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# CHANGES IN MEMBRANE PROPERTIES OF THE *DROSOPHILA* DORSAL LONGITUDINAL FLIGHT MUSCLE INDUCED BY SODIUM PUMP INHIBITORS

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SUMMARY

1. *Drosophila* dorsal longitudinal flight muscle fibres made anoxic by passing nitrogen through the tracheal system or treated with $10^{-5}$ M ouabain or strophanthidin show a reversible fall in resting membrane potential of 16·5 mV (S.E. 0·96), 13·7 mV (S.E. 0·87), and 17·0 mV (S.E. 2·8), respectively. The reversible depolarization obtained with these sodium pump blockers occurred within 10–15 min.

2. The depolarization of the muscle fibres was accompanied by a decrease in input resistance of 21·2% (S.E. 3·8) in anoxia, 21·4% in ouabain, and 25·6% (S.E. 6·7) in strophanthidin. The resistance decrease in strophanthidin and ouabain was transient and returned to above the resting level while the muscle fibres were still exposed to these agents.

3. Recovery of membrane potential in cells exposed to anoxia is biphasic. An initial 'fast' phase of recovery occurs within 15 s upon return to air followed by a late 'slow' phase lasting several minutes. Recovery of input resistance in cells exposed to $N_2$ coincided with the 'fast' phase of the recovery of resting membrane potential.

4. Recovery of membrane potential following exposure to strophanthidin is a long, slow process which occurs at conductance values at the resting level or below.

5. The tendency towards spontaneous action potentials was increased by anoxia and the action potentials occurring in anoxia were elongated into plateau potentials of about 18 s duration.

6. These results are consistent with the hypothesis that anoxia and cardioactive steroids inhibit a metabolic process, possibly an electrogenic ion pump, that is essential for maintenance of the resting membrane potential in *Drosophila* flight muscle. Exposure to these agents also results in changes in input resistance. Both of these effects could contribute to the depolarization and affect the excitable properties of the muscle fibre membrane.

## INTRODUCTION

The resting membrane potential of the *Drosophila* indirect flight muscle is acutely sensitive to anoxia (Ikeda & Kaplan, 1974). Unless oxygen is supplied to the muscle tracheoles of a dissected preparation, the fibres depolarize.

An electrogenic pump has been proposed to explain the large reduction of membrane potential by anoxia or metabolic inhibitors in other insect muscle (Huddart & Wood, 1966; Rheuben, 1972; Wareham, Duncan & Bowler, 1974*a*). The resting membrane potential of moth muscle was found to be maintained electrogenically to a level more negative than $E_K$ or $E_{Cl}$ so that inhibition of this pump depolarized the membrane to the level of $E_K$ (Rheuben, 1972). The cardiac glycoside, ouabain, which has been shown to inhibit electrogenic sodium pumping in a wide variety of tissues (De Weer, 1975) reduces the resting potential in cockroach muscle (Wareham *et al.* 1974*a*) but does not in moth muscle (Rheuben, 1972).

A loss of membrane potential could also occur as a result of change in membrane permeability. Huddart & Wood (1966) suggested that a change in the ionic permeability of cockroach and moth muscle fibres might explain the depolarization of fibres exposed to DNP, and Rheuben (1972) reported a small decrease in resistance associated with anoxia and DNP in moth muscle. Conductance changes occurring under anoxic conditions or in the presence of metabolic inhibitors have also been described in mammalian cortical neurones (Godfraind *et al.* 1971) and in crayfish muscle (Moody, 1978).

We have found that anoxia and the sodium pump inhibitors, ouabain and strophanthidin, reversibly depolarize the muscle fibres of the dorsal longitudinal flight muscle (DLM) in *Drosophila*, and that the depolarization is accompanied by a decrease in input resistance. Spontaneous muscle action potentials which occurred during or immediately after anoxia were greatly increased in duration compared to those occurring under aerobic conditions, suggesting that the intrinsic excitable properties of the DLM fibres were altered.

