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POTASSIUM:ATP RATIOS IN SMOOTH MUSCLE

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SUMMARY

K^+ and ATP contents were measured in the rat myometrium under different metabolic conditions. The linear correlation between ATP and K^+ contents, predicted by the association-induction model, did not always hold and alternative explanations have been suggested.

INTRODUCTION

That ATP is involved in the transport of Na^+ and K^+ by cells is well documented[1]. However, the mechanics of its regulatory function is disputed. On the basis of a membrane model of ion transport, hydrolysis of ATP by a membrane-bound ATPase provides the energy for extruding Na^+ and reaccumulating K^+ (ref. 2). However, on the basis of the "association-induction" hypothesis[3], selective accumulation of ions results from the association of ions at polarised sites on proteins, selectivity being determined by the associational energies of the interacting ions. Adsorption of small molecules at strategic locations could alter the binding of ions at many sites by altering the electron density throughout the protein matrix. ATP is thought to function by such an inductive effect[4]. On the basis of such a model, there should exist a quantitative relationship between the ATP concentration and the concentration of selectively accumulated ions like K^+ (refs 3 and 4). Using frog muscle, Ling[3] demonstrated such a relationship and argued that "each mole of ATP critically determined the selective accumulation of about 34 moles of K^+ ions". Although such a relationship appears to hold qualitatively in smooth muscle, there are no quantitative data. It has been suggested that measurements of ATP levels and smooth muscle K^+ content "under conditions of varying metabolic activity" would prove helpful in this regard[4].

MATERIALS AND METHODS

Tissue preparations

Female rats (Wistar strain) weighing 150–170 g were used in all experiments, after being pretreated with 50 µg 17β-estradiol, injected subcutaneously, three times

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a day for 2–3 days. After the animals had been killed by a blow on the head, their uterine horns were rapidly removed and dissected free of surrounding tissues. The endometrium was then removed to obtain myometrial pieces (primarily longitudinal muscle with some circular muscle attached). The tissues obtained thus were made Na^+-rich by incubating them at 4 °C for 18–20 h in glucose-free, K^+-free Krebs solution containing 140 mM Na^+. Such tissues reaccumulated K^+ and recovered spontaneous contractions on subsequent incubation in normal Krebs solution at 37 °C (140 mM Na^+, 4.6 mM K^+, 20 mM D-glucose). Fresh tissues were obtained by incubating myometrial pieces in normal Krebs solution for 45 min after dissection. Tissues were treated with iodoacetic acid and iodoacetamide in K^+-free Krebs for 30 min, and then allowed to recover in Krebs (4.6 mM K^+) containing either 20 mM D-glucose or 25 mM sodium pyruvate. Iodoacetic acid and iodoacetamide inhibit a variety of enzymes containing –SH groups, but selective inhibition of 3-phosphoglyceraldehyde dehydrogenase (and hence of glycolysis) can be obtained[5]. In that event pyruvate, but not glucose, can overcome the inhibition. Tissues were exposed to 2,4-dinitrophenol for a total period of 150 min. In the initial 30 min, the drug (2,4-dinitrophenol) was in a glucose-free, K^+-free medium, and subsequently in normal Krebs solution. The duration of exposure to K^+ was limited to 120 min, a steady state having been attained by that time.

Determination of ion contents

At the end of the experiment, tissues were rapidly removed, blotted and weighed. The tissues were dried in an oven at 105 °C for 36 h and the dried tissues digested with 35 % H_2O_2 and concentrated HNO_3 till a white residue was obtained. Blank test tubes were processed concurrently. The residues obtained were dissolved in de-ionised distilled water and the volumes made up to 25 ml. Ion contents were determined by flame photometry (only K^+ values are reported here). Further details were reported earlier[6].

Determination of ATP content of tissues

Tissues were rapidly removed at the end of experiment and plunged into hot glycine buffer (pH 10.5, 2 mM glycine) and heating continued for 10 min. The extraction by this method was virtually complete (98%). The ATP content of the tissues was then determined by using a firefly luciferase–luciferin method and a scintillation counting technique. A stock solution of ATP in 2 mM glycine buffer was prepared and frozen in small quantities. From this stock solution, ATP standards were prepared in glycine buffer about 1 h before assays were performed and stored in the cold till used. Luciferase-luciferin extract (Sigma) was obtained in vials and the enzyme solution made up in 0.1 mM arsenate buffer, pH 7.4, just before use. All solutions were kept on ice throughout the experiment. Further procedural details have been published earlier[7].

