Influence of Glucose on the Transmembrane Action Potential of Papillary Muscle

Effects of concentration, phlorizin and insulin, nonmetabolizable sugars, and stimulators of glycolysis

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ABSTRACT The action potential duration (APD) of isolated guinea pig papillary muscle is directly related to the medium glucose concentration regardless of the gas mixture with which it is in equilibrium. The APD can be maintained at control value for many hours by a glucose concentration of 50 mM in the complete absence of oxygen. Following reduction of the APD by incubation of the muscle in medium containing 5 mM glucose, adjustment of the glucose concentration to 50 mM will cause restoration of normal APD. Phlorizin has been shown to competitively interfere with the effect of glucose on the APD and insulin to prevent or reverse the effect of phlorizin. Nonmetabolizable sugars cannot produce glucose-like effects on the APD. Adrenaline, noradrenaline, and isopropylnoradrenaline increased the reduced APD of papillary muscles incubated in the absence of oxygen in a medium containing 5 mM glucose coincident with an increase in contractile force. The effect of isopropylnoradrenaline was blocked by acetylcholine and propranolol. In the presence of iodoacetate and 2-deoxyglucose, isopropylnoradrenaline increased contractile force but not the reduced APD. Aminophylline was found to produce changes in the reduced APD similar to those caused by the sympathomimetic amines. The findings clearly support the hypothesis that anaerobic metabolism utilizing either glycogen or exogenous glucose is capable of maintaining normal transmembrane electrical activity in guinea pig papillary muscle.

INTRODUCTION

Recently, it was reported by MacLeod and Daniel (1965) that a concentration of 50 mM glucose in the bath medium could prevent or reverse the
decrease in the action potential duration induced by anoxia in the cat papillary muscle. The effect of glucose could be partially duplicated by similar concentrations of the sugars, 2-deoxyglucose and xylose, but not by sucrose. Therefore, the effect of glucose was not due to an osmotic effect and did not seem to be due to a metabolic effect since 2-deoxyglucose and xylose, although transported by the glucose transport system (Kipnis et al., 1959; Fisher and Zachariah, 1961), are not metabolized to any extent. Since the decrease in action potential duration due to anoxia has been thought to be caused by an increased rate of repolarization resulting from an increase in the rate of potassium efflux during activity (Trautwein and Dudel, 1956; Webb and Hollander, 1956), MacLeod and Daniel (1965) proposed that glucose reduced the rate of potassium efflux. This proposal required that a carrier material be responsible for the transport of glucose both into and out of the cell and for at least the outward movement of potassium during repolarization. When the glucose concentration in the medium is low, all glucose carried into the cell is metabolized and its accompanying carrier left at the inner surface of the cell membrane available for the movement of potassium outward. When the glucose concentration in the medium is high, free glucose accumulates in the cell (Morgan et al., 1961). Similarly, with nonmetabolizable sugars there is an accumulation within the cell. Both these circumstances should create a back transport of sugar thereby occupying the carrier and reducing that available for outward potassium movement. Similarly, adrenaline, which has been shown to inhibit hexokinase (Kipnis and Cori, 1959) and therefore increase intracellular free glucose, was shown to increase the action potential duration of the anoxic muscle.

The present series of experiments was undertaken to investigate more fully the circumstances under which a reduction in action potential duration occurs. For this purpose, measurement was made of the action potential duration of papillary muscle incubated in modified Krebs solution containing either 0, 5, or 50 mM glucose equilibrated with either 95% O2:5% CO2, 95% N2:5% CO2 or an intermediate mixture, 60% N2:35% O2:5% CO2. Similarly, the effect of various concentrations of glucose on the action potential duration which had been reduced by incubation of the muscle in Krebs solution containing either 0 or 5 mM glucose was determined in the presence of the three gassing mixtures.

