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METABOLISM AND THE ELECTRICAL ACTIVITY OF ANOXIC VENTRICULAR MUSCLE

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SUMMARY

1. The action potential duration of anoxic guinea-pig ventricular muscle was related to ATP generated by glycolysis. In 50 mM glucose medium the action potential duration was maintained; in 5 mM glucose medium the action potential duration shortened, the glycolytic rate declined and the ATP content was reduced.

2. The action potential amplitude was related to the metabolic state of the muscle but not to the intracellular sodium concentration.

3. It is suggested that changes in the action potential duration and overshoot in anoxic muscle may be due to an influence of metabolism on the slow inward current.

4. Anoxic muscle incubated for 8 hr in 5 mM glucose medium had an E_m of $-77{\cdot}1$ mV compared to $-81{\cdot}1$ mV in fresh muscle. The calculated E_k of anoxic muscle was $-47{\cdot}4$ mV.

5. The resting potential of anoxic muscle was separated into two components, one dependent on potassium distribution and the other on the activity of an electrogenic sodium pump.

6. The electrogenic pump component was stimulated upon raising the glucose concentration of the medium or upon raising the external potassium concentration.

7. The electrogenic pump component was inhibited by ouabain or by reduction of the temperature from 35 to 8° C.

INTRODUCTION

Studies on the effects of metabolic depression on the electrical and contractile activity of mammalian ventricular muscle (MacLeod & Daniel, 1965; MacLeod & Prasad, 1969; Prasad & MacLeod, 1969) led to the proposal that a close relationship exists between the level of glycolytic

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activity and transmembrane electrical activity. Changes in electrical activity were thought to be related to changes in ATP derived from glycolysis, and this hypothesis was supported by measurements of the ATP content of cardiac muscle under various incubation conditions (McDonald, Hunter & MacLeod, 1971; McDonald & MacLeod, 1971*a*). In the present study these results are extended and both the action potential duration and ATP content of anoxic ventricular muscle are shown to be related to glycolytic rate.

During incubation of ventricular muscle in the absence of oxygen there is rapid depletion of ATP and a marked decline of developed tension (McDonald & MacLeod, 1971*a*). Major changes in transmembrane electrical activity occur only under conditions in which glycolytic activity is suboptimal. In medium containing 50 mM glucose, ventricular muscle may be incubated for at least 12 hr in the absence of oxygen with little change in transmembrane electrical activity. However, during prolonged anoxic incubation in medium containing 5 mM glucose there is a marked decrease in action potential duration, a diminution of the overshoot and a small decrease in resting potential. In glucose-free medium or in the presence of glycolytic inhibitors, all of these changes are exaggerated and the muscle becomes inexcitable. The reduced action potential amplitude and duration resulting from incubation in glucose-deficient media can be restored by raising the glucose concentration to 50 mM. A variety of other sugars were ineffective in this regard (MacLeod & Prasad, 1969).

A complete explanation for the various effects of anoxia on ventricular muscle is not presently available although it seems likely that changes in developed tension are closely related to the level of ATP (McDonald & MacLeod, 1971*a*). The reduction in action potential duration due to anoxia and suboptimal glycolysis may be due to a metabolic dependence of the mechanisms responsible for membrane regulation of ion permeabilities. Some authors have proposed that an increased potassium conductance leading to an increased rate of repolarization is responsible for the decreased duration (Webb & Hollander, 1956; Trautwein & Dudel, 1956; De Mello, 1959; MacLeod & Prasad, 1969). However, more recent results from experiments employing the voltage–clamp technique suggest that repolarization in ventricular muscle may be due to a time-dependent decrease of inward current rather than an increase of outward potassium current (Mascher & Peper, 1969; Beeler & Reuter, 1970; Giebisch & Weidmann, 1971). Thus, a more rapid decrease of inward current could reduce the action potential duration. The marked decrease in action potential amplitude which occurs during anoxic incubation of ventricular muscle in glucose-free medium also suggests a disturbance of inward currents. Since there is an accumulation of intracellular sodium during

anoxia, the relationship between action potential amplitude and the driving force for sodium has been investigated.

Two explanations can account for the striking lack of change in the resting potential of ventricular muscle during prolonged anoxic incubation and depletion of intracellular potassium. First, intracellular potassium may be compartmentalized such that the effective potassium gradient across the membrane is greater than that calculated assuming an even distribution. Secondly, the resting potential has an electrogenic sodium component. The latter explanation was favoured in a preliminary investigation (McDonald & MacLeod, 1971*b*) and the present results further support this view.

METHODS

Electrophysiology

Papillary muscles were obtained from the right ventricle of guinea-pig heart. Animals were killed by cervical dislocation and the heart removed as quickly as possible. Dissection of the muscles was carried out in cool modified Krebs solution (see below). Papillary muscles were usually selected from the same position within the right ventricle and were about 3–5 mm in length and 0·5 mm in diameter. The muscles were mounted horizontally in a jacketed 25 ml bath at 35° C. Resting tension was 100–200 mg. The muscles were held at one end in a plastic clamp and stimulated at 60 per minute through platinum electrodes attached to the clamp. The other end of the muscle was tied by a short length of silk thread to an insulated stainless steel rod connected to the head of a Statham force displacement transducer. The length of the muscle was adjusted through movement of the transducer by a micrometer screw. Gas was supplied to the bathing medium through a fritted disk 15 mm in diameter. This disk was situated about 10 mm below the muscle.

Single cell electrical activity was recorded using conventional micro-electrodes. Electrodes were mounted rigidly or floated using the technique of Woodbury & Brady (1956). Potential measurements were made through a Medistor negative capacitance electrometer, monitored on a Tektronix 504 oscilloscope, and recorded either on film or on a Grass polygraph. Action potential duration was measured at 75% repolarization. The criteria for acceptance of a particular resting potential measurement was a sharp change in potential upon impaling a cell, a steady potential for at least 5 sec, and a sharp return to base line upon withdrawing the electrode.

Solutions

Modified Krebs medium had the following composition in m-equiv/l.; Na 138·5, K 4·6, Ca 4·9, Mg 2·3, HCO_3 21·91, PO_4 3·48, Cl 124·91, and glucose 50 mM. Medium containing 50 mM glucose was equilibrated with either 95% O_2:5% CO_2 ($G_{50}O_2$) or 95% N_2:5% CO_2 ($G_{50}N_2$). Medium containing 5 mM glucose was equilibrated with 95% N_2:5% CO_2 (G_5N_2). Media with higher potassium concentration than normal were made by addition of KCl.

ATP determinations

ATP content was determined in right ventricular strips weighing between 15 and 25 mg. The method used was a modification of the firefly luminescence technique of Strehler & McElroy (1957). Complete details are provided elsewhere (McDonald & MacLeod, 1971*a*).

Lactate determinations

Right ventricular strips were incubated in micro-Buchner funnels (10 mm diameter) with fritted glass disks. The baths, containing 1·5 ml. of medium, were secured in a water-bath at 35° C. The equilibrating gas was fed into the stem of the funnel and marbles were placed on top of the funnels. After incubation the muscles were blotted with filter paper and weighed. Samples of 0·2 ml. were taken from the bathing medium and similar samples of stock medium served as blanks. Lactate production was also measured in papillary muscles incubated in a small volume bath (0·5 ml.) which allowed for electrical and mechanical recordings. Lactate concentration in the bathing medium was determined enzymatically (Hohorst, 1965) using the Boehringer lactate test combination.