Solutions

All solutions were made using double-distilled water. The Krebs solution used had the following ionic compositions (mM): NaCl 116; $NaHCO_3$ 22; NaH_2PO_4 1.2; KCl 4.6; $CaCl_2$ 1.5; $MgSO_4$ 1.2. Substrates were weighed out separately and added in concentrations of 20 mM to the final solution. K^+-free Krebs had the ionic composition of the solution shown above with the sole omission of K^+. Pyruvate Krebs was made by dissolving 25 mM sodium pyruvate in a modified Krebs solution containing 91 mM NaCl. All metabolic inhibitors were weighed out daily and added directly to the final solution. Whenever any substances were omitted from the solution, isosmolarity was maintained by sucrose. All solutions were equilibrated with O_2–CO_2 (95:5, v/v). The pH of all solutions was between 7.3 and 7.5. The pH of pyruvate Krebs was 7.8 but was reduced to 7.4 after equilibration with O_2–CO_2 (95:5, v/v).

Statistical analysis

The variability of samples is expressed as mean ± S. E.

RESULTS AND DISCUSSION

The tabulated results show the K^+ and ATP contents in terms of µmoles/g wet weight of tissues; K^+: ATP ratios are shown in the last column. Where the ATP contents are fairly high (Groups 1,3,7), the K^+:ATP ratios lie between 46 and 54 which, within limits of error, are constant. However, such a relationship clearly breaks down when tissues treated with iodoacetic acid or 2,4-dinitrophenol are considered. Such tissues accumulated significant amounts of K^+ with very low contents of ATP. Although there was a variable degree of swelling, K^+ contents and water gains were not correlated (P.K. Rangachari, unpublished).

The linear relationship between ATP and K^+ contents predicted by the association–induction hypothesis does not seem to hold under the conditions tested. There is, however, a relationship between ATP content and K^+ accumulation, since iodoacetic acid-treated tissues (Group 4) having virtually no ATP do not accumulate K^+ either. The results thus seem to suggest that a small fraction of total cellular ATP (approximately 35%) is sufficient for K^+ accumulation. This, however, may represent a significantly larger fraction in a localised site, *viz.* near the membrane. Alternatively, the rate or efficiency of utilisation of ATP for ion transport may be increased under the conditions tested. Thus the affinity of membrane ATPase for

TABLE I

ATP AND K^+ CONTENTS OF RAT MYOMETRIUM

All tissues were equilibrated with O_2–CO_2 (95:5, v/v).

Group	*Treatment*	*ATP* (*µmoles/g wet wt*)*	*K⁺* (*µmoles/g wet wt*)*	*K⁺:ATP***
1	Fresh tissues	0.94 ± 0.04 (7)	47.2 ± 2.8 (6)	50
2	Na⁺-rich (from the cold)	0.64 ± 0.06 (4)	1.6 ± 0.08 (6)	2
3	Na⁺-rich, recovering in normal Krebs	0.77 ± 0.15 (8)	41.7 ± 3.9 (6)	54
4	0.5 mM iodoacetic acid + glucose	nil (8)	3.6 ± 0.3 (6)	—
5	0.5 mM iodoacetic acid + pyruvate	0.21 ± 0.03 (8)	29.7 ± 2.5 (6)	141
6	1 mM 2,4-dinitrophenol	0.19 ± 0.02 (9)	25.1 ± 2.2 (9)	132
7	0.1 mM iodoacetamide + glucose	0.70 ± 0.15 (8)	32.2 ± 3.5 (6)	46

* Results are mean values ± S.E. Numbers in parentheses represent numbers of pieces analysed.

** Ratios calculated using mean values.

ATP may be increased so that a small amount of ATP is preferentially utilised for K^+ accumulation. While it is easy to propose a mechanism whereby a substantially lower level of ATP could function to pump Na^+ and accumulate K^+, it is more difficult to reconcile this finding with the association–induction hypothesis for it becomes necessary to argue that 65 % of the original ATP was unrelated to determination of the selectivity of proteins for ions.

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