Influence of Glucose on the Transmembrane Action Potential of Anoxic Papillary Muscle

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ABSTRACT The response of the cat papillary muscle to anoxia has been found to alter depending on the glucose concentration in the medium. At a glucose concentration of 5 mM anoxia caused a marked reduction in force of contraction and action potential duration within 20 minutes. At a glucose concentration of 50 mM anoxia induced similar changes in the force of contraction but little or no change in action potential duration. Elevation of glucose concentration during an anoxic interval reversed the anoxia-induced changes in action potential but had little effect on force of contraction. This effect of glucose could be partially duplicated by xylose and 2-deoxyglucose and in addition, 2-deoxyglucose has been found to prevent the effect of subsequently added glucose. These sugars appear to be transported by a system responsible for glucose transport but are not metabolized to any extent. It would appear therefore that transport of glucose is in some way related to transport of potassium as increased potassium permeability is thought by many to be responsible for anoxia-induced changes in action potential duration.

INTRODUCTION

The present study was prompted by the chance observation that the electrical activity of the cat papillary muscle became increasingly sensitive with time to relative oxygen lack in a medium containing 5 mM glucose but not in one containing 50 mM. Subsequent experiments showed that 50 mM glucose would in fact maintain single cell electrical activity at control levels during anoxic intervals of as long as 90 minutes although force of contraction decreased to the same degree as in 5 mM glucose.

Several studies have been carried out on the effects of anoxia, substrate depletion, or metabolic inhibitors on cardiac muscle (Trautwein and Dudel, 1956; Webb and Hollander, 1956; Kleinfield *et al.*, 1961, 1962; de Mello, 1959; Boulpaep, 1959; Lullmann, 1959). In both atrial and ventricular muscle the earliest effects were depression in force of contraction and reduc-

tion in action potential duration. Subsequently there were variable changes in overshoot and resting potential. Under most circumstances return of control conditions restored normal activity in the muscles. The alterations in electrical activity are usually explained by alterations in potassium permeability. Thus Trautwein and Dudel (1956) propose that anoxia causes increased potassium efflux and this could explain the loss of a plateau if potassium accumulation in the interstitial fluid initiated the increase in potassium permeability which is believed to cause repolarization (Weidmann, 1956).

As a result of experiments with rat atria Webb and Hollander (1956) proposed that ATP binding might influence the permeability characteristics of the membrane in such a manner that reduced ATP binding would result in a greater potassium efflux during activity but not during rest when electromotive restraint on diffusion is dominant. Since the membrane concentration of ATP is relatively low it would be more sensitive to decreased ATP production as might be expected to occur under a variety of "insufficiencies." In their paper Webb and Hollander (1956) reported effects of glucose which appeared to be independent of a generalized metabolic effect. Our present findings would indicate that this effect was due to an interrelationship between glucose transport and potassium permeability.

Recent evidence (Newey and Smyth, 1964) underlines the feasibility of investigation into possible common pathways of transport through cell membranes. In smooth muscle Daniel (1964) has proposed common carriers which are modified, probably by intracellular metabolic activity, for specific carrier activity. In this context increased transport of one substrate might decrease the availability of the carrier for another. In the present experiments generalized metabolic effects of glucose seem not to explain its activity on electrical activity of cardiac muscle.

METHODS

All experiments were carried out with papillary muscle obtained from the right ventricle of cat heart which was removed under ether anesthesia. Dissection of the muscles was carried out in cool modified Krebs-Ringer's solution of the following composition in milliequivalents per liter: Na 138.5, K 4.6, Ca 4.9, Mg 2.3, HCO_3 21.91, PO_4 3.48, SO_4 2.32, and glucose 50 mM equilibrated with 95 per cent O_2:5 per cent CO_2. The muscles were not selected as to size but the smallest conveniently handled muscle from any heart was used. The average weight of these muscles was 5.07 mg with a SD of ± 2.3 mg. The muscles were mounted horizontally at a resting tension of 1.5 gm in a jacketed 70 ml constant temperature bath at 37°C. Stimulation was at a rate of 60 per minute through platinum electrodes. Force of contraction was recorded on a Grass polygraph by means of a Statham force displacement transducer.

