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THE EFFECT OF VARYING GLUCOSE CONCENTRATIONS ON THE MAMMALIAN END-PLATE POTENTIAL

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Since relatively low concentrations of glucose were found to depress the manufacture of acetylcholine by tissue extracts *in vitro*, Feldberg (1945*a*, *b*, 1950) suggested that the concentration of glucose normally present in blood exerts a restraining influence on acetylcholine synthesis by nervous tissue, and he further suggested that the convulsions of insulin hypoglycaemia might be due to the removal of a restraining influence which blood glucose normally exerts upon the synthesis or release of acetylcholine (Feldberg, 1950).

The end-plate potential may be assumed to give a measure of the depolarization produced at the end-plates by the acetylcholine liberated during a nerve volley (Eccles & Macfarlane, 1949; Fatt & Katz, 1951). On the further assumption that the depolarizing action of acetylcholine is unaffected by the concentration of glucose, variations in the size of the end-plate potential may be taken to indicate variations in the liberation of acetylcholine under these conditions. The present study uses in this way the size of the end-plate potential to give an indirect estimate of the output and synthesis of acetylcholine. It is thus possible to test the hypothesis of Feldberg (1950) under *in vivo* conditions, in so far as it is applicable to acetylcholine synthesis in the motor nerve terminals.

METHOD

The left phrenic nerve and a 5 mm strip from the left hemi-diaphragm at the site of nerve entry were removed under ether anaesthesia from 3–4-month-old male albino rats of the Wistar strain. The strip was suspended between two Perspex fixation hooks in a sealed semi-cylindrical Perspex chamber (cf. R. M. Eccles, 1952). Rotation of the chamber permitted the preparation to be immersed in Krebs solution, or withdrawn from solution for stimulating and recording, without movement of the recording leads on the preparation.

Potentials were recorded between two platinum wire electrodes, one fixed at the costal attachment of the muscle, and one movable at the end-plate region; the nerve was mounted on two flamed platinum wire stimulating leads. A platinum earth plate was in contact with nerve and muscle near the central tendon attachment. Potentials were recording photographically from a cathode-ray oscillograph and amplifier with differential input.

The modified Krebs solution (Krebs & Henseleit, 1932) used throughout this work had the ionic composition shown in Table 1. The original concentration of bicarbonate (27·2mM) was reduced to 13·6 mM and 'carbogen' containing 5% CO_2 and 95% O_2 was bubbled through the solution. Glucose was added to the solution in varying concentrations. The temperature of the fluid in the chamber was thermostatically controlled at 38–39° C.

TABLE 1. Composition of modified Krebs solution (mM)

Na^+	138·0	Cl^-	134·6
K^+	6·2	$H_2PO_4^-$	1·2
Ca^{2+}	2·7	SO_4^{2-}	1·2
Mg^{2+}	1·2	HCO_3^-	13·6

D-Tubocurarine (Squibb) in a concentration of 4×10^{-6} M was used in each experiment in the Krebs solution in order to block neuromuscular transmission. Stimulation of the phrenic nerve then produced end-plate potentials. Where the solution was changed, as in decreasing the glucose concentration, the preparation was soaked for 30 min in the new solution before recordings were taken in order to ensure that concentration equilibrium had been reached (cf. Eccles & Macfarlane, 1949).

RESULTS

Single end-plate potentials with various glucose concentrations

Fig. 1*a* shows a typical end-plate potential which a phrenic nerve volley sets up in the isolated diaphragm strip, time to summit being 0·7 msec and half-decay from summit occupying 1·08 msec. These times are typical values when recording, as in these experiments, close to the end-plate region of the strip (Ludbrook & Whyte, unpublished). Previous records of mammalian end-plate potentials (Eccles, Katz & Kuffler, 1941; Hajdu & Knox, 1950) have been seriously distorted by being recorded in volume.

