

The effect of 2,4-dinitrophenol on electrical and mechanical activity, metabolism and ion movements in guinea-pig ventricular muscle

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Summary

1. DNP (2,4-dinitrophenol) reduced the duration of the action potential of guinea-pig ventricular muscle at a greater rate than did anoxia. The effect was dose-dependent and was modified by the concentration of glucose in the medium. DNP (0.1 mM) reduced the amplitude of the action potential of muscles incubated with 5 mM glucose; on raising the glucose concentration to 50 mM the effect was reversed.
2. A large dose-dependent loss of K^+ occurred within 15 min of incubation with DNP and was attributed to increased efflux. K^+ loss was not related to Na^+ gain during the first 60 min of incubation; during the first 30 min DNP-treated muscle did not gain any Na^+ . Although the shortening of the action potential by DNP during aerobic incubation was similar to that of muscles incubated under anaerobic conditions in glucose-free medium, the anaerobic incubation was not associated with increased ^{42}K efflux.
3. It was concluded that the reduction in duration of the action potential was not necessarily the result of an increased K^+ efflux. The effect of DNP on ^{42}K efflux is considered to result from a direct effect on the cell membrane; the effect on electrical activity may be a combination of the increase in K^+ efflux and a reduction in the inward current due to Na^+ and Ca^{++} previously assumed to be dependent on the glycolytic production of ATP.
4. Electrogenic Na^+ pumping may contribute to the maintenance of resting potential in K^+ -depleted, DNP-treated cardiac muscle.

Introduction

In earlier studies on the effect of anoxia on the transmembrane potential of guinea-pig papillary muscle it was found that the duration of the action potential was dependent on the glucose concentration in the medium (MacLeod & Prasad, 1969). The action potential shortened when the glucose concentration of the medium was 5 mM but was restored or maintained near control level when the concentration was raised to 50 mM. This effect was not due to the change in osmolarity and could not be duplicated with other sugars. McDonald, Hunter & MacLeod (1971) reported that the duration of the action potential of guinea-pig ventricular muscle was dependent on ATP produced glycolytically. In that study

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the effect of different concentrations of 2,4-dinitrophenol (DNP) on the duration of the action potential was studied, and it became apparent that at concentrations in excess of 0.001 mM the effect of DNP could not be explained on the basis of its uncoupling action. This was especially notable with 0.1 mM DNP which, during aerobic incubation of muscles in medium containing 5 mM glucose, reduced the duration of the action potential by 80% in 20 minutes. On the other hand, in muscles incubated anaerobically for 20 min in a similar medium without DNP, the duration of the action potential was within 10% of the control value. The ATP content of ventricular muscle exposed to 0.1 mM DNP declined more rapidly than that of muscle incubated anaerobically. Since this effect was not obtained by exposure to NaCN (1 mM), it was suggested that, in addition to inhibition of aerobic ATP production, DNP also caused an increased breakdown.

The present study was undertaken to compare more closely the effects of anoxia and DNP on cardiac muscle.

Methods

General procedures

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 of the following composition (mM): NaCl 115.5, KCl 4.6, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 21.9, and glucose 50. Papillary muscles were usually selected from the same position within the right ventricle and were about 5 mm in length and 0.5 mm in diameter. Ventricular strips (10–20 mg) were chosen from the right wall to obtain as thin a preparation as possible. Aerobic incubations were performed in media equilibrated with 95% O₂ and 5% CO₂; anaerobic conditions were obtained by gassing the medium with 95% N₂ and 5% CO₂. All experiments with DNP and NaCN were carried out with 95% O₂ and 5% CO₂. Stock solutions of DNP and NaCN were prepared in distilled water and added to the medium in the bath. The volume added never exceeded 1% of the bath volume. Since the addition of very acidic or basic solutions to the medium produced marked changes in muscle behaviour, the stock solution of DNP was neutralized by the addition of NaOH and that of NaCN by the addition of HCl.

Electrical and mechanical activity

Papillary muscles were mounted horizontally in a jacketed, constant temperature bath of 25 ml at 35° C. The muscles were held at one end in a plastic clamp and stimulated at 1 Hz through platinum electrodes attached to the clamp. The electrodes were close to the muscle but did not touch it. 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 Satham force-displacement transducer. The length of the muscle was adjusted by movement of the transducer with a micrometer head until a resting tension of 100–200 mg was obtained. The output of the transducer was recorded on a Grass polygraph. The bathing medium was bubbled with the gas mixture through a fritted disc 15 mm in diameter. The disc was situated about 10 mm below the muscle.