Single cell electrical activity was recorded by means of hand-pulled glass microelectrodes filled with 3 M KCl using the floating electrode technique of Woodbury and Brady (1961). Potential measurements were made through a Medistor negative

capacitance electrometer, monitored on a Tektronix 502 oscilloscope and recorded either on film or on a Grass polygraph. In most experiments anoxia was induced by replacing the O_2:CO_2 mixture with N_2:CO_2 but in some of the earlier experiments the O_2:CO_2 was simply shut off. In these early experiments the pH was checked at frequent intervals and no difference could be seen between the two techniques.

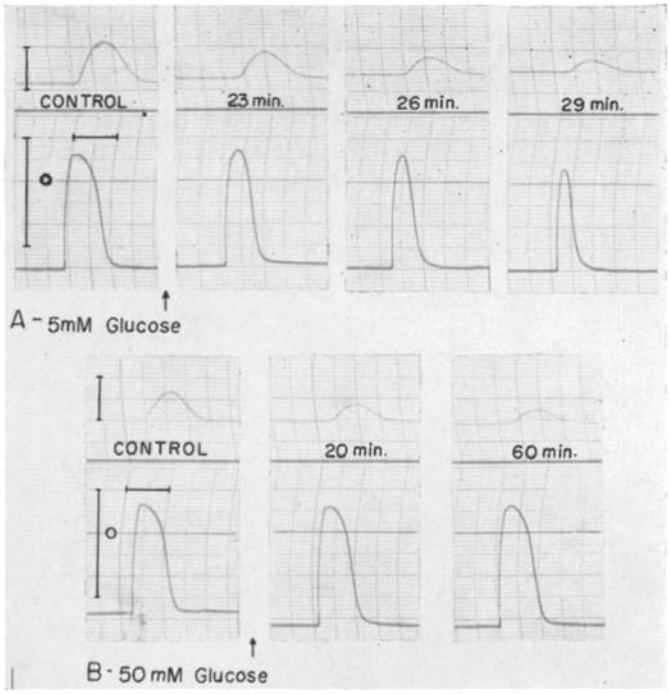

FIGURE 1. The effect of anoxia on the transmembrane action potential and force o contraction of a cat papillary muscle in modified Krebs-Ringer's solution containing 5 mM (A) and 50 mM (B) glucose. Time is measured from the beginning of the anoxic interval (arrows). Voltage calibration 100 mv, time calibration 200 msec., and force calibration 1 gm.

Action potential upstroke velocities were obtained by applying the output of the medistor electrometer to a Tektronix 555 oscilloscope, one channel of which differentiated the upstroke, and displaying the high sweep speed upstroke and its differential on a Tektronix 564 storage oscilloscope. When a series of records had been obtained on the storage scope, polaroid photographs were taken. Upstroke velocities could be obtained either by direct measurement on the photographs of the differential or by determining the slope of the upstroke and calculating maximum rate of rise.

RESULTS

Fig. 1 shows recordings of action potentials and mechanical activity of a cat papillary muscle during periods of anoxia. The upper four records show the response of the muscle to anoxia in a medium containing 5 mM glucose: the

lower three the response in 50 mM glucose. Although a decrease in the height of the action potential occurred after 29 minutes of anoxia in 5 mM glucose this change is not necessarily associated with the decrease in duration (see Figs. 2 and 6–9) that consistently occurs as a result of anoxia. Similarly, no consistent change occurred in the resting potential. Despite similar depression in force of contraction at both glucose concentrations the single cell electrical activity was maintained at control levels for 60 minutes by 50 mM glucose. That the effect of elevated glucose was not simply due to an osmotic effect is shown in Fig. 2. Here the same effect of anoxia was obtained although the medium contained 5 mM glucose plus 45 mM sucrose. Fig. 3 shows a graphic summation of our data to date on electrical and mechanical responses to anoxia in 5 and 50 mM glucose. Six trials in 5 experiments were carried out with 50 mM glucose, however, some 100 trials in 30 experiments are repre-