Increase in the concentration of glucose from 0·1 to 0·5% causes no significant change in the time course or size of the end-plate potential (Fig. 1*b*). However, decreasing the glucose concentration to zero causes a progressive decline of the end-plate potential, which is, however, fully reversed on restoration of glucose provided that the depression is not allowed to proceed too far (Fig. 1*c*). Since the time course of the end-plate potential showed no appreciable change, it may be assumed that the surface membrane of the muscle fibre was not seriously affected by the lack of glucose.

The effects of glucose concentration on the end-plate potentials of a tetanus

Fig. 2*a* is a typical repetitive end-plate potential evoked by tetanic nerve stimulation at 180 per sec. In Fig. 2*b* the successive end-plate potentials are plotted as a percentage of the first potential of the series. Before each stimulation the preparation had been soaked for 30 min periods in Krebs solutions containing 0·05, 0·16 and 0·36% glucose by weight, respectively. Typically there is no significant difference in the rate of decline of the successive end-plate potentials. During the course of this experiment there was

a 10% decrease in the height of the initial end-plate potential, presumably attributable to a general deterioration of the preparation over the two-hour period.

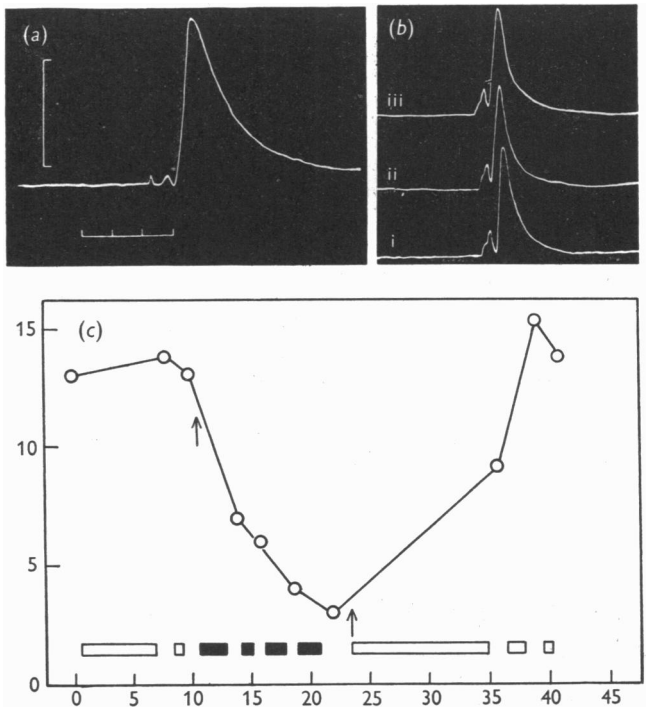

Fig. 1. (*a*) A single end-plate potential, showing the preceding intramuscular nerve volley. Voltage calibration, 0·2 mV. Time marks, msec. (*b*) Single end-plate potentials at different glucose concentrations: (i) 0·1%, (ii) 0·3%, (iii) 0·5% glucose (all w/v). Records retouched to improve contrast. (*c*) The graph plots the height of single end-plate potentials. Abscissa: time in min; ordinate: size of end-plate potential in arbitrary units. The initial potentials were recorded in Krebs solution containing 0·22% (w/v) glucose. At the first arrow, the preparation was removed to Krebs solution containing no glucose. At the second arrow, the preparation was re-immersed in Krebs solution containing 0·22% (w/v) glucose. The actual periods of immersion are indicated at the bottom of the figure, filled rectangles showing the periods of soaking in glucose-free solution.