Single cell electrical activity was recorded by means of conventional microelectrodes using the floating electrode technique of Woodbury & Brady (1956). Potentials

tial measurements were made through a Medistor negative capacitance electrometer, monitored by a Tektronix 504 oscilloscope and recorded either on film or on the polygraph. The duration of the action potential was measured at 75% repolarization.

Sodium and potassium analyses

Sodium and potassium contents were determined in right ventricular strips. Following incubation, muscles were rinsed briefly in isotonic choline chloride solution (161 mM), blotted between filter paper and weighed. The muscles were then dried in an oven, re-weighed and digested with concentrated HNO_3 in Hysil low-alkali test tubes placed in a heated aluminium block. After the acid had evaporated the residue was dissolved in a suitable volume of lithium chloride diluent (15 mM) and sodium and potassium were determined simultaneously on a digital read-out flame-photometer (Instrumentation Lab. Model 143).

^{42}K efflux was determined on right ventricular strips. ^{42}K was obtained as KCl from Union Carbide Corporation in a solution of 1 N HCl which was then neutralized with 2 N NaOH. The radioactivity of the loading medium (50 mM glucose, 95% O_2 and 5% CO_2) was about 2 $\mu\text{Ci/ml}$ and the concentration of K^+ did not exceed 5.5 mM. The muscles were loaded for 2–3 h at 35° C, in which time sufficient ^{42}K had been taken up for the purpose of these experiments. At the conclusion of the loading period the muscles were soaked for about 15 s in non-radioactive medium and impaled on the stimulating electrodes of a muscle bath (Fig. 1). The bath was inserted into a tight fitting test tube which was then placed in the well of a Picker Autowell II scintillation counter. All counts were corrected for decay and rate constants (Δ cpm/cpm) were calculated.

It is realized that the experimental procedure used to determine muscle radioactivity introduced a degree of error since not only was the radioactivity of the muscle counted but also the radioactivity of the bath solution and a small volume

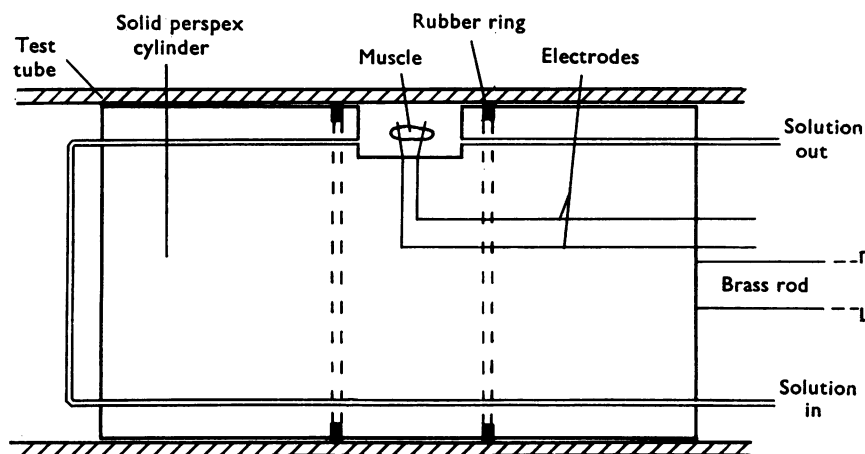


FIG. 1. Schematic diagram of the muscle bath used in ^{42}K efflux experiments. The bath was constructed from a solid cylinder of perspex about 4 cm in length and 3 cm in diameter by cutting out a section of approximately 0.25 ml volume. When the cylinder was pushed into a snug fitting test tube, the cut away section, now bounded by the wall of the test tube, served as a closed bath. Fine bore polyethylene tubing permitted continuous flow of medium through the bath. Perfusion rate was 10 ml/min at 35° C. Leaks from the bath were prevented by silicone grease and recessed rubber rings around the perspex cylinder.

of the perfusate. However, it is felt that this error presented a very small constant proportion of the total radioactivity and did not invalidate the results.

Lactate determination

Right ventricular strips were incubated in micro-Buchner funnels (10 mm diameter) with fritted glass discs. The baths, containing 1.5 ml medium, were placed 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 or 0.4 ml were taken from the bathing medium and similar samples of stock medium were used as blanks. Lactate concentration in the bathing medium was determined enzymatically (Hohorst, 1965) using the Boehringer lactate test combination.