Sodium and potassium determinations

Sodium and potassium were measured in right ventricular strips and papillary muscles. Following incubation, muscles were rinsed briefly in choline chloride solution (2·26%), blotted between filter paper and weighed. The muscles were then dried in an oven for 12 hr, reweighed and digested with conc. HNO_3 in Hysil low-alkali test tubes placed in a heated aluminium block. The residue was dissolved in a suitable volume of lithium chloride (15 mM) and sodium and potassium determined simultaneously on a digital read-out flame photometer (IL, Model 143). Intracellular ion concentrations were calculated assuming an extracellular space of 267 ml. per kg wet weight ([^{14}C]inulin space). This space does not change significantly during prolonged anoxic incubation (E. G. Hunter, T. F. McDonald and D. P. MacLeod, in preparation) and was assumed to remain constant during the cold cycles (see Taylor, Paton & Daniel, 1970).

Experimental procedure

All muscles were equilibrated in 50 mM glucose medium gassed with 95% O_2:5% CO_2 for 1 hr before beginning an experiment. Anoxic conditions were achieved by replacement with fresh medium and gassing with 95% N_2:5% CO_2. The fresh medium had previously been equilibrated with 95% N_2:5% CO_2. In long-term experiments, solutions were renewed every hour.

RESULTS

In the absence of oxygen, the action potential duration of ventricular muscle is particularly sensitive to the concentration of glucose in the medium. Fig. 1 shows typical action potentials recorded from papillary muscle incubated under anoxic conditions in medium containing 50, 5 or 0 mM glucose. The control action potential was recorded following an initial 1 hr equilibration period in 50 mM glucose medium gassed with oxygen ($G_{50}O_2$), and the other records were obtained following 1 hr in the absence of oxygen (N_2). At a glucose concentration of 50 mM, there was only a slight decrease in the action potential duration. However, at 5 mM glucose the action potential duration shortened markedly with a slight reduction in amplitude and both of these effects were exaggerated in glucose-free medium.

A summary of these experiments is shown in Fig. 2. Each value is the mean ± S.E. of six muscles. Individual muscle values for each point were obtained by averaging action potential durations recorded from different areas of the preparation. For any given muscle these durations were remarkably consistent and rarely varied by more than 5%. At a glucose

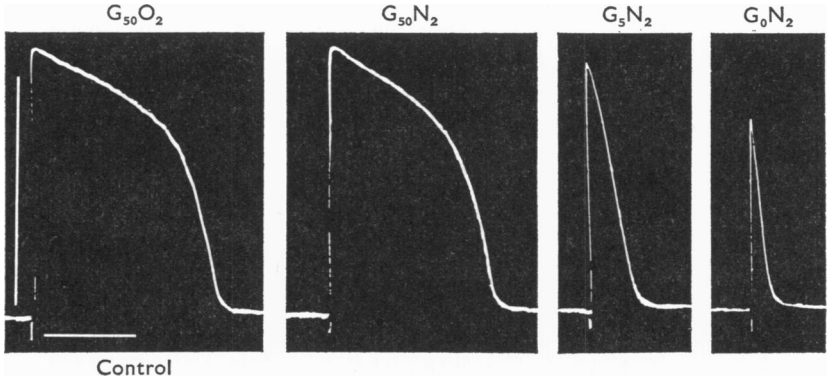

Fig. 1. Action potentials from anoxic guinea-pig papillary muscles. The control action potential was recorded following an initial 1 hr aerobic equilibration in medium containing 50 mM glucose ($G_{50}O_2$). The other action potentials were recorded following 1 hr of anoxic incubation in 50 mM glucose medium ($G_{50}N_2$), 5 mM glucose medium (G_5N_2) or glucose-free medium (G_0N_2). Vertical bars on control record indicates 100 mV, horizontal bar indicates 100 msec.

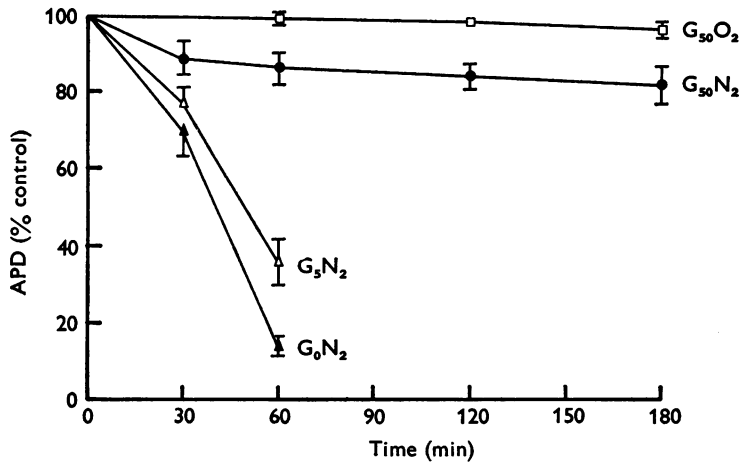

Fig. 2. The effect of glucose concentration on the action potential duration (APD) of guinea-pig papillary muscle incubated in the absence of oxygen (N_2). Note small decrease in duration in the presence of 50 mM glucose ($G_{50}N_2$) as compared to 5 mM (G_5N_2) and 0 mM glucose (G_0N_2). Points represent the mean of six experiments, vertical bars are S.E. of mean.

concentration of 50 mM the action potential duration remained within 20% of control during the 3 hr observation period. In 5 mM glucose, however, the action potential duration declined to about 35% of control in 1 hr, an effect which was more marked (14% control) in glucose-free medium. During prolonged anoxic incubation of up to 10 hr, muscle in 5 mM glucose maintained an action potential duration of about 20% of control. The action potential duration of muscle in glucose-free medium continued to decline precipitously until the muscle became inexcitable. Inexcitability usually occurred within 90 min of anoxic incubation.

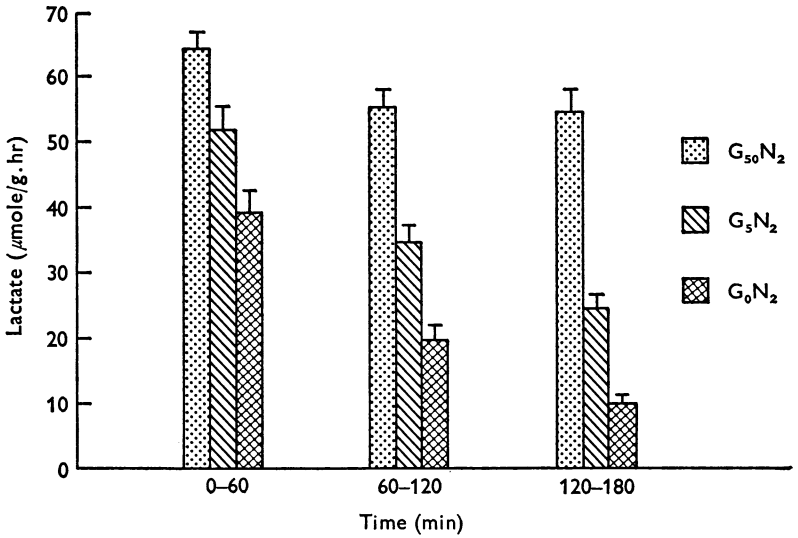

Fig. 3. The lactate production (μmole/g.hr) of ventricular strips incubated in the absence of oxygen. The medium contained 50 mM glucose ($G_{50}N_2$), 5 mM glucose (G_5N_2) or 0 mM glucose (G_0N_2). Mean ± S.E. of six determinations.