The recovery of end-plate potentials after prolonged tetanic stimulation

When stimulated at 180 per sec, the successive end-plate potentials initially declined rapidly to a level approximately 1/5 normal (Fig. 2*a* i), and then declined slowly to the level seen in Fig. 2*a* ii, when a steady state was reached. This steady state was maintained during stimulation for several minutes and presumably represents a condition in which the nerve terminals have a low store of diffusible acetylcholine, and acetylcholine is being synthesized at

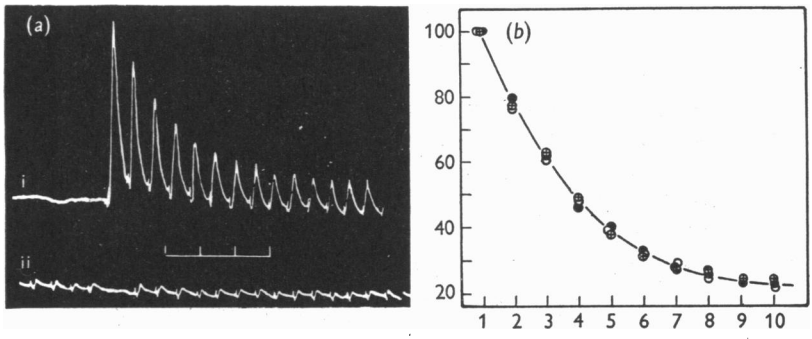

Fig. 2. (*a*) End-plate potentials during tetanic stimulation. (i) The potentials recorded at the beginning of a 180 per sec tetanus. (ii) Potentials after 45 sec tetanic stimulation. Time marks, 10 msec. Records retouched to improve contrast. (*b*) The graph plots the height of successive end-plate potentials at the start of tetanic stimulation of frequency 180 per sec. On the abscissa, the successive end-plate potentials are numbered serially from the first to the tenth; ordinates: size of end-plate potentials expressed as a percentage of the first. Observations after exposure to different glucose concentrations are plotted, the points being designated by different symbols as follows: ○, 0·05% glucose; ⊕, 0·16% glucose; ●, 0·36% glucose (all w/v).

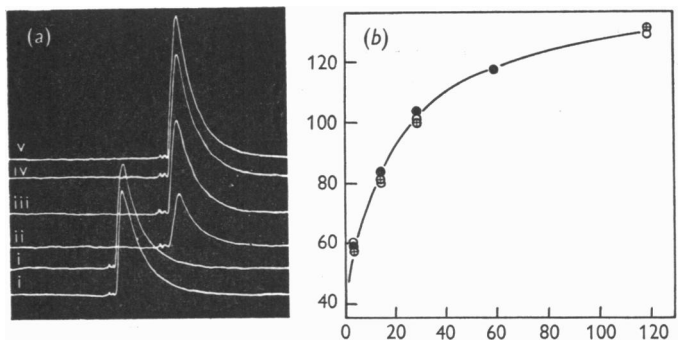

Fig. 3. (*a*) End-plate potentials recorded before and after a 1 min conditioning tetanus of frequency 180 per sec. (i) Control records before the tetanus. (ii), (iii), (iv) and (v) are records taken 5, 15, 30 and 60 sec respectively after cessation of the conditioning tetanus. Records retouched to improve contrast. (*b*) The graph shows the heights of testing single end-plate potentials after a 1 min, 180 per sec conditioning tetanus. Ordinates: size of end-plate potentials expressed as percentage of control end-plate potential before tetanic stimulation; abscissa: time in sec after cessation of conditioning tetanus. Results from tests with three different glucose concentrations are shown, the points being designated as follows: ⊕, 0·11% glucose; ○, 0·33% glucose; ●, 0·55% glucose (all w/v).

a rate which is equal to the liberation during activity. Recovery after such prolonged repetitive stimulation is revealed by testing with single nerve volleys at relatively infrequent intervals.

Fig. 3*a* shows control end-plate potentials and testing end-plate potentials, ii, iii, iv, v, recorded 5, 15, 30 and 60 sec respectively, after the conditioning tetanus.

In Fig. 3*b* the single end-plate potentials following a 1 min 180 per sec tetanus, expressed as a percentage of the control potential recorded before the tetanus, are plotted against the time interval between the end of the tetanus and the testing potential. The three values at each time interval represent glucose concentrations of 0·11, 0·33 and 0·55% respectively. At intervals of 5 and 15 sec the potential was below normal, at 30 sec in this experiment it reached normality, and at 60 and 120 sec intervals there was the characteristic post-tetanic potentiation (Liley & North, unpublished). There were no significant differences between the recoveries at the different glucose concentrations.

