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Effects of anisosmotic buffers on K^+ transport in isolated chicken enterocytes

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Cells isolated by hyaluronidase incubation from chicken small intestine were used to study the effects of anisosmotic buffers on K^+ transport. Hypo-osmolarity (200 mosmol · l^{-1}) reduced both the ouabain-sensitive and the ouabain-resistant, but bumetanide-sensitive, net K^+ influx and increased the K^+ efflux. The hypo-osmolarity induced K^+ efflux was prevented by quinine and unaffected by bumetanide. These results suggest that Ca^{2+}-activated K^+ channels may be involved in regulatory volume decrease in chicken enterocytes. Hyperosmotic conditions (400 mosmol · l^{-1}) increased the portion of net K^+ influx mediated by the Na^+/K^+-ATPase and that mediated by the bumetanide-sensitive K^+ transport system, and decreased the K^+ efflux.

Many cells are known to regulate their volume in anisosmotic media. It is now widely accepted that the volume regulation in most cells is achieved via dynamic and controlled changes of ion transport pathways (see Refs. 1–5 for reviews). The regulatory volume increase that occurs after hyperosmotic shrinkage of the cells appears to involve the entry of NaCl by activation of electroneutral cotransport systems, including either Na^+/Cl^- or $Na^+/Cl^-/K^+$ cotransport systems or Na^+/H^+ and Cl^-/HCO_3^- exchange systems. On the other hand, the regulatory volume decrease following hypo-osmotic swelling of the cells has been reported to be associated with a loss of cellular KCl in all vertebrate cell types investigated. The mechanisms involved in the KCl loss may include either separate K^+ and Cl^- conductances or electroneutral cotransport pathways such as the K^+/Cl^- cotransport system or a K^+/H^+ exchange coupled to a Cl^-/HCO_3^- exchange.

Using radioisotope flux studies we have previously demonstrated [6] in chicken enterocytes: (i) the presence of a K^+ permeability route activated by intracellular Ca^{2+} and (ii) that a small proportion of the ouabain-resistant net K^+ influx is mediated by a mechanism that shares many characteristics with the $Na^+/Cl^-/K^+$ cotransport system described in other cells [7,8], such as its sensitivity to loop-diuretics and to Na^+ or Cl^- removal from the bathing solutions.

The aim of the present work was to investigate the effects of anisosmotic media on the K^+ transport pathways present in chicken enterocytes.

4–6 week-old Hubbard chickens were used in the current study. Intestinal cells were isolated by hyaluronidase incubation following the method described by Kimmich [9]. After isolation the cells were washed twice with standard buffer of the following composition (mM); NaCl, 80; $CaCl_2$, 1; mannitol, 100; K_2HPO_4, 3; $MgCl_2$, 1; Tris-HCl (pH = 7.4), 20; 1 mg/ml bovine serum albumin, and resuspended in standard buffer.

A previous work has shown [6] that the maximum effect of ouabain on K^+ ($^{86}Rb^+$) uptake in chicken enterocytes is reached with 1 mM of the drug. This concentration was therefore chosen in the present experiments. As reported earlier the Na^+/K^+-ATPase is the major pathway for net K^+ uptake in chicken enterocytes exposed to isosmotic buffer (Fig. 1). Exposure of the cells to hypo-osmotic media (200 mosmol · l^{-1}) significantly reduced total net K^+ influx after 15 min incubation. Partition of the K^+ ($^{86}Rb^+$) net influx into ouabain-sensitive and loop-diuretic sensitive components showed that hypo-osmolarity decreased both, the ouabain-sensitive portion of the K^+ uptake (Na^+/K^+-ATPase) and the ouabain-resistant, bumetanide-sensitive influx pathway, and increased that component of K^+ net influx that is ouabain and bumetanide-resistant (residual influx).

