

QUANTITATIVE ANALYSIS OF RESTING MEMBRANE ELECTROGENESIS IN INSECT (DIPTERA) SKELETAL MUSCLE

I. INTRACELLULAR K^+ , Na^+ AND Cl^- ACTIVITIES, MEASURED USING LIQUID ION-EXCHANGER AND NEUTRAL ION-CARRIER MICROELECTRODES

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Summary

Electrophysiological properties of skeletal body-wall muscles of prepupal *Calliphora erythrocephala* were investigated using double-barrelled intracellular ion-sensitive microelectrodes. The most realistic estimate of the intracellular K^+ activity, obtained using K^+ -sensitive microelectrodes based on a neutral carrier, was 115 mmol l^{-1} . The K^+ equilibrium potential was consistently more negative than the prevailing resting potential, the average difference being -15 mV . The intracellular Na^+ activity and the Na^+ equilibrium potential were 7 mmol l^{-1} and $+46 \text{ mV}$ on average, respectively. The mean value of the intracellular Cl^- activity was 40 mmol l^{-1} , and this was apparently higher than that required for passive distribution of Cl^- . However, when reversibly exposed to a Ringer containing no Cl^- , cells could rapidly exchange most of their intracellular Cl^- , although the resting membrane potentials were only transiently affected. It is concluded that an anionic interferent exists inside muscles, that this artefactually elevates the measured intracellular Cl^- activities, and that Cl^- makes no contribution to resting membrane electrogenesis.

Introduction

Electrophysiological properties of insect muscles, particularly ionic parameters, have been studied for many years (for recent reviews see Piek & Nijio, 1979; Pichon & Ashcroft, 1985; Djamgoz, 1987). Insect skeletal muscles seem to be bathed directly in the haemocoelomic fluids, which have markedly different ionic compositions in the different orders. In most exopterygotes and carnivorous endopterygotes (e.g. Diptera), the ionic composition of the haemolymph is such that the $Na^+ : K^+$ ratio is high, the levels of Ca^{2+} and Mg^{2+} are relatively low, and the prevailing transmembrane ionic gradients are 'conventional'. In contrast, in

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many phytophagous insects, especially Lepidoptera, the haemolymph has a relatively low $\text{Na}^+:\text{K}^+$ ratio and is high in Ca^{2+} and Mg^{2+} , giving 'unconventional' ionic gradients (see Djamgoz, 1987). Thus, insects comprise interesting 'model' systems as regards the electrophysiology of their cell membranes. The neural inputs to insect muscles have been shown to involve both amino acid and modulatory peptide(s) (Usherwood & Cull-Candy, 1975; Piek, 1985). Consequently, insect muscle preparations are also proving to be useful in elucidating the actions and interactions of novel, endogenous neuroactive compounds, for which a thorough understanding of ionic properties is crucial. Finally, since many insect species are pests, such an understanding is also essential for evaluating the modes of action of insecticides. In these respects, the skeletal muscles of larval insects offer some unique experimental advantages. First, cells may be very large, measuring some hundreds of micrometres. Consequently, the cells can be impaled with microelectrodes (several, if necessary) without causing significant damage, and electrical problems associated with ultrafine microelectrodes can be avoided, making the interpretation of data straightforward. Second, many cells are superficially situated in the body, so they are readily accessible to microelectrodes. Furthermore, the cells are free of any connective tissue, and are therefore maximally exposed to bathing media and respond readily to solution changes.

In several early studies, the ionic contents of extracted myoplasm of insect muscles were studied using methods (such as flame photometry) which measured *concentrations* (see Djamgoz, 1987). Detailed knowledge of intracellular ion *activities* is surprisingly limited, however, and is lacking for Diptera muscle. This paper describes intracellular K^+ , Cl^- and Na^+ activity measurements in skeletal muscles of the larval blowfly, an example of an insect with a 'conventional' haemolymph. Together with the following paper, it leads to the quantitative testing of a model of resting membrane electrogenesis. Preliminary reports of these measurements have been given to the Physiological Society (Dawson & Djamgoz, 1983, 1984*a,b*).

Materials and methods

The preparation

Experiments were carried out on larvae of *Calliphora erythrocephala* Meig (Diptera). Final instar larvae approaching pupation (i.e. prepupae) were used. At this stage the muscle cells lose their ability to contract whilst apparently retaining normal resting potentials and general membrane responsiveness. Each maggot was dissected dorsally to expose the ventral body-wall muscles (Fig. 1). Preparations were bathed in Rice's saline, containing (in mmol l^{-1}): NaCl, 173; KCl, 13.5; MgCl_2 , 1.0; NaHCO_3 , 1.2; NaH_2PO_4 , 0.8; CaCl_2 , 0.9; glucose, 55.5 (pH 7.0, checked daily; Finlayson & Osborne, 1970). Preparations were allowed to equilibrate at room temperature (18–20°C) for at least 30 min in the saline prior to impalement of cells. Ventral longitudinal body-wall muscles 6A and 7A were used for all measurements (Crossley, 1965; Hardie, 1976; see Fig. 1).

