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Synaptic potentials recorded by the sucrose-gap method from the rabbit superior cervical ganglion

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Summary

1. Compound ganglionic potentials evoked by stimulation of the preganglionic nerves to the superior cervical ganglion of the rabbit were recorded by the sucrose-gap method.

2. When the distal part of the ganglion was bathed in flowing isotonic sucrose solution or sodium-deficient solutions, ganglionic action potentials were no longer evoked, only large synaptic potentials.

3. The compound synaptic potential, which remained unaltered for more than 1 h, originated in a population of cells at the interface between the Krebs and sucrose solutions. Hexamethonium reduced the size but did not alter the time course of the synaptic potential.

4. It is suggested that a higher concentration of sodium ions is required for the generation of ganglionic action potentials than for either conduction in the postganglionic axons or production of synaptic potentials.

5. When lithium replaced sodium in the solution bathing the distal part of the ganglion, the synaptic potential was greatly reduced in amplitude. Impulse propagation in the postganglionic axons was only slightly impaired when lithium replaced sodium in the solution bathing the axons.

6. A quantitative assessment of the potency of the ganglion-blocking drugs nicotine, pentolinium, hexamethonium and pempidine was made by measuring the depression of the synaptic potentials produced by bathing the distal part of the ganglion in flowing isotonic sucrose solution. The concentrations which produced a 50% depression were 8·1 μM nicotine, 26·5 μM pentolinium, 111 μM hexamethonium and 22·2 μM pempidine.

Introduction

In previous papers, the sucrose-gap method has been used to record evoked action and resting potentials from the isolated superior cervical ganglion of the rabbit (Kosterlitz & Wallis, 1966b; Kosterlitz, Lees & Wallis, 1968). Evidence is now presented that orthodromic stimulation of ganglion cells bathed in isotonic sucrose solution evokes large synaptic potentials but no action potentials. In frog skeletal muscle, the initiation of action potentials is blocked when 80% of the sodium ions of the bathing solution are replaced by an equivalent amount of

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sucrose, but large end-plate potentials are still obtained (Fatt & Katz, 1951). Similarly, it is possible that in the superior cervical ganglion also, lack of sodium ions prevents spike initiation. This technique makes possible an analysis of ganglionic synaptic potentials without the necessity of using ganglion-blocking drugs or of reducing the amount of transmitter released, for example, by altering the concentrations of calcium or magnesium ions.

Methods

Preparation. Superior cervical ganglia were removed from adult New Zealand White rabbits (2·7–4·6 kg) anaesthetized with urethane (1·25–1·5 g/kg) given intravenously as a 25% (w/v) solution. The ganglia were prepared for insertion into the sucrose-gap apparatus by the method previously described (Kosterlitz *et al.*, 1968).

Sucrose-gap apparatus. The apparatus was essentially that described by Kosterlitz *et al.* (1968) and Kosterlitz & Wallis (1966b) with the following modification: for the purpose of applying solutions of different compositions to the distal part of the ganglion, a second set of rubber membranes was used (Fig. 1). In order to achieve

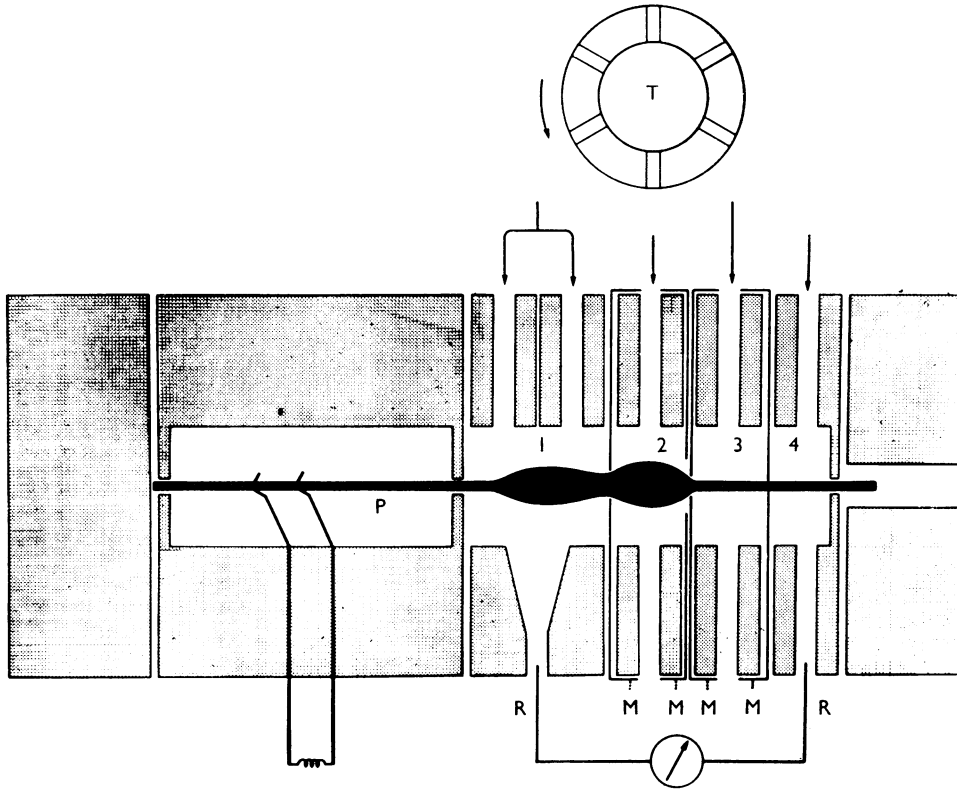

FIG. 1. Arrangement of components of sucrose-gap apparatus for recording evoked synaptic potentials. Note that the distal part of the ganglion lies in a separate chamber from the rest of the ganglion. Shaded areas indicate Perspex units which are held together tightly by means of a Perspex clamp. 1 and 4, Krebs or Locke solution; 2 and 3, isotonic sucrose solution; M, rubber membrane; P, paraffin pool with platinum stimulating electrodes on preganglionic trunk; R, recording electrodes; T, tap used to change solution flowing through 2.

a precise fit of the membrane around the appropriate portion of the ganglion, a punch of suitable size was selected after careful inspection of the desheathed ganglion. Irregularities in the contour of the ganglion made it almost impossible to attain as good a fit as on the internal carotid nerve. Thus, when solutions of altered composition flowed over only part of the ganglion, diffusion artefacts now contributed significantly to changes in resting potential. In this paper, reference will not be made to drug-induced changes in resting potential under these conditions. In most figures, a diagram is given of the arrangement for recording; the shape of the ganglion used in that experiment is drawn to scale with respect to the positions of the membranes on the ganglion and internal carotid nerve. The conditions for stimulation and recording were the same as those previously described.

Apparatus for recording with surface electrodes in a moist chamber

Antidromically conducted action potentials were recorded by platinum electrodes arranged in a moist chamber (Eccles, 1952a). The chamber, which contained Krebs solution, was partly immersed in a thermostatically controlled water bath at 37·5° ±0·5° C; not less than 90 s before the start of recording, the chamber was tilted to lift the preparation out of the Krebs solution. One of the pair of recording electrodes was movable and attached to a pointer which traversed a scale; whenever the electrode was moved to a new point of recording, its position on the scale was noted. At the end of the experiment, the ganglion was inspected under a microscope to determine the point on the scale which corresponded to the distal end of the ganglion. The recording electrodes were connected to the oscilloscope via a low grid-current cathode follower and an RC-coupled preamplifier with a time constant of 0·2 s.

Solutions. All solutions were made up with glass-distilled water. The Krebs solution had the following composition (mM): NaCl 118, KCl 4·75, $CaCl_2$ 2·54, KH_2PO_4 1·19, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 11; it was gassed with 5% carbon dioxide and 95% oxygen. In experiments in which the effects of alterations in Na^+ concentrations were examined, a modified Locke solution (mM) was used: NaCl 143·5, KCl 5·94, $CaCl_2$ 2·54, sodium phosphate buffer 1·0 (pH 7·3) and glucose 11: it was gassed with oxygen. NaCl was replaced by an equivalent amount of sucrose; choline chloride was not used because it depolarizes the ganglion cells and their postganglionic fibres in this preparation (Kosterlitz *et al.*, 1968). The concentration of the sucrose solution was 315 mM and taken to be isotonic. Experiments were carried out at temperatures between 22° and 27° C; in any one experiment, the temperature varied by less than 1° C.

