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ORTHODROMIC ACTIVATION OF SINGLE GANGLION CELLS

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Intracellular recording gives an opportunity for a more precise investigation of responses that have already been investigated with potential measurements from the whole ganglion. However, owing to the presence of a connective tissue sheath, this technique has proved to be peculiarly difficult in mammalian sympathetic ganglia (Eccles, 1955). The present account is concerned with the generation of impulses following orthodromic activation of sympathetic ganglion cells with the effect of post-tetanic potentiation and with the action of a ganglion-blocking agent on synaptic transmission.

METHODS

For this work superior cervical ganglia of rabbits were isolated by the technique already described in detail (Eccles, 1952*a*). The isolated ganglion preparation was set up at a constant temperature (35° C) in a Ringer–Locke solution whose composition has been already given (Table 1, Eccles, 1955). The preganglionic nerve trunk was led into a chamber containing paraffin oil and placed on platinum electrodes for stimulation.

The full details of the procedure whereby micro-electrodes are inserted into ganglion cells have been described (Eccles, 1955). The same differentiation procedure was employed as in Coombs, Curtis & Eccles (1957). In accordance with the terminology employed in investigations on the motoneurones of mammalian spinal cord, the initial depolarization, or synaptic potential, which is generated by preganglionic impulses, will be called the excitatory post-synaptic potential, EPSP.

RESULTS

Spike potentials generated by preganglionic volleys were reported to arise as a simple spike from the initiating EPSP (Eccles, 1955). This finding has been confirmed by differentiated records of the intracellular responses of single ganglion cells (Fig. 1), which showed that there was an inflexion only at the point where the spike potential arose sharply from the small preceding EPSP. No ganglion cell has ever shown another point of inflexion in the rising phase of the spike potential.

The effect of post-tetanic potentiation is shown in Fig. 2, where the potentials generated at the various intervals after the conditioning tetanus can be compared with the first record, which is the control response before conditioning. At 5–75 sec post-tetanically the after-hyperpolarization

observed after the control spike has been replaced by a small after-negativity. The spike potentials recorded 15 and 30 sec after the tetanus (61 and 63 mV, respectively) are greater than the original control (58 mV). Such changes in the responses of the testing volley are probably explicable as the result of superimposing a spike potential on the large positive after-potential which must follow the conditioning tetanus. However, after-negativity may also be produced by potentiation of the EPSP which may

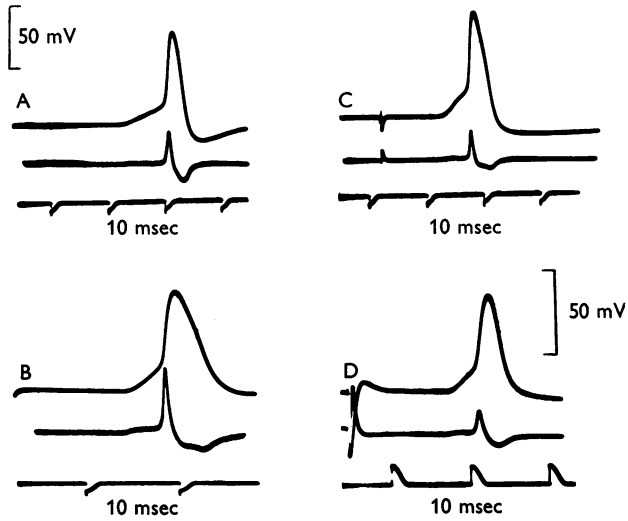

Fig. 1. A, B, C and D are the spikes generated in four ganglion cells following stimulation of the preganglionic nerve. Below each spike potential is the differentiated record and the appropriate time scale. A and B are from two cells in the same ganglion; C was recorded from the other ganglion of the same rabbit; D from another animal. Voltage scale on the left for A and B; on the right for C and D.