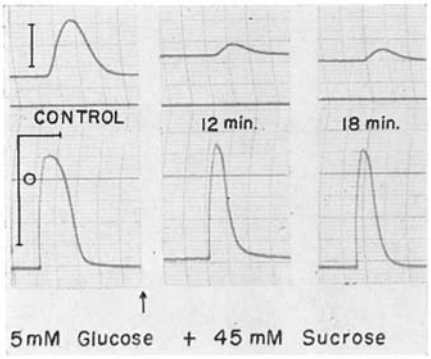

FIGURE 2. The effect of anoxia on the transmembrane action potential and force of contraction of cat papillary muscle in modified Krebs-Ringer's solution containing 5 mM glucose and 45 mM sucrose. Calibrations as in Fig. 1. Time is measured from the beginning of the anoxic interval (arrow).

sented in the 5 mM glucose curves. A small proportion of the experiments included in the 5 mM group were carried out in glucose-free solutions (10 trials in 5 experiments) or in 5 mM glucose plus 45 to 60 mM sucrose (5 trials in 2 experiments). A clear difference exists in the response of the single cell action potential to anoxia depending on the glucose concentration. In contrast to electrical activity, mechanical activity is depressed as much by anoxia in 50 as in 5 mM glucose although at 10 minutes from the beginning of the anoxic interval there is a difference ($P < 0.02$). The apparent difference at 30 minutes is not significant ($P > 0.2$) and may be accounted for by the small number of measurements (3) made at this time in 50 mM glucose.

The effects of anoxia on papillary muscle in 5 mM glucose or glucose-free media can be partially reversed as well as prevented by elevation of the glucose concentration to 50 mM. In Fig. 4 the effect is largely restricted to the electrical activity although some increase in force of contraction can be seen. A somewhat different response of the anoxic muscle to glucose can be seen in Fig. 5. In this experiment the muscle was made anoxic in 5 mM glucose plus