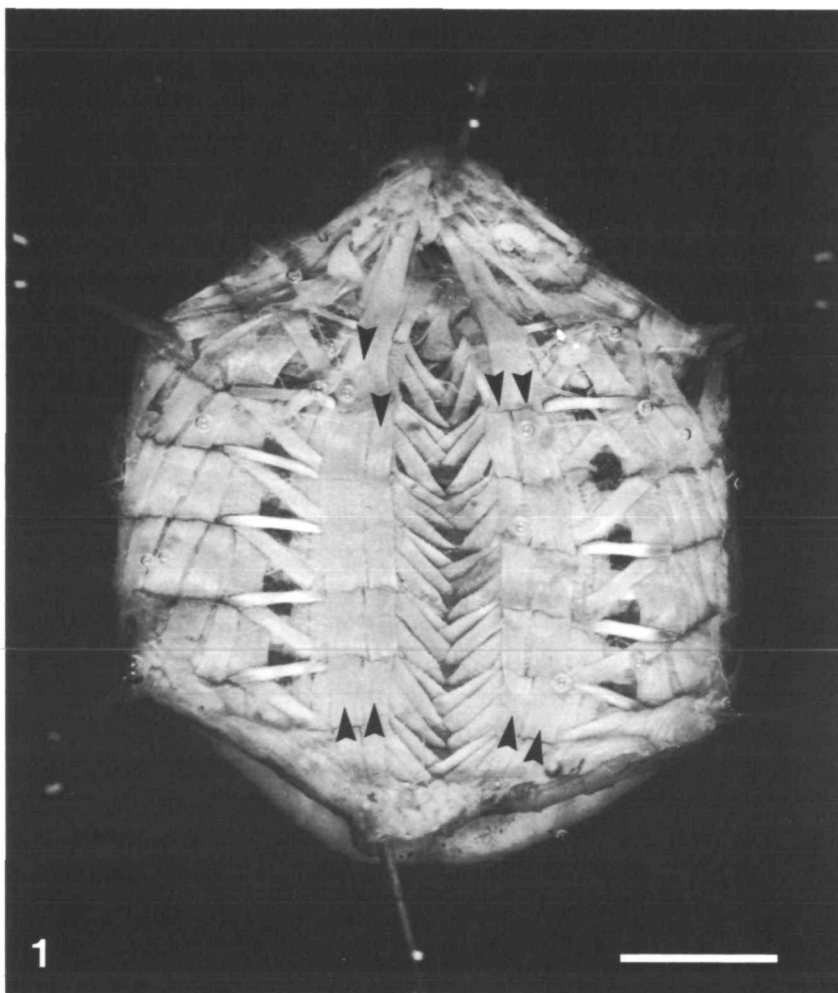


Fig. 1. A prepupal preparation of *Calliphora erythrocephala*. The prepupa was dissected from the dorsal side to expose the ventral body-wall muscles. The arrowheads denote the two pairs of double bands (6A and 7A) of longitudinal muscle cells used for this study. The preparation was immersed in Bouin's fixative for clarity of illustration. Scale bar, 2 mm.

Ion-sensitive microelectrodes

Double-barrelled ion-sensitive microelectrodes (ISMs), based on a variety of liquid sensors, were constructed and used as described in detail previously (Djamgoz & Dawson, 1986; Djamgoz & Laming, 1987*a,b*). Briefly, lengths of borosilicate glass capillary tube (1.0 and 1.5 mm, o.d.) were glued together with Araldite, twisted through 180° in the middle in a vertical electrode puller and then pulled in a modified Livingstone-type horizontal puller. The inside of the larger barrel was silanized by exposing the opening to the vapour of dimethyldichlorosilane (Sigma). The tip of this barrel was plugged with a column of liquid ion

sensor (0.1 mm to a few mm in length) and back-filled with the appropriate salt solution (see below). The filamented reference barrel was filled by direct injection of solution using a 30 gauge needle and syringe. Details of the liquid sensors and back-filling solutions for the three types of ISM were as follows.

K⁺-sensitive microelectrodes

Both the 'classical' ion-exchanger (LIX; Corning 477317) and the valinomycin-based neutral carrier (NIC) were used (Walker, 1971; Oehme & Simon, 1976; Tsien, 1980). 0.5 mol l⁻¹ KCl was used as the back-filling solution in the active barrel, and reference barrels were filled with 5 mol l⁻¹ LiCl.

Cl⁻-sensitive microelectrodes

The improved Cl⁻ exchanger, Corning 477913, was used (Baumgarten, 1981). Active barrels were back-filled with 0.5 mol l⁻¹ KCl. The reference liquid ion exchanger (RLIE) solution of Thomas & Cohen (1981) was found to be ideal for the reference barrels (see Djamgoz & Dawson, 1986).

Na⁺-sensitive microelectrodes

The neutral Na⁺ carrier ETH227 was used (Steiner, Oehme, Ammann & Simon, 1979). The active barrel was back-filled with 0.5 mol l⁻¹ NaCl, and the reference barrel was filled with 1 mol l⁻¹ KCl.

Active barrels employing ion-exchangers and neutral carriers had tip resistances in the ranges 10–90 GΩ and 60–160 GΩ, respectively. Reference barrels filled with inorganic electrolyte solutions, and those filled with the RLIE, had tip resistances in the ranges 50–200 MΩ and 10–50 GΩ, respectively. Ion-sensitive microelectrodes were connected to a dual/differential electrometer (WPI 223A), the outputs of which were monitored on a chart recorder. The reference electrode was a short length of 1.5 mm o.d. capillary tubing filled with 'solid' 3 % agar in Rice's saline into which the chlorided end of a silver wire was inserted and connected to earth. The same electrode was used for the calibration of the ISM and earthing of the preparation. The potential difference between the earth electrode and the reference barrel of a given ISM was stable to within ±2 mV in all ionic substitution solutions used in a given experiment.