The lactate production of anoxic ventricular muscle was measured during a 3 hr incubation in medium containing 50, 5 or 0 mM glucose (Fig. 3). In the presence of 50 mM glucose, lactate production remained fairly stable during the observation period, while muscles in 5 or 0 mM glucose medium had a declining lactate production over the same period. Since the lactate produced in muscle incubated without exogenous substrate must be presumed to arise primarily from glycogen, the difference between the 5 and 0 mM glucose groups can be considered to be due to the external glucose supply. It is somewhat surprising that in the absence of glucose, lactate was still being produced during the third hour of incubation. In further measurements taken during 4 and 5 hr of incubation, no lactate was produced by muscles in glucose-free medium while muscles

in 5 mM glucose appeared to reach a steady-state production of 15–25 μmole/g.hr.

The assumption has been made that the link between glycolytic activity and transmembrane electrical activity is ATP (McDonald *et al.* 1971). It was important therefore to establish a relationship between glycolytic activity and muscle ATP content. Fig. 4 shows the lactate production and ATP content of anoxic ventricular strips incubated under one of the following conditions: (1) 1 hr in 50 mM glucose medium, (2) 1 hr in 5 mM glucose medium followed by 1 hr in 50 mM glucose medium, (3) 1 hr in 5 mM glucose medium and (4) 2 hr in 5 mM glucose medium. The data indicate a close relationship between the two parameters.

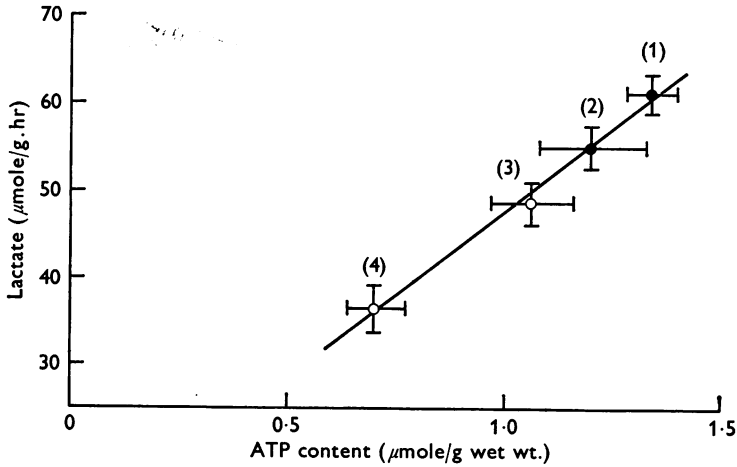

Fig. 4. The relationship between lactate production and ATP content of anoxic right ventricular strips incubated under several different experimental conditions: (1) 1 hr 50 mM glucose, (2) 1 hr 50 mM glucose, then 1 hr 5 mM glucose, (3) 1 hr 5 mM glucose, (4) 2 hr 5 mM glucose. Lactate production was measured during the final hour of incubation; ATP content determined after the incubation. Values are mean ± S.E. of eight to twenty determinations.

In an attempt to relate more directly the glycolytic activity, action potential duration and contractile activity, experiments were done in which these parameters were measured simultaneously in the same preparation. The result of one such experiment is shown in Fig. 5. During the first hour of anoxic incubation in 50 mM glucose medium the action potential duration fell only slightly, developed tension declined to about one third and lactate production was constant. When the glucose concentration was lowered to 5 mM there was a marked decrease in action potential duration, a continued fall in developed tension and a decrease in

lactate production. Upon restoration of the glucose concentration to 50 mM the action potential duration returned to control level coincident with increased lactate production. However, only a small increase in developed tension was observed.

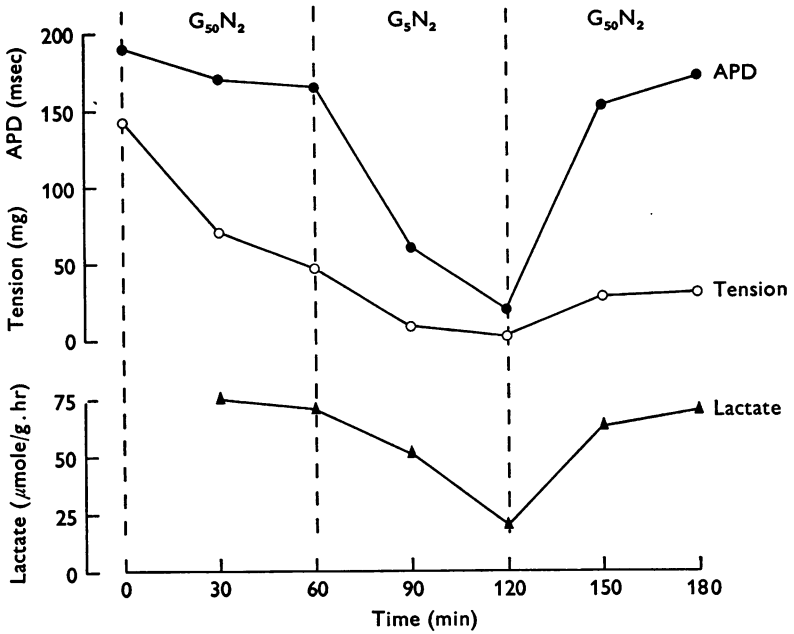

Fig. 5. The effect of glucose concentration on the action potential duration (APD), developed tension and lactate production of a papillary muscle during anoxia. Note decline in all parameters when the medium contained 5 mM glucose (G_5N_2).

The effect of glucose on the action potential of anoxic muscle was not restricted to the duration. Changes in action potential amplitude were also apparent, particularly during prolonged experiments. Since there appears to be a relationship in some tissues between action potential amplitude and the driving force of sodium across the cell membrane (Hodgkin, 1951), it was of interest to determine the intracellular sodium concentration of anoxic ventricular muscle under conditions in which the action potential amplitude was both at control value and reduced to varying degrees. Four such situations were studied: *A*, following 60 min of incubation in 50 mM glucose medium, *B*, following 75 min of incubation in glucose-free medium, *C*, following 8 hr of incubation in 5 mM glucose medium and *D*, following 8 hr of incubation in 5 mM glucose medium and 15 min in 50 mM glucose medium. Fig. 6 shows typical action potentials recorded after these incubations, and the intracellular sodium concentrations $[Na]_i$ of ventricular

muscle incubated under similar conditions are shown below the records. It is apparent that action potential amplitude was not correlated with intracellular sodium concentration. The factor which did appear to correlate with the amplitude was the glucose concentration in the medium; in the presence of 50 mM glucose a normal overshoot was observed during