On the other hand, hyperosmotic (400 mosmol · l^{-1}) conditions (Fig. 2) slightly increased total K^+ net uptake and also modified the contribution of the different

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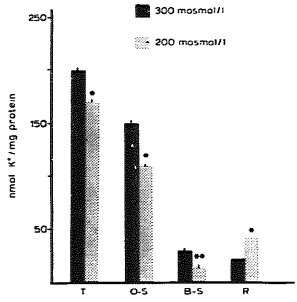

Fig. 1. Effect of hypo-osmolarity on K^+ influx (15 min flux measurement) by isolated chicken enterocytes. K^+ uptake was measured at 37 °C using cells at a final concentration of 15–25 mg cell protein/ml. Incubations were started by adding 1 ml of cell suspension to 4 ml of buffer (standard or hypo-osmotic medium), containing 0.25 μCi/ml ^{86}Rb as a tracer for K^+ and 0.1 μCi/ml ^{14}C-PEG 4000 as an extracellular space marker. The standard incubation medium (300 mosmol·l^{-1}) had the composition indicated within the test. Hypo-osmolarity (200 mosm·l^{-1}) was obtained by removing the mannitol from the standard buffer. Uptake was terminated by diluting 200 μl cell suspension in 500 μl ice-cold buffer and the cells separated by centrifugation (10000× g, 20 s) through a 250 μl layer of the oil mixture di-*n*-butyl phthalate/dinonyl phthalate (3:2, v/v). The cell pellets were lysed in 1 ml distilled water, and the content of ^{86}Rb in the incubation media and solubilized pellets was estimated by measuring its Cerenkov radiation. The amount of ^{86} Rb uptake was calculated taking into account the trapped extracellular volume estimated from the amount of ^{14}C-PEG 4000 associated with the pellet. The protein content of the pellet was determined by the Lowry assay [14]. All the modifiers were present in the incubation buffer from the start of the incubation period. The modifiers had the following final concentration: ouabain 1 mM, bumetanide 0.1 mM. The ouabain-sensitive component (O-S) was calculated by subtracting in each experiment the ouabain-resistant portion from the total ^{86}Rb uptake (B-S) is the difference in the uptake of K^+ in the presence and absence of bumetanide. Residual flux (R) is that portion of K^+ uptake obtained in the presence of ouabain and bumetanide. Data are the mean ± S.E. of five determinations. * $P < 0.001$; ** $P < 0.025$ for the differences from control (300 mosmol·l^{-1}), as judged by unpaired *t*-test.

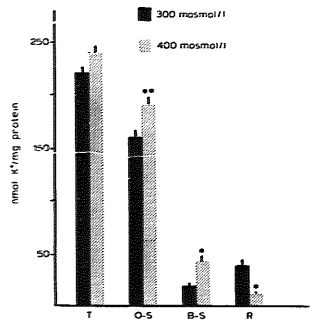

Fig. 2. Effect of hyperosmotic media on K^+ influx (15 min flux measurement) by isolated chicken enterocytes. Hyperosmolarity (400 mosmol·l^{-1}) was obtained by adding 100 mM mannitol to the standard buffer. Other details as in Fig. 1. Data are the mean ± S.E. of five determinations. * $P < 0.001$; ** $P < 0.010$ for the differences from control (300 mosmol·l^{-1}, as judged by unpaired *t*-test).

transfer routes to the total net K^+ influx. Thus, as compared to isosmotic media, hyperosmotic media stimulated both the ouabain-sensitive and the bumetanide-sensitive $^{86}Rb^+$ influx, and significantly reduced the residual net K^+ influx.

We have previously shown [6] that the efflux of K^+ from preloaded (^{86}Rb) chicken enterocytes is inhibited by Ba^{2+} and quinine, stimulated by A23187 and, as now shown in Fig. 3 is not affected by bumetanide. These findings suggest that the efflux of K^+ from chicken enterocytes may be mediated by Ca^{2+}-activated K^+ channels. The effects of anisosmotic media on K^+ efflux are summarized in Figs. 3 and 4. As can be