K⁺-sensitive microelectrodes (K⁺-SMs) were calibrated in a series of solutions based on Rice's saline, in which the K⁺ concentration varied from 1.35 to 135 mmol l⁻¹, using Na⁺ as the substitute. Intracellular K⁺ activities were derived by direct reference of the K⁺-SM potential to the microelectrode's calibration curve. Cl⁻-sensitive microelectrodes (Cl⁻-SMs) were calibrated similarly using gluconate (sodium salt) as the substitute; extra Ca²⁺ was added to compensate for sequestration of Ca²⁺ by gluconate (Kenyon & Gibbons, 1977; Vaughan-Jones, 1979). Intracellular Cl⁻ activities were derived again by direct reference of the Cl⁻-SM potential to the respective calibration curve. Calibration solutions for Na⁺-sensitive microelectrodes (Na⁺-SMs) were not based on Ringer's solutions, however, since the latter contained a high level of Ca²⁺ which is known to interfere

with the Na^+ carrier ETH227 (Steiner *et al.* 1979). Instead, the calibration solutions were designed to include a constant background of interference approximating the intracellular medium, i.e. $152.8 \text{ mmol l}^{-1}$ KCl, 2.8 mmol l^{-1} MgCl_2 , $0.014 \text{ mmol l}^{-1}$ CaCl_2 and 2.8 mmol l^{-1} Hepes (Deitmer & Schlue, 1983). Since normal Rice's saline was not included as one of the steps in the calibration solutions, the potential recorded by the Na^+ -SM in the medium bathing the preparation was meaningless. It was therefore necessary to take the *difference* between the reference level, 0 mV (corresponding to 100 mmol l^{-1} Na^+ , the highest Na^+ concentration in the calibration solutions) and the intracellular potential recorded by the Na^+ -SM, and refer this directly to the calibration graph to give the intracellular Na^+ activity. All ISMs used in this study responded to a 10-fold change in the activity of the primary ion sensed by giving a potential change of 50 mV or greater in the range $10\text{--}100 \text{ mmol l}^{-1}$, and 35 mV or greater in the range $1\text{--}10 \text{ mmol l}^{-1}$. Importantly, the calibration of a given ISM before and after an experiment agreed to within $\pm 5 \text{ mV}$ in all solutions, such that the 'shallow' end of a calibration could be used reliably (also, see below). *Activity* is a fraction of a given concentration, representing mobile ions that contribute to electrochemical phenomena. ISMs measure activities directly, and throughout the text activity values have been given for consistency. However, assuming that the intracellular activity coefficient for K^+ , Na^+ and Cl^- is the same as in Rice's saline (i.e. 0.72), then corresponding concentration values can be derived by simple division (Weast, 1971). All data in the text are given as means \pm standard errors.

Criteria for the selection of results

The following conditions were met in selecting and evaluating the results obtained with ion-sensitive microelectrodes. (i) The calibrations of a given ISM before and after experiments were within $\pm 5 \text{ mV}$ of each other at all points. Most calibrations agreed to within $\pm 2 \text{ mV}$. (ii) Prior to the impalement of a cell, the levels of potential and ionic activity in the recording chamber were steady to within $\pm 1 \text{ mV}$ for at least 2 min, again to ensure that the ISM was giving a steady response. (iii) The changes in the voltage readings of the ISM upon impalement of a cell were abrupt, indicating a non-damaging penetration. (iv) The intracellular potentials recorded were steady for longer than 2 min to ensure that the membrane had formed a tight seal around the ISM. In fact, steady impalements and recordings were frequently obtained for several hours. (v) Cells had membrane potentials of at least -30 mV . (vi) The means and distributions of membrane potentials obtained from a given preparation were similar when measured using a conventional microelectrode or an ISM, again to indicate the absence of any damage by the relatively coarse double-barrelled ISMs.

Results

Intracellular potassium activities

A recording of the impalement of a maggot muscle cell with a double-barrelled NIC-type K^+ -SM is illustrated in Fig. 2. The lower trace shows the membrane

potential (E_M), which was -40 mV for this cell, and the upper trace records the intracellular K^+ activity (aK_i), 115 mmol l^{-1} . Averages and frequency distributions of data obtained with both NIC and LIX-type K^+ -SMs are given in Table 1 and Fig. 3. aK_i values measured using NIC-type K^+ -SMs were distributed normally in the range $68\text{--}210 \text{ mmol l}^{-1}$ (98 % of values falling between 68 and 170 mmol l^{-1}), with a mean value of 115 mmol l^{-1} . However, using LIX-type K^+ -SMs, a much wider range ($150\text{--}760 \text{ mmol l}^{-1}$) and a much higher mean value of aK_i (382 mmol l^{-1}) were recorded (Fig. 3; Table 1). The latter measurements are highly likely to have been erroneous and a possible cause of this is given in the Discussion.