Results

Action potential, lactate production and ATP content

When guinea-pig papillary muscle was incubated aerobically in medium containing 50 mM glucose and 0.01 mM DNP, there was a steady decline in the duration of the action potential (Fig. 2). After 90 min incubation the duration had declined to 55% of the control values. However, when the medium contained 5 mM glucose, the same concentration of DNP reduced the duration of the action potential to less than 20% of the control value in 60 minutes. If at this point the glucose concentration was raised to 50 mM the action potential lengthened to 55% of the control in the course of 30 minutes.

At a concentration of 0.1 mM, DNP caused a rapid decrease in the duration of the action potential (Fig. 2) to approximately 35% of the control in either 5 or

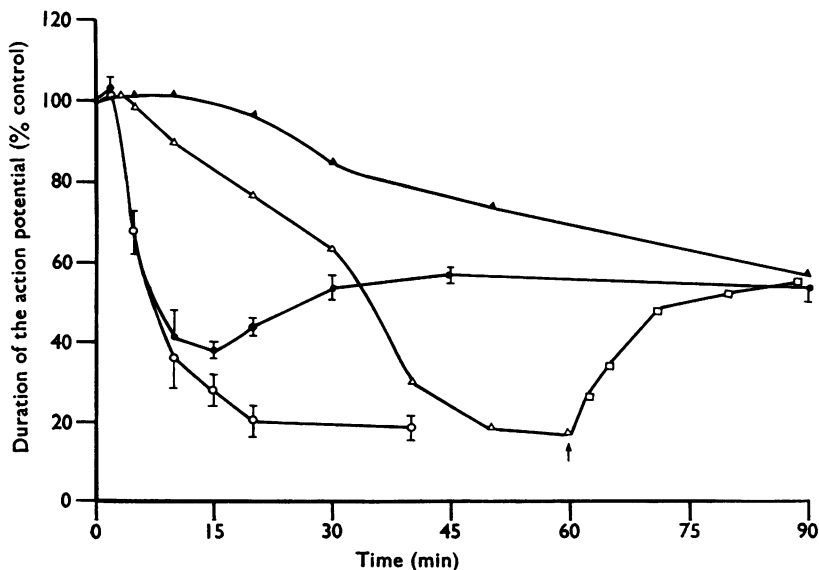


FIG. 2. The effect of DNP on the duration of the action potential of guinea-pig papillary muscle incubated in medium containing either 5 mM (open symbols) or 50 mM glucose (closed symbols). Concentrations of DNP were 0.01 mM (Δ , \square , \blacktriangle , 1 experiment) and 0.1 mM (\circ , \bullet , 7 experiments); the vertical bars indicate \pm S.E.M. Note effect of raising the glucose concentration to 50 mM at arrow.

50 mM glucose medium. Subsequently, the action potential continued to shorten when the glucose concentration was 5 mM; in 50 mM glucose there was a recovery of the duration of the action potential to 55% of the control during the ensuing 15 to 30 minutes.

Glycolytic activity was studied by measuring the lactate production of ventricular muscle strips exposed to 0.1 mM DNP. Strips were used in preference to papillary muscle because of their larger mass and therefore larger production of lactate; improved techniques utilizing papillary muscles have yielded similar results (McDonald & MacLeod, unpublished observations). Lactate production by anoxic muscles was significantly greater ($P < 0.01$) in 50 mM than in 5 mM glucose (Table 1). In the presence of oxygen, muscle treated with DNP also produced more lactate ($P < 0.01$) in 50 mM than in 5 mM glucose. Whereas DNP-treated strips incubated aerobically produced more lactate than anoxic strips in 50 mM glucose ($P < 0.01$), there was no such difference at a glucose concentration of 5 mM. This

TABLE 1. *Effect of glucose concentration on lactate production of right ventricular strips exposed to DNP*

	Number of observations	Mean lactate production ($\mu\text{mol/g/h} \pm \text{S.E.M.}$)		
		0-15 min	15-45 min	0-60 min
5 mM glucose, anaerobic	10			47.3 ± 3.3
5 mM glucose, aerobic, 0.1 mM DNP	9-10	36.3 ± 1.7	46.1 ± 1.8	47.2 ± 1.9
50 mM glucose, anaerobic	7			62.7 ± 1.2
50 mM glucose, aerobic, 0.1 mM DNP	7-9	39.8 ± 2.5	59.6 ± 3.0	73.6 ± 3.3

Mean lactate production during the 15 and 30 min experiments was lower than during the 60 min experiments. The discrepancy may arise in part from the large blank reading obtained with the green DNP medium; during experiments lasting for 15 or 30 min the contribution of the blank to the sample reading was considerably increased due to the smaller quantity of lactate produced.