## MATERIALS AND METHODS

Four-day-old Canton-S flies were mounted laterally so that the right thoracic spiracle lay over the opening of a plastic tube 3·05 mm in diameter. The fly was sealed in wax to the tube so that only the thorax was exposed. Air, or nitrogen to make the cells anoxic, was supplied through a smaller tube (1·1 mm diameter) which was inserted into the larger tube. The larger tube was closed at the end. The size of the smaller tube permitted the free flow of air between the two tubes so that air entering the larger tube from the smaller tube rapidly displaced the residual air. With a regulated line pressure of 1 p.s.i., the flow of air was monitored by placing the end of the 1·1 mm tubing into a beaker of water and adjusting the flow until a constant stream of bubbles was observed.

The DLM on the opposite side of the thorax was exposed, following the procedure of Ikeda & Kaplan (1974), and perfused with saline. The saline was a modified Bodenstein solution for *Drosophila*, consisting of: 128 mM-$Na^+$, 4·7 mM-$K^+$, 1·8 mM-$Ca^{2+}$, 4 mM-$Mg^{2+}$, 136·3 mM-$Cl^-$, and 4 mM Tris (hydroxymethyl) aminomethane titrated to pH 7·4 with HCl. Ouabain (Strophanthin-G) and strophanthidin were obtained from Sigma Chemical Co.

Muscle fibres I–III, the largest and most ventral of the six pairs of DLM fibres, were selected for study because of their relative similarity of size and input resistance. The posterior dorsal mesothoracic nerve (PDMN) was cut about 50 $\mu$m from the thoracic ganglion.

Table 1. *Effects of anoxia and ouabain* on resting membrane potential*

| Treatment | *n* | $\Delta E$ (mV) 1–180 s | $\Delta E$ (mV) Total | Recovery (mV) 1–15 s | Recovery Total (mV) | % Recovery |
|---|---|---|---|---|---|---|
| Nitrogen | 15 | 0·13 ± 3·0† | 16·5 ± 3·7 | 5·3 ± 3·4 | 10·1 ± 5·1 | 67·9 ± 27 |
| Ouabain* | 9 | 2·3 ± 0·5 | 13·7 ± 2·6 | 0 | 6·6 ± 6·0 | 47·1 ± 41 |

* $10^{-5}$ and $10^{-4}$ M. † S.D.

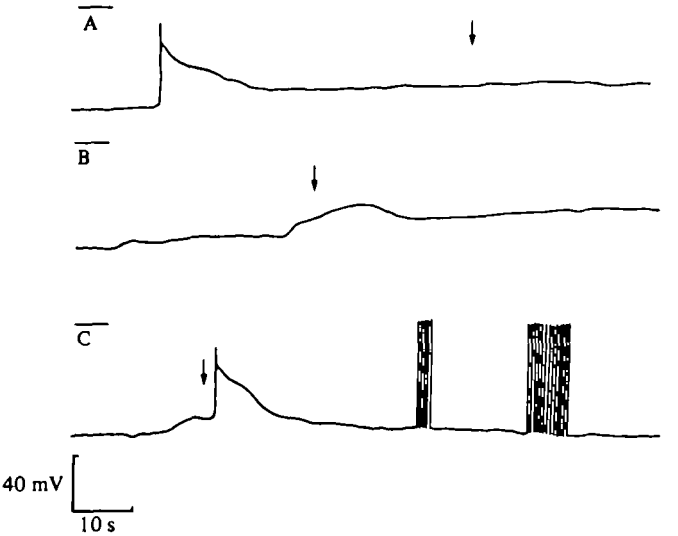


Fig. 1. Spontaneous action potentials in DLM fibres exposed to anoxia. Arrows indicate time at which air was returned to the preparation. Horizontal bars indicate zero potential for each record. (A) Plateau potential. (B) Fast depolarization without spike. (C) Plateau potential just after end of $N_2$ followed by trains of spontaneous action potentials occurring in air. Vertical calibration, 40 mV; horizontal calibration, 10 s.