DISCUSSION

Studies on brain metabolism indicate that the primary energy source of nervous tissue is the oxidation of blood glucose (McIlwain, 1950). Studies of acetylcholine synthesis by nervous tissue in a divided state *in vitro*, have shown that acetylcholine synthesis requires a source of energy and that this energy is normally derived from aerobic glucose metabolism (Feldberg, 1945*a*; Mann, Tennenbaum & Quastel, 1938, 1939). Thus it is to be expected that there would be a depressed production of acetylcholine in the nerve fibres of an isolated neuromuscular preparation soaked in a glucose-free solution, thereby providing an explanation for the depression of end-plate potentials illustrated in Fig. 1*c*. There is also evidence that the fine non-medullated nerve terminals of the isolated neuromuscular preparation are more readily depressed by glucose deficiency than are the medullated fibres of a nerve trunk or muscle fibres (Hajdu & McDowall, 1949).

Mann *et al.* (1938, 1939) first showed that glucose stimulated acetylcholine synthesis by brain slices. In the absence of glucose there was little acetylcholine formation. At a glucose level of 0·02% there was an almost maximal synthesis of acetylcholine, with a further slight increase at higher glucose levels.

Feldberg (1945*a*), using 0·1% glucose, likewise observed the accelerating effect of glucose on acetylcholine synthesis. However, he found that when homogenized brain was incubated in eserine-saline (5 ml./g of ground tissue), the addition of 0·1% glucose caused an 18% depression of acetylcholine synthesis, while with 0·8% glucose there was a depression of 85%. Hence Feldberg (1950) suggested that blood glucose normally exerts a restraining effect on the continuous synthesis or release of acetylcholine in the central

nervous system, and that lowering of the blood glucose, in removing the 'glucose brake', permits synthesis and release to proceed at an abnormal rate. He further suggested that the convulsions of hypoglycaemia are thus due to an increased synthesis and release of acetylcholine in the central nervous system.

The present experimental investigation indicates that Feldberg's hypothesis cannot be extended to motor nerve terminals *in vivo*, for the following findings are contrary to prediction from the hypothesis: (i) Increase of glucose concentration from 0·1 to 0·5% causes no significant change in the end-plate potential (Fig. 1*b*), whereas a depression would be predicted. (ii) On removal from a solution with 0·22% glucose to a glucose-free solution (Fig. 1*c*), the first change that is observed is depression of the end-plate potential, whereas according to the hypothesis a transient potentiation would be expected as the glucose concentration was lowered to the optimum value for acetylcholine synthesis in the motor-nerve terminals. (iii) The time course of decay of the successive end-plate potentials during a tetanus is not affected by increasing the glucose concentration from 0·05 to 0·36% (Fig. 2), where, according to the hypothesis, there should be a more rapid decay at higher glucose concentrations due to depression of acetylcholine synthesis. (iv) The time course of recovery of end-plate potentials after a prolonged tetanus is not appreciably affected by increase in the glucose concentration from 0·11 to 0·55% (Fig. 3), whereas, according to the hypothesis, depression of acetylcholine synthesis should cause recovery to be slower with higher concentrations of glucose.

Thus it can be concluded that neither a normal blood glucose level of 0·1%, nor glucose concentrations higher than this, exerts an inhibitory influence on the synthesis and release of acetylcholine by the axonal terminals of living motoneurones. Concentrations of glucose less than the normal blood level, presumably produce effects on acetylcholine synthesis only when nerve metabolism is impaired by a reduction in the available energy below the needs of that metabolism. At such concentrations a depression of acetylcholine synthesis will occur.

SUMMARY

It has been shown, from investigation of the end-plate potential of an isolated mammalian preparation, that glucose in normal blood concentrations does not exert a continuous depressant influence on acetylcholine synthesis and release by the axons of motoneurones.

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