Drugs. The drugs used were hexamethonium bromide, pentolinium tartrate, pempidine tartrate, nicotine hydrogen tartrate and (+)-tubocurarine chloride. The stock solutions were made with modified Locke solution without sodium phosphate buffer. The concentrations (μM) refer to the bases.

Results

Effects of sucrose solution on ganglionic potentials

When the distal part of the ganglion was bathed in flowing isotonic sucrose solution, a large, broad-peaked, slowly decaying potential was evoked by stimulation

of the preganglionic nerves. Figure 2 illustrates the development of this synaptic-like potential from the action potential (a). After 5 min exposure to sucrose solution (b), the rate of rise and particularly the rate of decay of the potential were decreased. At a later stage (c), the rising phase showed a pronounced inflection between the apparent synaptic potential and the residuum of the action potential; the synaptic-like potential was fully established after 30 min exposure to the sucrose solution (d) and remained unchanged in amplitude and shape for a further 80 min (e). When the synaptic-like potential was fully developed, the mean time of half-decay was at least twice that observed for the action potential before exposure to sucrose. The mean amplitude of the evoked action potential was $13{\cdot}07 \pm 0{\cdot}36$ (S.E. of mean) mV and the mean amplitude of the synaptic-like potential was $5{\cdot}56 \pm 0{\cdot}28$ mV ($n=38$). The shape of this potential closely resembled synaptic potentials recorded with conventional surface-electrodes from this ganglion when transmission was prevented by a ganglion-blocking agent (Eccles, 1952a, b; Eccles & Libet, 1961; Libet, 1964, 1967; Kosterlitz & Wallis, 1966a).

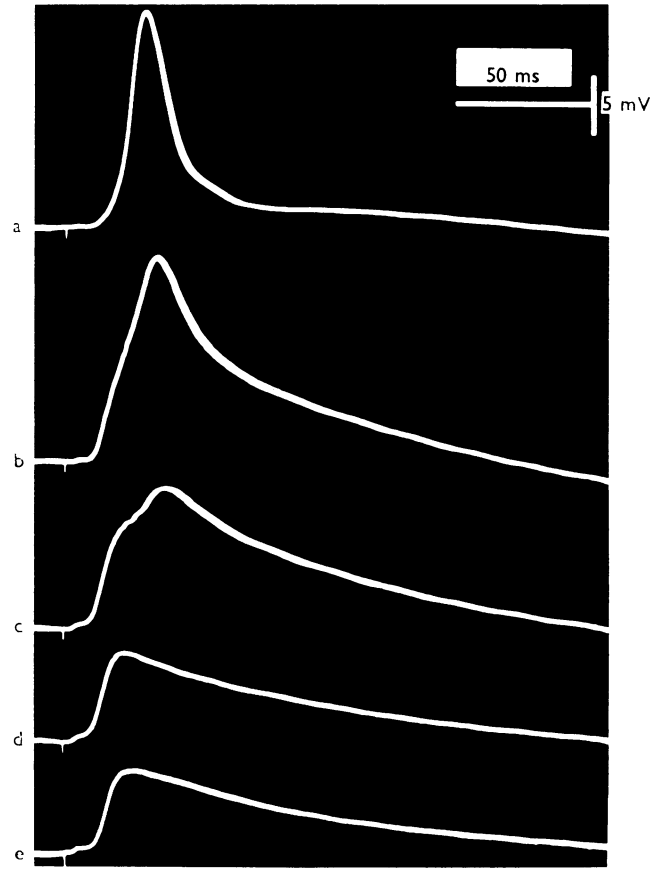

FIG. 2. Alteration in shape of the evoked potential from that typical of a ganglionic action potential to that of a synaptic potential when isotonic sucrose solution flows through chamber 2. Fig. 1 shows the experimental arrangement. a, Krebs solution in 2, record of ganglionic action potential; b, c, d and e, 5, 11, 30 and 110 min after exposure of distal part of the ganglion to flowing isotonic sucrose solution. Stimulus initially maximal for Sa deflection, parameters constant throughout experiment.

If the assumption is correct that the synaptic-like potential is an excitatory post-synaptic potential, hexamethonium ought to diminish its amplitude without altering its shape. Indeed, hexamethonium had this effect (Fig. 3B). On the other hand, when a ganglionic action potential was recorded, for example while surrounding the ganglion with Krebs solution and the internal carotid nerve with isotonic sucrose solution, hexamethonium not only reduced the amplitude of the potential but also greatly altered its shape (Fig. 3A).

A characteristic feature of a synaptic potential is the decrement of its amplitude as it spreads electrotonically from its site of origin. If it is true that the exposure of the distal part of the ganglion to isotonic sucrose solution blocks generation of propagated responses, then postganglionic action potentials will not be recorded from the postganglionic fibres, notwithstanding the presence of large synaptic potentials. In order to test this hypothesis, the ganglion and the proximal part of the internal carotid nerve were suspended across chambers 1 to 4 as usual, while the remainder of the nerve lay on a pair of platinum recording electrodes in paraffin; for this type of experiment, ganglia with exceptionally long internal carotid nerves (12–15 mm) were required. The results of one of two experiments are given in Fig. 4. Record (a) shows, in the upper trace, a ganglionic potential which is small and biphasic because chamber 3 contained Krebs solution and not isotonic sucrose solution and, in the lower trace, a large propagated postganglionic response. On bathing the distal part of the ganglion in sucrose solution (chamber 2), the propagated response in the internal carotid nerve was greatly diminished (b, lower trace) and a synaptic-like potential was recorded from the ganglion (b, upper trace). This effect of sucrose solution on the distal part of the ganglion was reversible (c). When sucrose solution flowed through chamber 3 only, a ganglionic action potential was recorded (d, upper trace) and the propagated response in the internal

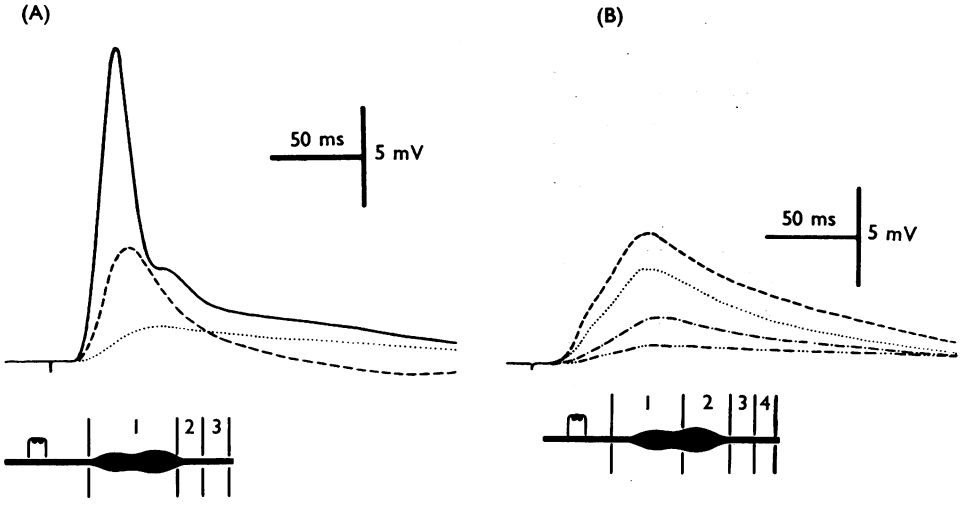

FIG. 3. Effect of hexamethonium on ganglionic action potential (A) and synaptic-like potential (B). (A) and (B) from different preparations. (A), Sucrose in chamber 2 throughout experiment. ——, control; - - - -, hexamethonium (550 μM) in chamber 1 for 10 min; . . . . , hexamethonium in chamber 1 for 68 min. (B), Sucrose in chambers 2 and 3 throughout experiment. - - - -, control; . . . . , hexamethonium (550 μM) in chamber 1 for 5 min; - . - . , hexamethonium in chamber 1 for 10 min; - . . . - , hexamethonium in chamber 1 for 27 min. Stimulus maximal for Sa deflection.

carotid nerve was abolished (d, lower trace). When, now, sucrose solution also surrounded the distal part of the ganglion, the synaptic-like potential reappeared (e, upper trace).