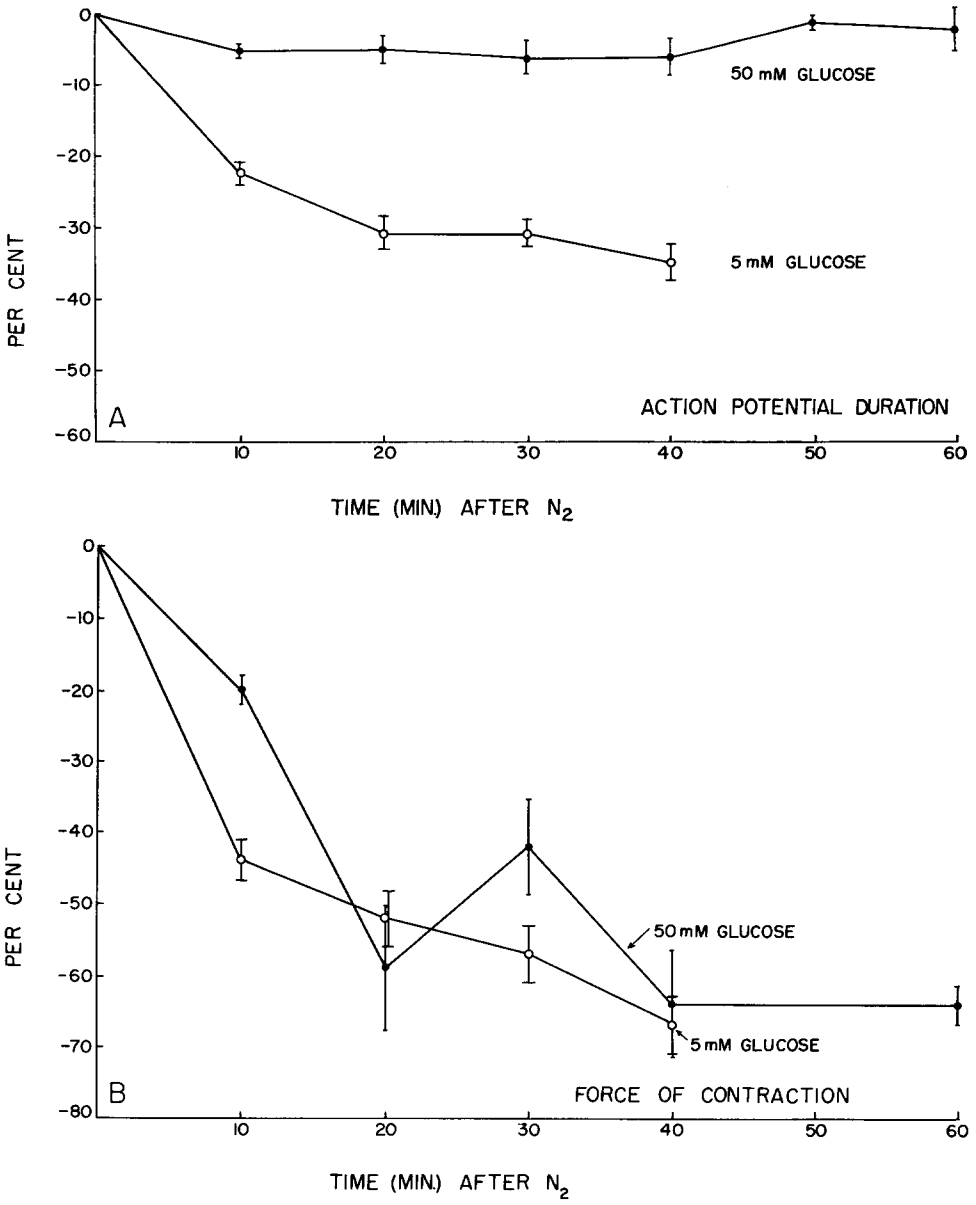

FIGURE 3. A, per cent change in transmembrane action potential duration of papillary muscle with time as a result of anoxia in 5 and 50 mM glucose. B, per cent change in force of contraction of papillary muscle with time as a result of anoxia in 5 and 50 mM glucose. Vertical bars represent the standard error of the mean.

45 mM sucrose and there was a considerable latent period in the response to glucose as compared to the response shown in Fig. 4. In the course of this series of experiments the delay prior to responses to glucose varied between

1 and 15 minutes whether the reduction in action potential duration was caused by turning off the oxygen or by incubating in 95 per cent N_2:5 per cent CO_2. In addition the response to the high concentration of glucose was not

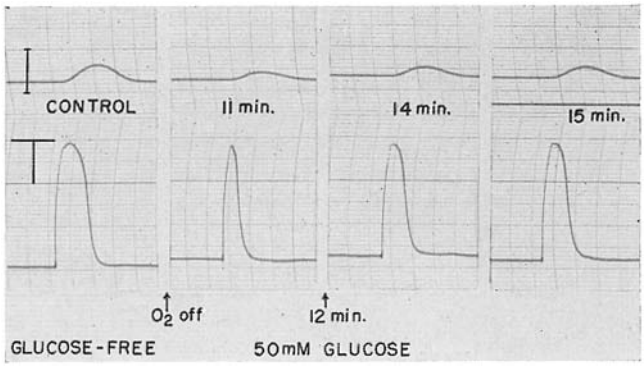

FIGURE 4. The effect of 50 mM glucose on the transmembrane action potential and force of contraction of a cat papillary muscle made anoxic in glucose-free Krebs-Ringer's solution. Glucose was added 12 minutes from the beginning of the anoxic interval. Voltage calibration 40 mv, force calibration 1 gm, time calibration 200 msec. Time measured from beginning of anoxic interval.

