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SHORT COMMUNICATION

RESTING POTENTIAL AND POTASSIUM-SELECTIVE ELECTRODE MEASUREMENTS IN LOCUST SKELETAL MUSCLES

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The membrane of locust skeletal muscle fibres is permeable to both potassium and chloride ions in the resting state and has a potassium selectivity similar to that of a potassium electrode (Usherwood, 1967). The experiments described here were performed on the isolated *retractor unguis* muscle of the locust *Schistocerca gregaria*. The composition of the standard locust saline was similar to that of Hoyle (1953) except that in most experiments the HPO_4/HCO_3 buffer was replaced by Tris-maleate (pH 6·8). Conventional electrophysiological techniques were used and electrodes had tip potentials of less than 5 mV.

The relationship between membrane potential (E_M) and external potassium concentration ($[K]_o$) is accurately described by a simplified version of the Goldman–Hodgkin–Katz equation (Hodgkin & Horowicz, 1959):

$$E_M = \frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o}{[K]_i}, \quad (1)$$

where $\alpha = P_{Na}/P_K$ and the subscripts o and i refer to extra- and intracellular respectively.

Fig. 1 shows the effect of changing $[K]_o$ (by substitution for Na) on E_M. Muscles were soaked in different salines and the resting potential of a number of fibres measured in each solution. Muscles were bathed in each solution for at least 10 min before recording. Rectification did not affect these results. The data was fitted by equation 1 assuming $\alpha = 0{\cdot}016$ and $[K]_i = 130\,\text{mmol}\,l^{-1}$. Use of HPO_4/HCO_3 or Tris-maleate as the buffer did not affect membrane potential, unlike for cockroach muscle (Wareham, Duncan & Bowler, 1973).

In the experiments of Fig. 1, the $[K]_o \times [Cl]_o$ product changed, resulting in swelling or shrinkage of the fibres with an asymmetric time course (Usherwood, 1967). To avoid this complication, experiments were repeated in Cl-free (SO_4 substituted) salines (Fig. 2). These data were also fitted by equation 1, assuming $[K]_i = 140\,\text{mmol}\,l^{-1}$ and $\alpha = 0{\cdot}055$, for experiments at room temperature. There was

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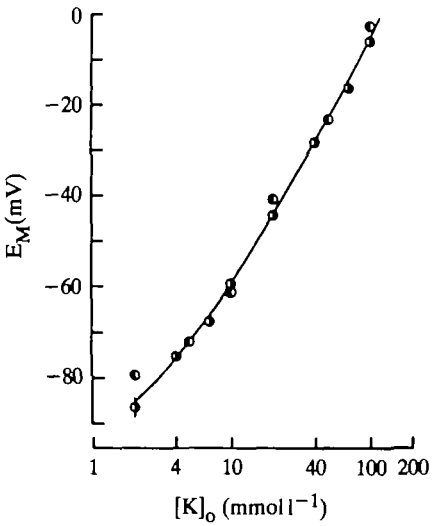

Fig. 1. The effect of changing $[K]_o$ on E_M at room temperature. Results from experiments in HPO_4/HCO_3 (◑) or Tris-maleate (◐) buffered salines are shown. Each point represents the mean (±S.E.M.) for at least 15 fibres. The line was drawn according to equation 1 assuming $[K]_i = 130$ mmol l^{-1} and $\alpha = 0{\cdot}016$. Normal saline was (in mmol l^{-1}) NaCl, 140; KCl, 10; $CaCl_2$, 2; $MgCl_2$, 2; Tris-maleate, 10 (pH 6·8).

a component of the resting potential which was sensitive to temperature, with E_M gradually falling by 10–20 mV on cooling from room temperature (approx. 20°C) to approx. 5°C. When $[K]_o$ is changed in Cl-free solutions at low temperature the data could be fitted (equation 1) assuming $[K]_i = 140$ mmol l^{-1} and $\alpha = 0{\cdot}104$. $[Na]_i$ was not measured but would be expected to rise on cooling. An increase in P_{Na}/P_K has also been noted in cockroach muscle at low temperature (Wareham, Duncan & Bowler, 1974).

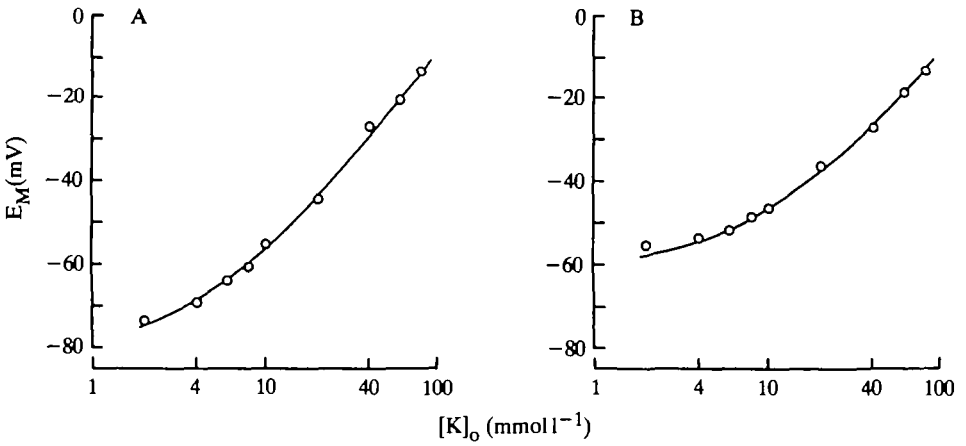

Fig. 2. The effect of changing $[K]_o$ on E_M in Cl-free salines. Sulphate was used to substitute for chloride in Tris-maleate buffered salines. (A) Experiments performed at room temperature, line drawn according to equation 1 with $[K]_i = 140$ mmol l^{-1} and $\alpha = 0{\cdot}055$ ($N = 20$). (B) Experiments at 5°C, line drawn according to equation 1 with $[K]_i = 140$ mmol l^{-1} and $\alpha = 0{\cdot}104$ ($N = 20$).

