However as has already been pointed out (Pascoe, 1956; Perry, 1957) because of the temporal dispersion of the action potentials of the individual cells of a multicellular preparation, the ratio between a steady depolarization and the amplitude of a compound action cannot be expected to give a reliable estimate of the ratio between the magnitudes of the action potential and depolarization of a single cell.

It may also be noted (cf. Paton & Perry, 1953) that the degree of blockade of transmission during depolarization cannot readily be deduced from the reduction in the amplitude of the action potential recorded from the whole ganglion with external electrodes; this follows from the fact that the amplitude of the orthodromic action potential of a single cell body is reduced during depolarization, although it is propagated along the post-synaptic axon (presumably with a normal amplitude).

The second difference concerns the action of tetramethylammonium. This drug occupied a special position among the ganglion blocking agents tested by Paton and Perry in that its action was confined to the period during which a depolarization was evident. In the present experiments, all the depolarizing drugs tested produced a persistent blockade, in the presence of the drug, which outlasted the depolarization. It is not clear if this discrepancy represents a difference between the properties of sympathetic ganglion cells of the cat and those of the frog, but it is of interest in this connexion that tetramethylammonium causes a persistent blockade of transmission which outlasts the depolarization in the isolated superior cervical ganglion of the rat (Shand, 1962).

No evidence has been found to suggest that the depolarizing and stimulating actions of the drugs were due to an effect on the presynaptic nerve.

Thus, there appeared to be no obvious increase in the frequency of spontaneous synaptic potentials associated with the depolarization, and the 'drug-induced' action potentials were distinct from those which resulted from orthodromic stimulation; they could therefore not have been due to stimulation of the preganglionic nerve by the drug.

SUMMARY

1. A study has been made of the electrical changes, in sympathetic ganglion cells of the frog, caused by the application of a number of drugs and recorded with intracellular electrodes.

2. Acetylcholine, carbachol, nicotine and tetramethylammonium depolarized the ganglion cells and depressed synaptic transmission. In the continued presence of any of these drugs the depolarization subsided but the depression of synaptic transmission persisted. Succinyl choline and decamethonium in higher concentrations depressed transmission without causing any initial depolarization.

3. The depolarization was often accompanied by small oscillatory changes in membrane potential and by action potentials. These responses were apparently a direct result of the depolarization and were not due to a 'drug-induced' release of transmitter from the presynaptic nerve.

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