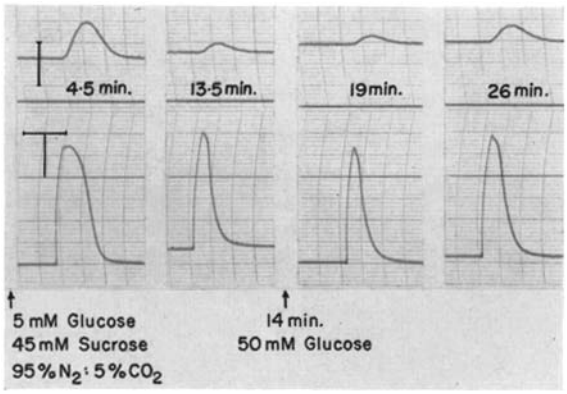

FIGURE 5. The effect of an elevation of glucose concentration from 5 to 50 mM on the transmembrane action potential and force of contraction of a cat papillary muscle made anoxic in Krebs-Ringer's solution containing 45 mM sucrose. Calibrations as in Fig. 4. Time is measured from the beginning of the anoxic interval.

consistently altered by the presence of 45 mM sucrose + 5 mM glucose or by 5 mM glucose alone.

As there appeared to be a marked separation of electrical and mechanical events during anoxia in 50 mM glucose, it is unlikely that the action of glucose

was due to a generalized metabolic effect. In order to test this hypothesis experiments were carried out in which the effect of xylose and 2-deoxyglucose on the muscle made anoxic in low glucose was determined. Both these sugars are thought (Battaglia and Randle, 1960; Kipnis and Cori, 1959 *a*) to be transported by a system responsible for glucose transport, 2-deoxyglucose is phosphorylated but xylose is not, and neither is further metabolized to any extent. Fig. 6 shows the effect of 50 mM xylose on the electrical and mechanical activity of a muscle made anoxic in 5 mM glucose. A rapid response to xylose occurred. In 3 of 15 trials this response was transitory in comparison to that to glucose but additional trials will be necessary to define the conditions under which this occurs. The responses to xylose were not so consistent as

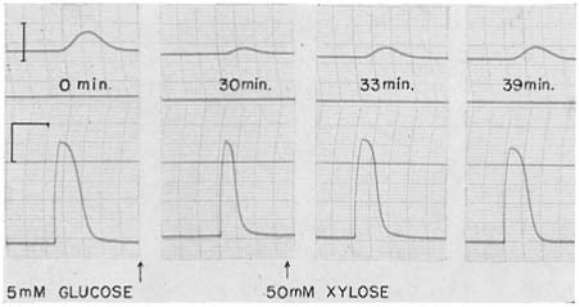

FIGURE 6. The effect of 50 mM xylose on the transmembrane action potential and force of contraction of a papillary muscle made anoxic by incubation in 95 per cent N_2:5 per cent CO_2 in Krebs-Ringer's solution containing 5 mM glucose. Calibrations as in Fig. 4. Time is measured from the beginning of the anoxic interval (first arrow).

were those to glucose and in 4 of the trials a response could be obtained to glucose but not to xylose.

The response of an anoxic muscle to 50 mM 2-deoxyglucose is shown in Fig. 7. Although a definite response to this sugar occurred, the effect was transitory and despite the addition of glucose and return of aerobic conditions the action potential duration continued to decrease and eventually the muscle developed spontaneous activity and finally asystole. The initial response of anoxic muscles to 2-deoxyglucose varied even more than the response to xylose; however, in contrast to xylose, it consistently blocked the effects of subsequently added glucose.

Fig. 8 shows the results of an experiment in which simultaneous records of upstroke, first derivative of the upstroke, and complete action potential were made. No change in upstroke velocity occurred during anoxia-induced reduction in action potential duration or following the increase in action potential duration due to the elevation of glucose concentration to 50 mM. The results

indicate that the glucose effect on electrical activity is not due to a generalized membrane stabilization for in such a situation a reduction of depolarization rate would be expected.