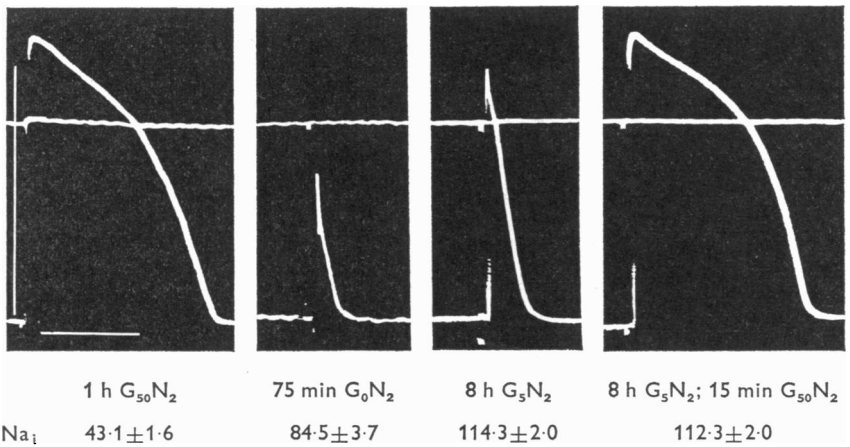

Fig. 6. Action potentials and resting potentials recorded from guinea-pig papillary muscles following incubation procedures designed to alter the intracellular sodium concentration. Left to right: anoxic incubation in 50 mM glucose medium for 1 hr; in glucose-free medium for 75 min; in 5 mM glucose medium for 8 hr; in 5 mM glucose medium for 8 hr followed by 15 min in 50 mM glucose medium. Horizontal 0 mV line obtained by withdrawing the electrode from the cell. Intracellular sodium concentrations (m-mole/kg fibre water) are from right ventricular strips, mean ± S.E. of eight to twelve determinations. Vertical bar in left-most panel indicates 100 mV; horizontal bar indicates 100 msec.

experiments lasting for up to 12 hr. In 5 mM glucose medium there was a reduction in overshoot while in glucose-free medium the overshoot was completely absent. The reduced overshoot of muscles incubated in 5 mM glucose medium for 8 hr was restored upon raising the glucose concentration to 50 mM. Although the record in Fig. 6 was obtained 15 min after raising the glucose concentration to 50 mM, the recovery of overshoot was usually complete within 1 min.

A diminution in the overshoot of the cardiac action potential, induced by either a lowering of the resting potential (Weidmann, 1955) or a reduction in the extracellular sodium concentration (Yeh & Hoffman, 1968) is usually accompanied by a decrease in the maximum rate of rise of the depolarizing spike. While a comparison of the upstroke velocity in oxygenated and anoxic muscle has not yet been conducted in a quantitative way, fast-sweep records of the upstroke have not revealed any significant

reductions during anoxia. Similarly, under conditions where the action potential duration and overshoot are declining in low glucose medium, the rate of rise of the upstroke did not appear to be reduced. Fig. 7 illustrates this point. Fast sweep records were taken over a 5 min period while the action potential was declining in glucose-free medium. The superimposed records indicate that the initial rapid depolarizing phase did not

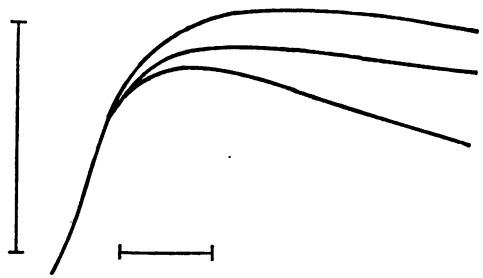

Fig. 7. Superimposed fast-sweep records of action potential upstrokes during anoxic incubation in glucose-free medium. Records were obtained over a 5 min interval during which the action potential duration declined from about 160 msec to less than 20 msec. Note that the decline in amplitude is not accompanied by a reduction in the rate of the rapid depolarizing phase. Calibrations represent 100 mV and 1 msec.

change during a time when the action potential duration declined from 160 msec to less than 20 msec and the amplitude declined from about 122–100 mV. In this particular case the muscle had been anoxic for about 3 hr before the introduction of glucose-free medium. This accounts for the rapid changes in duration and amplitude since previous work has demonstrated that the sensitivity of the action potential to alterations in the glucose concentration increases with anoxic pre-incubation (MacLeod & Prasard, 1969; McDonald & MacLeod, 1972*a*).

From Fig. 6 it can be seen that the resting potential of anoxic ventricular muscle was maintained during prolonged incubation in 5 mM glucose medium. During an 8 hr anoxic incubation in 5 mM glucose, the mean resting potential was $-77{\cdot}1$ mV even though the potassium content of these muscles declined from 54·2 to 16·7 mV/kg wet wt. and the calculated potassium equilibrium potential, E_k, was $-47{\cdot}4$ mV (McDonald & MacLeod, 1971*b*).

There are at least two possible explanations for the high resting potential in the face of a low E_k: (1) the intracellular potassium of anoxic muscle is compartmentalized in such a way that the potassium gradient between this compartment and the extracellular phase can account for the observed resting potential; (2) a component of the resting potential of anoxic muscle is produced by an electrogenic pump. An electrogenic sodium pump has

been demonstrated in guinea-pig atria (Glitsch, 1969) and cat papillary muscle (Page & Storm, 1965) following hypothermia. If the large resting potentials observed in anoxic muscle were due to an electrogenic mechanism, then a sudden cessation of energy production should be accompanied by a decline in the resting potential. Upon return to control conditions, the resting potential should recover. Therefore, the resting potential of muscle was measured while the temperature of the bathing medium was reduced to 8° C for a 15 min period and then returned to 35° C.

Fig. 8 illustrates the effect of cooling on the resting potential (E_m) of papillary muscle before and after 8 hr of anoxic incubation. Also shown are the intracellular concentrations of sodium $[Na]_i$ and potassium $[K]_i$ measured in ventricular muscle incubated under similar conditions, and E_k calculated from the mean potassium values. After the initial equilibration period E_k was slightly greater than E_m, and following 15 min of anoxic incubation in 50 mM glucose medium at 8° C, E_m had declined from $-81{\cdot}1$ to $-67{\cdot}9$ mV and E_k from $-82{\cdot}6$ to $-69{\cdot}3$ mV. These changes reflect the temperature change and subsequent loss of intracellular potassium during the incubation at 8° C. The temperature was then raised to 35° C and after 15 min E_m was $-81{\cdot}8$ mV but E_k increased only to $-74{\cdot}0$ mV since the potassium lost during incubation at 8° C was not reaccumulated. During the incubation at 8° C the intracellular sodium concentration increased from 36·7 to 64·8 mM. Fifteen minutes after restoring the temperature to 35° C, the intracellular sodium concentration had declined non-significantly to 60·2 mM. Muscles were then incubated under anoxic conditions in 5 mM glucose medium at 35° C for a further $7\frac{1}{2}$ hr. During this time intracellular sodium increased to 132·6 mM while intracellular potassium declined to 33·8 mM. As a result of this potassium loss, E_k declined to $-53{\cdot}1$ mV although E_m declined only slightly to $-76{\cdot}4$ mV. At this point the glucose concentration was raised to 50 mM and the temperature lowered to 8° C for 15 min during which E_k declined to $-45{\cdot}1$ mV as a result of the temperature change and the decline of potassium to 28·8 mM. During this second incubation at 8° C, E_m declined rapidly to $-46{\cdot}4$ mV, a value in close agreement with the calculated E_k. Fifteen minutes after restoring the temperature to 35° C there was a large disparity between E_m and E_k. E_m increased to $-85{\cdot}9$ mV whereas E_k increased only to $-47{\cdot}0$ mV. Intracellular potassium did not change during this period but intracellular sodium declined significantly ($P < 0{\cdot}05$) to 122·8 mM.