Nature of the recorded potential

Site of recording. The evidence presented so far strongly suggests that the evoked potential, which is recorded when the distal part of the ganglion is bathed in sucrose solution, is a synaptic potential. Since this synaptic potential has its origin not in one cell but in a population of cells, it is a compound potential. Histological studies have not yet located the ganglion cells whose axons form the internal carotid nerve, because it is difficult to trace cell processes within the ganglion for long distances (Huber, 1899 ; Ranson & Billingsley, 1918 ; Elfvin, 1963). With the sucrose-gap method, one would expect the principal site of recording to be at the liquid junction formed by the saline and sucrose solutions. That this is so was confirmed by two kinds of experiments ; in the first, recordings were made with a

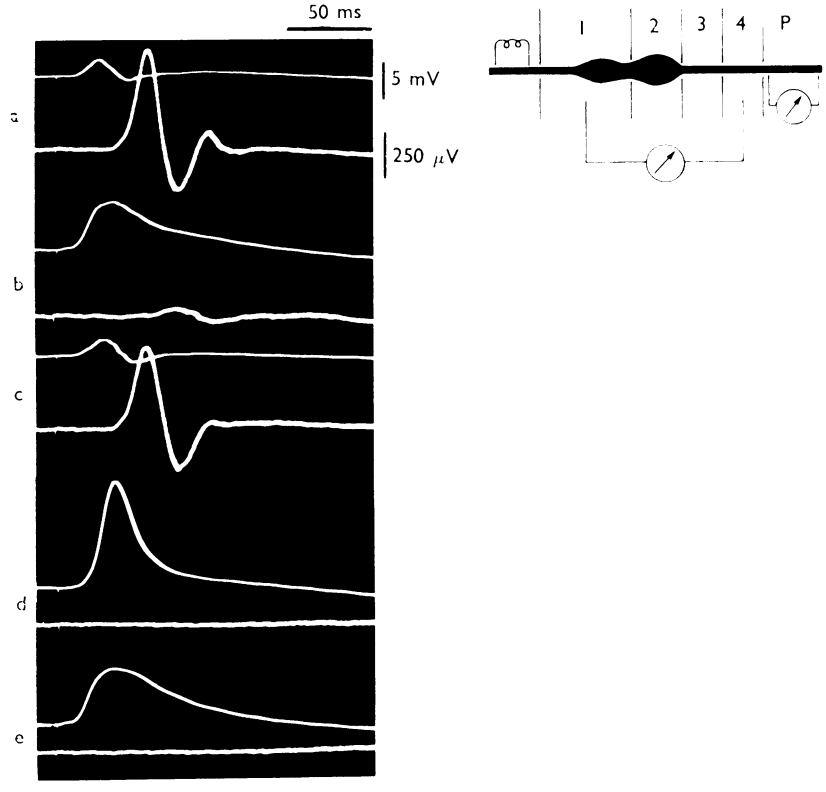

FIG. 4. Effect of altered ionic composition of bathing solutions on generation of propagated action potentials. a, b, c, d and e, five pairs of tracings: upper trace, recording from ganglion (chamber 1) with reference to middle part of internal carotid nerve (chamber 4) ; lower trace, recording from distal part of internal carotid nerve with platinum electrodes in paraffin pool, P. Chambers 1 and 4 contained phosphate-buffered Locke solution in each part of experiment. a, Locke solution in chambers 2 and 3 ; b, isotonic sucrose solution in chamber 2, Locke solution in chamber 3 ; c, Locke solution in chambers 2 and 3 ; d, Locke solution in chamber 2, sucrose solution in chamber 3 ; e, sucrose solution in chambers 2 and 3. Upper voltage calibration signal applies to all upper traces ; lower voltage calibration signal applies to all lower traces. Stimulus maximal for Sa deflection. For details see text.

small discrete platinum electrode from different parts of the ganglion with the saline-sucrose interface in a constant position ; in the other, the position of the interface between sucrose and saline solutions was altered while continuing to lead from the same two chambers.

The first type of experiment is illustrated in Fig. 5. For discrete recording a thin platinum wire was insulated with closely fitting polythene tubing, which projected just beyond the tip of the wire ; the cut end of the tubing was placed gently on the ganglion. This electrode was placed in turn on the parts of the ganglion in chambers 1, 2 and 3 indicated by the white spots, while the reference platinum electrode touched the wick draining chamber 6. The potentials obtained in this way were compared with those recorded from the calomel electrodes touching the wicks draining chambers 1 and 6. It was found that the potential recorded by the discrete electrode was comparable to that recorded across the calomel electrodes only when

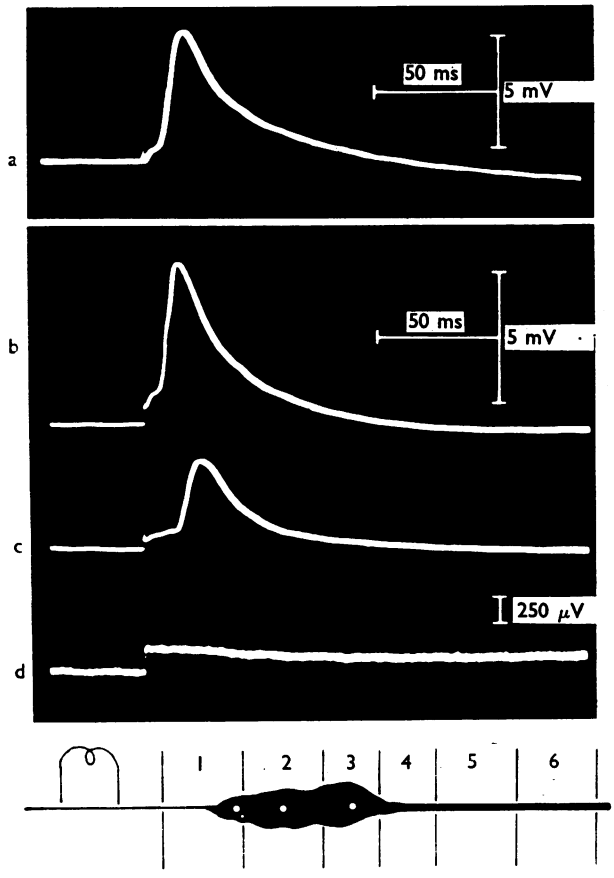

FIG. 5. Comparison of potentials recorded with two types of extracellular electrode across the sucrose gap. For each trace, chambers 1 and 6 contained Krebs solution while chambers 2, 3, 4 and 5 contained isotonic sucrose solution. a, Record obtained through calomel electrode touching wick leading from chamber 1 with reference to electrode touching wick from chamber 6 ; potential constant in amplitude and shape throughout experiment. b, c and d, Potentials recorded from different parts of the surface of the ganglion with a discrete electrode (see text) ; the points of recording are indicated by white dots in chambers 1, 2 and 3 respectively, reference electrode leading from chamber 6 for all three traces. In lower panel, upper calibration signals apply to b and c ; time calibration for b, c and d. Parameters of stimulation constant throughout experiment.

the discrete electrode was placed as close as possible to the interface between 1 and 2 (compare Fig. 5a and b). In chamber 1 the position of the discrete electrode was near but not at the saline-sucrose interface, a fact which accounts for the slight difference in the times to peak of the deflection in records a and b. These findings agree well with the observations of Julian, Moore & Goldman (1962), who compared the potentials recorded by the sucrose-gap method with the potentials obtained with a microelectrode inserted into a lobster giant axon in a single position, which was as little as 50 μm from the sea water-sucrose interface. They found that the potentials recorded by these procedures were identical.

