ROLE OF TAURINE AND THE CYTOSKELETON IN REGULATORY VOLUME DECREASE IN RECTAL GLAND CELLS OF SHARK (SQUALUS ACANTHIAS)

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In hypotonic media, cells rapidly gain water and increase their volume. In response to continued hypotonic stress, most (but not all) cells are also capable of gradually decreasing their volume close to control values. This regulatory volume decrease (RVD) is mediated by a net loss of intracellular solute content, which then results in water loss and restoration of cell volume. Typically, RVD involves the loss of cell K (and Cl) via K-Cl symport, separate K and Cl conductive channels and/or the parallel operation of K-H and Cl-HCO_3 antiports (Eveloff & Warnock, Am. J. Physiol. 247:C495, 1984). Alternatively (or additionally), in cells with a high content of organic osmolytes (e.g. elasmobranchs), extrusion of these molecules may also contribute to the RVD (Leite & Goldstein, J. Exp. Zool. 242:95, 1987).

The response of shark rectal gland cells (RGC) in media made hypotonic by reducing the NaCl concentration remains unexplored. Previous studies were confined to the special instance of lowering the tonicity by omitting urea form the incubating medium; here the magnitude of RGC swelling was less than predicted from the behavior of a prefect osmometer, a consequence of enhanced urea efflux (Kleinzeller & J. Goldstein, J. Comp. Physiol. 154:561, 1984). The process was also characterized by a net gain, rather than loss, of K content of RGC.

In this study, we first demonstrated that RGC were capable of RVD upon exposure to hypotonic low-NaCl medium. To elucidate the underlying mechanism(s) (K loss vs organic osmolyte loss), we measured the cellular fluxes of K (or ^{86}Rb) and of taurine, a major intracellular osmolyte (50 mM) in this tissue. The methods for work with tissue slices were as previously described (Kleinzeller & J. Goldstein, loc. cit.; Ziyadeh et al., Biochim. Biophys. Acta 943:43, 1988). Media osmolality was reduced from 920 to 600 mosM by decreasing NaCl concentration or by omitting urea. Cell water (H_2O_i) and electrolyte concentrations/content were corrected for solute and water content of the extracellular (^3H-polyethylene glycol) space. All values are for means ± SE, n = 4-5.

Upon incubation in the hypotonic low-NaCl media, peak swelling occurred within 10 min, and was inversely proportional to medium tonicity. Peak H_2O_i values were close to those predicted by the behavior of an ideal osmometer. In 600 mosM, H_2O_i was 170 ± 9% of control at 10 min. With prolonged exposure to hypotonic medium, H_2O_i decreased slowly, exhibiting a gradual RVD. By 4-5 h, H_2O_i stabilized at 120 ± 11% of control. However, and in contrast to the K loss associated with RVD in many other cell types, RGC ^{86}Rb fluxes and net K content were not different from controls. At 5 h, intracellular K content was 341 ± 9 and 331 ± 12 mEq/kg dry weight in the hypotonic and the isotonic medium, respectively. The profiles of ^{86}Rb efflux were similar in the two media; by 1 h, 85% and 82% of the label was washed out from ^{86}Rb-loaded RGC in the hypotonic and the isotonic medium, respectively. Similarly, ^{86}Rb uptake was not significantly altered. During the swelling phase, 'zero-time' uptake was 1.55 ± 0.16 μEq/h · kg dry weight in the hypotonic medium (vs 1.98 ± 0.16 in isotonic medium). During the RVD phase (e.g. at 2 h), the uptake was 1.81 ± 0.16 in the hypotonic medium (vs 1.76 ± 0.17 in isotonic medium).

In contrast to K fluxes, taurine uptake and efflux were significantly perturbed during RVD. ^{14}C-Taurine uptake was decreased in hypotonic media: 50 ± 4% and 36 ± 3% inhibition after 3h incubation in the low-NaCl and the urea-free hypotonic media, respectively. While a reduction in the Na concentration of the low-NaCl medium contributed to the inhibition of the Na-dependent taurine uptake system in RGC (cf. Ziyadeh et al., loc. cit.), the data suggested that hypotonicity per se (e.g. urea-free medium) also inhibited taurine uptake.

^{14}C-taurine efflux was markedly accelerated in the hypotonic low-NaCl medium; the slow rate-constant corresponding to the largest cellular pool (Ziyadeh et al., loc. cit.) was approximately 20-fold higher than in isotonic medium. By 2 h, only 7 ± 1% of label was retained in the intracellular compartment during incubation in the hypotonic medium, compared with 86 ± 3% in

controls. Comparable hypotonicity produced by omission of urea from the medium, or isotonic salines in which the omitted Na was replaced by Li, choline or N-methyl-gulcamine did not accelerate taurine efflux. Thus, taurine efflux was increased only in the low-NaCl hypotonic medium, i.e. when RVD was concomitantly expressed.

While the increase in taurine efflux in low-NaCl hypotonic medium is driven by the large outwardly-directed transmembrane taurine gradient, the mechanism responsible for the increase in membrane permeability to taurine (but not to K) during RVD, remains to be elucidated. In RGC, taurine efflux was not significantly modified by the addition of a cAMP analogue, phorbol esters or Ca-ionophore (Ziyadeh et al., MDIBL Bull. 28:52, 1989). In contrast, phorbol esters and the Ca-ionophore markedly accelerated taurine efflux in skate erythrocytes (Leite & Goldstein, loc. cit.). Furthermore, increased taurine efflux in RGC need not always be a consequence of hypotonicity and/or cell swelling. Thus, as shown above, hypotonicity per se (e.g. urea-free medium) did not increase taurine efflux. We also previously demonstrated that taurine efflux is not altered when RGC were massively swollen in isotonic propionate medium (Ziyadeh et al., BBA, loc. cit.).

We next analyzed cellular morphology and the distribution of F-actin (cf. Mills et al., MDIBL Bull. 26:13, 1986; Feldman et al. Am. J. Physiol. 257:C377, 1989), in order to assess the relationship between cytoskeletal disruption and taurine efflux. We found that taurine efflux was accelerated only in conditions which also caused a disorganization in F-actin distribution: low NaCl-hypotonicity, high KCl medium or the addition of 1 mM p-chloromercuribenzene sulfonate. Under these conditions, the cells were also swollen. Moreover, even in the absence of cell swelling (e.g. high K gluconate medium) F-actin disorganization was noted, and was also associated with marked acceleration of taurine efflux.

Conversely, when cytoskeletal architecture was preserved, taurine efflux was not increased despite the occurrence of significant cell swelling, e.g. urea-free hypotonicity or propionate-induced swelling. These observations indicate that the integrity of the cytoskeleton (particularly F-actin fibers in close association with the cytoplasmic face of the membrane) is important in maintaining the low permeability of the membrane to taurine. When the interaction between the membrane and F-actin fibers is disrupted, the membrane permeability to organic osmolytes is markedly increased, thus allowing for accelerated osmolyte efflux.

In summary, taurine uptake is reduced during hypotonic stress. Moverover, increased efflux of organic osmolytes (e.g. taurine) appears to be a predominant mechanism for RVD in RGC. Efflux of K is not accelerated during this response. We suggest that disruption of F-actin fibers may allow the cell membrane to increase its permeability to taurine (but not to K).

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