The values of the K^+ equilibrium potential (E_K) obtained using NIC-type K^+ -SMs were in the range -49 to -77 mV (mean value, -62 mV). Resting potentials (E_M) varied between -34 and -63 mV (mean value, -47 mV) (Fig. 4). For every

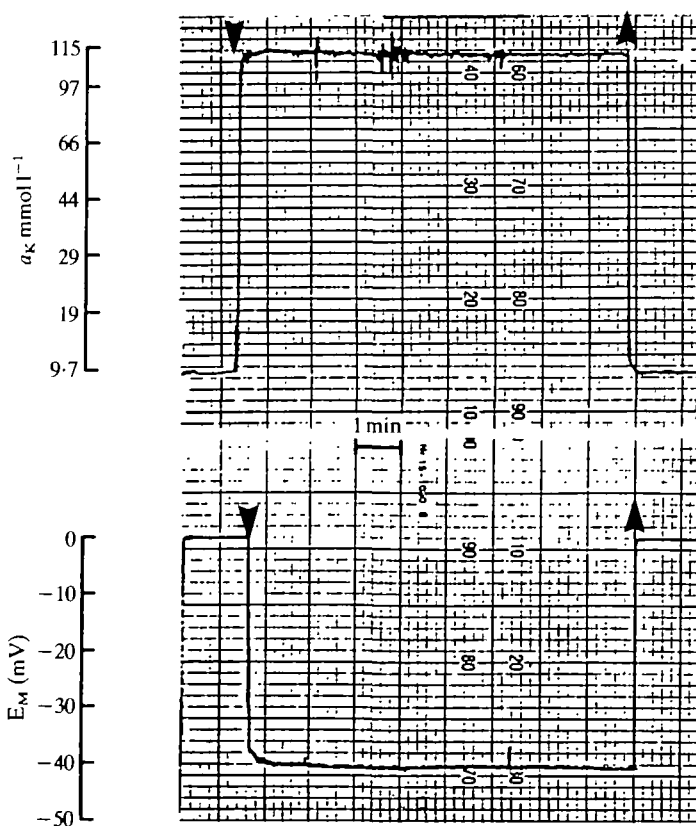


Fig. 2. Chart recording of the impalement of a prepupal *Calliphora* muscle cell with a double-barrelled NIC-type K^+ -sensitive microelectrode. Upper trace, K^+ activity (a_K); lower trace, membrane potential (E_M). The moment of impalement of the cell is marked by downward-pointing arrowheads (these do not coincide due to an offset in the positions of the recorder pens). At the point marked by upward-pointing arrowheads, the microelectrode was withdrawn from the cell.

Table 1. Intracellular ionic activity data from *Calliphora prepupae*

	NIC	LIX
aK_i (mmol l ⁻¹)	115 ± 3	382 ± 12
E_K (mV)	-62 ± 1	—
E_M (mV)	-47 ± 1	-50 ± 1
$E_K - E_M$ (mV)	-15 ± 1	—
aNa_i (mmol l ⁻¹)	7 ± 1	
E_{Na} (mV)	76 ± 2	
E_M (mV)	46 ± 1	
$E_{Na} - E_M$ (mV)	122 ± 3	
aCl_i (mmol l ⁻¹)	40 ± 2	
E_{Cl} (mV)	-31 ± 1	
E_M (mV)	-50 ± 1	
$E_{Cl} - E_M$ (mV)	19 ± 1	

Symbols have been defined in the text.

K⁺ data from 96 cells, seven preparations (LIX-type K⁺-SMs); 85 cells, 10 preparations (NIC-type K⁺-SMs).

Na⁺ data from 43 cells, six preparations.

Cl⁻ data from 35 cells, six preparations. All data are given as means ± s.e.

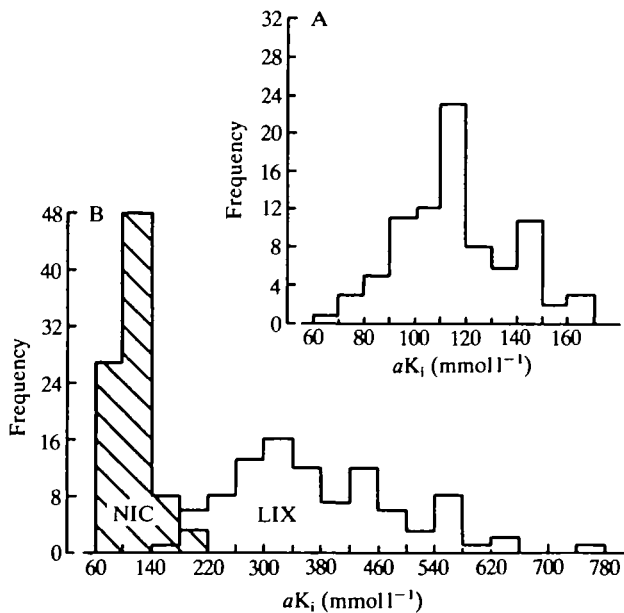


Fig. 3. Frequency histograms illustrating: (A) the distributions of intracellular K⁺ activity (aK_i) values measured using NIC-type K⁺-sensitive microelectrodes; and (B) a comparison of aK_i values obtained using LIX- and NIC-type K⁺-SMs, plotted on the same abscissa.