In the second type of experiment, the saline-sucrose interface was moved progressively nearer the stimulating electrodes on the preganglionic nerve. When isotonic sucrose solution replaced Krebs solution round the distal part of the ganglion (chamber 3, Fig. 6), the evoked ganglionic potential (Fig. 6a) was replaced by a synaptic potential (Fig. 6b). By the inclusion of more of the ganglion in the sucrose gap the delay to peak of the deflection was shortened (Fig. 6c); the amplitude of the potential was reduced although shunting between the recording electrodes was decreased and temporal dispersion diminished. This finding suggested that, as the interface was moved proximally, fewer ganglion cells were in continuity with the fibres of the internal carotid nerve to contribute to the recorded potentials. Presumably, some of the proximal ganglion cells send axons into the external

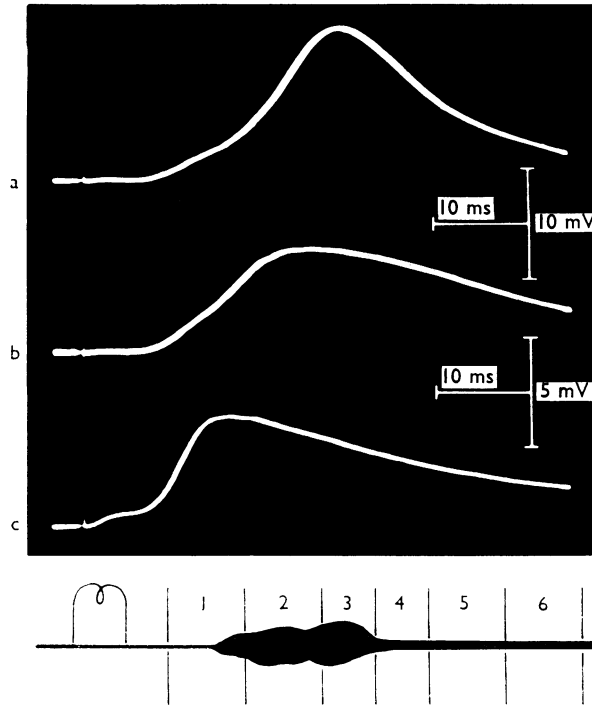

FIG. 6. Alteration in latency to peak of potential with changes in position of Krebs-sucrose solution interface. Leading throughout experiment from chambers 1 and 6, which contained Krebs solution. Isotonic sucrose solution in chambers 4 and 5 throughout experiment. a, Krebs solution in chambers 2 and 3. b, Krebs solution in chamber 2, sucrose solution in chamber 3. c, Sucrose solution in chambers 2 and 3. Upper calibration signals apply to a and b, lower signals to c. Parameters of stimulation same in a, b and c.

carotid nerve or other branches from the ganglion. To test this conclusion, the extent of the antidromic invasion from the internal carotid nerve was investigated in four ganglia of the most commonly occurring shapes. The preparation was mounted in a moist chamber; the arrangements of the stimulating and recording electrodes are illustrated in Fig. 7. In each preparation, the amplitude of the antidromic action potential diminished greatly as the movable recording electrode was shifted from the distal to the proximal part of the ganglion, with the reference electrode on the preganglionic nerve.

It should be noted that, to produce a synaptic potential, the saline-sucrose interface had to be moved in a proximal direction from the distal pole of the ganglion (Fig. 2); for this reason the synaptic potential is recorded from a cell population different from that giving rise to the ganglionic action potential, which is recorded with sucrose solution flowing around the proximal part of the internal carotid nerve. This view is supported by the somewhat low correlation (0·436, $P<0·02$) between the mean amplitudes of the synaptic and action potentials.

Some ionic requirements for the generation of action potentials and the P wave

Because of the presence of rubber membranes, which acted as closely fitting external barriers, the interface between the saline and sucrose solutions was almost

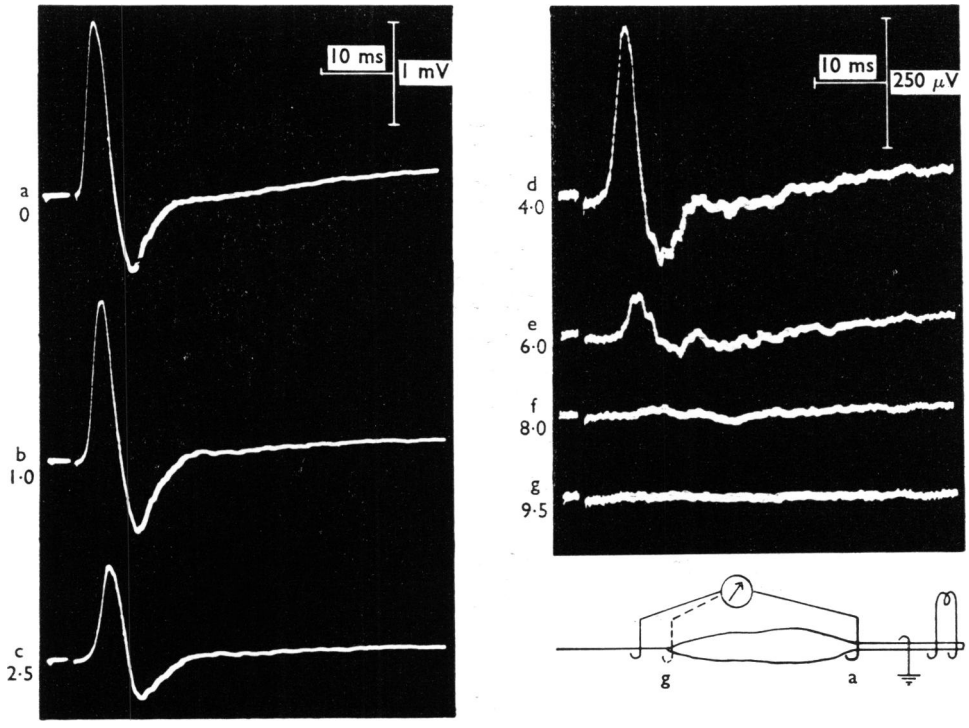

FIG. 7. Extent of antidromic invasion of ganglion. The arrangement for recording with surface electrodes in a moist chamber is shown in the diagram; stimulating electrodes on the internal carotid nerve. Letters refer to the position on the ganglion of the movable recording electrode and to the traces recorded at that position. a, Extreme distal end of ganglion; g, proximal pole of ganglion; b, c, d, e and f, intermediate recording positions. Numbers below letters refer to distances (mm) of recording position from distal end of ganglion (a). Positions c and e correspond to widest portion of distal and proximal parts of the ganglion respectively. Note that amplification was increased ×5 for records d–g. Stimulus supramaximal.

exclusively within the tissue. Thus it was assumed that there would be a region in the tissue without sharply defined boundary in which the ionic concentrations of the extracellular fluid were lowered. It was, therefore, decided to investigate the effects of graded reductions in the concentrations of Na^+ on the recorded potentials.

In four experiments, ganglionic potentials were recorded by the sucrose-gap method during exposure of the distal part of the ganglion to 144, 60, 30 or 15 mM Na^+-Locke solutions. In each preparation, slow depolarizations of 3–7 mV occurred under these conditions. When 30 or 15 mM Na^+-Locke was used, the action potential was replaced by a synaptic potential (Fig. 8). The P wave was more depressed than the preceding negative potential. In four experiments, the amplitude of the P wave was reduced by $36{\cdot}8 \pm 3{\cdot}3$, $64{\cdot}1 \pm 5{\cdot}3$ and $70{\cdot}5 \pm 11{\cdot}4\%$ ($\pm$S.E.) in 60, 30 and 15 mM Na^+-Locke solutions, respectively, while the amplitude of the original action potential was decreased by $14{\cdot}3 \pm 2{\cdot}8$, $30{\cdot}0 \pm 9{\cdot}4$ and $46{\cdot}2 \pm 17{\cdot}3\%$ ($\pm$S.E.). When isotonic sucrose solution bathed the distal part of the ganglion, the P wave was less than one-tenth of its original size (Fig. 9).