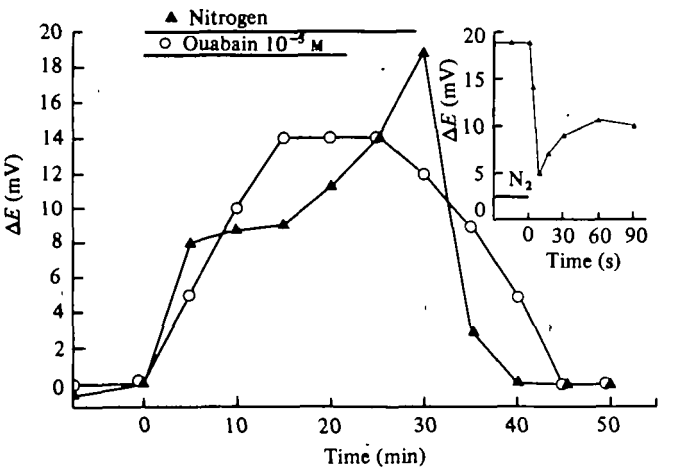


Fig. 2. Changes in resting membrane potential, $\Delta E$, during anoxia (▲) and exposure to $10^{-5}$ M oubain (O). Initial resting potentials were —91 and —89 mV, respectively. The depolarizing direction is shown as upwards. Bars indicate duration of exposure. Inset shows expanded time scale of recovery of membrane potential of the cell exposed to $N_2$ for 28 min, 49 s upon return to air. Note biphasic recovery of resting potential.

Table 2. *Effects of anoxia, strophanthidin, and ouabain on input resistance and resting membrane potential*

| Cell no. | $E_m$ (mV) | $\Delta E_m$ (mV) | $R_1 \times 10^6\ \Omega$ | $R_2 \times 10^6\ \Omega$ | % $\Delta R$ | $R_3 \times 10^6\ \Omega$ |
|---|---|---|---|---|---|---|
| | | | Nitrogen | | | |
| 1 | −83 | 12 | 2·91 | 2·27 | 22 | 3·00 |
| 2 | −85 | 16 | 2·82 | 2·45 | 13 | 2·91 |
| 3 | −92 | 17 | 1·82 | 1·42 | 22 | 1·82 |
| 4 | −93 | 23 | 2·20 | 1·40 | 36 | 2·18 |
| 5 | −90 | 22 | 2·19 | 1·90 | 13 | 2·73 |
| Mean | −88·6 | 18 | 2·39 | 1·88 | 21·2 | 2·53 |
| S.D. | 3·9 | 4·1 | 0·41 | 0·42 | 8·4 | 0·45 |
| | | | Strophanthidin $10^{-5}$ M | | | |
| 1 | −85 | 42 | 2·56 | 1·49 | 42 | 2·56 |
| 2 | −86 | 26 | 3·40 | 2·84 | 17 | 3·30 |
| 3 | −85 | 12 | 1·80 | 1·47 | 18 | 2·18 |
| Mean | −85·3 | 26·7 | 2·59 | 1·93 | 25·6 | 2·68 |
| S.D. | 0·47 | 12·3 | 0·65 | 0·64 | 11·6 | 0·46 |
| | | | Ouabain $10^{-5}$ M | | | |
| 1 | −83 | 21 | 1·87 | 1·47 | 21·4 | 2·27 |

Data from the individual fibres are presented from left to right.
$R_1$ is the initial input resistance before application of drug or exposure to N.
$R_2$ is the input resistance measured at the time of maximum change in resistance.
$R_3$ is the input resistance measured 500 s after termination of treatment.

Resting potentials were recorded differentially between a 20–30 M Ω glass micropipette filled with 3 M-KCl and a similar electrode placed in the bath, using two BAK ELS preamplifiers and a Tektronix 565 oscilloscope. A silver-silver chloride electrode in the bath served as ground.

## RESULTS

The mean amplitude of the DLM resting membrane potential was —89 mV (S.E. 0·59) with a range of —83 to —96 mV. The membrane potential was stable for periods of 1 h or longer.