If transport of glucose per se is responsible for its effect on repolarization, then it was essential to show that insulin, which has been shown (Fisher and Lindsay, 1956; Morgan et al., 1961) to facilitate glucose transport in cardiac muscle, should also increase its effect on electrical activity. Similarly, phlorizin, which has been accepted as a specific inhibitor of the glucose transport process in various tissues (Lotspeich, 1960–61; Diedrich, 1966), should decrease the effect of glucose on the action potential duration. It should be noted that although both insulin and high glucose concentration in the
medium increase inward movement of glucose, there is some difference in the fate of the glucose so transported. In the case of increased uptake due to insulin, more of the extra glucose is found as glycogen. When the increased uptake is due to a high external glucose concentration, more of the extra glucose is directly metabolized (Villar-Palasi and Larner, 1958, 1961).

An extended series of experiments has been carried out with the non-metabolizable sugars, D-xylose, 2-deoxyglucose, arabinose, 3-O-methylglucose, D-galactose, L-glucose, and α-methyl-D-glucoside. This series of experiments has demonstrated beyond reasonable doubt that no one of these sugars can duplicate the effect of glucose on the reduced action potential duration.

An alternative explanation for the effect of glucose on the reduced action potential duration was thought to be a stimulation of anaerobic glycolysis. If such an increase in glycolysis is responsible for the effect of glucose on the action potential duration, then it follows that a similar effect would be produced by a stimulation of glycogenolysis. Sympathomimetic amines have been shown to stimulate glycogenolysis by promoting the accumulation of cyclic 3',5'-adenosine monophosphate (3',5'-AMP), which in turn stimulates phosphorylase b kinase which transforms (inactive) phosphorylase b to (active) phosphorylase a (Rall and Sutherland, 1958; Krebs et al., 1958; Butcher and Sutherland, 1962). A close temporal relationship between the increases in phosphorylase activity and contractile force following adrenalin administration has been shown by Hess and Haugaard (1958) and Cotten and Moran (1961). Since glycogenolysis is related to phosphorylase activity in the heart (Ellis et al., 1957) and if increased glycogenolysis causes an increase in APD, then amine-induced increases in contractile force should be correlated with changes in the action potential duration. Experiments were carried out to determine whether such a correlation exists.

Finally, a short series of experiments was carried out in which the effect of aminophylline on the reduced action potential duration and force of contraction of papillary muscle was studied. The action of aminophylline in increasing glycogenolysis is also mediated by an accumulation of cyclic 3',5'-AMP due to inhibition of phosphodiesterase which is normally responsible for its breakdown (Butcher and Sutherland, 1962).

**METHODS**

All experiments were carried out with papillary muscle obtained from the right ventricle of guinea pig heart. The heart was removed from the animal after it had been rendered unconscious by a single blow on the head. Dissection of the muscles was carried out in cool modified Krebs-Ringer solution of the following composition in milliequivalents per liter: Na 138.5, K 4.6, Ca 4.9, Mg 2.3, HCO3 21.91, PO4 3.48, SO4 2.32, Cl 125, and glucose 50 mM equilibrated with 95% O2:5% CO2 (carbogen). Guinea pig papillary muscle tended to be flat, 6-8 mm in length, 1-2 mm wide, and 0.5-1 mm thick. The muscles were mounted horizontally at a resting tension of 500
mg in a jacketed 100 ml constant temperature bath at 37°C. Stimulation was at a rate of 60 per min through platinum electrodes which approximated but did not touch the muscles. Force of contraction was recorded on a Grass polygraph by means of a Grass force displacement transducer.

Single cell electrical activity was recorded by means of hand-pulled glass micro-electrodes filled with 3 m KCl using the floating electrode technique of Woodbury and Brady (1956). 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.

PRELIMINARY experiments showed that the action potential duration of papillary muscle decreased in response to incubation in Krebs solution containing 5 mM glucose equilibrated with 95% N₂:5% CO₂ to the same degree when duration was measured at either 30, 60, or 90% repolarization. Where quantitative measurements are given, they will apply to the duration as measured at 60% repolarization.

RESULTS

The previously reported finding (MacLeod and Daniel, 1965) that a glucose concentration of 50 mM in the bath medium could maintain a normal action potential duration (APD) in cat papillary muscle in the absence of oxygen was confirmed in guinea pig papillary muscle. In addition, it was found that the APD could be maintained at control level for as long as 8 hr in the complete absence of oxygen if the medium contained 50 mM glucose (Fig. 1). If during incubation of a muscle in the absence of oxygen the glucose concentration was reduced to 5 mM, there was a reduction in APD which could
be completely reversed by reintroducing medium containing 50 mM glucose (Fig. 2).