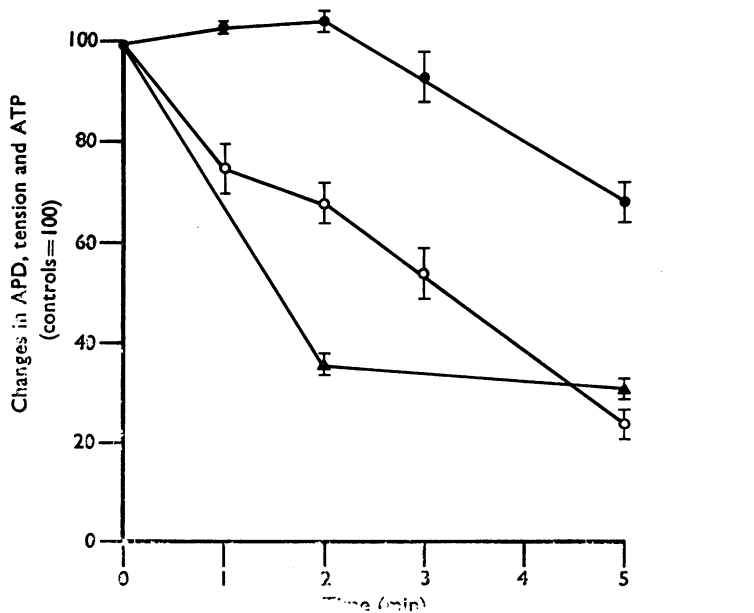


FIG. 3. The effects of 0.1 mM DNP on the action potential duration (APD, ●) and tension (○) of guinea-pig papillary muscles (5 experiments) during the first 5 min of exposure. The ATP contents (▲) were those obtained in earlier experiments on ventricular strips (McDonald *et al.*, 1971). The vertical bars indicate $\pm \text{S.E.M.}$

latter finding may be the basis for the different effects of DNP on the duration of the action potential in 5 and 50 mM glucose. However, it was necessary to measure lactate production at shorter time intervals to ascertain whether the recovery in the duration of the action potential in 50 mM glucose and 0.1 mM DNP, between 15 and 45 min (Fig. 2), could be correlated with increased glycolysis. During the first 15 min of incubation there was no difference between DNP-treated strips incubated in 5 or 50 mM glucose, but during the 15–45 min period lactate production was significantly higher ($P < 0.01$) in the 50 mM than in the 5 mM glucose medium (Table 1).

DNP had a marked effect on the contractility of papillary muscle. Fig. 3 compares early changes in the duration of the action potential and the tension developed by papillary muscles treated with 0.1 mM DNP, as well as the ATP content of ventricular strips similarly treated. During the first few minutes there was a marked fall both in total muscle ATP and in tension while the action potential duration was maintained or even slightly increased. The decreased tension was accompanied by a rapid rate of decrease in time of tension development and a slower decrease in the rate of tension development. This depression was not the result of pH changes since the addition of 0.1 mM DNP to the medium did not alter pH by more than 0.05 units. The most likely explanation of these early changes in the duration of the action potential and in tension is the rapid decline in total muscle ATP content. It has previously been proposed (McDonald *et al.*, 1971; McDonald & MacLeod, 1971a) that the tension developed by ventricular muscle is closely associated with the total muscle ATP content but that the duration of the action potential is associated with only a portion of muscle ATP content, probably that portion produced glycolytically.

It was noted that not only the duration but also the amplitude of action potentials declined after exposure of papillary muscles to 0.1 mM DNP in 5 mM glucose medium. After 65 min of exposure to 0.1 mM DNP the action potential amplitude was reduced by about 40 mV although the resting potential was essentially unchanged (Fig. 4). Raising the glucose concentration to 50 mM restored the ampli-