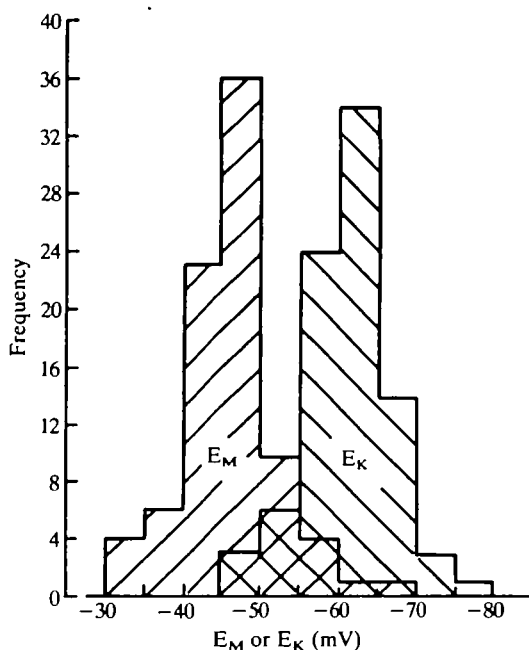


Fig. 4. Frequency histograms illustrating the distributions of the resting membrane potential (E_M) and K^+ equilibrium potential (E_K) values, measured using NIC-type K^+ -SMs. Data are plotted on the same abscissa for comparison.

cell tested, however, E_K was more negative than the corresponding E_M , the average difference being -15 mV (Table 1). E_K values have not been calculated for the data obtained using LIX-type K^+ -SMs, since the latter are not likely to reflect the real situation in these cells, as already noted.

Thus, since E_K values were consistently and significantly more negative than E_M values, K^+ cannot be the only ion involved in resting membrane electrogenesis. At least one other ion with an equilibrium potential more *positive* than E_M must also play a part.

Intracellular sodium activities

A successful impalement of a cell with a double-barrelled Na^+ -SM is shown in Fig. 5. As in Fig. 2, the lower trace gives the membrane potential, and the upper trace corresponds to Na^+ activity. This cell had an E_M of -46 mV, and its intracellular Na^+ activity (aNa_i) was 9 mmol l^{-1} . The frequency distribution of the population of aNa_i values measured is shown in Fig. 6. The values of aNa_i varied from 1 to 15 mmol l^{-1} , with a mean value of 7 mmol l^{-1} (Table 1). The calculated values of the Na^+ equilibrium potential (E_{Na}) were consistently positive, covering a wide range (57 – 117 mV). The mean value of E_{Na} was 76 mV and the average difference between E_{Na} and E_M was 122 mV (Table 1).

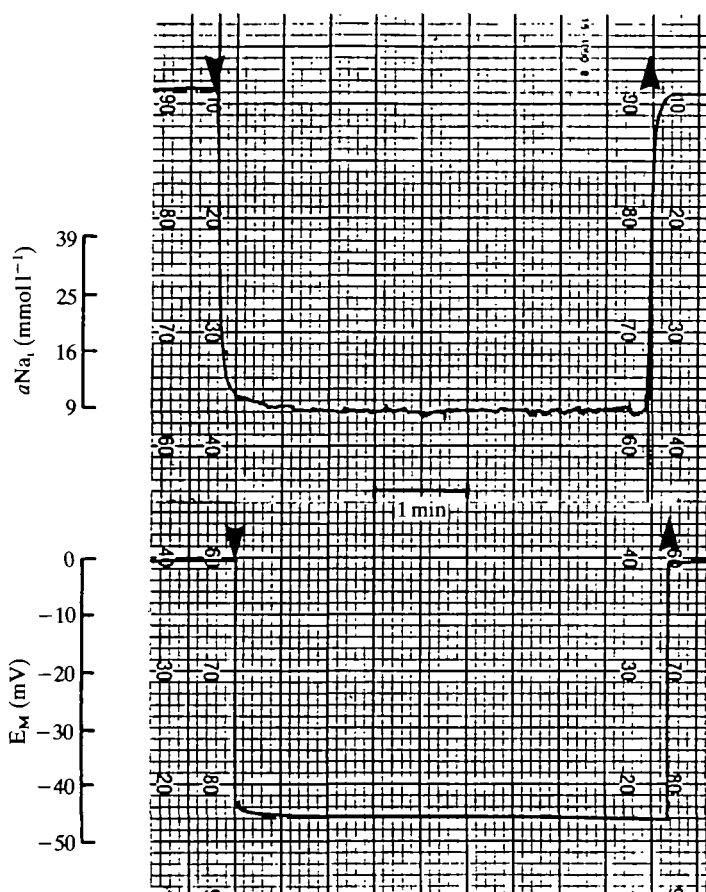


Fig. 5. Chart recording of the impalement of a *Calliphora* prepupal muscle cell with a double-barrelled Na^+ -sensitive microelectrode. Details of symbols etc. are as in the legend of Fig. 2.

Intracellular chloride activities and the effect of reducing extracellular chloride activity

A typical, simultaneous recording of membrane potential and intracellular Cl^- activity ($a\text{Cl}_i$) in a cell is shown in Fig. 7. Again, the lower trace denotes the membrane potential (-48 mV), and the upper trace records the $a\text{Cl}_i$ (30 mmol l^{-1}). Average data are given in Table 1, and the frequency distribution of the $a\text{Cl}_i$ values found is shown in Fig. 8. The histogram has a range of $29\text{--}66\text{ mmol l}^{-1}$, with all values but one falling between 29 and 53 mmol l^{-1} , and a mean value of 40 mmol l^{-1} (Fig. 8; Table 1). The calculated values of Cl^- equilibrium potentials (E_{Cl}) were in the range -18 to -39 mV , such that for every cell E_{Cl} was apparently more positive than the corresponding E_{M} . However, there is no clear relationship between E_{Cl} and E_{M} (Fig. 9).