It may be noted that there was a greater loss of intracellular potassium during the initial 15 min cold period than during the 15 min cold period after 8 hr of anoxia (Fig. 8). This finding has been confirmed in a further series of experiments. Potassium loss during a 15 min period at 8° C was

measured in fresh muscles and in muscles whose intracellular potassium had been reduced by about 33 or 66 % following 3 or 8 hr of anoxia incubation at 35° C. The potassium loss was less in potassium-depleted muscles than in fresh muscles, and the relationship between the rate of loss and the intracellular potassium concentration before cooling was found to be linear.

The results indicated that the resting potential of anoxic muscle had at least two components. When energetic pathways were inhibited (cold) E_m approximated E_k; when energy was available E_m was greater than E_k.

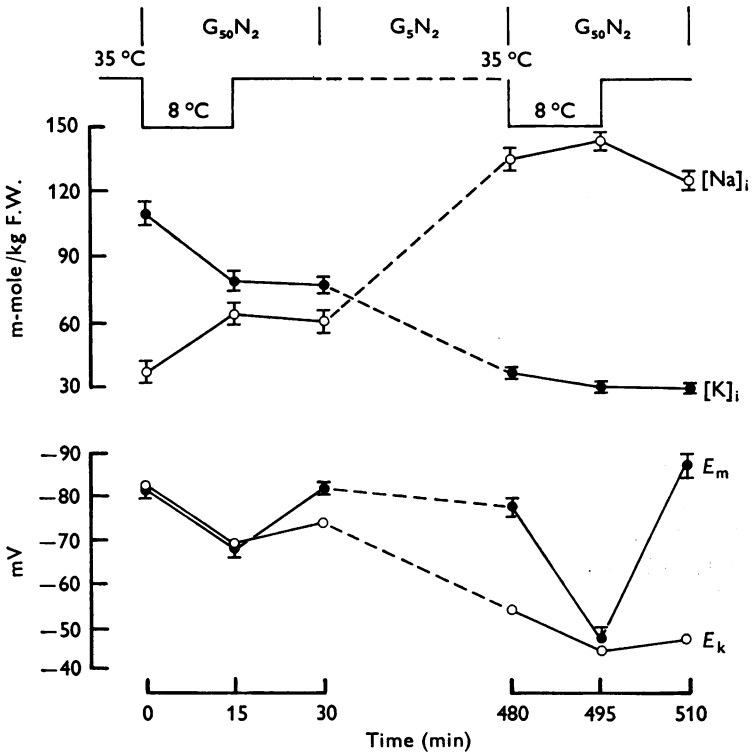

Fig. 8. The effect of temperature change on anoxic ventricular muscle. Incubation was in 50 mM glucose medium ($G_{50}N_2$) for the first 30 min, in 5 mM glucose (G_5N_2) for 450 min (dashed lines), and in 50 mM glucose for the remaining 30 min. The temperature was lowered from 35 to 8° C for 15 min during the periods 0–15 min and 480–495 min. Intracellular ion concentrations (m-mole/kg fibre water) of sodium, $[Na]_i$ and potassium, $[K]_i$ were determined on right ventricular strips. Values are mean ± S.E. of twenty-four determinations. E_k was calculated from mean potassium values. The resting potential (E_m) was measured in four papillary muscles. Values are mean ± S.E. of twenty to twenty-five penetrations. Note agreement between E_m and E_k during cold cycles, divergence upon rewarming to 35° C.

This point was further demonstrated in additional experiments where muscle was incubated under anoxic conditions for 0, 3 or 8 hr before lowering the temperature to 8° C for 15 min. Fig. 9 shows the time course of the changes in E_m (open circles) during the cold/warm cycles. Also shown are the E_k values (filled triangles) based on potassium determinations immediately before and at the end of similar cold cycles. The data

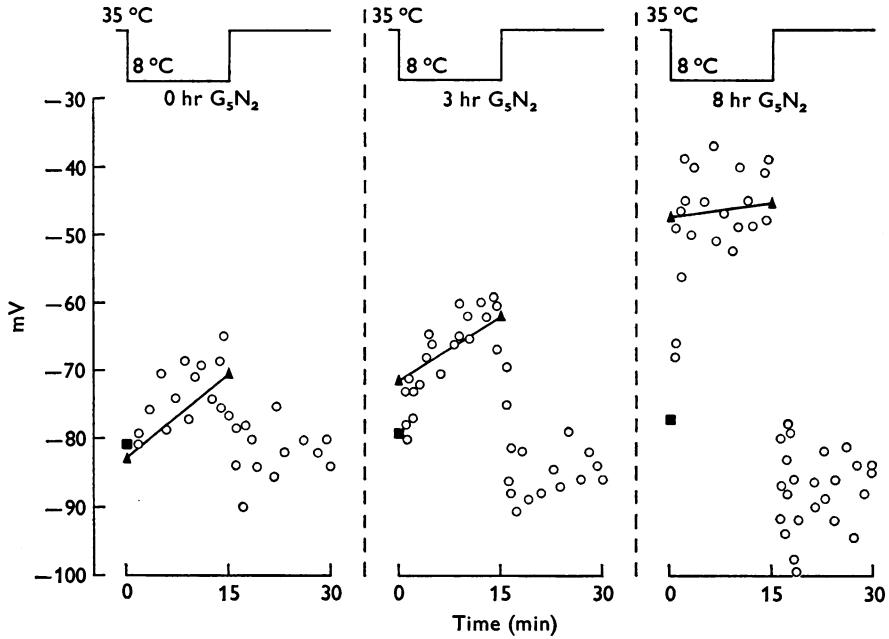

Fig. 9. The resting potential (E_m) and E_k of anoxic ventricular muscle during 15 min cold (8° C)-warm (35° C) cycles following 0, 3, or 8 hr of anoxic incubation in 5 mM glucose medium (G_5N_2). Incubation at 8° C was in 5 mM glucose medium while incubation at 35° C following the cold was in 50 mM glucose medium. E_m was measured in three papillary muscles; mean control E_m immediately before the cold (filled squares), during the cold/warm cycles (open circles). E_k (filled triangles) was calculated from the mean potassium contents of right ventricular strips (n = 8–13) immediately before and at the end of the cold incubations.

again indicate a close relationship between E_k and E_m during cold incubation. E_m declined rapidly towards E_k when the temperature was lowered, and then declined at a slower rate as E_k reflected a further loss of potassium. The membrane hyperpolarized immediately upon returning the temperature to 35° C and the magnitude of the hyperpolarization appeared to be greater with increasing anoxic pre-incubation. This may well reflect a relationship between pump activity and the level of intracellular sodium (Thomas, 1972).

