J. Phys. (1950) 111, 43–49 612.813.611.26

THE END-PLATE POTENTIALS OF THE RAT DIAPHRAGM PREPARATION

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(*Received* 24 *August* 1949)

Preparations of mammalian muscle from which end-plate potentials may be obtained have been described by Eccles & O'Connor (1939) and by Brown & Burns (1949). In both cases, however, the circulation to the muscle was intact and drugs and other modifying agents were injected into it. For some purposes, and particularly for the investigation of ionic effects, it would be useful to have an isolated mammalian preparation which would give reasonably large end-plate potentials. We therefore investigated the suitability of the isolated phrenic-diaphragm preparation of the rat for this purpose.

METHODS

The preparation was made by the technique described by Bülbring (1946), but the strip of muscle was usually cut from the right side of the diaphragm as this contains more muscle and less tendon than the more usual left side preparation, though the nerve is slightly more difficult to dissect.

The apparatus consisted of a Perspex bath in which the muscle could be fixed horizontally, the tendon being attached either to a lever system or to a crystal pick-up as a tension recorder. An outer water-bath maintained a constant temperature.

The recording electrodes were of silver wire fused into glass capillary tubing and were about 80–100 μ in diameter at the tip. They were cleaned by honing before each experiment. These electrodes were mounted on the outer bath and two screw adjustments were provided giving vertical and horizontal motions, so that records could be taken every $\frac{1}{2}$ mm. along the muscle with the two electrodes a fixed distance apart, their position being recorded on a millimetre scale. In certain experiments only one such travelling electrode was employed, the indifferent electrode being a silver wire immersed in the fluid in the bath. A lateral traverse of the muscle was obtained by a sliding movement of the inner Perspex bath.

The phrenic nerve was pulled through two silver loop electrodes and was stimulated by an electronic square wave generator. The stimulus was usually 0·5–1·0 V. with a duration of 0·1 msec., which was found to be supramaximal for nerve stimulation. The action potentials of the muscle were fed into a capacity-coupled balanced amplifier followed by a compressor stage, and were displayed on a double-beam cathode ray tube. The overall time-constant of the amplifier was 75 msec.

In the experiments on summation of the end-plate potentials, the output of the square-wave stimulator was passed through the primary of an air-cored induction coil, while the secondary was

connected to the stimulating electrodes. The time between the two stimulating voltage spikes could then be varied by altering the duration of the square-wave.

In all cases the muscle was suspended in Krebs solution through which a mixture of 95% oxygen and 5% CO_2 was bubbled. The entire muscle and nerve were beneath the surface of the solution.

RESULTS

Longitudinal traverses of the preparation in normal Krebs solution with the electrodes about 2 mm. apart showed action potentials, diphasic or multiphasic, at all points as would be expected. The peak voltages of the spikes varied considerably during a traverse, presumably mainly due to summation effects, but the usual value was about 5–10 mV.

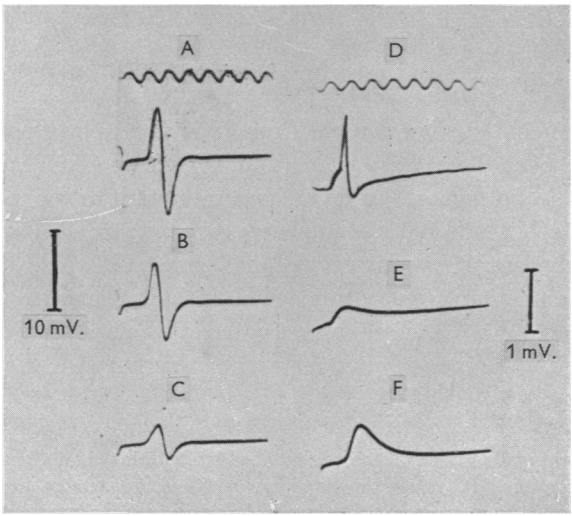

Fig. 1. Muscle action potentials of isolated rat diaphragm showing successive stages after the addition of tubocurarine (1 μg./c.c.) between frames A and B. Between frames C and D the amplification was increased about ten times and between frames E and F the electrodes were moved slightly to find the optimum end-plate potential. Recording electrodes 2·5 mm. apart. Time, 1000 cyc./sec.

