ABSENCE OF SIGNIFICANT REGULATORY VOLUME INCREASE IN RECTAL GLAND CELLS OF SHARK (SQUALUS ACANTHIAS)

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We examined the response of shark rectal gland cells (RGC) to hypertonicity and whether these cells were capable of regulatory volume increase (RVI) in vitro. The localization of Na-K-2Cl carrier in the basolateral membrane of RGC is a potential pathway for increased NaCl influx in vivo and hence RVI. Long-term adaptation to hypertonicity may also involve the uptake of organic osmolytes. The methods for work with tissue slices were as described (Kleinzeller & J. Goldstein, J. Comp. Physiol. 154:561, 1984). Media osmolality was increased from 920 up to 1300 mosM by adding mannitol or increasing NaCl concentration; in some experiments Na was replaced by N-methyl-glucamine (NMG), and Cl by gluconate or sulfate. Cell H_2O (H_2O_i) and electrolyte concentrations and content (means ± SE, n=4) were corrected for solute and H_2O content of the extracellular (^{3}H-polyethylene glycol) space. Measurements were at 5-15 min intervals during 5h.

By 10 min of incubation in the various hypertonic media, H_2O_i decreased in direct proportion to medium tonicity, close to values predicted by the behavior of an ideal osmometer; e.g. H_2O_i was 73 ± 4% of control in 1300 mosM. At 30 min, an apparently anomalous response was observed in that cells shrank further, to 64 ± 6% of control; following H_2O efflux, the outward transmembrane urea gradient resulted in urea efflux and hence further H_2O loss. The relatively slow equilibration of urea (30 min), vs H_2O (10 min), is consistent with the kinetics of ^{14}C-urea and ^3H_2O fluxes in RGC (Kleinzeller & J. Goldstein, loc. cit.). In contrast, the efflux in hypertonic medium of the relatively impermeant taurine remained unchanged.

RGC incubated up to 5 h in hypertonic media (mannitol, Na gluconate, Na sulfate or NMG-Cl) did not exhibit any RVI. This was because the shrinkage-induced increase in intracellular electrolyte concentrations altered the combined chemical potential of Na, K and Cl, thus favoring net efflux rather than influx of solute via the Na-K-2Cl carrier. However, when RGC were bathed in hypertonic NaCl medium (1300 mosM), they exhibited a small RVI : steady-state H_2O_i increased to 73 ± 3% of control. This was associated with a relatively small but significant increase in Na content of the intracellular compartment (from 120 ± 9 mEq/kg dry weight to 375 ± 18) and Cl content (from 251 ± 14 to 510 ± 22) while cell K content decreased from 375 ± 14 to 239 ± 17. Since fluxes via the Na-K-2Cl cotransporter are sensitive to small changes in medium K we increased the latter from 6 to 18 mM in the hypertonic NaCl medium. Here, H_2O_i increased further but only to 77 ± 5% of control. Bumetanide (0.2 mM) abolished the RVI-related electrolyte uptake (H_2O_i was 69 ± 6% of control). Thus, the small degree of RVI in RGC is mostly due to electrolyte uptake via the Na-K-2Cl carrier.

In contrast to cAMP-stimulation of RVI in the medullary thick ascending limb of mouse nephron (Hebert, Am. J. Physiol. 250:C907, 1986), the addition of 1mM dibutyryl-cAMP plus isobutylmethylxanthine to the hypertonic NaCl medium (+18 mM K) was not associated with significant changes in RGC volume (H_2O_i remained at 78 ± 6% of control). Thus, while addition of cAMP and/or increasing media tonicity bring about an increased density of Na-K-2Cl cotransporters in RGC, as measured by labeled benzmetanide membrane binding (Lytle et al., this Bulletin), these maneuvers do not significantly increase net electrolyte uptake or induce RVI.

RVI was also absent when cells were transferred to standard isotonic medium following pre-equilibration in hypotonic medium (600 mosM, low NaCl). RGC exhibited regulatory volume decrease (RVD) in response to hypotonic stress, mostly due to efflux of taurine and possibly other organic osmolytes (Ziyadeh et al., this Bulletin). At 10 min following transfer to isotonic medium, an overshoot in cell shrinkage was seen (H_2O_i decreased from 125 ± 7% to 87 ± 4% of control) reflecting prior osmolyte loss. Upon prolonged incubation in isotonic medium, cell volume remained unchanged (absence of RVI post-RVD). Supplementation of the medium with 70 mM TMAO, 4 mM betaine and 2 mM taurine (final osmolarity = 920 mosM) was associated with only slight RVI (H_2O_i was 90 ± 3% of control at 5 h) reflecting the slow uptake of these osmolytes.

In summary, short-term adaptation of RGC to hypertonic stress in vitro is characterized by little, if any, RVI.

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