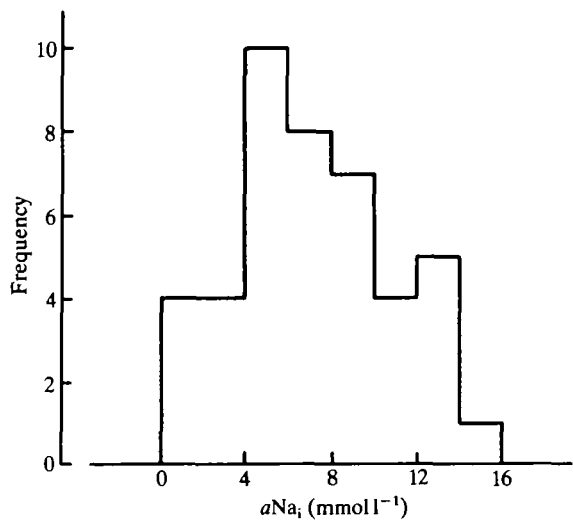


Fig. 6. Frequency histogram illustrating the distribution of intracellular Na⁺ activity ($a\text{Na}_i$) values, measured using a double-barrelled Na⁺-sensitive microelectrode.

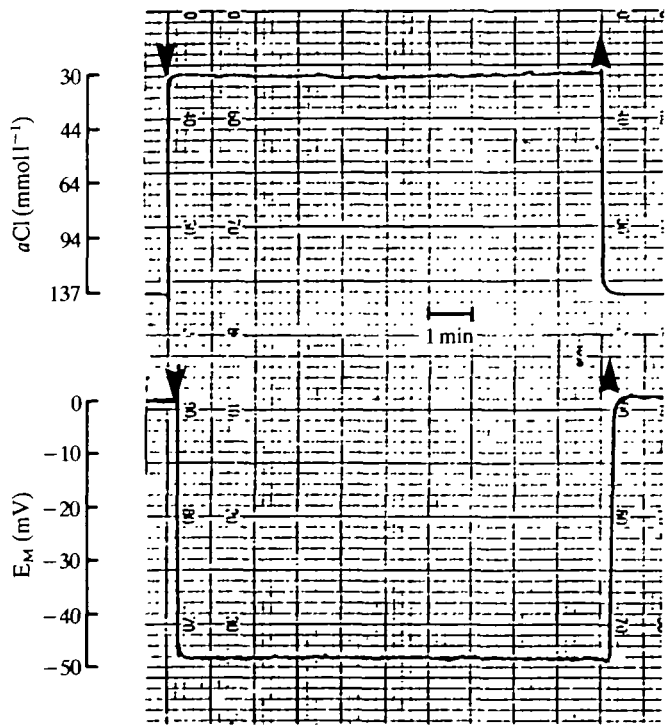


Fig. 7. Chart recording of the impalement of a *Calliphora* prepupal muscle cell with a double-barrelled Cl⁻-sensitive microelectrode with a RLIE-filled reference barrel. Details of symbols etc. are as in the legend of Fig. 2.

To test the possibility that Cl^- was free to move across the membrane, the effect of removing all external Cl^- on $a\text{Cl}_i$ and E_M was investigated (Fig. 10). For this cell, $a\text{Cl}_i$ was 58 mmol l^{-1} and E_M was -44 mV . When Cl^- -free Ringer was applied, E_M immediately hyperpolarized by about 10 mV , and then gradually recovered back to the original level over about 1 min . $a\text{Cl}_i$ decreased steadily, however, and reached a final value of 13 mmol l^{-1} , which was maintained. Upon washing with normal Ringer, E_M again hyperpolarized transiently, by about the

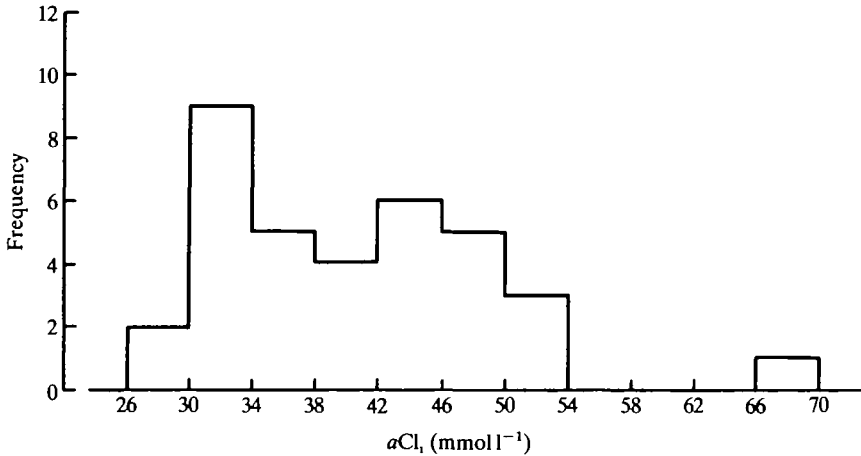


Fig. 8. Frequency histogram illustrating the distribution of intracellular Cl^- activity ($a\text{Cl}_i$) values, measured using double-barrelled Cl^- -sensitive microelectrodes.