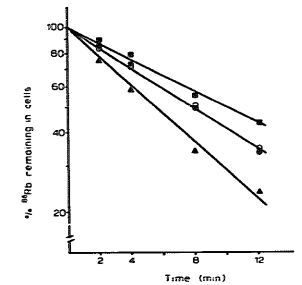

Fig. 3. K^+ efflux from preloaded (^{86}Rb) chicken enterocytes. Cell suspension (40–60 mg cell protein/ml) was first preloaded by incubation at 37 °C in a shaking water bath for 30 min in the presence of ^{86}Rb (12–14 μCi/ml). The cells were then washed twice in radioisotope-free, ice-cold buffer and resuspended in buffer to a final concentration of 20–30 mg cell protein/ml. The rate of ^{86}Rb loss from the cells was then measured by diluting 0.5 ml of the preloaded cell suspension into 4.5 ml of radioisotope-free buffer (iso-, hyper- or hypo-osmotic buffer) kept at 37 °C. Aliquots (200 μl) were taken at 2, 4, 8 and 12 min and the ^{86}Rb in the cell pellet was estimated as indicated above. K^+ efflux was calculated as percent of ^{86}Rb remaining in the cells and expressed as an apparent efflux rate coefficient. Throughout this paper this efflux rate is referred to as 'K^+ efflux' and has the units of min^{-1}. The percentage of ^{86}Rb content remaining in the cells is plotted on a logarithmic scale against time. 100% is the initial radioactivity present in the samples taken at 0 min. Results are means of five experiments. The experiments were performed in isotonic media in the presence (○) and absence (●) of 0.1 mM bumetanide, in hypo-osmotic buffer (▲) or in hyperosmotic buffer (■).

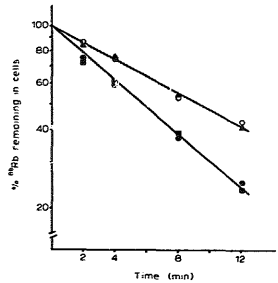

Fig. 4. Effects of quinine and bumetanide on the hypo-osmolarity-dependent increase in K^+ efflux. The experiment was performed as that in Fig. 3 but in isosmotic (open symbols) or hypo-osmotic media (filled symbols) and in the presence of either 125 μM quinine (▲) or 0.1 mM bumetanide (■). Results are means of five experiments. Other details as in Fig. 3.

observed hypo-osmotic conditions raised the rate constant of K^+ efflux from 0.089 ± 0.007 to 0.110 ± 0.008/min ($P < 0.001$ by unpaired t-test). On the other hand hyperosmotic conditions (Fig. 3) reduced the rate constant of efflux from its base level of 0.089/min to a new rate of 0.069 ± 0.008/min ($P < 0.050$ by unpaired t-test). The increase in K^+ efflux induced by hypo-osmotic conditions was prevented by quinine and it was not modified by bumetanide (Fig. 4), suggesting that, as in other cell types [10–12], Ca^{2+}-activated K^+ channels may be involved in the regulatory volume decrease in chicken enterocytes.

Taken together these findings indicate that the separate transfer routes for K^+ transport present in the plasma-membrane of chicken enterocytes, are significantly affected by changes in the tonicity of the external media.

Some of the present findings agree with previous reports showing that (i) hypo-osmolarity may result in an increased K^+ (^{86}Rb) efflux through Ca^{2+}-activated K^+ channels [10–12] and (ii) that hyperosmotic solutions lead to a stimulation of K^+ influx via a bumetanide-sensitive K^+ transport system [5] and to a loss of K^+ conductance [14]. However, in addition to these changes, the loop-diuretic-inhibitable K^+ route was inhibited by hypo-osmolarity.

Gagnon et al. [13] reported that the proximal convolute tubule appears to utilize the Na^+/K^+ pump for regulatory volume control. The present results also shown that the ouabain-sensitive K^+ uptake is modified by changes in the osmolarity of the external media. However, the observed changes could also result either from initial variations in the intracellular Na^+ concentration (thus hypo-osmotic media, by increasing cell volume, will decrease cell Na^+ concentration and hence the activity of the Na^+ pump. The opposite will be true in hyperosmotic media) or from variations in the cell Na^+ concentration secondary to changes in Na^+ uptake via the $Na^+/Cl^-/K^+$ cotransport system.

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