When curare was added to the bath to give a concentration of 1–2 μg./c.c. the muscle action potentials became progressively smaller and disappeared. At this stage, if the electrodes were moved along the muscle it was possible to record at certain points a monophasic potential, more prolonged than the muscle action-potential spike and about 0·3–0·6 mV. in magnitude. This is shown in Fig. 1, the duration of the spike potential being 1 msec., whereas that of the monophasic potential is at least 4 msec. This latter potential was not conducted as it disappeared on moving the active electrode a few mm.

Fig. 2, taken from a different experiment with unipolar recording, shows that on removing the curare block gradually by progressive dilution and replace-

ment of the solution, a muscle action-potential spike grew up on the monophasic potential. The addition of eserine (0·2 μg./c.c.) to the curarized muscle caused a similar effect.

By moving the electrodes along the diaphragm from tendon to ribs in parallel traverses after curarization, it was found that these low voltage monophasic potentials were confined to certain regions of the preparation, though

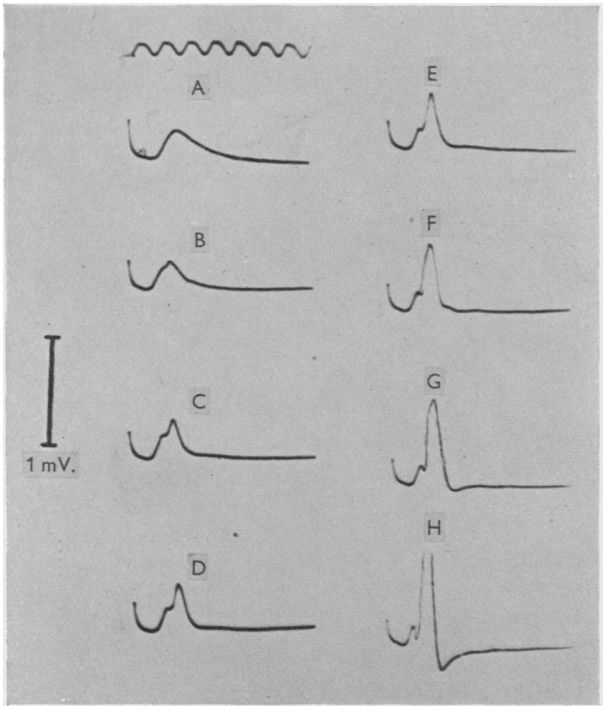

Fig. 2. Frame A shows the end-plate potential of the curarized rat-diaphragm. Between frames A and B the bath fluid was partially replaced to remove the curare block, and frames B–H show successive stages in the growth of the muscle action-potential spike. Unipolar recording. Time, 1000 cyc./sec.

there were slight variations from muscle to muscle. Fig. 3 gives a diagram of the upper surface of the preparation from the right half of the rat diaphragm showing the area over which these potentials are most likely to be found. It forms a strip running across the muscle, approximately midway between tendon and ribs, and situated a few mm. on the rib side of the point of entry of the phrenic nerve. The potentials are usually most readily obtained towards the medial side of the preparation and are difficult to pick up near the lateral edge as the diagram indicates. The same description also holds good for the left-side preparation which, of course, is in the form of a mirror-image of the present diagram.

Fig. 4 shows a small portion of a typical longitudinal traverse of the curarized muscle with the electrodes 3 mm. apart and moved 0·5 mm. between each frame. As the end-plate region was approached by the leading electrode, the monophasic potential appeared and increased to a maximum within 1·5 mm., showing that it was not conducted. Similar results were obtained using a single exploring electrode and an indifferent electrode.