*Effects of anoxia on spontaneous action potentials.* Exposure to nitrogen increased the occurrence of spontaneous muscle action potentials (Fig. 1). Spontaneous action potentials occurred between 190 and 300 s of exposure to $N_2$ in those preparations in which the rate of depolarization was most rapid (see below). The spontaneous potentials were comparable to the plateau potentials described by Patlak (1976) in *Sarcophaga* and averaged 18·3 s ($n = 7$) in length compared to about 30 ms required for a normal action potential to return to baseline. Plateau potentials of shorter duration (average = 2·87 s, $n = 12$) sometimes followed the initial long plateau, and these tended to occur within the first 300 s in air after exposure to $N_2$. Few preparations had spontaneous action potentials outside of these time periods; but in those cases, the action potentials tended to occur in trains and were of normal duration. In 13 cells, fast depolarizations of about 10 mV were seen. These fast depolarizations were of similar duration to the long plateau potentials and occurred within the same time interval (190–300 s in $N_2$). In the present study, two preparations had spon

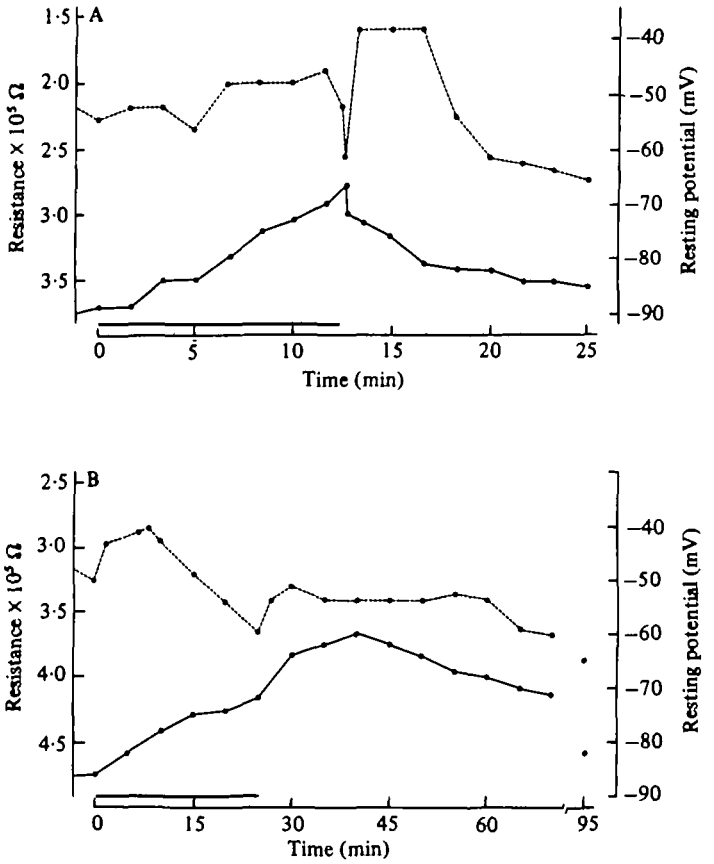


Fig. 3. Effect of anoxia (A) and $10^{-6}$ M strophanthidin (B) on resting membrane potential, $E_m$ (solid lines), and input resistance, $\Delta E/i$ (dashed lines). Resistance was plotted with lower values uppermost to illustrate correlation of decreases in resistance with decreases in membrane potential. Bars indicate duration of treatment.

taneous action potentials of normal duration within 2 min of return to air following the occurrence of long plateau potentials in anoxia (Fig. 1).

*Effects of anoxia on resting membrane potential* (Table 1). Eight of 15 muscle fibres examined for the effects of anoxia on resting membrane potential became hyperpolarized by 1–5 mV during the initial 3 min of anoxia. By 3 min, all cells had begun to depolarize, and peak depolarizations of 9–23 mV ($\Delta E$) were reached by 240–1730 s. The maximum value of $\Delta E$ from which a full recovery of membrane potential occurred was 19 mV.