In order to study more fully the relationship between glucose concentration in the medium and transmembrane electrical activity, it was desirable to have...
information on the part played by the oxygen level in the gassing mixture employed. Experiments were therefore carried out in which papillary muscles were incubated in Krebs solution containing either 0, 5, or 50 mM glucose equilibrated with either 95% O₂:5% CO₂ (carbogen), 95% N₂:5% CO₂ or an intermediate mixture, 60% N₂:35% O₂:5% CO₂. As many be seen in Fig. 3, there was a decrease in APD when the muscles were incubated in Krebs solution containing 5 mM glucose regardless of the gas mixture with which the medium was in equilibrium. Although there was some difference in the rate of reduction of the APD depending upon whether the oxygen was completely or partially replaced by nitrogen, the effect at the end of 1 hr incubation was similar. The reduction in APD which occurred when the medium was equilibrated with carbogen was surprisingly quite marked. The effect occurred slowly at the beginning of an experiment (Fig. 4 A) but with repeated periods of incubation in Krebs solution containing 5 mM glucose alternating with periods of incubation in Krebs solution containing 50 mM glucose, during which the APD returned to control level, the rate of reduction of the APD in response to lowered glucose concentration increased (Fig. 4 B). In all instances a more rapid decrease in APD occurred in the complete absence of glucose and the APD could be maintained at or returned to control levels by a glucose concentration of 50 mM. The glucose-induced increase in APD was the same regardless of the gassing mixture employed during the reduction in APD.

These experiments demonstrate the importance of the concentration of glucose in the medium for the maintenance of normal APD in papillary muscle. That the effect is not dependent upon changes in osmotic pressure has been previously shown by MacLeod and Daniel (1965). To further demonstrate that glucose uptake must be involved in its effect on APD, experiments were conducted in which the APD was reduced to between 30 and 40% of control in Krebs solution containing 5 mM glucose and equilibrated with 95% N₂:5% CO₂ after which various concentrations of glucose were added to the bath. It will be noted in Fig. 5 that glucose in all the concentrations used increased the APD. The proportionately greater effect at lower concentrations of glucose and subsequent plateau at higher concentrations can most readily be explained by an increasing occupancy of a combining site necessary for glucose transport.

Since the transport of glucose appeared to be necessary for its effect on the reduced APD, experiments were conducted in which the effect of the glucose transport inhibitor, phlorizin, on the APD was determined. In 15 experiments, it was found that phlorizin (0.25–4 mM) consistently increased the rate of decrease in APD of muscles incubated in Krebs solution containing 5 mM glucose and equilibrated with any of the three gas mixtures. Phlorizin has also been shown to consistently reduce the effects of elevated glucose
Figure 4 A. Decrease in action potential duration and contractile force of papillary muscle incubated in Krebs solution containing 5 mM glucose and equilibrated with 95% O₂:5% CO₂ (G5/O₂) early in an experiment.

Figure 4 B. Incubation of same muscle under conditions as described in A but later in the same experiment and following a period of incubation in Krebs solution containing 50 mM glucose. Note much more rapid changes (30 min vs. 80 min) in B than in A. Calibration as in Fig. 2.
concentration on the reduced APD. The effect of phlorizin was found to be inversely proportional to the concentration of glucose as can be seen in Fig. 6. The parallelism of the % inhibition curves indicates competitive antagonism between glucose and phlorizin.

Since insulin has been shown to increase glucose uptake in cardiac muscle (Fisher and Lindsay, 1956; Morgan et al., 1961), it seemed reasonable to suspect that it might alter the APD by itself or alter the effect of glucose on the APD. In a large number of trials, insulin itself was not found to measurably alter the reduced APD of papillary muscle incubated in the absence of oxygen in Krebs solution containing 5 mM glucose. It was also not possible to measure any significant difference in the effect of glucose on the reduced APD of concentrations of insulin from 0.2–120 milliunits/ml (compare bars A and B in Fig. 8 for effect of 40 milliunits/ml insulin on the action of 25 mM glucose).