When the glucose concentration of the medium is raised from 5 to 50 mM, anoxic ventricular muscle responds with an increased glycolytic rate and ATP production. Under these circumstances we might expect a stimulation of the electrogenic component of the resting potential. Papillary muscle was therefore incubated under anoxic conditions in 5 mM glucose medium for 8–10 hr following which the glucose concentration in the medium was raised to 50 mM. E_m was monitored immediately before and for 15 min after the addition of glucose. In six experiments the mean increase in resting potential, 2 min after raising the glucose concentration, was 8·6 mV. After 15 min the mean resting potential was 3·9 mV higher than that recorded in 5 mM glucose medium. Sucrose (50 mM) was ineffective in this regard.

It has been shown in other tissues that ouabain inhibits electrogenic sodium pumping (e.g. Rang & Ritchie, 1968), and it was therefore essential to show that ouabain could prevent hyperpolarization above E_k in the present study. Muscles were incubated anaerobically for 3 hr at 35° C and for 1 hr at 8° C, after which the temperature was raised to 35° C for an additional 30 min. During the last 10 min at 8° C and the subsequent 30 min at 35° C, 10^{-5} M ouabain was added to the medium. The membrane potential was measured in papillary muscles and the sodium and potassium content in ventricular strips. The results are shown in Fig. 10. During the 10 min of cold, the resting potential (filled circles) was approximately -48 mV and was in good agreement with the calculated E_k (open triangles). During the subsequent 30 min of incubation at 35° C, the presence of ouabain effectively blocked the usual hyperpolarization (E_m control). As might also be expected on the basis of an electrogenic pump hypothesis, ouabain prevented the sodium extrusion seen in control muscles during the rewarming period.

The cold/warm cycle experiments indicated that during anoxic incubation the presumed electrogenic component of the resting potential was negligible initially and 30–40 mV after 8 hr. This being the case, it was expected that exposure to ouabain at the beginning of anoxic incubation would have little effect on the resting potential, while after 8 hr of anoxia there would be a significant reduction in potential. The results of three experiments in which 10^{-4} M ouabain was added to the medium, either 10 min or 8 hr after the onset of anoxia, are shown in Fig. 11. Following 20 min in medium containing ouabain, the resting potential of 'fresh' muscle declined by about 5 mV, presumably reflecting the net loss of potassium during this period (McDonald & MacLeod, 1972*a*). The resting potential of anoxic muscle incubated for 8 hr and then exposed to ouabain, declined from about -80 to -45 mV. A similar finding has been reported in rat myometrium where ouabain had a small effect on the resting

potential of fresh muscle but a large effect on the hyperpolarization of muscles recovering from 18 hr of cold incubation (Taylor *et al.* 1970).

An increase in the external potassium concentration has been shown to stimulate electrogenic sodium pumping (Tamai & Kagiyama, 1968; Taylor *et al.* 1970). It seemed likely that increasing the external potassium

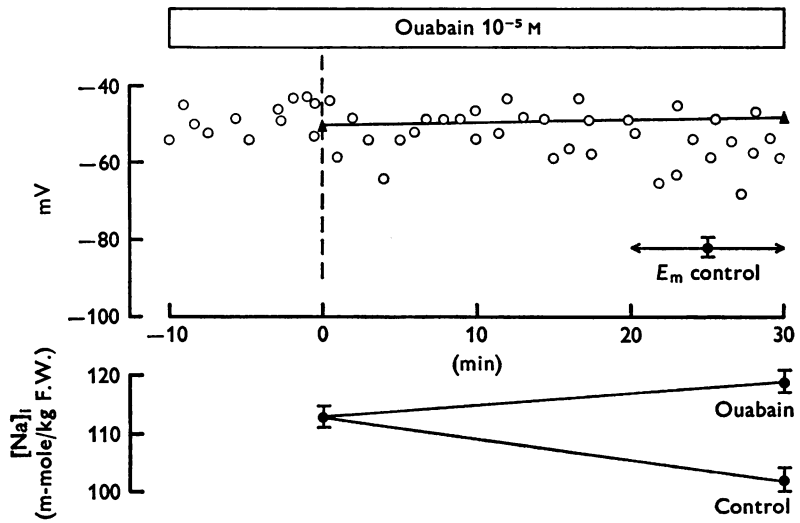

Fig. 10. The effect of 10^{-5} M ouabain on the recovery of anoxic ventricular muscle from a 1 hr cold period. Muscle was incubated anaerobically in 5 mM glucose medium for 3 hr at 35° C and then in 50 mM glucose medium ($G_{50}N_2$) for 1 hr at 8° C. Upper graph, measured resting potentials (open circles) during the last 10 min at 8° C and 30 min at 35° C in the presence of ouabain. Data from four papillary muscles. The resting potential, in the absence of ouabain measured between 20 and 30 min after recovery from cold, is also shown (E_m control, mean ± S.E., twenty-six penetrations, four muscles). The potassium equilibrium potential (E_k, filled triangles) is based on the mean potassium content of twelve ventricular strips. Lower graph, the effect of 10^{-5} M ouabain on the sodium extrusion of right ventricular strips recovering from cold. Same incubation procedure as upper graph. Mean ± S.E. of twelve to sixteen determinations.

concentration, at a time when the pump was apparently quite active (during recovery from hypothermia), would result in a greater hyperpolarization above E_k than a similar potassium increase when the pump was much less active (aerobic incubation). Muscles were incubated aerobically for 1 hr in 50 mM glucose medium containing 4·6 mM potassium, following which the external potassium concentration was raised to 15 mM and then reduced again to 4·6 mM. Following 3 hr of anoxia at 35° C, 1 hr at 8° C and 30 min at 35° C, the external potassium concentration was again raised to 15 mM. Membrane potential measurements

were made on four papillary muscles and potassium equilibrium potentials were calculated from determinations in twelve ventricular strips at each time period. The results of these experiments are shown in Fig. 12. Raising the external potassium concentration resulted in only a slight gain in intracellular potassium and gains in total muscle content were mainly due to the increased potassium in the extracellular space. The resting potentials recorded during high potassium incubation of 'fresh' muscle were in

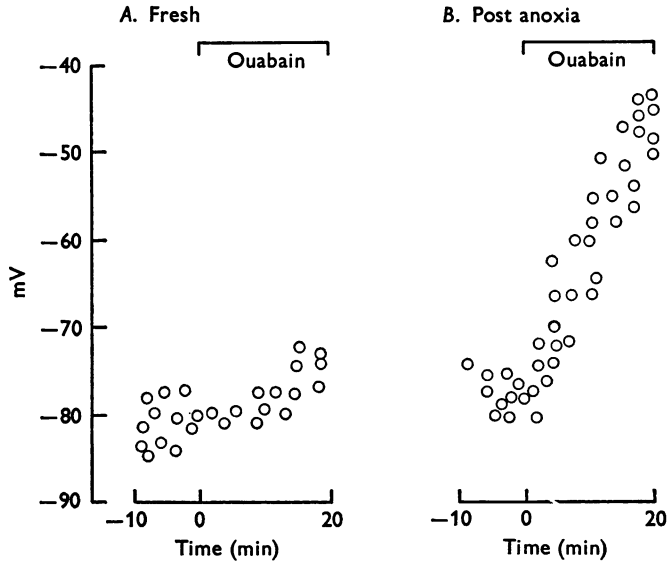

Fig. 11. The effect of 10^{-4} M ouabain on the membrane potential of anoxic papillary muscle incubated in 5 mM glucose medium. *A*, after 10 min of incubation (fresh) and, *B*, after 8 hr of incubation.