In four experiments, the effects of low Na^+ concentrations on the axons and the ganglion cells were compared. Propagated action potentials evoked by stimulation of the preganglionic nerve were recorded from the distal part of the internal carotid nerve. By means of partitions, sodium-deficient solutions flowed round either the distal part of the ganglion or the proximal part of the internal carotid nerve. The effects of modified Locke solutions, containing 144, 60, 30 and 15 mM Na^+, were tested on the initiation of the ganglionic action potential and on impulse conduction in the axons. The reduction in amplitude of the action potential and

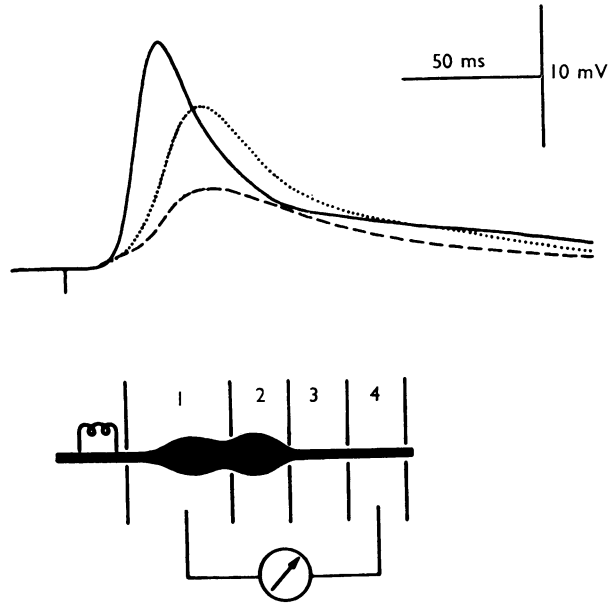

FIG. 8. Effect of low Na^+-Locke solutions on shape of evoked potential recorded by sucrose-gap method. Recording throughout experiment from chambers 1 and 4, which contained Na^+-Locke solution; isotonic sucrose solution in chamber 3 throughout experiment. ——, 144 mM Na^+-Locke solution in chamber 2; . . . . , 30 mM Na^+-Locke solution in chamber 2 for 40 min; - - - -, 15 mM Na^+-Locke in chamber 2 for 18 min. Stimulus maximal for Sa deflection.

the increase in latency to peak of the response were always less when sodium-deficient solutions flowed round the internal carotid nerve (Fig. 10A) than when they flowed round the ganglion (Fig. 10B).

In two experiments of the types just described, Li^+ replaced Na^+ in the Locke solution and single stimuli were applied every 5 min. Ganglionic transmission became progressively blocked, the amplitude of the synaptic potential was reduced by 80% and the P wave was abolished; these findings were obtained after 20–30 min exposure to Li^+, even when, during this period, the preganglionic nerve was not stimulated prior to the test stimulus at the end of this period. On the other hand, when Li^+-Locke bathed the internal carotid nerve, the amplitude of the nerve action potential was reduced by not more than 30%.

Effect of ganglion-blocking agents on synaptic potentials

It is possible to assess quantitatively the relative potencies of ganglion-blocking drugs of different types on the synaptic potentials obtained by bathing the distal

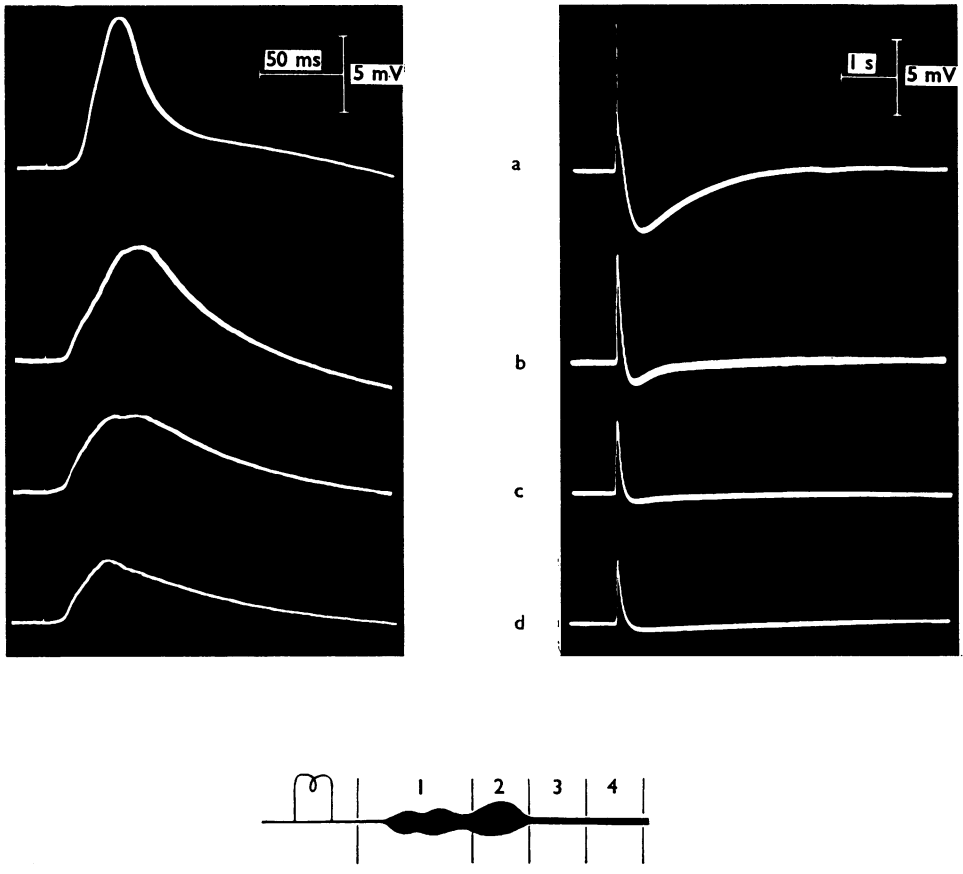

FIG. 9. Effect of isotonic sucrose solution on ganglionic action potential and P wave. Recording from chambers 1 and 4 throughout, slower sweep in right-hand panel to show P wave. Krebs solution in chambers 1 and 4 throughout experiment; isotonic sucrose solution in chamber 3 throughout experiment; a, Krebs solution in chamber 2; b, c and d, isotonic sucrose solution in chamber 2 for 10, 15 and 30 min respectively. Stimulus maximal for Sa deflection.