Since the failure of insulin to increase the action of glucose on the reduced APD might have been due to an already maximum stimulation of uptake by...
anoxia coupled with high glucose concentrations, the experiments were repeated in the presence of oxygen. As can be seen in Fig. 7, an increase in the action of 10 mM glucose on the reduced APD of papillary muscle incubated in Krebs solution containing 5 mM glucose equilibrated with carbogen could be observed in the presence of insulin. This difference of about 10% was also observed with 15 mM glucose.

Although insulin failed to significantly influence the action of glucose on the reduced APD in the absence of oxygen, it was found to prevent or reverse the inhibiting effect of phlorizin on the action of glucose. Fig. 8 shows that insulin was capable of completely antagonizing the effect of 0.5 mM phlorizin but not that of 2 mM phlorizin.

Our findings to this point appeared to demonstrate that the APD is a
function of glucose uptake. Also, the results of the experiments with insulin suggested that the subsequent fate of the glucose taken up was important in its effect on APD.

In an attempt to separate the effects of the transport of glucose from the effects of its subsequent metabolism, experiments were carried out employing a number of nonmetabolizable sugars. It has been reported by MacLeod and Daniel (1965) that d-xylose and 2-deoxyglucose which are thought (Kipnis and Cori, 1959; Fisher and Zachariah, 1961) to be transported by the glucose transport system, could only partially duplicate the effect of glucose on the reduced APD. Since it seemed to be essential to decide whether or not a transported but nonmetabolizable sugar could produce an effect on the APD, these experiments were extended to include a number of other sugars which are thought (Fisher and Zachariah, 1961; Battaglia and Randle, 1960; Morgan et al., 1964) to be also transported by the glucose transport system (l-arabinose, 3-O-methylglucose, d-galactose). For comparative purposes l-glucose and α-methyl-d-glucoside were included in the experiments as sugars which are transported by a system other than the glucose transport system or not transported at all (Battaglia and Randle, 1960; Henderson, 1964; Morgan et al., 1964). The experiments were carried out by incubating muscles in
Krebs solution containing 0 or 5 mM glucose and equilibrated with one of the three gas mixtures until the APD was reduced to between 35 and 45 % of the control and then adding the test sugar. If the APD increased to more than 75 % of the control during 60 min following addition of the sugar, it was arbitrarily considered to have a glucose-like activity. At the same time, the effect of glucose on the APD was determined in the presence and absence of each of the nonmetabolizable sugars. As can be seen from Table I, which summarizes all the data from these experiments, no sugar was found to possess a significant action on the reduced APD in the absence of oxygen. Some effects from xylose were observed but in general they were minimal. When one considers the total number of observations made when the gas mixture was either 95 N₂:5 % CO₂ or 60 % N₂:35 % O₂:5 % CO₂ it seems clear that the effect of glucose on the reduced APD cannot be duplicated by nonmetabolizable sugars. The effects of D-xylose and L-arabinose in the presence of oxygen were surprising and at present we can offer no explanation for these findings. However, both sugars are thought to be metabolized to some degree (Krahl, 1961). That these nonmetabolizable sugars are in fact transported by the glucose transport system may be assumed from the data presented in Table I and Fig. 9. It can be seen that all sugars with the exception of L-glucose and α-methyl-D-glucose antagonized the action of glucose on the reduced APD. These latter two sugars produced no change in the