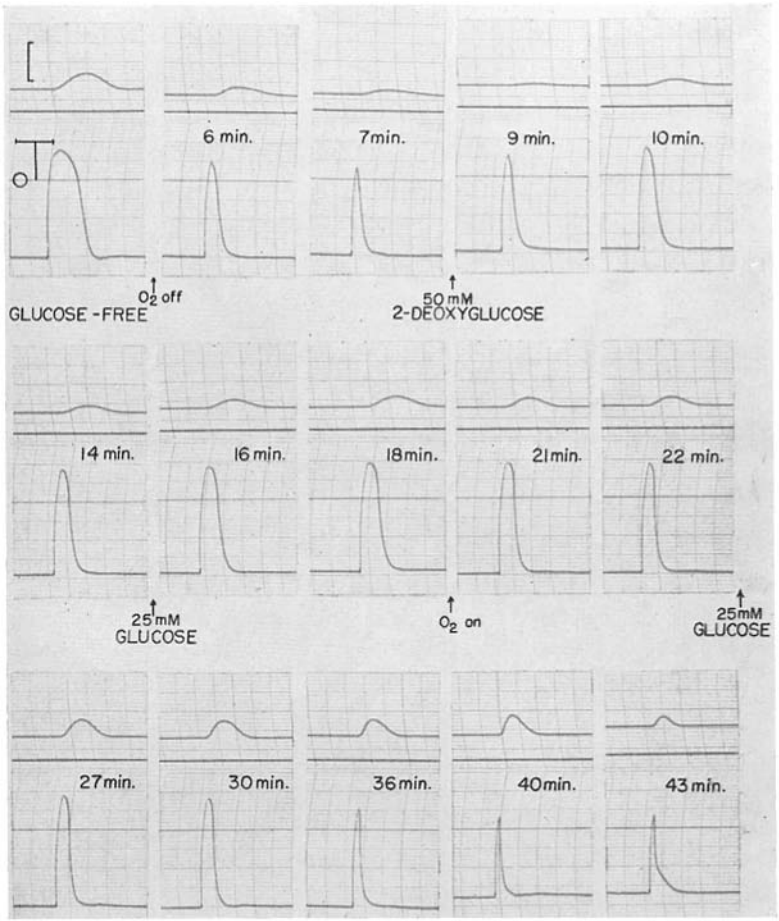

FIGURE 7. The effect of 50 mM 2-deoxyglucose on the transmembrane action potential and force of contraction of a cat papillary muscle made anoxic in glucose-free Krebs-Ringer's solution. Subsequent additions of glucose at 15 and 23 minutes to make a final concentration of 50 mM produced only transient increases in action potential duration despite return of aerobic conditions at 19 minutes.

In contrast to the sugars which affect the electrical activity of anoxic muscle to a much greater degree than mechanical activity, the effect of adrenalin is predominantly on mechanical activity (Fig. 9). It would appear that although a definite increase in action potential occurred this effect was more transient than the change in force of contraction. Following wash of the

preparation with 5 mM Krebs which had been equilibrated with 95 per cent N_2:5 per cent CO_2 mixture the action potential duration had returned to pre-adrenalin levels, whereas the force of contraction was still greater than

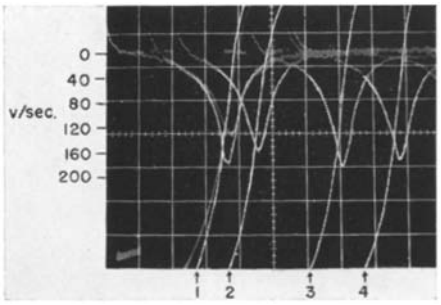

FIGURE 8A

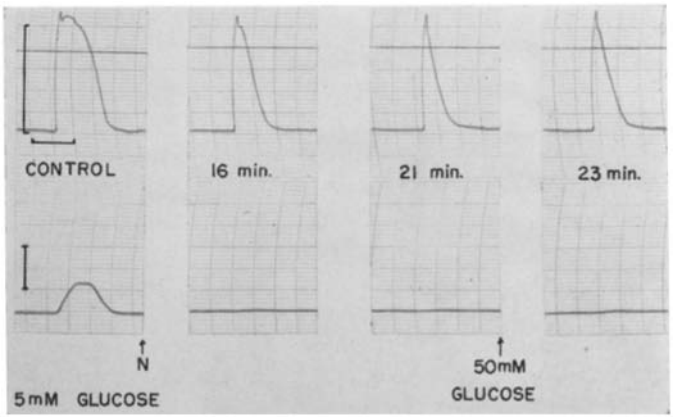

FIGURE 8B

FIGURE 8. Simultaneous recordings of action potential upstroke, first derivative of the upstroke, and complete action potential during anoxia-induced decrease in action potential duration and subsequent response to glucose. Voltage calibration 100 mv, time calibration 200 msec. Force calibration 1 gm.

before exposure to adrenalin. In addition, in 4 of 8 trials using 0.2 microgram/ml adrenalin the action potential duration was increased by less than 10 per cent whereas the force of contraction was increased from 6 to 20 times.