The recovery of resting membrane potential following a period of anoxia appears to consist of two distinct components (Fig. 2). An initial 'fast' phase of recovery occurred within the first 15 s on return to air. The amount of recovery within this period ranged from 2–14 mV with a mean of 5·3 mV (S.E. 0·88), compared to the total mean recovery of 10·1 mV (S.E. 1·3). The second phase of recovery was much slower and required a period of several minutes (mean = 7 min, 5 s) for peak recovery.

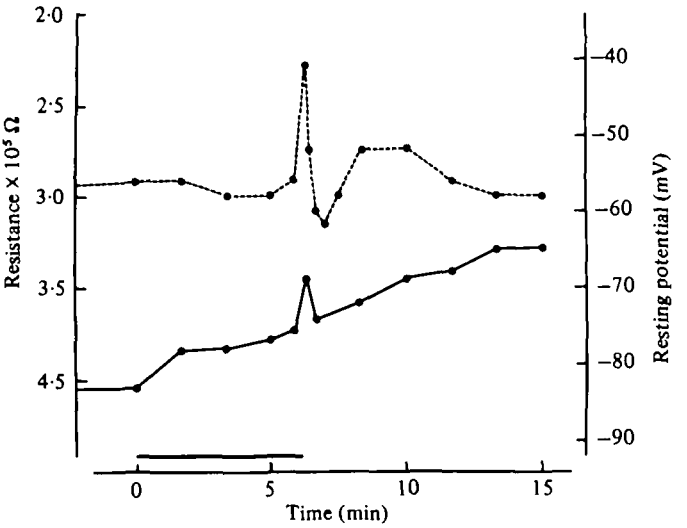


Fig. 4. Effect of anoxia on resting membrane potential (solid line) and resistance (dashed line). Resistance was plotted with lower values uppermost to illustrate concomitant changes in resistance and potential. This fibre shows 'fast' phase of resting membrane potential recovery, but no 'slow' phase. Bar shows duration of exposure to nitrogen.

While all of the cells examined showed some recovery during the initial 'fast' phase, only 10 of the 15 cells showed recovery during the longer 'slow' phase.

*Resting membrane potential changes in ouabain* (Table 1). Cells treated by bath application of $10^{-5}$ to $10^{-3}$ M ouabain began to depolarize immediately. The amount of depolarization occurring within the first 3 min of exposure was 2–3 mV. None of the cells showed the hyperpolarization in this time period that was seen in cells exposed to nitrogen. Peak depolarizations of 9–19 mV (mean = 13·7 mV; S.E. 0·87) were attained within 10–15 min, and a steady membrane potential was maintained for a period of several minutes in some preparations (Fig. 2).

A period of several minutes elapsed after ouabain had been removed from the preparation before any of the fibres began to recover. There was no 'fast' component to the recovery from oubain. When recovery did occur, it was a slow, gradual process comparable to the slow phase of recovery from anoxia, but of longer duration (mean = 21 min, 30 s). Six of the nine cells from which data were taken showed some recovery while four cells recovered 80% or more of the initial resting potential.

*Effects of anoxia and cardioactive steroids on input resistance* (Table 2). Measurements of input resistance ($\Delta E/i$) were obtained by placing 2 KCl electrodes into a single muscle fibre and passing 30 ms hyperpolarizing pulses at a frequency of 0·5 Hz through one electrode while recording changes in membrane potential with the other. Current was monitored by a current-to-voltage converter circuit in series with ground. The continuous monitoring of input resistance during treatment and recovery permitted a direct comparison between changes in resistance and concomitant changes in resting potential.

The mean initial input resistance for all cells examined was $2{\cdot}4 \times 10^5\ \Omega$ ($n = 9$) an average room teperature of 25 °C. Although the initial values of resistance taken

from the individual fibres showed considerable variation from cell to cell, consecutive measurements of resistance taken from the same cell were highly consistent. The DLM fibres showed an initial decline in input resistance of 21·2% (S.E. 3·8) in $N_2$, 25·6% (S.E. 6·7) in strophanthidin, and 21·5% (one cell) in ouabain (Fig. 3). These changes were significant at the $P < 0{\cdot}01$ level by a one-tailed Student's $t$ test when 10 consecutive measurements in the same cell before treatment were compared to 10 consecutive resistance measurements at the time of the peak conductance change.