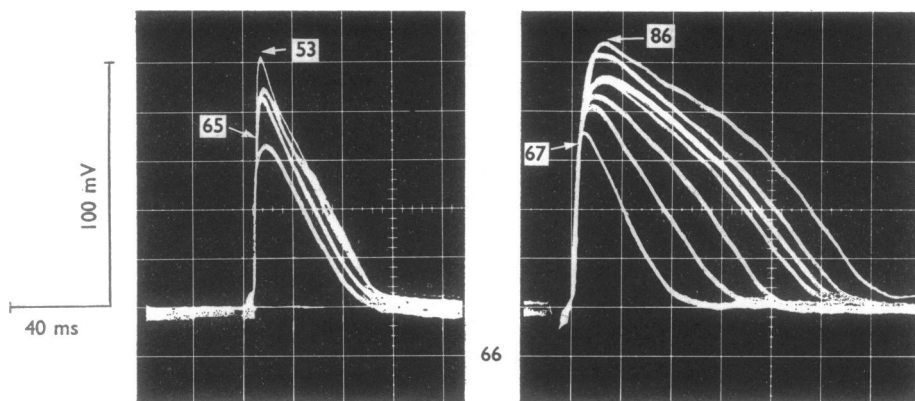


FIG. 4. Intracellular recordings from the same single cell of a papillary muscle illustrating the effect of 0.1 mM DNP on the amplitude and duration of the action potential. The numbers indicate time (min) after the addition of DNP. Records in the left-hand panel were obtained in 5 mM glucose; at 66 min, glucose concentration was raised to 50 mM resulting in the increased amplitude and duration seen in the right-hand panel. The rounding off in overshoots during recovery was probably due to gradual deterioration of the electrode tip.

tude and lengthened the duration, an effect which was also observed in muscles treated with 0.1 mM DNP for as long as 3 hours. In four experiments not shown the addition of ATP to give concentrations of 0.05 to 0.5 mM did not affect the reduced duration of the action potential of muscle incubated in 5 mM glucose anaerobically or in the presence of 0.1 mM DNP.

Sodium and potassium contents, ⁴³K efflux

During aerobic incubation there was no significant change in the Na⁺ content of ventricular strips during the first 30 min of exposure to 0.1 mM DNP but after 120 min a net gain of 15 mmol/kg wet weight was observed (Fig. 5). Muscles incubated in the presence of 0.01 mM DNP showed no change in Na⁺ content during the first 60 min but gained 7 mol/kg after 120 minutes. Changes in K⁺

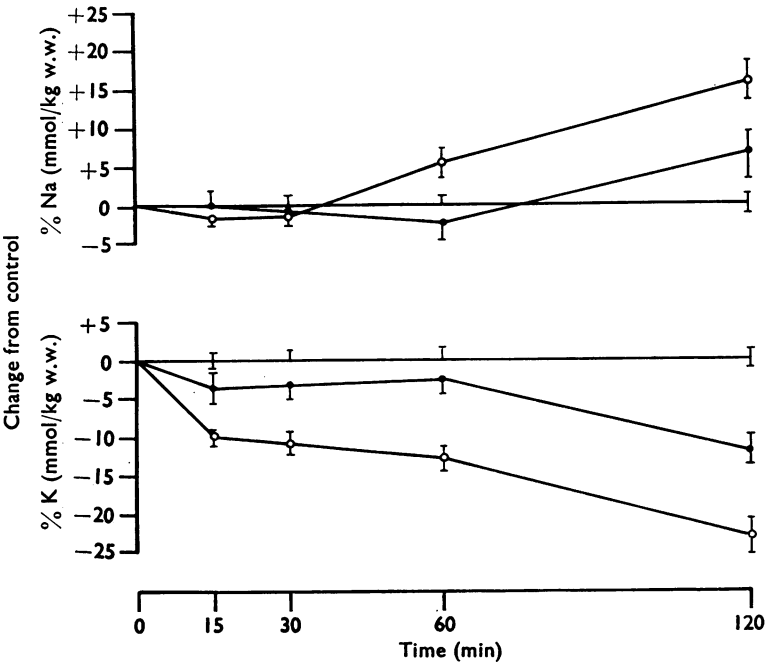


FIG. 5. Effect of DNP on the Na⁺ and K⁺ contents (mmol/kg wet weight) of right ventricular strips incubated aerobically in 50 mM glucose and 0.01 mM DNP (●) or 50 mM glucose and 0.1 mM DNP (○). The points indicate mean differences between experimental and control values of 16–20 measurements and the vertical bars are the S.E. of the means of the actual observations. S.E.M. of the control observations are indicated by vertical bars on the horizontal line through zero.

TABLE 2. Effect of DNP and ouabain on the Na⁺ and K⁺ contents of right ventricular strips incubated aerobically in 50 mM glucose medium

	Mean contents (mmol/kg wet weight ± S.E.M.) after incubation of							
	Na ⁺	15 min	K ⁺		Na ⁺	60 min	K ⁺	
Controls	55.3 ± 1.0	(24)	53.2 ± 0.9	(24)	56.7 ± 1.0	(24)	51.4 ± 1.4	(24)
0.1 mM DNP	53.8 ± 1.1	(16)	43.2 ± 1.0	(16)	62.2 ± 1.8	(16)	37.5 ± 1.4	(16)
0.1 mM ouabain	60.7 ± 2.0	(6)	47.8 ± 2.2	(6)	89.2 ± 1.2	(6)	28.8 ± 1.3	(6)