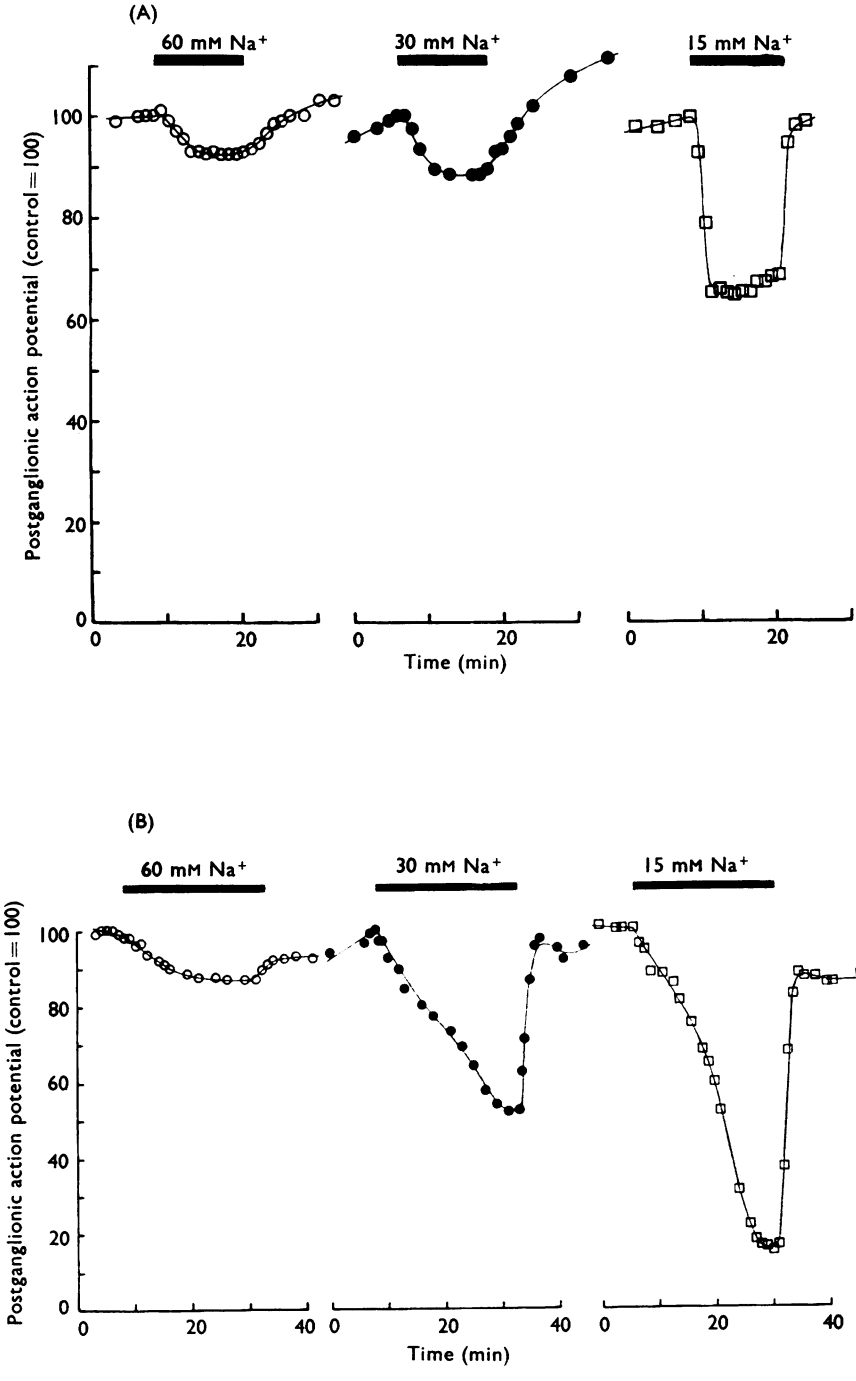

FIG. 10. Effect of low Na^+-Locke solutions on axonal conduction (A) and ganglionic transmission (B) in the same preparation. Recording from distal part of internal carotid nerve with platinum electrodes in paraffin in A and B. Ordinates, amplitude of propagated action potential as percentage of control; abscissae, time (min). 60, 30 and 15 mM Na^+-Locke solutions applied to internal carotid nerve in chamber 3 (A) and to distal part of ganglion in chamber 2 (B), for periods indicated by black bars. Stimulus maximal for Sa deflection throughout experiment.

part of the ganglion with isotonic sucrose solution. Quantitative assessment of potency cannot be made when action potentials are present, because, in any one cell, spike initiation occurs until the synaptic potential is depressed below the threshold level for initiation ; reduction in the amplitude of the synaptic potential below this threshold is probably proportional to the concentration of the drug.

In order to compare the relative potencies of some ganglion-blocking drugs, concentration-response curves were constructed. However, for such curves to be valid, the amplitude of the synaptic potentials must remain constant sufficiently long to allow exposure to a number of different concentrations of drug. Figure 11 shows that the amplitude of the synaptic potentials remained almost unchanged for at least an hour. In other experiments, the amplitude was sufficiently constant for 3 h.

Concentration-response curves for nicotine, pentolinium, hexamethonium and pempidine are shown in Figs. 12 and 13. Since complete recovery from the depressant effects was slow, it was necessary to expose the ganglion successively to higher concentrations without washing out between exposures. The slopes of the concentration-response curves for nicotine, pentolinium and hexamethonium were not significantly different from each other, but were each significantly different from the slope of the curve for pempidine. The concentrations which produced a 50% reduction in the amplitude of the synaptic potential were 8·1 μM nicotine, 26·5 μM pentolinium, 111 μM hexamethonium and 22·2 μM pempidine. The threshold concentration of (+)-tubocurarine was about 100 μM.

By varying the strength of stimulus to the preganglionic nerve, synaptic potentials of varying size can be elicited. The lowest stimulus strengths will evoke synaptic potentials in only those ganglion cells, or some of them, with which the pre-

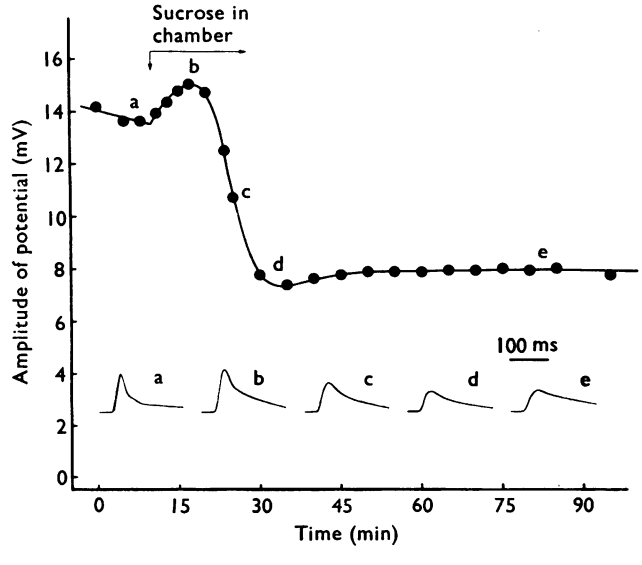

FIG. 11. Constancy of synaptic potential. Ordinate, amplitude of potential (mV). Abscissa, time (min). Experimental arrangement as in Fig. 1 ; leading from chambers 1 and 4 containing Krebs solution, throughout the experiment. Isotonic sucrose solution in chamber 3 throughout. a, Ganglionic action potential ; b, c, d and e, potentials recorded when isotonic sucrose solution also present in chamber 2, from arrow. Potentials a–e recorded at times indicated on graph. Stimulus maximal for Sa deflection.

ganglionic B fibres synapse. Further, since cells receive multiple preganglionic inputs, the lowest stimulus strengths might be expected to excite only one of a number of fibres synapsing with some cells, so that small synaptic potentials would be evoked in these cells. As stimulus strength increases, the number of cells displaying submaximal synaptic potentials decreases and the number of cells excited increases. In view of these considerations, we felt that qualitative differences in the modes of action of different ganglion-blocking drugs might possibly be unmasked by investigating their effects on synaptic potentials of varying amplitude, evoked by graded stimulation of the preganglionic nerve. However, it was found that hexamethonium and pempidine had identical effects: the amplitudes of the synaptic potentials evoked by the weaker stimuli were more reduced than those of the responses to near-maximal and maximal stimulation (Figs. 14 and 15). Similarly, the area under the potentials evoked by submaximal stimulation was more affected