<table>
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<tr>
<th>Table 1</th>
<th>The Effect of Nonmetabolizable Sugars on the Reduced APD and on the Effect of Glucose on the Reduced APD of Papillary Muscle</th>
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<tr>
<td><strong>Concentration</strong></td>
<td>95 % O₂: 5 % CO₂</td>
</tr>
<tr>
<td><strong>No. of trials in which sugar demonstrated glucose-like activity/number of trials conducted with each of 3 gassing mixtures</strong></td>
<td><strong>Reduction of glucose action on APD,</strong></td>
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<tr>
<td><strong>Sugars</strong></td>
<td><strong>95 % O₂: 5 % CO₂</strong></td>
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<tr>
<td>D-Xylose</td>
<td>45</td>
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<tr>
<td>L-Arabinose</td>
<td>45</td>
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<tr>
<td>3-O-methyl-Dglucose</td>
<td>25</td>
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<tr>
<td>2-Deoxyglucose</td>
<td>45</td>
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<tr>
<td>D-Galactose</td>
<td>45</td>
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<tr>
<td>L-Glucose</td>
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<tr>
<td>α-Methyl-D-glucoside</td>
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* Experiments conducted with 60 % N₂:35 % O₂:5 % CO₂ as gassing mixture.
† Reduction of the action of glucose by 20% or greater.
Figure 9. The effect of 25 mM glucose (G25) on the reduced APD of papillary muscle incubated in Krebs solution containing 5 mM glucose and equilibrated with 60% N₂:35% O₂:5% CO₂ (N₂:O₂) in the absence and presence of various nonmetabolizable sugars. The upper records in each quarter of the figure show the control effect of glucose. The lower records in each quarter show the effect of glucose in the presence of arabino-nose (A), α-galactose (B), 2-deoxyglucose (C), and α-methyl-β-glucoside (D) reduced the effect of glucose. Voltage calibration 100 mV; time calibration 200 msec.

action of glucose on the reduced APD. The effects were quite marked especially with 2-deoxyglucose (Fig. 9). The fact that these nonmetabolizable sugars were found to reduce the ability of glucose to increase the reduced APD of papillary muscle is taken to indicate that they utilize at least some
portion of the glucose transport system and are in fact transported. It is clear, however, that their transport per se does not increase the reduced APD of papillary muscles as does glucose.

As was previously reported for adrenaline (ADR) (MacLeod and Daniel, 1965), isopropylnoradrenaline (INA) and noradrenaline (NA) were found to increase the reduced APD of muscles incubated in Krebs solution containing 5 mM glucose equilibrated with either 95% N₂:5% CO₂, 60% N₂:35% O₂:5% CO₂ or carbogen. As can be seen in Fig. 10, the effect of INA on the APD was concentration-dependent.

It has been demonstrated that there is a simultaneous increase in myocardial contractile force and phosphorylase activity after administration of ADR (Hess and Haugaard, 1958; Cotten and Moran, 1961). Also, since increased phosphorylase activity is necessary for an increase in glycogenolysis (Ellis et al., 1957), then it follows that a relationship should exist between sympathomimetic amine-induced increases in contractile force and APD.
In 15 experiments, the time course of the effect of 0.5 μg per ml INA on the reduced APD and contractile force of papillary muscle was studied. It was observed that the increase in the APD was synchronous with an increase in the contractile force as may be seen in Fig. 11. The effect of INA on both parameters began within 5 sec (fifth beat), reached its peak simultaneously (thirty-fifth beat), and even decayed on a similar time course. It may be pointed out, however, that in most trials with INA and ADR the APD was found to decrease more rapidly than the contractile activity as the effect of the amines decayed (see Discussion).

It has been reported that only those sympathomimetic amines which activate phosphorylase produce positive inotropic effects in cardiac muscle (Hess and Haugaard, 1958; Kukovetz et al., 1959; Mayer and Moran, 1960; Drummond et al., 1966). These authors showed that INA, ADR, and NA, which increase contractile force, also activate phosphorylase; phenylephrine, which even in high concentrations was capable of producing only a moderate effect on the contractile force, produced a small increase in phosphorylase activity. Finally, methoxamine which has virtually no positive inotropic action, failed to activate phosphorylase even at the highest concen-
trations. In five experiments in the present study, the effects of 5, 10, and 20 μg per ml of phenylephrine on the reduced APD and force of contraction of papillary muscle were compared to that produced by 0.5 μg per ml INA. At the two lowest concentrations, phenylephrine was without effect on either

the force of contraction or the APD but had some effect at a concentration of 20 μg per ml. In three experiments, each with 250, 500, and 1,000 μg per ml methoxamine, however, no effect on either force of contraction or APD could be observed. In their effects on either contractile force or phosphorylase activation the potency of INA, NA, and ADR was related in a 5:2:1 ratio (Kukovetz et al., 1959). In 14 experiments carried out in the present series, the effects of 0.25, 0.5, and 1.0 μg per ml each of ADR, NA, and INA on the