Number of measurements in parentheses.

content were more pronounced and muscles treated with 0.1 mM DNP lost 10 mmol/kg within 15 min; when muscles were incubated anaerobically without DNP less than 2 mmol/kg of K^+ were lost in 15 minutes. During the next 45 min of incubation in 0.1 mM DNP, the K^+ content decreased only slightly but after 120 min the loss was 23 mmol/kg. Similar, though less severe, losses of K^+ were observed in the presence of 0.01 mM DNP. Table 2 compares the changes in Na^+ and K^+ contents of ventricular strips incubated with 0.1 mM DNP or 0.1 mM ouabain. Although during the first 15 min DNP induced a greater loss of K^+ than ouabain, ouabain but not DNP caused a gain in Na^+ . After 60 min exposure to ouabain, the gain in Na^+ and loss of K^+ were much greater than after 60 min exposure to DNP. The loss of K^+ during the first 15 min exposure of muscles to DNP appeared to be greater than could be accounted for by reduced uptake. Exploratory experiments with ^{42}K did not show a significantly reduced uptake over that of muscles incubated anaerobically in 50 mM glucose medium.

DNP increased ^{42}K efflux in a dose-dependent manner whereas 0.1 mM NaCN had little effect on efflux (Fig. 6A, B). In addition, the effect of 0.1 mM DNP on the duration of the action potential was much greater than that of 0.1 mM NaCN (Fig. 6C). An increase in the NaCN concentration to 1 mM gave similar results. The increase in efflux tended to occur rapidly and tail off during exposure to DNP.

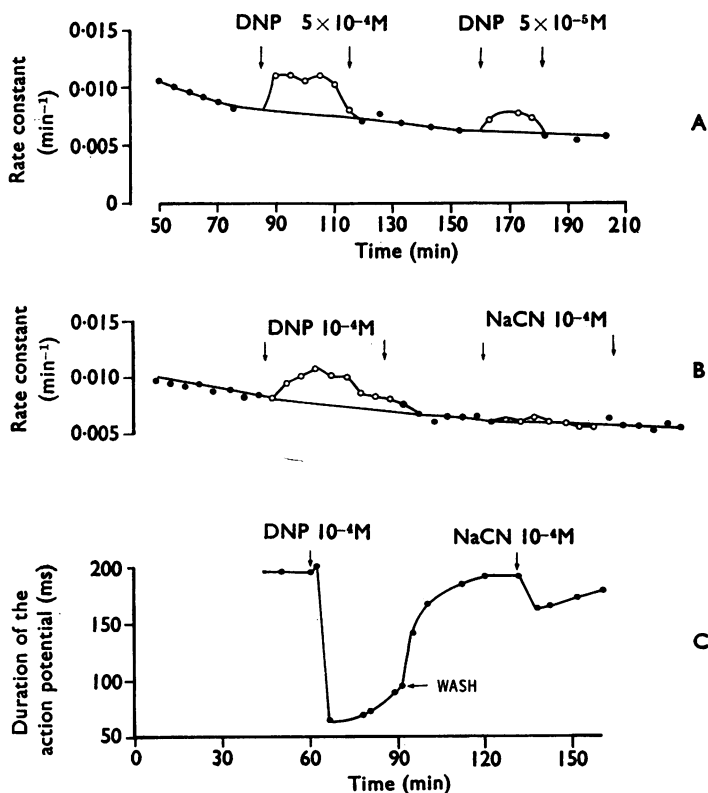


FIG. 6. A. Effect of DNP on ^{42}K efflux from a ventricular strip incubated aerobically in medium containing 50 mM glucose. B. Comparison of the effects of 0.1 mM DNP and 0.1 mM NaCN on a ventricular strip incubated as in A. A and B share the same time axis. C. Comparison of the effects of 0.1 mM DNP and 0.1 mM NaCN on the duration of the action potential of a papillary muscle incubated as in A.

A similar response has been observed in DNP-treated frog atria (Haas, Kern & Einwachter, 1970).

As noted earlier DNP reduced the duration of the action potential of ventricular muscle at a much greater rate than did anoxia. DNP (0.1 mM) also reduced the ATP content of ventricular muscle much more rapidly than did anoxia (McDonald *et al.*, 1971). The question arose as to whether the increase in ^{42}K efflux observed with DNP was associated with the reduction in ATP. In order to answer this question, experiments were carried out under conditions in which the duration of the action potential and the ATP content were rapidly decreased without the use of DNP. When papillary muscle was incubated anaerobically for a prolonged period, the duration of the action potential became much more sensitive to alterations in the glucose concentration (McDonald *et al.*, 1971). Therefore it was expected that following a 2 h anaerobic incubation in medium containing 50 mM glucose, removal of glucose from the medium would result in a shortening of the action potential at a rate comparable to that seen with DNP. Since ATP is severely reduced during anaerobic incubation with 50 mM glucose, the lack of glucose would reduce ATP even more.