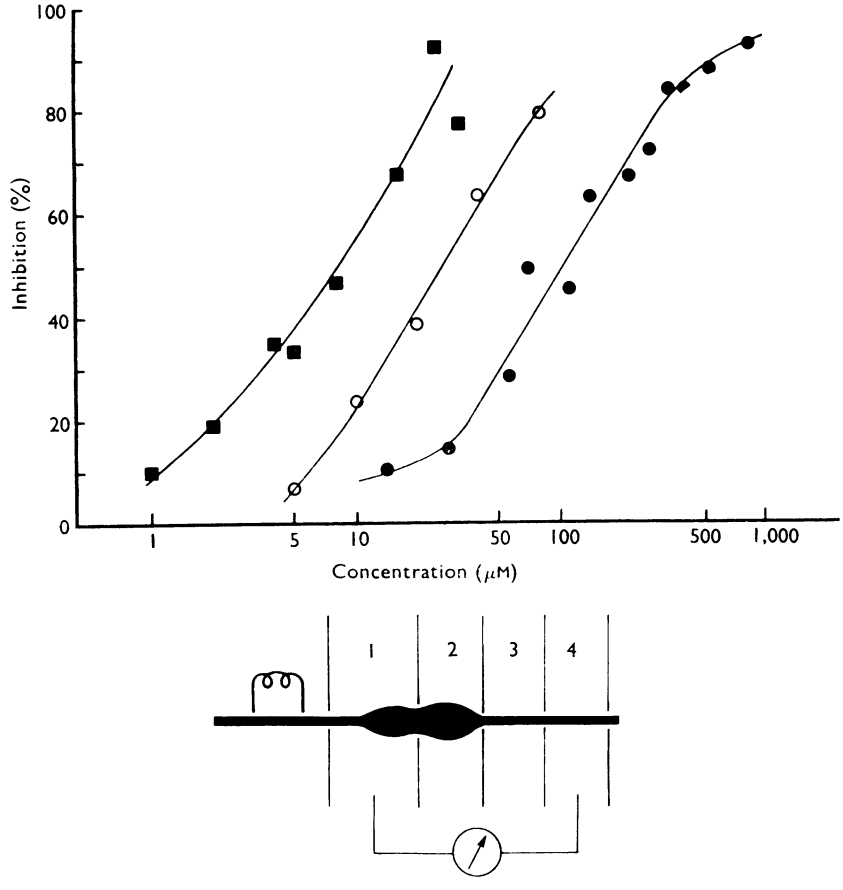

FIG. 12. Comparison of effects of ganglion-blocking drugs on synaptic potential. Synaptic potential produced by exposing ganglion in chamber 2 to flowing isotonic sucrose solution. Diagram shows experimental arrangement; Krebs solution was present in chambers 1 and 4 and sucrose solution in chambers 2 and 3 throughout each experiment. Ordinate, inhibition of synaptic potential as percentage of control. Abscissa, concentration of ganglion-blocking drug (μM). All drugs added to chamber 1 only. Points at each concentration represent mean values obtained from three experiments with nicotine (■) and six experiments with hexamethonium (●); there was only one experiment with pentolinium (○).

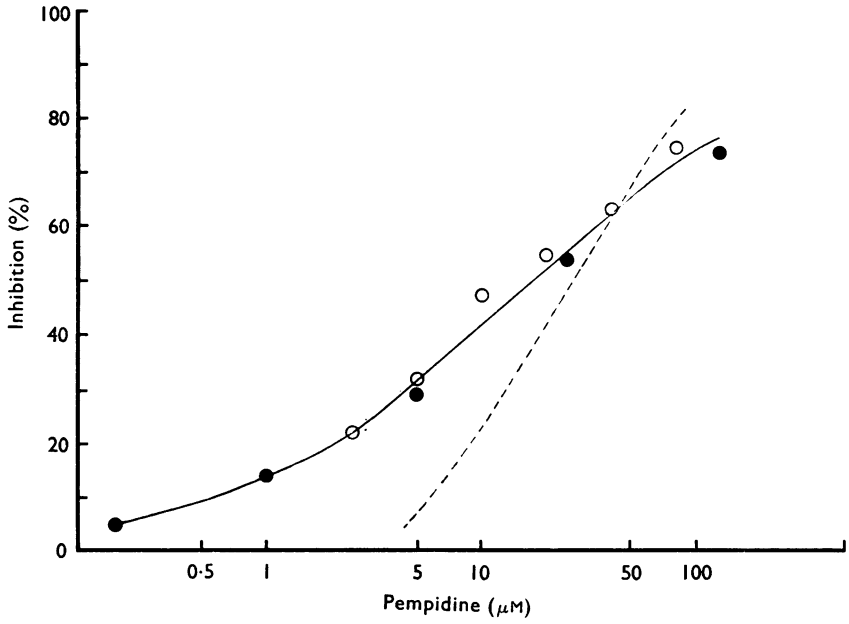

FIG. 13. Comparison of effects of ganglion-blocking drugs on synaptic potential. Arrangements same as in Fig. 12. Ordinate, inhibition of synaptic potential as percentage of control. Abscissa, concentration of pempidine (μM). Pempidine added to chamber 1 only. ●——●, ○——○, Results from two experiments. - - - -, Concentration-response curve for pentolinium (Fig. 12) is shown to facilitate comparison of slopes.

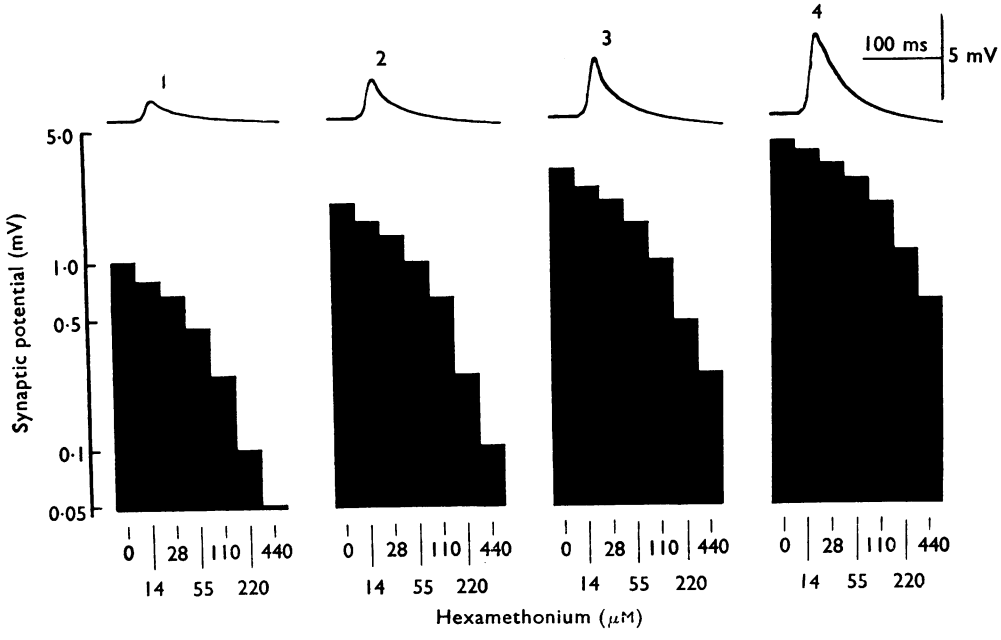

FIG. 14. Effect of hexamethonium on synaptic potentials of graded size. Synaptic potentials produced by exposure of distal part of ganglion to isotonic sucrose solution. Tracings illustrate control responses to submaximal stimulation (1, 2 and 3) and to maximal stimulation (4) of preganglionic nerves. Stimuli all 0·1 ms duration ; stimulus strengths were: 1, 3·8 V ; 2, 4·6 V ; 3, 6 V ; 4, 15 V which gave responses 23, 48, 72 and 100% of maximum respectively. Histograms: ordinates, height of synaptic potential (mV), log scale ; abscissae, concentration of hexamethonium (μM), log scale.

by both drugs than the area under the potential which resulted from maximal stimulation.

It should be noted that the effects of ganglion-blocking agents on synaptic potentials in these experiments were observed at 23°–25° C; in experiments with surface electrodes in the moist chamber, it was found that a lowering of the temperature from 37·5° C to 31°–33° C resulted in a reduction of the blocking effect of hexamethonium. This observation is similar to the findings of Holmes, Jenden & Taylor (1951) and Bigland, Goetzee, Maclagan & Zaimis (1958) for the competitive block at the neuromuscular junction by tubocurarine.