The resistance decline in cells exposed to strophanthidin and ouabain reached a peak at 200–500 s of exposure. Resistance then showed a sustained increase, beyond the resting level, throughout exposure to the drugs. The magnitude, duration, and direction of these changes were highly consistent from one preparation to the next.

When air was returned to a preparation following exposure to $N_2$, there was a fast recovery of resistance during the first 15 s in 4 out of 5 cells, coinciding with the 'fast' phase of the recovery of the resting potential (Fig. 4). Resting levels of input resistance were attained at about 500 s after the return of air, and the resistance was maintained at an elevated level for a sustained period after this time (Fig. 3).

## DISCUSSION

The 19–22 mV reversible decline in the resting membrane potential seen with anoxia and the cardioactive steroids is similar in size to the 18 mV active component observed by Wareham *et al.* (1974*a*) for cockroach muscle. The reduction of the resting potential by ouabain and strophanthidin at $10^{-5}$ M as well as by anoxia suggests that an electrogenic sodium pump could be contributing to the resting membrane potential of the *Drosophila* DLM, but this is by no means the only possibility (Wareham *et al.* 1974*a*; Barker & Gainer, 1975). At least part of the observed decline in resting membrane potential might occur as a result of the decrease in input resistance associated with both anoxia and the more specific blockers of the Na-K-ATPase. This suggestion is supported by the coincidence of the time of onset of the depolarization with that of the decline in membrane resistance produced by both anoxia (3–6 min) and the cardioactive steroids (1–2 min) and by the close correlation of the 'fast' phase of resting potential recovery following periods of anoxia with recovery of input resistance. The slow phase of recovery of membrane potential following anoxia and recovery of resting potential following exposure to cardioactive steroids have time courses more typical of the type of recovery expected following inhibition of active sodium pumping (Thomas, 1972).

It is interesting to note that recovery of membrane potential from strophanthidin begins at a time when the resistance is increased above the resting level and continues for a period of several minutes at a stable resistance level. It is possible that recovery of membrane potential could occur as a result of a decrease in the resting conductance to some ion, but the data are consistent with observations in frog skeletal muscle (Livengood & Geduldig, 1974) and rat skeletal muscle (Clausen & Flatman, 1977) where a decrease in conductance is associated with active sodium pumping.

There is good evidence for the involvement of an electrogenic pump. However, it seems clear that the depolarization cannot be explained simply on the basis of blocking an electrogenic component of the resting potential. It does seem likely that the altered state of ion distribution resulting from inhibition of active transport

could produce changes in membrane resistance and lead to alterations in membrane excitability (Gorman & Marmor, 1974; Wareham, Duncan & Bowler, 1974*b*; Delaleu & Holley, 1976; Lieberman & Nosek, 1976; Blaustein, 1977). Low concentrations of strophanthidin have been shown to increase the slow inward $Ca^{2+}$ current and the action potential plateau in cardiac Purkinje fibres (Weingart, Kass & Tsien, 1978). The action potential of the *Sarcophaga* flight muscle results from a permeability increase to both $Na^+$ and $Ca^{2+}$, but Patlak (1976) found that the plateau potential is supported by $Na^+$ and that the duration of the plateau increases with the external $Na^+$ concentration.

The greatly increased duration of spontaneous action potentials in anoxia could be explained if the internal $Na^+$ concentration in the DLM were raised by inhibition of an active $Na^+$ pump (Thomas, 1972).

It is hoped that further investigation of the effects of anoxia and cardioactive steroids on the membrane properties of the *Drosophila* DLM will help to explain the interactions between metabolic activity, active ion transport, and regulation of the intrinsic factors controlling membrane excitability.

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