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RAPID COMMUNICATION

Participation of Electrogenic Na^+-K^+-ATPase in the Membrane Potential of Earthworm Body Wall Muscles

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Summary

The effect of Na^+-K^+-ATPase inhibitor ouabain on the resting membrane potential (V_m) was studied by glass microelectrodes in isolated somatic longitudinal muscles of the earthworm *Lumbricus terrestris* and compared with frog sartorius muscle. In earthworm muscle, V_m was –49 mV (inside negative) in a reference external solution with 4 mmol/l K^+. The electrogenic participation of Na^+-K^+-ATPase was absent in solutions with very low concentrations of 0.01 mmol/l K^+, higher in 4 and 8 mmol/l K^+ (4-5 mV) and maximal (13 mV) in solutions containing 12 mmol/l K^+ where V_m was –46 mV in the absence and –33 mV in the presence of $1x10^{-4}$ M ouabain. The electrogenic participation of Na^+-K^+-ATPase was much smaller in m. sartorius of the frog *Rana temporaria* bathed in 8 and 12 mmol/l K^+. The results indicate that the Na^+-K^+-ATPase is an important electrogenic factor in earthworm longitudinal muscle fibres and that its contribution to V_m depends directly on the concentration of K^+ in the bathing solution.

Key words
Na^+-K^+-ATPase • Resting membrane potential • Ouabain • Earthworm • Frog

Introduction

The resting membrane potential (V_m) in skeletal muscle fibre is the result of several integrative mechanisms (Siegenbeek van Heulekom *et al.* 1994): the electrochemical potential of electrogenic ions (Edwards 1982, Edwards and Vyskočil 1984), selective ionic permeability (Shabunova and Vyskočil 1982), pH (Volkov 1983), membrane ionic pumps such as Na^+-K^+-ATPase (Kernan 1962,Vyskočil *et al.* 1995) and the furosemide-sensitive Cl^- transporter (Volkov *et al.* 1987, Urazaev *et al.* 1998). V_m is also controlled by an osmotic

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state of the cell (Edwards 1982, Lang *et al.* 1995), hormones (Zemková *et al.* 1982) and by long-lasting transmitter release regulated by second messengers, nitric oxide (Urazaev *et al.* 1998, Mukhtarov *et al.* 1999, Nikolsky *et al.* 1999) and presynaptic autoreceptors (Bukharaeva *et al.* 1999). Ion pumps, Na^+-K^+-ATPase and Cl^- transporter in particular, have dual effects on the V_m of muscle fibres: they stabilize the transmembrane ion gradients and they are electrogenic *per se.* In vertebrates, the electrogenic component represents 3-30 % of the total V_m value (Martin and Levinson 1985). Despite the fact that the role of V_m for impulse transmission and muscle contraction is very important in invertebrates as well as in vertebrates (Chang 1969, Walker *et al.* 1993), the extent of pump electrogenicity in developmentally important phyllum *Annelidae* is not known. For this reason, we studied the V_m of earthworm somatic muscles and its dependence on a mild increase of potassium in the bathing solution under conditions of active and inhibited Na^+-K^+ pump and compared it with the resting electrogenic contribution of the pump in frog *Rana temporaria.*

Material and Methods

Experiments were performed on isolated neuromuscular preparations of the longitudinal somatic muscles of the earthworm *Lumbricus terrestris* (Drewes and Pax 1974) and m. sartorius of the frog *Rana temporaria* in winter period (February - March).

The earthworm Drewes-Pax solution contained (mM): NaCl 163; KCl 4; $CaCl_2$ 6; sucrose 167; Tris 2; pH 7.2-7.4. The frog Ringer solution contained (mM): NaCl 116; KCl 2.0; $CaCl_2$ 1.8; $NaHCO_3$ 1.0; $MgCl_2$ 2.0; pH 7.2-7.4. Strips of muscle, approximately 10 segments in length, were prepared from the body wall of the earthworm, from which the nerve cord and viscera had been removed. The electrogenic contribution of the Na^+-K^+ pump to V_m, which causes hyperolarization of muscle fibres, was statistically quantified by impaling, with glass microelectrodes (2.5 mol/l KCl, 15-20 MΩ) 20 or more fibres during a 5 min period before, and another 20 or more fibres 5-10 min after the addition of $1x10^{-4}$ mol/l ouabain (Sigma, USA) to the medium. The difference between the mean resting membrane potentials under these two conditions is generally considered to be due to electrogenic activity of the pump (Kernan 1962, Zemková *et al.* 1982).

Microcal Origin version 3.5 (Microcal Software, Inc. 1991-1994) was used for statistical analyses. Parametric analysis of variance (ANOVA) of the experimental groups versus the control group was made by multiple comparison using the Bonferroni t-test. Throughout the text, statistically significant differences between mean ± S.E.M. of two groups are indicated at the given level of probability.