Discussion

In order to analyse the evoked ganglionic potentials, it is necessary to establish the principal site of recording and the population of cells contributing to them. On theoretical grounds, it is to be expected that the potentials recorded with the sucrose-gap technique arise principally from ganglion cells and their processes at

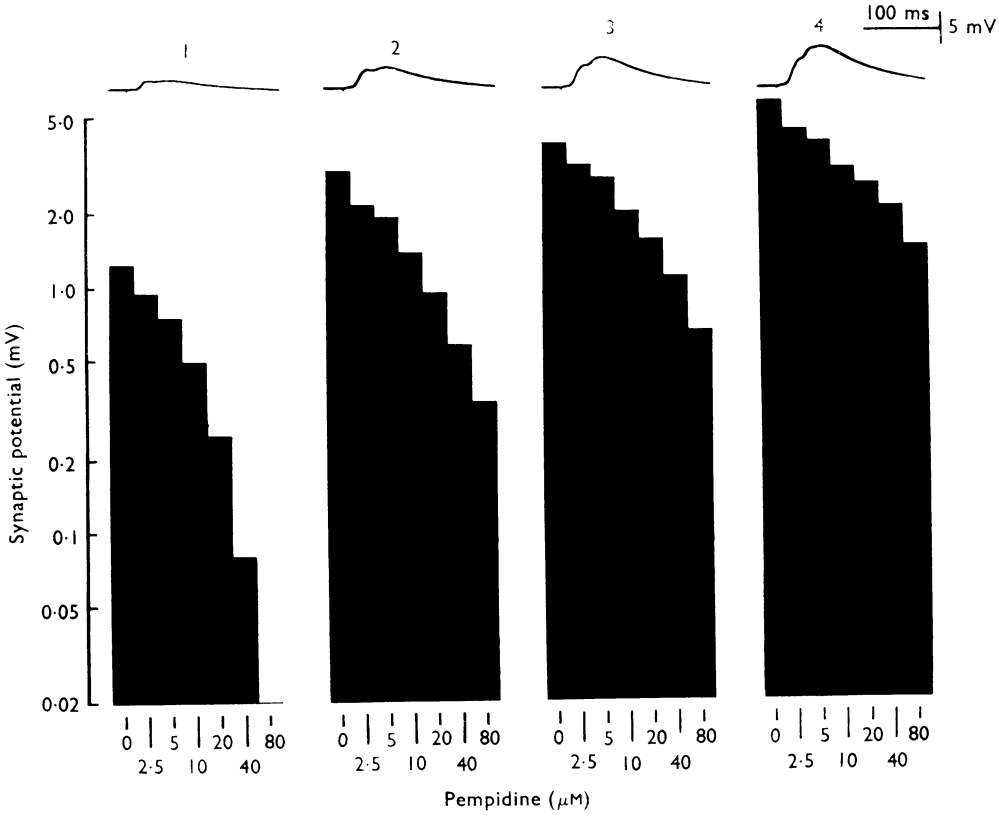

FIG. 15. Effect of pempidine on synaptic potentials of graded size. Synaptic potentials produced by exposure of distal part of ganglion to isotonic sucrose solution. Tracings illustrate control responses to submaximal stimulation (1, 2 and 3) and to maximal stimulation (4) of preganglionic nerves. Stimuli all 0·1 ms duration; stimulus strengths were: 1, 4 V; 2, 5 V; 3, 9 V; 4, 15 V which gave responses 37, 55, 69 and 100% of maximum respectively. Histogram: ordinates, height of synaptic potential (mV), log scale; abscissae, concentration of pempidine (μM), log scale.

the interfaces of the saline and sucrose solutions ; this is now confirmed by our experimental observations. Further, it is clear from the experiments in which antidromic invasion was investigated, that most of the cells whose axons leave the ganglion to form the internal carotid nerve lie in the distal 3 mm or so of the ganglion.

No single piece of evidence proves conclusively that the evoked potentials, recorded when the distal part of the ganglion is bathed in sucrose solution, are synaptic ; but, when the evidence is considered as a whole, the weight of the argument in favour of this view is substantial. First, the evoked potential recorded across the sucrose-gap has a shape and time-course similar to the synaptic potentials obtained by exposure of this ganglion to a ganglion-blocking agent. Second, this synaptic-like potential is not altered in shape during exposure of the ganglion to hexamethonium. A true synaptic potential would be reduced in amplitude but not altered in shape by hexamethonium, whereas a collection of temporally dispersed action potentials would be both reduced in amplitude and altered in shape as the action potentials are replaced by synaptic potentials. The third piece of evidence depends on the interpretation of the results of the experiment in which propagation of the synaptic-like potential into the internal carotid nerve was investigated (Fig. 4). It can be argued that evoked action potentials are not recorded from the internal carotid nerve because, in all likelihood, the extracellular concentration of cations is so low round the ganglion cells that initiation of action potentials is suppressed ; if this is correct, the evoked potential recorded from the ganglion is a synaptic potential. An alternative interpretation is that spike initiation persists but that, a short distance along the axons, conduction ceases and the ganglionic record is that of temporally dispersed action potentials. The latter possibility seems to be ruled out by the evidence obtained from the experiments with hexamethonium which decreased the amplitude of the potential but did not alter its shape. Pappano & Volle (1966a) have also recorded synaptic potentials when, in the cat *in situ*, the ionic concentration of the extracellular fluid of the superior cervical ganglion was reduced by sucrose solution.

The failure of spike initiation in the ganglion cells probably reflects a rise in the threshold for spike initiation due to diminished sodium conductance. Thus, for a given change in permeability to sodium ions, less current will flow because of the smaller number of sodium ions available to move into the cell and the lower concentration gradient. In normal circumstances, a spike is initiated before the synaptic potential reaches its maximum amplitude. A selective depression of spike initiation would therefore explain the large amplitude of the synaptic potentials. Moreover, synaptic potentials, being of relatively long duration, are less subject to temporal dispersion ; therefore, compound synaptic potentials are larger relative to the compound action potential than the synaptic potential of a single cell relative to its action potential.

Although there is undoubtedly a great reduction in the concentration of sodium ions in the extracellular spaces of those parts of the ganglion exposed to a sodium-deficient solution, the sodium concentration is still sufficient to release transmitter and to support the development of synaptic potentials. This observation is in contrast to the findings of Nastuk (1953, 1954) and del Castillo & Katz (1955) who, in similar experiments on the neuromuscular junction, could not obtain end-plate potentials when the extracellular sodium concentration was very low ; since at this

junction sodium ions are not necessary for the release of acetylcholine (Katz & Miledi, 1965, 1967a, b), the failure must have been postsynaptic.

The experiments in which sodium-deficient solutions were used show that the P wave is more readily reduced in amplitude than the ganglionic action potential or the synaptic potential. The P wave consists of two components—a true positive after-potential and a slower positive deflection (IPSP) still present when spike initiation has been abolished by ganglion-blocking drugs (Eccles, 1952b; Eccles & Libet, 1961). Both components seem to be depressed in sodium-deficient solutions.

The finding that lithium ions may substitute for sodium ions in axonal conduction confirms the observation of Ritchie & Straub (1957) for mammalian non-myelinated fibres. In agreement with the findings of Pappano & Volle (1966b, 1967) we have found that lithium ions do not substitute for sodium ions in synaptic transmission in sympathetic ganglia; however, the effect of lithium may be partly presynaptic. The rapidity with which Li^+ interferes with ganglionic transmission, even in the unstimulated preparation, is in agreement with the observations of Woodward, Bianchi & Erulkar (1969). These authors found that, in the rabbit superior cervical ganglion, the sodium content is reduced at 30 min by 94% and the potassium content by 69% when Na^+ of the perfusion solution is replaced by Li^+.

Since the synaptic potentials produced by bathing the distal part of the ganglion are large and remain constant over long periods, it is possible to determine the potencies of ganglion-blocking drugs. The finding that the slopes of the concentration-response curves for the depressant actions of hexamethonium and pempidine are dissimilar may lend support to the view that the modes of action of these substances are different, but further analysis is required. In particular, it is not permissible to conclude from the parallel concentration-response curves that hexamethonium, pentolinium and nicotine have the same mode of action.

The results of Hertzler (1961) and Kosterlitz & Wallis (1966a) would suggest that the ganglion cells, Sa_1, excited by stimulation of the most rapidly conducting preganglionic nerve fibres, are the most resistant to ganglion-blocking agents. The findings in this paper, however, suggest that the synaptic potentials evoked by the weakest submaximal stimuli, involving the Sa_1 cells, are more susceptible. This is only an apparent paradox because Hertzler and Kosterlitz & Wallis used maximal or near maximal stimuli which would have excited most, if not all, of the terminals ending on the Sa_1 cells, thus producing very large synaptic potentials with a large safety factor for spike initiation. In consequence, the threshold level for spike initiation was probably exceeded even when the effectiveness of the transmitter was reduced by the ganglion-blocking drug. On the other hand, selective excitation of Sa_1 cells by submaximal stimuli excites only few terminals on these cells, there is no safety margin of transmission and the effectiveness of ganglion-blocking agents is, therefore, relatively great.

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