Ventricular strips were loaded with ^{42}K during aerobic incubation in medium with 50 mM glucose for 90 min; this was followed by anaerobic incubation in the same medium for 60 minutes. The muscles were then incubated anaerobically in the absence of ^{42}K , namely in medium with 50 mM glucose for 80 min, in glucose-free medium for 40 min and finally in 50 mM glucose for 40 minutes. Transmembrane electrical activity of papillary muscles exposed to the same procedure was measured. The results from a single experiment are shown in Fig. 7. Al-

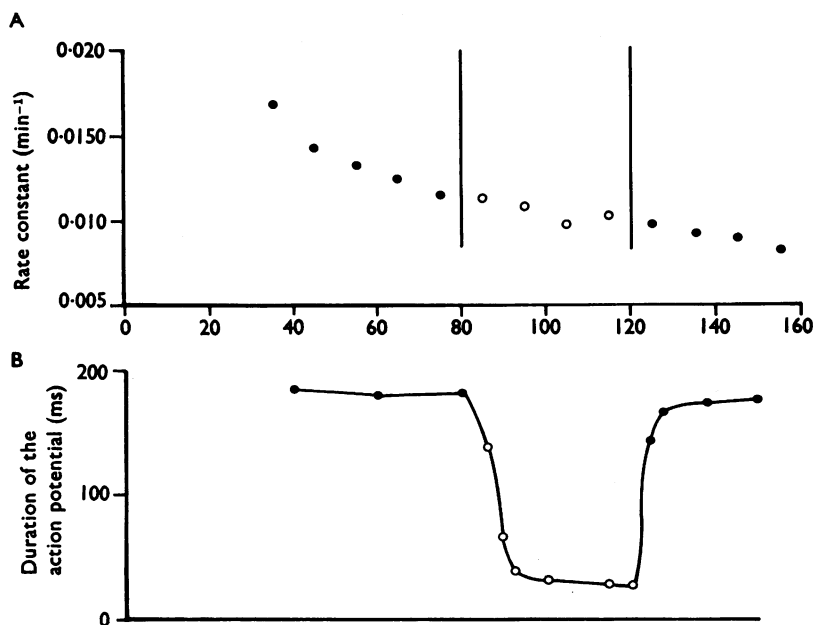


FIG. 7. Effect of anaerobic incubation in glucose-free medium on (A) ^{42}K efflux from a right ventricular strip and (B) duration of the action potential of a papillary muscle. The abscissae (time, min) and the vertical lines apply to both experiments. (●), Anaerobic incubation with 50 mM glucose; (○) anaerobic incubation with glucose-free medium.

though marked and rapid changes occurred in the duration of the action potential, no increase in the efflux of ^{42}K was observed during anaerobic incubation in glucose-free medium. Similar results were obtained in six other experiments. In addition, the effects of anaerobic incubation in medium containing 50 or 5 mM glucose were examined following aerobic incubation in medium with 50 mM glucose. In six experiments, anaerobic incubation did not induce a detectable increase in ^{42}K efflux.

Discussion

The effect of DNP in reducing the duration of the action potential is dose-dependent. Whereas 0.01 mM DNP gradually shortened the action potential, 0.1 mM DNP reduced its duration to 40% control within 10 minutes. Continued exposure to 0.1 mM DNP further shortened the action potential when the medium contained 5 mM glucose but the action potential lengthened to 50% of the control value when the medium contained 50 mM glucose. This observation may be compared to a recovery in duration to 90% of the control when glucose is increased to 50 mM during anaerobic incubation of papillary muscle (McDonald *et al.*, 1971).