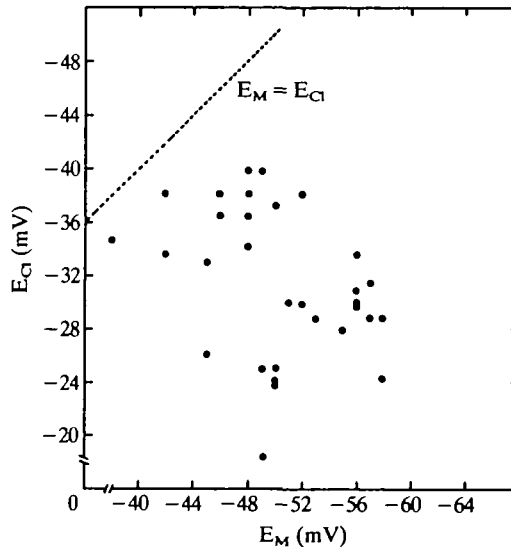


Fig. 9. Graph to illustrate the relationship between the Cl^- equilibrium potential (E_{Cl}) and resting membrane potential (E_M) in *Calliphora* prepupal muscle cell. The dashed line denotes the equality $E_{\text{Cl}} = E_M$, showing that for all cells E_{Cl} is consistently more positive than E_M and that there is no obvious relationship between E_{Cl} and E_M .

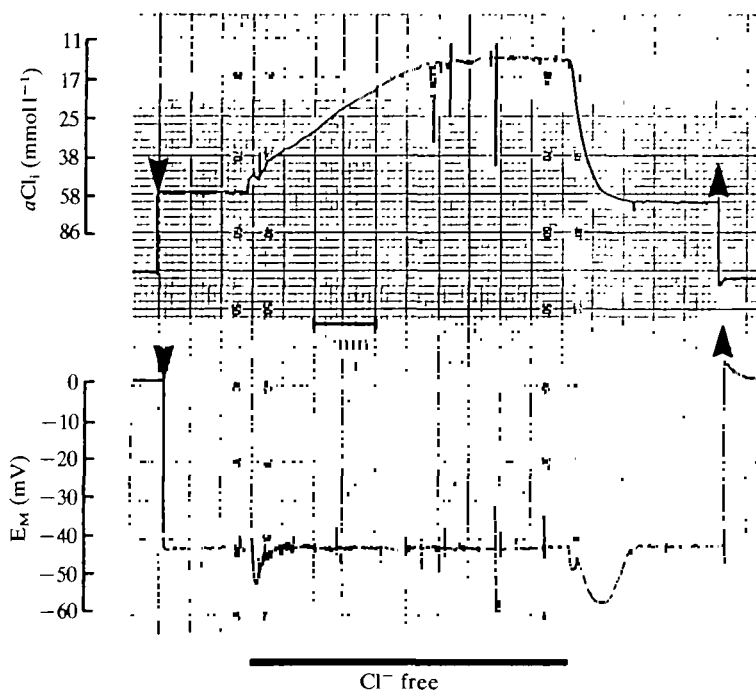


Fig. 10. Chart recording of a typical experiment demonstrating the effects of a Cl^- -free medium on E_M (resting membrane potential) and $a\text{Cl}_i$ (intracellular Cl^- activity). Gluconate salts were used as substitutes to remove all Cl^- from the bathing medium; the duration of application of the Cl^- -free medium is indicated by the thick horizontal bar. At both changes, E_M hyperpolarizes only transiently, but during sustained application, there is a gradual loss of intracellular Cl^- . Arrowheads, as in the legend of Fig. 2.

same magnitude; $a\text{Cl}_i$ returned to the original 'resting' value, but the time course of the cell's reloading with Cl^- was much faster than that of its unloading (Fig. 10). Similar results were obtained from seven cells from seven different preparations. On average, for this group of cells, E_M was -50 ± 3 mV and $a\text{Cl}_i$ was 43 ± 5 mmol l^{-1} , and changing external Cl^- concentration produced only transient E_M hyperpolarizations of 9–16 mV. In zero external Cl^- , the cells rapidly lost their intracellular Cl^- , leaving an apparent $a\text{Cl}_i$ of 10 ± 1 mmol l^{-1} . When the Cl^- -free Ringer was introduced with the ISM tip in the bath, the potential difference between the reference barrel of the ISM and the earth electrode did not change significantly. Thus, the changes in E_M observed could not be due to any change in junction potential(s).

Discussion

The intracellular K^+ activities ($a\text{K}_i$) of prepupal muscle cells of *Calliphora* have been measured using K^+ -SMs for the first time. The values obtained with K^+ -SMs

employing the ion-exchanger were found to be considerably higher than those obtained with K^+ -SMs based on the neutral ion-carrier. This probably reflects the presence of certain interfering organic compound(s), such as quarternary amines (Oehme & Simon, 1976). Thus, the average value of 115 mmol l^{-1} obtained using NIC-type K^+ -SMs is certainly a more realistic measurement in these cells.

Intracellular Na^+ concentrations of skeletal muscles of *Periplaneta*, *Locusta* and *Schistocerca*, measured by flame photometry, lie in the range $21\text{--}62 \text{ mmol l}^{-1}$ (Tobias, 1948a,b; Wood, 1963). Assuming that aNa_i values of these insect muscles are close to that of *Calliphora*, since all are bathed in haemolymphs with similar ionic contents, it follows that a substantial amount of bound Na^+ exists in the skeletal muscles of the insects studied earlier. However, X-ray microanalysis of *Calliphora* rectal muscles has given an overall intracellular Na^+ concentration of only 11 mmol l^{-1} (Gupta, Wall, Oschman & Hall, 1980). Thus, either *Calliphora* muscles have relatively low intracellular Na^+ concentrations compared with the other insects (i.e. the above assumption is not valid), or visceral and skeletal muscles of *Calliphora* are markedly different in their Na^+ contents. Further work is required on *Calliphora* to clarify this point.