DISCUSSION

The early response of the cat papillary muscle to incubation in 95 per cent N_2:5 per cent CO_2 or to cessation of oxygenation appeared to be limited to a decrease in force of contraction and a shortening of the action potential dura-

tion. No consistent change in resting potential was observed during anoxia-induced shortening of the action potential duration beyond the limits of error in our observations. Similarly, as can be seen in Figs. 2 and 6–9, marked shortening of the action potential duration can occur as a result of anoxia with no change in the amplitude of the action potential. These findings are in agreement with those of Webb and Hollander (1956) for rat atrium and Trautwein and Dudel (1956) for cat ventricle. These authors have proposed that the decrease in action potential duration is due to an increased rate of repolarization as a result of enhanced potassium efflux during activity. Changes in amplitude of the action potential and resting potential are thought

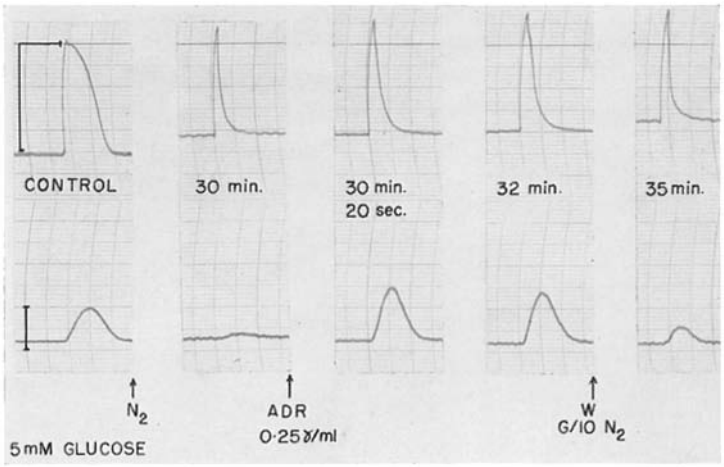

FIGURE 9. The effect of adrenalin (ADR) on the transmembrane action potential and force of contraction of an anoxic papillary muscle. The experiment was carried out in 5 mM glucose. At *W* the preparation was washed with Krebs-Ringer's solution containing 5 mM glucose equilibrated with 95 per cent N_2:5 per cent CO_2. Calibrations as in Fig. 8.

to occur only as a consequence of altered chemical gradients to potassium or sodium. It is significant that anoxia-induced potassium loss and sodium gain in rat ventricle is enhanced by stimulation (Hercus, MacDowall, and Mendel, 1955).

The results of this study indicate that glucose and certain of its analogues can oppose and partially reverse the effects of anoxia on the duration of the action potential in papillary muscle. An increase in energy supply, possibly consequent to increased glycolysis is a conceivable mechanism of action. Since it has been proposed (Trautwein and Dudel, 1956; Webb and Hollander, 1956) that increased potassium permeability during anoxia is responsible for the increase in repolarization rate, an effect of glucose on potassium permeability is another conceivable mechanism of action. The effectiveness of two non-metabolizable sugars (xylose and 2-deoxyglucose) in substituting for glu-

cose makes it unlikely that the effect of glucose is the result of increased energy production due to increased glucose uptake. The failure of restoration of contractions by glucose is incompatible with any hypothesis of a generalized increase in energy supply. Also, glucose uptake, intracellular glucose levels, and glucose phosphorylation rates at external glucose concentrations of 5 and 50 mM have recently been reported (Morgan *et al.*, 1960) in the perfused rat heart under anaerobic conditions; the only striking difference appeared to be increased levels of intracellular free glucose at the higher concentration. If the papillary muscles we studied are similar to the perfused rat heart, then increased external glucose concentrations would not increase the rate of glycolysis.