The values of $[K]_i$ estimated from these experiments are considerably higher than the value of 49 mmol l^{-1} measured by Piek (1975) in muscle homogenates using ion-selective sensors. In view of the indirect nature of these estimates, it was decided to measure $[K]_i$ using ion-selective intracellular electrodes. Single barrelled potassium electrodes were made using standard techniques (see Thomas, 1978) with Corning 477317 resin. Electrodes were calibrated in solutions where [K] was changed by substitution for Na at constant [K]+[Na]. Fig. 3 shows the distribution of values for $[K]_i$ measured in three different salines. No correction for interfering ions was made and ion activity has been taken to equal ion concentration. With these assumptions, mean values (±S.E.M.) for $[K]_i$ of $140{\cdot}7 \pm 3{\cdot}1$ mmol l^{-1} ($N = 40$), $139{\cdot}5 \pm 3{\cdot}1$ mmol l^{-1} ($N = 31$) and $138{\cdot}5 \pm 3{\cdot}4$ mmol l^{-1} ($N = 37$) were obtained under the different conditions (at room temperature). These values are not significantly different and are in close agreement with estimates from resting potential experiments. At low temperature (approx. 5 °C), $[K]_i$ was significantly lower at $111{\cdot}6 \pm 2{\cdot}1$ mmol l^{-1} ($N = 46$). This value is lower than that used to fit the curve of Fig. 2B.

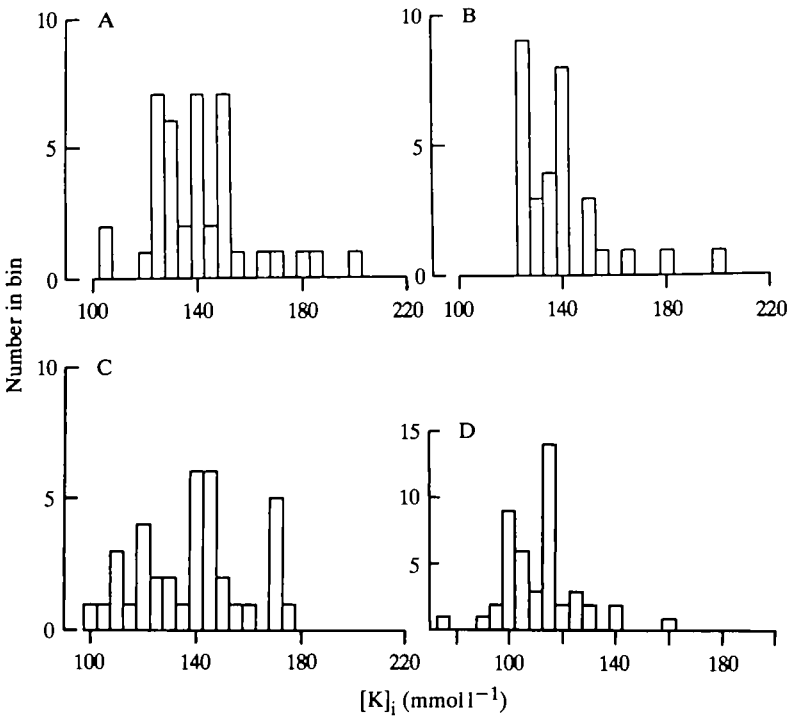

Fig. 3. Distribution of values of $[K]_i$ measured using K-selective electrodes. Values have been divided into 5 mmol l^{-1} bins. (A) Measurements at room temperature in normal saline (10 mmol l^{-1} [K], Cl). Mean (±S.E.M.) value $140{\cdot}7 \pm 3{\cdot}1$ mmol l^{-1} ($N = 40$). (B) Measurements at room temperature in 20 mmol l^{-1} [K], Cl saline. $[K]_i$= $139{\cdot}5 \pm 3{\cdot}1$ mmol l^{-1} ($N = 31$). (C) Measurements at room temperature in 10 mmol l^{-1} [K], Cl-free saline. $[K]_i = 138{\cdot}5 \pm 3{\cdot}4$ mmol l^{-1} ($N = 37$). (D) Measurements at low temperature (5 °C) in 10 mmol l^{-1}, [K], Cl-free saline. $[K]_i = 111{\cdot}6 \pm 2{\cdot}1$ mmol l^{-1} ($N = 46$). The three values measured at room temperature are not significantly different from each other while that at 5 °C is significantly lower ($P < 0{\cdot}001$).

At normal $[K]_o$ (10 mmol l^{-1}) the reduction in E_M on cooling is close to the fall in E_K calculated from ion-selective electrode measurements.

Thus the membrane potential of locust skeletal muscle is accurately described by a simplified version of the Goldman–Hodgkin–Katz equation and estimates of $[K]_i$ extrapolated from this were in close agreement with ion-selective electrode measurements indicating a value of about 140 mmol l^{-1}.

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