DETERMINANTS OF REGULATORY VOLUME DECREASE IN RECTAL GLAND CELLS OF SQUALUS ACANTHIAS

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Anisotonic cell volume regulation requires a change in cell solute content, often resulting from adjustments in membrane transport rates. These phenomena are cell- and solute-specific (Kleinzeller and Ziyadeh, Comp. Physiol. 4:59, 1990). For instance, in shark rectal gland cells (RGC), regulatory volume increase is virtually absent in vitro because of insufficient solute gain in hypertonic saline (Ziyadeh and McCallum, Bull. MDIBL 29:73, 1990). However, in hypotonic saline there is substantial regulatory volume decrease (RVD), despite constancy of K^+ content and ^{86}Rb fluxes, largely due to marked acceleration of osmolyte efflux, e.g. taurine (Ziyadeh et al., Bull. MDIBL 29:68, 1990) and betaine (Ziyadeh, Bull. MDIBL 29:70, 1990). These observations are here expanded to account for the fate of other solutes upon hypotonic challenge. In particular, we examine taurine uptake and possible mechanisms for enhanced taurine efflux during RVD. In addition to tissue slices, we used freshly prepared tubule suspensions obtained from collagenase-treated slices as first reported by us (Feldman et al. Am. J. Physiol. 257:C377, 1989).

Medium osmolarity was reduced from 920 to 600 mosM by lowering Na^+ to 130 mM, Cl^- to 135 mM and keeping urea 350 mM. Transferring tissue slices to this medium increased cell water to a peak level, 75% above control values by 10 min. While this osmometric response was close to the predicted behavior of an ideal osmometer, two additional events occurred concomitantly, with roughly opposite consequences on cell water changes : with dilution of cellular contents, net uptake of the permeant urea likely occurred, tending to increase the swelling phase; however, due to reduced NaCl concentration in the medium, intracellular Na^+ and Cl^- content were reduced which minimized swelling. Cell Na^+ content was decreased from 160 ± 9 to 64 ± 5 mmol/kg dry wt, and Cl^- from 239 ± 9 to 141 ± 7 by 10 min (n=4). Subsequently, and up to 5 h in hypotonic medium, while cell water decreased to 20% above control values, there was no further reduction in cell Na^+ and Cl^- content. Moreover, throughout hypotonic exposure, cell K^+ remained constant (320 ± 7 mmol/kg dry wt at 5 h). Thus the RVD phase for RGC is due to loss of osmolytes other than Na^+, K^+ and Cl^-. Of note, the cell membrane potential at 5 h in hypotonic saline (85.9 ± 1.4 mV, n=4) was similar to that in isotonic saline (83.4 ± 1.3 mV). Voltage was determined from the steady-state distribution of the lipophilic cation, triphenylmethyl phosphonium, as described (Kleinzeller and J. Goldstein, J. Comp. Physiol. B154:561, 1984).

The abundant trimethyl-N-oxide (TMAO) in RGC (70 mM), while in apparent equilibrium with blood levels, is an effective osmolyte since cell levels remain unchanged when tissues are incubated in TMAO-free isotonic Ringer (Kleinzeller, J. Exp. Zool. 236:11, 1985). Thus we tested whether TMAO-free hypotonic Ringer can stimulate TMAO efflux, as a component of the RVD. To achieve effective ^{14}C-TMAO loading prior to efflux studies, and owing to the very slow uptake in isotonic saline (Ziyadeh et al., Bull. MDIBL 27:44, 1987), slices were first depleted of TMAO in KCl-Ringer for 90 min (Kleinzeller, loc. cit.), followed by ^{14}C-TMAO loading for 4 h in isotonic NaCl-Ringer. Efflux was then examined; under isotonic conditions, ^{14}C-TMAO efflux was quite slow (slow rate constant

$k' = 0.00194\ min^{-1}$) consistent with osmolyte behavior. However, in hypotonic medium, efflux was accelerated ≈ 10-fold ($k' = 0.0178\ min^{-1}$). Thus TMAO loss contributes to the RVD.

Loss of RGC taurine in hypotonic saline may partly be due to decreased influx, in addition to the accelerated efflux: ^{14}C-taurine uptake at 3 h was reduced by 55 ± 4% (n=5). The following studies revealed that this decrement is a consequence of two phenomena: hypotonicity per se as well as the reduction in Na^+-dependent taurine uptake due to reduced external Na^+. Thus when the hypotonic medium was made isotonic by choline Cl or LiCl supplementation, uptake was reduced, but by only 35 ± 5%, contributed by the lowering of medium Na^+ (Ziyadeh et al., Biochim. Biophys. Acta 943:43, 1988). On the other hand, when the standard saline was made hypotonic by omitting urea (keeping Na^+ concentration equal to that of isotonic saline), uptake was also decreased (by 30 ± 6%) due to an effect attributed to hypotonicity per se.

The mechanism of enhanced taurine efflux remains to be defined. In skate erythrocytes, hypotonicity stimulates phospholipase D activity, resulting in release of the protein kinase C activator, diacylglycerol (Musch and Goldstein, J. Biol. Chem. 265:13055, 1990). We found no effect on efflux in isotonic medium by treatment with phorbol 12-myristate 13-acetate (10^{-6} M), Ca^{++}-ionophore A23187 (10^{-5} M), dibutyryl-cAMP (0.4 mM) or by the omission of Ca^{++} from the medium (plus 0.2 mM EGTA). None of these agents altered taurine efflux from RGC in hypotonic media. Thus, the signal-transduction pathway involved in activation of taurine efflux remains to be elucidated. Our previous structure-function correlates implicated a role for the cytoskeleton (particularly fibers or molecules in close association with the cytoplasmic face of the membrane) in maintaining the low permeability of the membrane to taurine (Ziyadeh et al., Bull MDIBL 29:68, 1990). Disruption of F-actin fibers, or other cytoskeletal elements, independent of cell swelling, may trigger an increase in membrane permeability allowing taurine to extrude from the cell. This increase in permeability appears to be selective or specific since other solutes, e.g. K^+, are not affected.

We could not confirm the major reduction in taurine efflux from RGC by the stilbene anion-exchange inhibitors (Goldstein et al., J. Exp. Zool. 254:114, 1990). Thus, ^{14}C-taurine efflux in hypotonic media was reduced by only 8 ± 3% at 2h in tissue slices (n=3) and by only 12 ± 4% at 30 min in tubule suspensions (n=3) treated with either 0.5 mM DIDS or 0.2 mM DNDS. These agents did not significantly alter cell volume and the RVD response. In skate hepatocytes, it was reported that 0.5 mM DIDS partially inhibited taurine efflux in hypotonic medium (Ballatori et al., Bull. MDIBL 29:71, 1990). Other studies indicated that the anionic form of taurine is not the predominantly transported moiety during efflux: the addition of 5mM NH_4Cl to the hypotonic medium, which should alkalinize the cell and increase taurine dissociation, did not stimulate the efflux; conversely, the addition of 10 mM propionate to the hypotonic medium, which should acidify the cell and decrease taurine dissociation, did not inhibit the efflux. Thus, non-dissociated taurine is the predominant moiety extruded from RGC.

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