good agreement with the calculated E_k (Fig. 12*A*). During the 30 min post-anoxia cold period at normal external potassium (Fig. 12*B*), the membrane hyperpolarized above E_k in a manner similar to that seen after prolonged anoxia and 15 min cold. In this case, however, where the cold period was 1 hr instead of 15 min, the largest hyperpolarizations were seen after 10–12 min of rewarming as opposed to 2–5 min. At the end of the 30 min period the resting potential had resumed a steady value of approximately −80 mV. At this point, raising the external potassium to 15 mM provoked a marked depolarization approaching the new value of E_k. During the next 10 min the membrane hyperpolarized markedly, reaching a value some 40 mV above E_k, and gradually assumed a steady value approximately 25 mV above the calculated E_k. If one compares the two periods of hyperpolarization at 10–20 min after hypothermia and 10–20 min after raising the potassium, the difference between E_k and mean E_m

is 30·6 and 34·9 mV respectively. Similarly, comparing the hyperpolarization immediately before the introduction of high potassium, and the mean hyperpolarization after 10–20 min of high potassium, the difference is more evident, 27 mV *vs.* 34·9 mV.

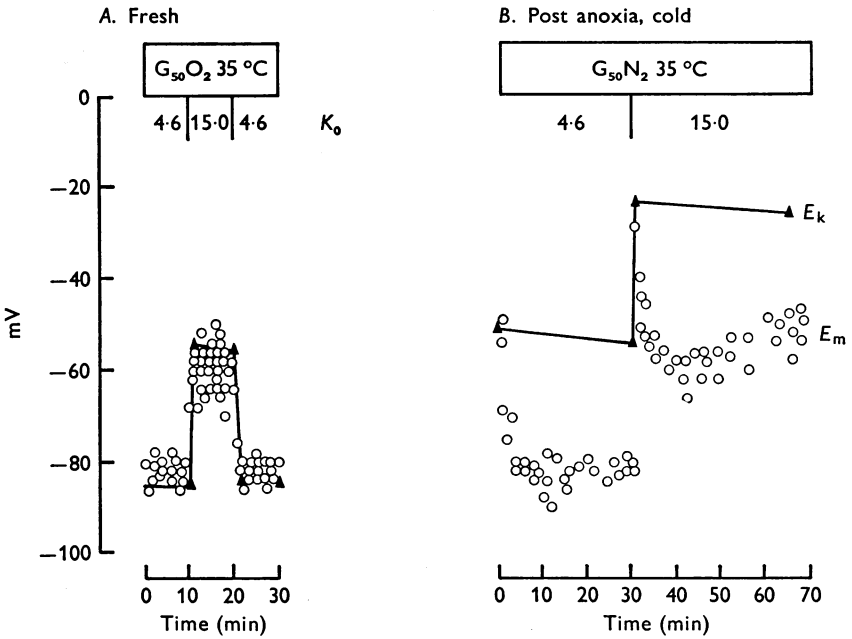

Fig. 12. The effect of high potassium on the resting potential of (*A*) fresh papillary muscle and (*B*) anoxic papillary muscle recovering from cold. Resting potential (E_m, open circles) was measured in four papillary muscles during (*A*) 10 min aerobic incubation in 50 mM glucose medium ($G_{50}O_2$) at external potassium concentration of 4·6 mM, 10 min at 15 mM, and a further 10 min at 4·6 mM. Muscles were then incubated anaerobically in 5 mM glucose medium for 3 hr at 35° C and in 50 mM glucose ($G_{50}N_2$) for 1 hr at 8° C. Recovery from cold (*B*) took place in $G_{50}N_2$ medium at 35° C, for 30 min at 4·6 mM potassium and for a further 30 min at 15 mM potassium. The potassium equilibrium potential (E_k, filled triangles was calculated from the mean potassium content of twelve right ventricular strips.

In four additional experiments muscles were incubated anaerobically for 8 hr in 5 mM glucose medium, external potassium concentration increased from 4·6 to 45 mM and the membrane potential measured 2–3 min later. The membrane potential recorded was $-38{\cdot}8 \pm 2{\cdot}8$ mV (mean ± S.E. of twenty-six penetrations). Assuming a reasonable rate of potassium accumulation under these conditions (see Taylor *et al.* 1970) E_k was approximately +7 mV. This hyperpolarization of 46 mV above E_k may be compared with the hyperpolarization of about 31 mV before the potassium

concentration was raised. It seems permissible therefore to conclude that raising the external potassium concentration stimulates electrogenic hyperpolarization in anoxic ventricular muscle.

DISCUSSION

The metabolic basis of electrical activity in anoxic guinea-pig ventricular muscle has been investigated. The evidence indicates that the action potential duration and the action potential overshoot are related to the availability of glycolytic ATP. The resting potential can be separated into at least two components, one dependent on the potassium distribution and the second on the activity of an electrogenic sodium pump.

Previous work on the relationship between glucose concentration and the action potential duration (MacLeod & Prasad, 1969; McDonald *et al.* 1971) has been verified and extended. The action potential duration of anoxic muscle was maintained near control level in 50 mM glucose medium but declined in 5 mM glucose medium and declined at a more rapid rate in glucose-free medium. Changes in the glucose concentration of the medium were followed by changes in the glycolytic rate, as measured by lactate production. Changes in the glucose concentration of the medium also provoked changes in muscle ATP content and there was a linear relationship between lactate production and ATP content.

The amplitude of the action potential was also dependent on the metabolic state of the muscle. In the presence of 50 mM glucose the amplitude was never reduced below that seen during the initial aerobic incubation. In 5 mM glucose medium the reduction in amplitude was in the order of 5–20 mV while in glucose-free medium the amplitude was progressively reduced until, following prolonged incubation, the muscle became inexcitable. Raising the glucose concentration to 50 mM always restored the action potential amplitude of anoxic muscle which had been incubated in 5 mM glucose medium, and also restored the amplitude of anoxic muscle in glucose-free medium provided the latter incubation had not caused inexcitability.

There is a relationship between the level of the resting potential and the magnitude of the overshoot in nerve (Hodgkin & Huxley, 1952) and in cardiac muscle (Weidmann, 1955). As the resting potential is reduced, the overshoot declines, and this response has been attributed to a decreased availability of the sodium-carrying system. This inactivation of the sodium system also results in a reduced spike velocity (Weidmann, 1955; Yeh & Hoffman, 1968). The reduced overshoot of anoxic ventricular muscle incubated in 5 or 0 mM glucose medium would not appear to be due to a similar inactivation. With the exception of those periods immediately

before inexcitability in glucose-free medium, the decline of the action potential duration and amplitude was characterized by a decline in the secondary rising phase with no apparent change in the initial rapid phase.