DNP reduces the ATP content of guinea-pig ventricular muscle to a greater degree than can be explained on the basis of its uncoupling action on oxidative phosphorylation (McDonald *et al.*, 1971) and the present study indicates that this reduction is not due to a decreased rate of glycolysis. Similar results have been reported for DNP-treated turtle bladder (Klahr, Bourgoignie & Bricker, 1968). Since the duration of the action potential is related to glycolytically produced ATP (McDonald *et al.*, 1971), the results suggest the following interpretation. The early transient shortening of the action potential by 0.1 mM DNP may be due to a rapid depletion of muscle ATP and to a direct effect on processes in the cell membrane resulting in an increased K^+ efflux. The ensuing partial recovery of the duration of the action potential in 50 mM glucose can be explained by an increased glycolytic activity due in part, perhaps, to lowered ATP levels releasing phosphofructokinase from inhibition (Burlington, Whitten, Sidel, Posiviata & Salkovitz, 1970). A rapid depression of contractility followed by partial recovery was reported by Paton (1968) when rabbit detrusor muscle is incubated in medium containing 50 mM glucose and 1 mM DNP. A high-glucose medium also modifies the effect of DNP on the ion content and the contractility of rat uterus (Daniel, Carroll, Robinson & Graham, 1967).

The amplitude of the action potential of papillary muscle declines after prolonged (>1 h) exposure to 0.1 mM DNP in medium containing 5 mM glucose. Similar findings have been reported for rat atria (Webb & Hollander, 1956) and rabbit atria (deMello, 1959). In many excitable cells the amplitude of the action potential is dependent on the driving force for Na^+ (Hodgkin, 1951). However, it has been suggested that the inward movement of Ca^{++} is responsible for a major portion of the overshoot in guinea-pig ventricle (Coraboeuf & Vassort, 1968). In the present experiments, the accumulation of Na^+ , leading to a reduced driving force, was insufficient to account for the decline in the amplitude of the action potential. Furthermore, after two exposures of 3 h to 0.1 mM DNP and subsequent anaerobic incubation for 1 h in medium containing 50 mM glucose, papillary muscles had normal action potentials (McDonald & MacLeod, unpublished observations) although under such conditions intracellular sodium can be assumed to

have at least doubled (McDonald & MacLeod, 1971b). Haas *et al.* (1970) found that DNP partially blocks Na^+ channels in frog atria and thus reduces the early inward Na^+ current. Although no effect of DNP on the late inward Na^+ and Ca^{++} currents in guinea-pig ventricular muscle (Ochi, 1970) has so far been reported, the changes in the duration and amplitude of the action potential may possibly be due to a reduction in these currents. A relationship between glycolytic ATP, the duration and amplitude of the action potential, and the slow inward current has previously been postulated (McDonald & MacLeod, 1971a). It is therefore pertinent that raising the glucose concentration to 50 mM during incubation of DNP-treated muscle increases the glycolytic rate, restores the amplitude of the action potential and partly restores its duration.

Increased K^+ efflux may be a contributing factor to the DNP-induced shortening of the action potential. DNP increases K^+ efflux in frog atria (Haas, Hantsch, Otter & Siegel, 1967) and also increases membrane P_K in cortical neurones (Krnjevic, Godfraine, Pumain & Provini, 1970). In the present experiments, the increase in efflux caused by DNP (0.1 mM) contributed to a loss of nearly 20% of muscle K^+ after 15 min, and there seemed to be a relationship between the rate and extent of K^+ efflux and the rate and extent of decline in the duration of the action potential. This relationship is probably not based on cause and effect since, in glucose-free medium, there is a rapid decline in the duration of the action potential without any significant increase in ^{42}K efflux. Moreover, the amplitude of the action potential also decreases in glucose-free medium and raising of the glucose concentration to 50 mM restores both amplitude and duration without affecting ^{42}K efflux.

The effect of DNP on the Na^+ content of ventricular muscle was unexpected. The Na^+ content was consistently lowered by exposure to 0.01 mM DNP for 1 h, during which time a loss of K^+ occurred. With 0.1 mM DNP, a small loss of Na^+ was apparent during the first 30 min while there was a large loss of K^+ during the same period. A comparison of the effects of 0.1 mM DNP and 0.1 mM ouabain after exposure for 15 min emphasized that Na^+ pumping was not hindered by DNP. It is interesting that Conway (1963) noted a net extrusion of Na^+ from DNP-treated frog skeletal muscle, particularly at lower concentrations. Haas *et al.* (1970) reported that DNP causes a slight hyperpolarization of frog atrial membrane during the first 15 min and suggested that this is the result of an increased outward movement of charge carried by K^+ . On the other hand, there is evidence that in guinea-pig atria (Glitsch, 1969) and in cat papillary muscle (Page & Storm, 1965) there is an electrogenic Na^+ pump which also contributes to the maintenance of the resting potential in K^+ -depleted anoxic cardiac muscle of guinea-pig (McDonald & MacLeod, 1971b). In view of the ion movements observed, a similar mechanism may contribute to the maintenance or slight hyperpolarization of the membrane potential in DNP-treated cardiac muscle.

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