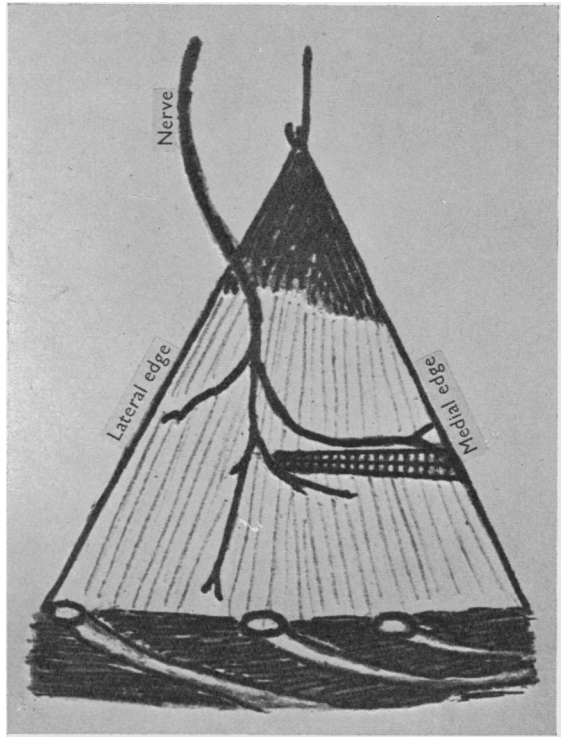

Fig. 3. Diagram of the upper (thoracic) surface of a preparation from the right half of the rat diaphragm. The area over which end-plate potentials are most likely to be obtained is shown cross-hatched.

When two stimuli were applied to the nerve at increasing intervals, two monophasic potentials appeared when the interval was greater than about 0·8 msec. With deep curarization, though summation was present and the second potential was greater than the first, no conducted action potential appeared as a result. With light curarization, however, the second potential became great enough to set up a conducted spike, as shown in Fig. 5.

It was later found that the most rapid and convenient method of locating the end-plate region was to add a very small amount of tubocurarine to the bath to

give a concentration of 0·5 μg./c.c. or less. At the usual frequency of stimulation of 12 per min. a large conducted muscle action potential remained after this dose. If now, however, the frequency of nerve stimulation was increased

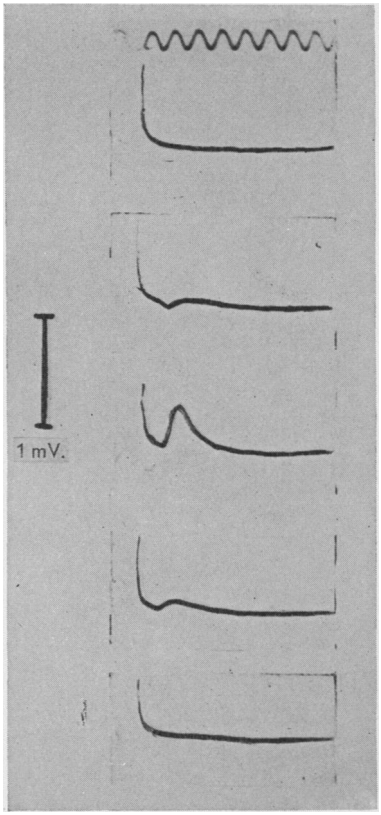

Fig. 4.

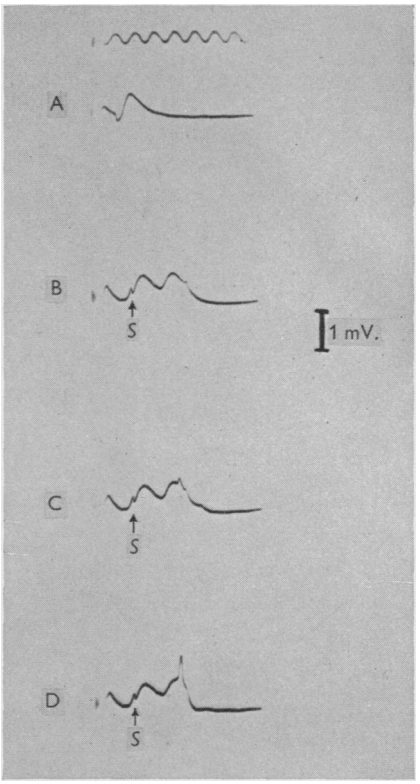

Fig. 5.

Fig. 4. Successive frames from part of a longitudinal traverse of the curarized rat-diaphragm preparation. Recording electrodes 3 mm. apart and moved 0·5 mm. towards the ribs between each frame. Time, 1000 cyc./sec.