The amplitude of the action potential was not related to the intracellular concentration of sodium, but was dependent on the glucose concentration in the medium. Normal amplitudes of 110 to 125 mV were always observed in 50 mM glucose medium even under conditions which raised the intracellular sodium concentration as high as 112 mM. There is strong evidence in favour of a two-component system during the rising phase of the action potential in frog and guinea-pig cardiac muscle. Based on the selective block of sodium channels by tetrodotoxin, the inhibition of slow inward sodium/calcium current by manganese, and experiments involving the manipulation of sodium and calcium ion concentrations in the medium, it has been proposed that the initial phase of the cardiac action potential upstroke is due to the fast inward current (sodium), and the slower more positive portion is due to the slow inward current carried by sodium and/or calcium (Niedergerke & Orkand, 1966; Coraboeuf & Vassort, 1968; Rougier *et al.* 1969; Tarr, 1971). Further, a decrease in the slow inward current was accompanied by a decrease in the action potential duration (Rougier *et al.* 1969; Vitek & Trautwein, 1970) whereas an increase lengthened the action potential duration (Vassort *et al.* 1969).

The evidence suggests that the cardiac action potential amplitude and duration is dependent on the slow inward current and that this current can be influenced by metabolism. This interpretation implies that the reduced action potential of anoxic muscle is related to a decreased inward current rather than an increased potassium outward current. Further support for this theory is as follows: (1) under conditions of rapid decline in the action potential duration (glucose-free medium) there was no increase in the rate constant of ^{42}K efflux (McDonald & MacLeod, 1972*a*); (2) recent experiments (Giebisch & Weidmann, 1971) suggest that normal repolarization in sheep ventricular muscle depends on a time-dependent decrease of inward current (sodium, calcium) in the face of constant outward current rather than on a time-dependent increase of potassium outward current which appears to be the case in Purkinje fibres (Noble & Tsien, 1969*a*, *b*); (3) manganese, which blocks the slow inward current in guinea-pig ventricular muscle (Ochi, 1970) reduces the action potential duration and amplitude of anoxic muscle in 50 mM glucose medium (McDonald & MacLeod, 1972*b*); (4) isopropylnoradrenaline (INA), which increases the slow inward current in cardiac muscle (Vassort *et al.* 1969), stimulates glycolysis and increases the duration of the reduced action potential in anoxic muscle. The increased slow inward current due to INA is blocked by manganese (Vassort *et al.* 1969). Similarly, the INA-induced

restoration of the action potential duration in anoxic muscle is blocked by manganese (McDonald & MacLeod, 1972*b*).

The maintenance of the action potential plateau in cardiac muscle is clearly dependent on a fine balance between inward and outward currents. A voltage-clamp study of anoxic ventricular muscle should help to clarify the interpretations above and further extend our knowledge of the basic processes underlying cardiac electrical activity.

It should be emphasized that there are marked differences in the behaviour of cardiac muscle depressed by anoxia and muscle depressed by 2,4-dinitrophenol (DNP). Haas, Kern & Einwächter (1970) have reported that frog atrial muscle treated with 0·5 mM-DNP exhibited a decreased rate of rise of the action potential preceding a shortening of the duration, and a concomitant increase in steady-state outward current and ^{42}K efflux. In guinea-pig ventricular muscle we have noted (McDonald & MacLeod, 1972*a*) several differences between anoxia and DNP treatment: (1) DNP (10^{-4} M) reduced the action potential duration of aerobic muscle in 5 mM glucose medium at a rate some 6 times faster than anoxia; (2) DNP treated muscle had a lower ATP content than anoxic muscle; (3) DNP always induced an increase in the efflux of ^{42}K while anoxia did not; (4) DNP rapidly reduced the ATP content and action potential duration of muscle incubated under anoxic conditions in 50 mM glucose medium (unpublished observations). Sodium cyanide, another widely used inhibitor of oxidative metabolism, does not induce these 'extra' effects seen with DNP. It seems likely that DNP, in addition to uncoupling oxidative phosphorylation, increases membrane conductance (Bielawski, Thompson & Lehninger, 1966; Godfraind, Krnjević & Pumain, 1970) and actively promotes an 'energy leak', as demonstrated in anaerobic turtle bladder by Klahr, Bourgoignie & Bricker (1968).

Guinea-pig ventricular muscle lost potassium and gained sodium during prolonged anoxic incubation. Following 8 hr of incubation in 5 mM glucose medium the resting potential was within 5 mV of control values but 30–35 mV greater than E_k. Two possible interpretations of the data were considered. The first of these was that potassium was compartmentalized in such a way that the potassium gradient between this compartment and the extracellular phase could support the observed potentials. Compartmentalization of potassium in cardiac muscle has been suggested by Schrieber (1956) and more recently by Vick *et al.* (1970). Maintenance of the resting potential by potassium compartmentalization can be tentatively ruled out on the following grounds. First, if potassium was not distributed throughout the cell (and throughout the muscle mass), we would not expect to see a good correlation between E_m and E_k, the calculation of which is based on total muscle content. During incubation at 8° C, and

also in the presence of ouabain, E_m and E_k were in good agreement. Secondly, if the potassium concentration in the compartment remained nearly constant throughout the anoxic incubation, a sudden lowering of temperature of 8° C should reduce the resting potential to the same value regardless of when this cold period took place. This should be true, particularly at earlier times, whether or not the maintenance of potassium in this compartment was energy dependent. This was clearly not the case since E_m rapidly declined to a progressively lower level with increasing time of anoxia before the cold period.

The data support the interpretation that the resting potential of anoxic ventricular muscle consists of two components, one determined by potassium distribution and the other by the activity of an electrogenic sodium pump. Marmor & Gorman (1970) have proposed that the resting potential of *Anisodoris* giant neurone can be separated into an ionic dependent component and a metabolic dependent component. The properties exhibited by the pump in anoxic muscle are similar to those of the Na^+–K^+-activated ATPase as outlined by Skou (1965). It is energy-dependent, depressed by ouabain and stimulated by high concentrations of intracellular sodium and extracellular potassium. In this respect, the behaviour is similar to pumps in other tissues (e.g. Keynes & Steinhardt, 1968; Taylor *et al.* 1970; Thomas, 1972). It appears that the rate of ATP production by anoxic muscle in 5 mM glucose medium is sufficient to maintain a normal resting potential for periods of at least 8 hr. Yet, during this period there is a continuous loss of potassium and gain of sodium so that although the rate of ATP production is adequate for the activity of an electrogenic pump and the maintenance of resting potential, it does not prevent large scale ion changes. An electrogenic pump dependent on energy from glycolysis has been observed in mammalian non-myelinated nerve fibres (den Hertog *et al.* 1969).

The data indicate the extent to which cardiac muscle can be 'abused' and still retain characteristic function. Thus after prolonged anoxia, guinea-pig ventricular muscle responds to electrical stimulation with action potentials of normal amplitude and contractile events of normal appearance although greatly reduced in strength. These functions survive in spite of a three- to fourfold increase in intracellular sodium and a similar reduction in intracellular potassium. It is interesting to note that Page & Storm (1965) observed repetitive muscle contractions in cat ventricular muscle recovering from hypothermia. Although they did not measure electrical activity, they concluded that action potentials were possible under conditions where there was no difference between the extracellular and intracellular concentrations of sodium.

The repolarizing phase of the action potential in potassium depleted

muscles did not appear to differ from that seen in normal muscle. In view of the low E_k in anoxic muscle it would be extremely interesting to determine the nature of the ionic currents underlying this phase of the action potential.

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