A common property of those sugars which have been found to alter activity is that they are thought to be transported into the cell by a common mechanism. According to Morgan *et al.* (1964) a carrier-mediated sugar transport system has been identified in cardiac and skeletal muscle by the demonstration of countertransport of the non-metabolizable sugar, *o*-methyl glucose. The model usually presented includes the complexing of a sugar with a carrier, the movement of the complex through the membrane, and the release of the sugar at the inner surface of the membrane. The process is assumed to be reversible and has been found to conform to Michalis-Menten kinetic relationships. At low glucose concentrations under either aerobic or anaerobic conditions free glucose may not accumulate in the cell due to the rapid phosphorylation rate and glucose transport may be rate-limiting. In this situation the carrier must return to the outer surface of the membrane empty or carrying some other passenger molecule. At higher glucose concentrations under anaerobic conditions phosphorylation may become rate-limiting and free glucose accumulates in the cell. Under these circumstances inward and outward transport of glucose utilizing the carrier would eventually approach equilibrium. Such a situation would occur with 50 mM glucose under anaerobic conditions. Now, if the carrier can be modified in some manner as suggested by Daniel (1964) to carry more than one molecule or ion, it is conceivable that an interrelationship could exist between glucose and potassium transport. If this concept is employed, increased potassium efflux during anoxia in normal glucose could be explained by the increase in carrier at the intracellular surface of the membrane due to the increased uptake and utilization of glucose which accompanies anoxia. When the external glucose concentration is increased sufficiently so that intracellular levels of free glucose rise and back transport occurs, the availability of the carrier for potassium might be decreased and the repolarization rate slowed. In the case of xylose which is not phosphorylated or utilized, transport would be in both directions at all concentrations but back transport would become important only after significant intracellular levels of xylose had been attained usually after a delay.

Although the experiment has not been done, it should be possible for xylose to exert an effect on the electrical activity of the anoxic muscle at lower concentrations than glucose since back transport will occur with virtually any external concentration of xylose because it is non-metabolized.

With 2-deoxyglucose the situation is complicated by the inconsistency of its initial effect on action potential duration. It is also phosphorylated and it apparently inhibits the effect of subsequently added glucose. It can, however, be fitted into the present model on the following basis. An initial effect on action potential duration would be expected if its phosphorylation rate were sufficiently slow to allow some intracellular accumulation of free sugar to compete with potassium for carrier. An initial effect would not be expected if phosphorylation rates were sufficiently high to prevent intracellular accumulation and hence back transport of the sugar. The accumulation of 2-deoxyglucose-6-phosphate has been shown by Kipnis and Cori (1959 *b*) to inhibit the transport of glucose and several other sugars and on this basis the lack of response to glucose following exposure to 2-deoxyglucose may be explained.

The small but definite prolongation of the action potential of the anoxic muscle following adrenalin may be explained on the basis of its reported action on hexokinase. It has been shown by Kipnis and Cori (1959 *a*) that although adrenalin did not influence the uptake of 2-deoxyglucose in the rat diaphragm it did cause a marked decrease in phosphorylation rate and a corresponding intracellular accumulation of free sugar. Since in these experiments 2-deoxyglucose was being used as a marker for mechanisms normally involved with glucose, it may be supposed that similar effects occur with glucose. Therefore if adrenalin does cause an accumulation of intracellular free glucose under circumstances where accumulation would not normally occur, back transport would result, carrier available for potassium would be decreased, and repolarization rate slowed.

On the basis of theories that suggest the efflux of potassium to be simply diffusion through water-filled pores in a depolarized membrane down a concentration gradient it is difficult to explain our findings. That membrane levels of ATP control this efflux and can be altered by local glycolysis must be ruled out due to the fact that xylose is effective though not phosphorylated. Whether the sugar:carrier complex might be substituted for ATP as a controller of diffusion downhill cannot be answered until something is known about the charge distribution of the complex.

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