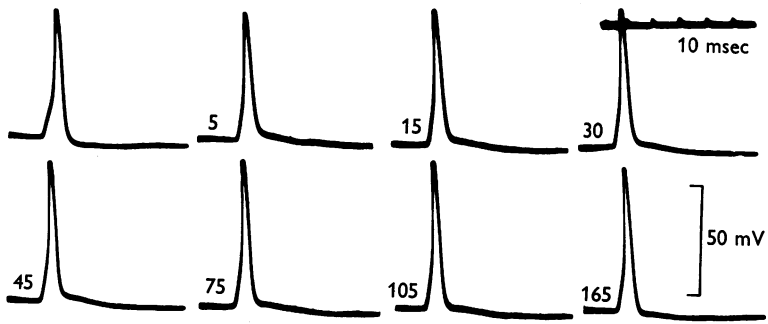

Fig. 2. This figure is a series of spikes recorded intracellularly from a ganglion cell; one (a control) before, and then the others at intervals after the end of a train of repetitive preganglionic volleys (64/sec for 15 sec). The figures indicate the appropriate time, in seconds, after the end of the repetitive stimulation. All records were taken at the same amplification and sweep speed.

in part survive the spike (cf. Eccles, 1961). This potentiation of the EPSP is seen in the more rapid rate of rise of the synaptic potential and an earlier initiation of the spike (e.g. the records of 5 and 15 sec after cessation of the conditioning tetanus).

Figure 3 illustrates the effect of a ganglion-blocking agent on synaptic transmission. A small amount of dihydro-β-erythroidine hydrobromide was added carefully to a corner of the bath and A was recorded immediately. The amount of blocking agent added was sufficient to block transmission when it was evenly distributed through the pool of 15 ml. Three minutes after addition of the drug (Fig. 3C) there was a slight alteration

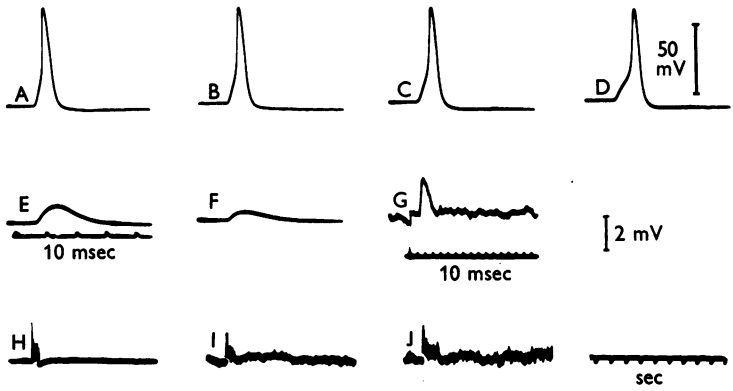

Fig. 3. A series of records taken intracellularly from a ganglion cell on preganglionic stimulation. A was the control spike just as dihydro-β-erythroidine hydrobromide was added to the bath. Four minutes later the record D was obtained. E and F show progressive curarization and were recorded at approximately 3 and 5 min respectively after C. The voltage scale and time scale was constant from A to F. The response at 8 min (G) shows a small synaptic potential (note the new potential and time scales). The third row (H–J) shows the effect of dihydro-β-erythroidine on the responses to repetitive preganglionic volleys at 40/sec. H is the control taken at the low amplification and I, J are trains of volleys (0·5 and 1·0 sec in duration) taken at the same amplification as G and shortly after record G. The time scale for H–J is in seconds.

in the size and time course of the spike. After 4 min the full-sized spike was initiated later from a much less steeply rising EPSP, but the threshold level of depolarization was unaltered. As the concentrations of dihydro-β-erythroidine increased in the extracellular regions the EPSP eventually was unable to reach the threshold depolarization for generation of an impulse (Fig. 3E), and finally was itself depressed in size, though its time course was virtually unchanged (Fig. 3F). Even 8 min after addition of the blocking agent, a synaptic potential of about 2 mV could still be recorded (G). Such an action of a ganglion-blocking agent was expected