Removing all external Cl^- clearly shows that (i) intracellular Cl^- is lost rapidly, and (ii) the resting membrane potential changes only transiently, i.e. Cl^- is free to be exchanged rapidly across the cell membrane in either direction and makes no net direct contribution to the generation of the membrane potential. Taken together, these results strongly suggest that the transmembrane distribution of Cl^- is in passive equilibrium with the resting potential, possibly in accordance with a Donnan system. In turn, this implies that aCl_i has been overestimated, probably due to the presence of an anionic interferent in prepupal cells (see Deisz & Lux, 1982). Since the cells on which Cl^- depletion experiments were carried out had an average E_M of -50 mV and aCl_i of 43 mmol l^{-1} , and Cl^- appears to be passively distributed, the true aCl_i would be expected to have an average value of 19 mmol l^{-1} , with the interfering ion(s) accounting for some 24 mmol l^{-1} 'apparent' aCl_i . The identity of the interferent(s) is not known, however.

The response of the cell membrane to lowered extracellular Cl^- level was somewhat unexpected. Instead of the transient *depolarization* predicted from a simple Donnan equilibrium, a *hyperpolarization* was observed. This hyperpolarization of the membrane may be attributable to a number of mechanisms. First, Cl^- may be involved in the regulation of intracellular pH, and a reduction in extracellular Cl^- might therefore change intracellular pH by affecting the Cl^-/HCO_3^- exchange mechanism (Thomas, 1977, 1984). Intracellular pH controls a wide spectrum of membrane functions (Moody, 1984) and a change might have an indirect effect on E_M (see Washio, 1971; Jan & Jan, 1976; Piek, Mantel & Wijsman, 1977). Second, lowering extracellular Cl^- level may indeed lead to a very small depolarization which may be sufficient under the prevailing experimental conditions to increase the permeability of the cell membrane to an ion such as K^+ , thus causing the E_M to hyperpolarize. This could occur *via* voltage-sensitive, self-inactivating K^+ channels such as those found in *Drosophila* flight muscle

(Salkoff & Wyman, 1983). Such channels would be activated immediately following the onset of a small depolarization and might result in the observed transient hyperpolarization of E_M . This seems unlikely, however, since these channels operate on a time scale of milliseconds, and there was no hint of even a slight depolarization when cells were first exposed to a Cl^- -free Ringer. Irrespective of the mechanism of this effect, Cl^- is clearly not involved in resting membrane electrogenesis in *Calliphora* muscles. A similar conclusion was reached by Jan & Jan (1976) in analogous muscles of prepupal *Drosophila*, since replacing all Cl^- in the bathing medium with sulphate was similarly found to affect the membrane potential only transiently by 10–20 mV. On returning to normal saline, 80–90 % of the resting potentials recovered within 10 min. Sulphate was taken to be an impermeant ion, since replacing the sucrose present in the saline with Tris(hydroxylaminomethane sulphate) had no effect on the membrane potential.

In conclusion, these observations taken together strongly suggest that Cl^- is passively distributed in Diptera skeletal muscles, and does not, therefore, directly generate any part of the resting membrane potential. This is very similar to the situation in Orthoptera skeletal muscles, which are also bathed in haemolymphs with 'conventional' ion contents (Usherwood, 1969; Lea & Usherwood, 1973). The intracellular Cl^- concentrations of *Locusta* skeletal muscles were originally measured, using a classical microtitration method, by Wood (1965). In freshly dissected muscles (bathed in a haemolymph Cl^- concentration of 94 mmol l^{-1}), the intracellular Cl^- concentration was 14 mmol l^{-1} . The intracellular Cl^- concentration measurements were repeated and the resting potentials also recorded after soaking the muscles in an artificial saline containing $150 \text{ mmol l}^{-1} \text{ Cl}^-$, giving values of 44 mmol l^{-1} and -56 mV , respectively (Wood, 1965). These results have two implications. (i) Cl^- is 'free' to enter the cells once the extracellular Cl^- concentration is raised. This is consistent with the results of Lea & Usherwood (1973). (ii) A substantial amount of 'bound' Cl^- exists in these muscles such that the E_{Cl} , calculated using a Cl^- concentration value from homogenized muscle, is apparently more positive than the resting potential (Wood, 1965; Piek, 1975). Further aspects of Cl^- in insect muscles have been discussed in considerable detail by Djamgoz (1987).

Assuming that the intracellular activity coefficient for K^+ is the same as the extracellular one (i.e. 0.72), the average value of aK_i found is equivalent to a K^+ concentration of 160 mmol l^{-1} , which is comparable to the value of 140 mmol l^{-1} reported in isolated retractor unguis muscles of *Schistocerca gregaria* (Leech, 1986). The value of aK_i in *Calliphora* corresponds to an average E_K of -62 mV , which is considerably more negative than the average resting potential of -47 mV . This, in turn, implies that K^+ is not the only ion contributing to resting membrane electrogenesis, and that at least one other ion with an equilibrium potential more positive than E_M must also be involved. This additional ion cannot be Cl^- , but it may be Na^+ . In the following paper (Djamgoz & Dawson, 1988), a quantitative model of resting membrane electrogenesis, based on partial contributions from K^+ and Na^+ , is formulated and its predictions are tested by a variety of experiments.

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