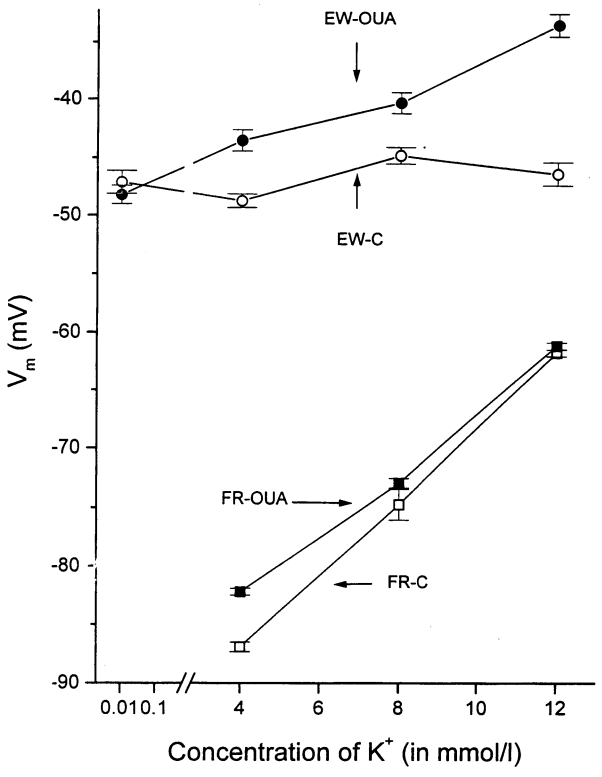

Fig. 1. *Resting membrane potential V_m in mV (ordinate) of earthworm longitudinal muscles (EW, circles) and frog sartorius muscles (FR, squares) before (controls, C) and after application of $1x10^{-4}$ mol/l ouabain (OUA) bathed in saline with different concentrations of potassium ions (abscissa).*

Results and Discussion

V_m of earthworm muscle fibres was –48.7±0.6 mV (inside negative, n=300, Fig. 1, open circles) in Drewes-Pax solution with a normal concentration of K^+ (4 mmol/l). This value of V_m is much smaller than the membrane potential in frog sartorius muscle, which was –82.2±0.3 mV (n=60) in Ringer solution with 4 mmol/l of K^+ (Fig. 1, solid squares). Even in solutions containing

a very low concentration of K^+ (0.01 mmol/l), V_m of earthworm muscle fibres was –47.1±1.0 mV (n=140) which does not differ significantly (P>0.05) from values in 4 mmol/l K^+ solutions.

One possibility is that the low value of V_m in earthworm muscles is due to substantial participation of resting permeability to other ions, in particular chlorides (Dulhunty 1978). Substitution of 90 mmol/ NO_3^- anion for a corresponding amount of Cl^-, however, did not significantly hyperpolarize the V_m of earthworm muscles which was –41.1±1.0 mV (n=80, P>0.05) one hour after this ion exchange.

The increase of K^+ concentration from 4 to 8 mmol/l depolarized the muscle fibres substantially in the frog; V_m of the sartorius muscle was –76.8±0.6 mV, (n=60) in 8 mmol/l K^+. However, this concentration of potassium caused only slight depolarization to –45.0±0.7 mV (n=220) in earthworm muscles. The increase of K^+ up to 12 mmol/l further depolarized the frog muscles; V_m was –61.3±0.6 mV, (n=60) in the sartorius muscle (Fig. 1, squares). Contrary to this, the 12 mmol/l K^+ solution did not depolarize the V_m in earthworm muscle fibres, which was –46.3 mV (P>0.05; Fig. 1, open circles). This evidently contradicts the expected depolarization which should develop according to the Goldman-Hodgkin-Katz equation describing the relationship between membrane potential and ion concentrations across the membrane (Hodgkin and Horowitz 1959). This absence of depolarization can be explained either by the small participation of K^+ permeability in V_m of earthworm muscles or by activation of electrogenic membrane Na^+-K^+-ATPase by higher extracellular potassium (Martin and Levinson 1985) which compensates the K^+-induced depolarization.

If the latter possibility is true, then the inhibition of Na^+-K^+-ATPase would remove the „compensatory" hyperpolarization and the K^+ dependence would be closer to Goldman-Hodgkin-Katz prediction.

The Na^+-K^+- ATPase was therefore inhibited by $1x10^{-4}$ M ouabain which was applied to the earthworm muscle bath. Measurements of V_m were performed in the time window between 5-10 min of the presence of ouabain, to minimize the late depolarization due to loss of ion gradients across the membrane expected after inhibition of the Na^+-K^+-pump. In the presence of ouabain, V_m became depolarized in solutions with increased extracellular K^+ (Fig. 1, solid circles) with a coefficient of 11.25 mV per 10 mmol/l change in the concentration of extracellular potassium ions. This is already a reasonable K^+ dependence, but still much smaller than in the frog, where it was 24 mV per 10 mmol/l K^+. In frog, in solutions with 8 and 12 mmol/l potassium, ouabain was much less potent and pump electrogenic contribution of about 5 mV was significant only in 4 mmol/l K^+ (Fig. 1, squares).

The experiments with ouabain provided us with two important pieces of information: First, the K^+ transmembrane gradients do participate in V_m of earthworm muscles, which are therefore no exceptions to the general rule. Secondly, the development of V_m depolarization by K^+ ions in muscles with inhibited Na^+-K^+-ATPase by ouabain demonstrates that the Na^+-K^+ pump is strongly electrogenic in resting muscle fibres and its absolute contribution to V_m rises with higher K^+ concentrations: it is 5-6 mV in 4 and 8 mmol/l K^+, and as much as 13 mV in the medium with 12 mmol/l K^+. This value is close to the maximum, theoretically calculated for electrogenic contribution of the Na^+-K^+ pump (Martin and Levinson 1985) and much higher than in frog muscle. Experiments are in progress with the aim to characterize further the role of Na^+-K^+-ATPase and to compare the properties of resting membrane potential components in the earthworm with other animal species.

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Reprint requests

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