![Figure 12](image-url). Effect of 0.5 μg/ml of INA on the reduced APD and force of contraction of papillary muscle before and during exposure of the muscle to 10 μg/ml of acetylcholine (ACh). The muscle was incubated in Krebs solution containing 5 mM glucose equilibrated with 60% N₂:35% O₂:5% CO₂ (G5/N₂:O₂). Calibration as in Fig. 2.
APD of papillary muscle incubated in Krebs solution containing 5 mM of glucose and equilibrated with 95% N₂:5% CO₂ were determined. In all instances, the APD was reduced to approximately one-third of the control before exposure of the muscle to an amine. It was found that the % change in APD induced by 0.25 μg/ml each of INA, NA, and ADR produced a ratio of 4.4:1.5:1. These values are remarkably similar to those reported by Kukovetz et al., referred to above.

Complete antagonism of the glycogenolytic effect of adrenaline by acetylcholine in perfused guinea pig heart has been reported by Vincent and Ellis (1963). They also reported that acetylcholine alone did not significantly increase the cardiac glycogen level. Four experiments were therefore carried out in which papillary muscle was exposed to 10 μg per ml acetylcholine for 1–2 min after the APD had been reduced to approximately one-third of the control and the effect of 0.5 μg per ml INA on the APD and force of contraction determined. It may be seen in Fig. 12 that acetylcholine markedly antagonized the effect of INA on the APD but not on contractile activity.

Sympathomimetic amine-induced activation of phosphorylase has been shown to be blocked by various β-adrenergic blocking agents, AY 64043 (propranolol), pronethalol, and dichloroisoproterenol (DCI), while α-adrenergic blocking agents, piperoxan and phenoxybenzamine, were ineffective (Mayer and Moran, 1960; Dhalla, 1966). In six experiments it was found that the effect of 2 μg per ml of ADR on the reduced APD was blocked by 3 μg per ml of propranolol. In an additional six experiments with either 1 or 5 μg per ml priscoline, an α-adrenergic blocking agent, no antagonism to the effect of ADR on APD could be observed.

The evidence obtained in the preceding series of experiments strongly supported the hypothesis that the effect of sympathomimetic amines on the APD was mediated by their stimulation of glycolysis. On this basis it was reasonable to suppose that the effect of sympathomimetic amines on the reduced APD should be prevented by the glycolysis-blocking agent, iodoacetic acid (IAA), but not necessarily their effect on contractile activity. In four experiments, the effect of 0.5 μg per ml INA on the reduced APD and force of contraction of papillary muscle was studied before and during exposure of the muscle to 10⁻⁶ M or 0.5 × 10⁻³ M IAA. In both concentrations IAA completely antagonized the effect of INA on the APD but was only partially able to antagonize the effect on contractile activity (Fig. 13). The latter observation is in agreement with recently reported results of Horn et al. (1967). Similar results were obtained in the presence of 2-deoxyglucose (2-DG), which is thought to inhibit phosphoglucoisomerase (Wick et al., 1957; Ferrari et al., 1959) and so inhibit glycolysis.

If catecholamines are able to increase the reduced APD by increasing glycogenolysis, then depletion of glycogen stores should abolish this effect. In
five experiments muscles were incubated in glucose-free Krebs solution equilibrated with 95% N₂: 5% CO₂ until the APD was markedly reduced and the mechanical activity virtually absent. At this point it was assumed that the glycogen store would be depleted or much reduced. Under these circum-

stances, ADR was found to increase contractile activity but to have no effect on the APD.

Although the weight of evidence supported an increase in glycogenolysis as an explanation for amine-induced increases in APD, the possibility of an effect on glucose transport still existed. Since Williamson (1964) reported that ADR increases the transport of glucose in rat heart whereas Kipnis and Cori (1959) reported an inhibition of transport, it was of interest to study the effect of ADR and INA on the reduced APD in the presence of phlorizin. In five experiments it was found that 1 mM of phlorizin did not qualitatively alter the
effect of these amines on the reduced APD (Fig. 14), although the effect of glucose was abolished.