from recording of potentials evoked in the whole sympathetic ganglion, where dihydro-β-erythroidine led to partial or complete suppression of impulse transmission though preganglionic impulses still set up synaptic potentials on the ganglion cells. Repetitive stimulation produced a train of synaptic potentials (Fig. 3I, J). Under these conditions at the slow sweep speed the synaptic potentials appeared to be fused. Following the summed synaptic potentials there was no sign of the late positivity (P wave) or late negativity (LN wave) which are observed when the potentials of the curarized ganglion are recorded between the ganglion itself and the whole of the post-ganglionic trunk (Eccles, 1952*b*; Eccles & Libet, 1961).

DISCUSSION

Generation of impulses in ganglion cells does not present the same pattern of events as that recorded in motoneurones (Coombs *et al.* 1957; Fatt, 1957), but resembles the potentials of interneurones (Eccles, Eccles & Lundberg, 1960) and of frog sympathetic ganglia (Nishi & Koketsu, 1960). The threshold for firing impulses in ganglion cells with normal resting potentials is likely to be considerably higher than the 15–20 mV range for depolarized ganglion cells (Fig. 1), probably 25–40 mV.

The ganglion-blocking drug, dihydro-β-erythroidine, prevents the generation of impulses by reducing the effective depolarization produced by a preganglionic volley. There is no appreciable alteration in the latency of the initial depolarization nor in the resting potential, but a marked reduction in the rate of rise of the EPSP to the critical level for generation of an impulse (compare Fig. 3A with C). Eventually the initial depolarization appears without any spike superimposed on it (Fig. 3D) and any further increase in the concentration of the drug reduced the rate of rise, and the size of the synaptic potential without affecting its time course (Fig. 3E). This agrees with the hypothesis that tubocurarine and drugs allied to it act competitively to reduce the effectiveness of the depolarizing action of acetylcholine liberated from the preganglionic terminals by impulses in the preganglionic fibres. In the presence of ganglion-blocking agents the synaptic potential from an excised mammalian ganglion is followed by a late positive (P) and late negative (LN) waves (Eccles, 1952*b*; Eccles & Libet, 1961). No definite trace of these potentials was recorded intracellularly (Fig. 3I, J). In the previous study (Eccles & Libet, 1961) it was stated that there was no evidence on the location of the LN receptor region, which may be situated on special ganglion cells.

SUMMARY

1. Since differentiation of synaptically generated spike potentials from sympathetic ganglion cells does not reveal a step on the rising phase, it is concluded that synaptic stimulation of sympathetic ganglion cells directly evokes an impulse in the cell body.

2. Spike potentials evoked on post-tetanic potentiation revealed an earlier initiation of the spike as well as a reduction of the after-hyperpolarization.

3. A curare-like substance, dihydro-β-erythroidine hydrobromide, prevented generation of impulses post-synaptically by depressing the initial depolarization, the EPSP, produced by a single preganglionic volley.

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REFERENCES

COOMBS, J. S., CURTIS, D. R. & ECCLES, J. C. (1957). The interpretation of spike potentials of motoneurones. *J. Physiol.* **139**, 198–231.

ECCLES, J. C. (1961). Membrane time constants of cat motoneurones and time courses of synaptic action. *Exp. Neurol.* **4**, 1–22.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1960). Types of neurone in and around the intermediate nucleus of the lumbosacral cord. *J. Physiol.* **154**, 89–114.

ECCLES, R. M. (1952*a*). Action potentials of isolated mammalian sympathetic ganglia. *J. Physiol.* **117**, 181–195.

ECCLES, R. M. (1952*b*). Responses of isolated curarized sympathetic ganglia. *J. Physiol.* **117**, 196–217.

ECCLES, R. M. (1955). Intracellular potentials recorded from a mammalian sympathetic ganglion. *J. Physiol.* **130**, 572–584.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol.* **157**, 484–503.

FATT, P. (1957). Sequence of events in synaptic activation of a motoneurone. *J. Neurophysiol.* **20**, 61–80.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.