Fig. 5. Potentials from curarized rat-diaphragm preparation when two stimuli were applied to the phrenic nerve. Frame A, single end-plate potential when stimuli were 0·7 msec. apart. Frames B, C and D show successive stages of summation of two end-plate potentials to set up a spike action potential when the interval between the stimuli was 1·6 msec. *S*, stimulus artefact of second stimulus to nerve. Time, 1000 cyc./sec. Unipolar recording.

to 100 per sec., the conducted action potential rapidly disappeared and with the electrode over the end-plate region a pure end-plate potential was obtained. As at this frequency a standing image was present on the oscilloscope it was easy to find the end-plate region by rapidly traversing the muscle until the end-plate potential appeared. Fig. 6 shows that by varying the frequency of

stimulation all stages between a muscle action potential and an end-plate potential could be obtained. With continued stimulation at 100 per sec. the end-plate potential remained constant for considerable periods up to 1½ hr. or more.

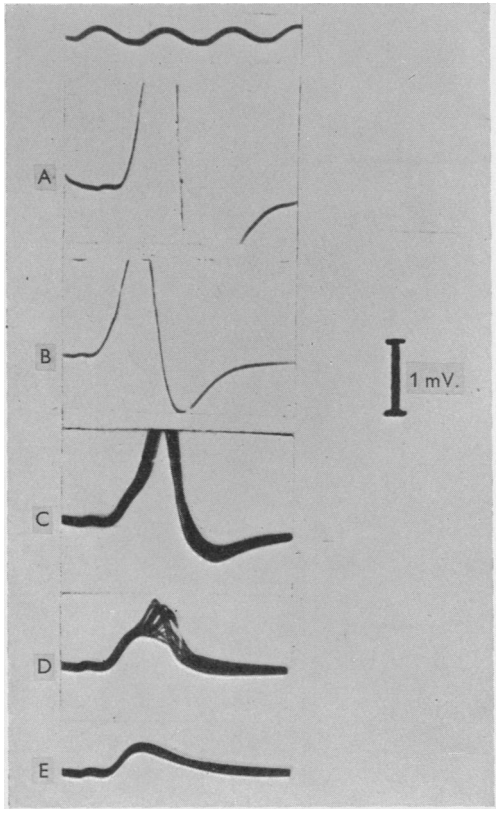

Fig. 6. Rat diaphragm preparation lightly curarized (0·5 μg. tubocurarine/c.c.). Frame A, nerve stimulated at a frequency of 12 per min. Frame B, stimulated at 2 per sec. Frame C, at 10 per sec. Frame D, at 50 per sec. Frame E, at 100 per sec. Time, 1000 cyc./sec. Frames C, D and E show several sweeps superimposed.

DISCUSSION

There seems little doubt that the monophasic potentials obtained were true end-plate potentials as they satisfied the following criteria:

(1) They were observed after partial curarization.

(2) They were confined to definite locations on the muscle.

(3) They were not conducted.

(4) On removal of the curare block a conducted muscle action potential spike appeared on the broader low voltage potential.

(5) On applying two stimuli to the nerve at an appropriate interval, summation of the potentials occurred.

There would, therefore, appear to be a concentration of end-plates in the rat diaphragm along a strip about mid-way between the central tendon and the ribs. This is rather surprising in view of the statement by Brown, Bülbring & Burns (1948) that the fibres of this preparation are usually only about 5 mm. in length.

The observation that during very light curarization progressive increase in the frequency of nerve stimulation first abolishes the action potential and then, with still higher frequencies, depresses the end-plate potential itself, obviously requires further investigation. Presumably there is a critical frequency at which the end-plate potential is depressed below the threshold required for triggering the muscle action potential. It is possible that end-plate potentials may be obtainable by this method in uncurarized muscle, but we were unable to test this satisfactorily owing to the limited frequency range of our apparatus. This phenomenon may perhaps be concerned in the mechanism of Wedensky inhibition as was suggested by Feng (1940, 1941), following somewhat similar experiments on amphibian preparations. In Feng's experiments, however, the volley of nerve impulses was continued only for some 100 msec., but within that period he found that the amphibian end-plate potentials were able to follow the stimulus frequency up to the limit set by the refractory period of the nerve itself.

The fact that no change in the end-plate potential was apparent over long periods at stimulus frequencies up to 100 per sec. was unexpected in view of the usual theories of neuromuscular 'fatigue'.

SUMMARY

1. A method of recording end-plate potentials from the isolated rat phrenic-diaphragm preparation is described.

2. End-plate potentials can be most easily observed in a region midway between the central tendon and the ribs.

3. Preliminary experiments on the effect of frequency of nerve stimulation on the end-plate potentials of this preparation are briefly discussed.

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