Theophylline, like catecholamines, has been found to elevate phosphorylase activity in rat, rabbit, and frog heart (Hess and Haugaard, 1958; Mayer and Moran, 1960; Kukovetz and Pöch, 1962; Dhalla and McLain, 1966). This effect is thought to be mediated through the accumulation of cyclic AMP due to inhibition of phosphodiesterase which is normally responsible for its breakdown (Butcher and Sutherland, 1962). Theophylline has also been shown to increase glycogenolysis in the isolated perfused guinea pig heart (Vincent and Ellis, 1963). In five experiments the soluble salt of theophylline, aminophyl-
line, was found to increase the APD and contractile force of papillary muscle incubated in low glucose Krebs solution equilibrated with 60% N₂:35% \( \text{O}_2:5\% \text{CO}_2 \) (Fig. 15). In contrast to their effect on sympathomimetic amines, \( \beta \)-adrenergic blocking agents have not been found to block the effects of aminophylline on contractile activity or phosphorylase activation in frog heart (Dhalla and McLain, 1966). It was not surprising therefore to find in three experiments that 3 \( \mu \)g per ml of propranolol in no way altered the effect of aminophylline on the APD and contractile force of papillary muscle.

**Figure 15.** Effect of 100 \( \mu \)g/ml of aminophylline on the reduced APD and force of contraction of papillary muscle incubated in Krebs solution containing 5 mm glucose and equilibrated with 60% N₂:35% \( \text{O}_2:5\% \text{CO}_2 \) (G5/N₂:O₂). Voltage calibration 100 mv, time calibration 200 msec, force calibration 500 mg.

**DISCUSSION**

The foregoing data allow certain general conclusions to be drawn and in addition offer new proposals for further investigation. It is evident that studies of the effects of substrate depletion, metabolic inhibition, or oxygen lack on the activity of cardiac muscle must include measurements of both electrical and mechanical activity. It is evident from this and earlier work that changes in the force of contraction may or may not be paralleled by changes in transmembrane electrical activity. It is clear that the effect of anoxia on the electrical activity varies with the external glucose concentration as is the fact that anoxia-induced changes in the force of contraction are not appreciably altered by the glucose concentration. For these reasons, when discussing reported changes in the transmembrane electrical activity, it is the glucose concentration of the medium and not the oxygen content of the gassing mixture which is important. There is no doubt, however, that the oxygen content of the medium qualitatively influences the changes in APD occurring as a result of incubation.
of a muscle in a medium containing little or no glucose. Since the majority of the studies on the effects of oxygen lack or substrate depletion have been conducted with 5 mM glucose as the reference or control concentration of glucose, a decrease in APD has always been associated with a decrease in force of contraction of a variety of cardiac muscle preparations (Webb and Hollander, 1956; Yang, 1963; Wallon et al., 1960; Trautwein et al., 1954; Trautwein and Dudel, 1956).

The present work has confirmed some of the findings of MacLeod and Daniel (1965) and disproved others. That a concentration of 50 mM glucose in the bath medium could maintain normal transmembrane electrical but not contractile activity of papillary muscle in the complete absence of oxygen was confirmed for periods up to 10 hr at a stimulation rate of one per sec. The importance of the glucose concentration in the bath medium for the maintenance of a “normal” APD was further demonstrated in those experiments in which muscles were incubated in Krebs solution containing 0 or 5 mM glucose and equilibrated with gassing mixtures containing different levels of oxygen. Changes in electrical and mechanical activity occurring with different gassing mixtures were qualitatively similar whether the media contained 5 or 0 mM glucose but the rate of change in glucose-free Krebs solution was more rapid. It was surprising, however, that there was so marked a reduction in the APD when the muscles were incubated in Krebs solution containing either 0 or 5 mM glucose equilibrated with carbogen. Although these effects were not so well-marked as when the muscles were incubated under complete or partial anoxia, they did occur. It was found that the sensitivity of any particular muscle to the effect of low glucose increased with repeated incubation periods in medium containing 5 mM glucose. This increasing sensitivity to oxygen lack and/or a decreased glucose concentration in the medium has been reported previously by Trautwein and Dudel (1956) and MacLeod and Daniel (1965) who considered the effect to be an increasing sensitivity to oxygen lack. The present findings with glucose deprivation indicate the probability that a number of factors reducing metabolic activity produce greater effects with repeated administration. That a progressive decline in glycogen content might be a possible explanation would seem to be unlikely in view of the marked increase in the reduced APD produced by the sympathomimetic amines and aminophylline. However, these agents may be able to produce their effects at low glycogen levels.

The lack of a measurable effect of insulin on the APD in the absence of oxygen or on the effect of glucose on the APD was unexpected whereas the findings with phlorizin were as might be expected. In the light of many reports on the effect of insulin on glucose uptake in cardiac muscle (Fisher and Lindsay, 1956; Morgan et al., 1961), it seemed reasonable to expect that insulin would produce an effect on the reduced APD similar to the effect of an increase in
glucose concentration. The observation by Villar-Palasi and Larner (1958, 1961) that more of the extra glucose taken up under the influence of insulin is found as glycogen, whereas more of the extra glucose taken up because of an increase in extracellular concentration is directly metabolized may offer an explanation as to why insulin was not found to alter the action of glucose. If the increase in glycolysis is associated with an increase in the reduced APD, then the effect of an increase in extracellular glucose concentration would be more likely to increase the reduced APD. An alternative explanation is that the conditions under which the experiments were conducted were not conducive to the demonstration of an insulin effect. If for instance transport was not the limiting factor in glucose metabolism when insulin was tested, it would not produce an effect. Since insulin had a small but consistent effect on the action of glucose on the APD in the presence of oxygen, the latter explanation seems a distinct possibility.

In any event, it seems likely that some aspect of glucose metabolism is responsible for its effect on electrical activity in cardiac muscle. Since the effect is produced under anoxic conditions, aerobic metabolism may be tentatively excluded and attention centered on the glycolytic pathway of glucose metabolism.

Present results show that catecholamines produce a rapid increase in APD and mechanical activity which reaches a maximum in 30 sec and lasts for 1-2 min, after which both parameters decrease simultaneously. In perfused rat heart Williamson (1964) has reported a rapid catecholamine-induced increase in phosphorylase activity with a maximum at 20 sec for 1 to 2 min and falling to control over the next 30 min; similar changes were found to occur in the formation of lactic acid. That the lactate formed was derived from glycogen and that glycogenolysis and lactate production paralleled phosphorylase activity were shown in the same study. Although force of contraction was not measured in Williamson’s (1964) study, he reported that epinephrine-induced increases in heart rate remained constant during a 30 min perfusion period despite the relatively transient effect of epinephrine on glycogenolysis. He concluded that subsequent compensations in aerobic metabolism occurred to maintain the catecholamine-induced increase in heart work. In the present study, such adjustments in aerobic metabolism were not possible and it must be concluded that catecholamine-induced changes in both APD and force of contraction parallel the changes in glycogenolysis.

The evidence obtained in all the experiments conducted in the present study supports the proposal that the effect of catecholamines on APD is mediated by an increase in glycolysis although much of the evidence is indirect, since it has been obtained by showing that sympathomimetic amines affect the APD in our experiments in a direction similar to their reported effects on phosphorylase activity. However, the observation that IAA and 2-DG inter-
fered with the action of the amines on APD but not the force of contraction seems conclusive. Obviously an intact glycolytic pathway is not necessary for a sympathomimetic amine-induced increase in contractile activity but it is necessary for an increase in APD.

It follows that a relationship must be established between glycolytic activity and cell membrane function. The most likely connection is through ATP production and utilization. Since there seems to have been no association between changes in mechanical and electrical activity throughout these experiments, it does seem that although contractile activity and membrane function may utilize ATP produced aerobically, that produced by anaerobic mechanisms appears to be restricted to membrane activity. Although we have no evidence to support such a proposal, it may be suggested that ATP produced by glycolysis is largely restricted to the cell membrane since it is produced there as opposed to production in the cytoplasm and then being preferentially moved to the membrane.

Finally, it must be shown whether the ATP involved in changes in cell membrane electrical activity produces such changes in a passive way as suggested by Webb and Hollander (1956) or as a result of being utilized in some further process. Evidence to be presented in a subsequent report supports the position that changes in action potential configuration, especially duration, may result from either a reduction of available ATP or a restriction in its utilization. Conversely an increase in the APD of a muscle incubated in low glucose may be produced by increasing available ATP or increasing its utilization.

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