OSMOLYTE FUNCTION OF MYO-INOSITOL IN DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND

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To study the osmolyte function of myo-inositol (MI) in shark rectal gland, we measured MI concentration, uptake, and efflux under several experimental conditions. A specific enzymatic assay was used to determine [MI] in serum and tissues (MacGregor and Matschinsky, Anal. Biochem. 141:382-389, 1984).

MI is maintained in normal rectal gland approximately 40 fold higher than serum levels (fresh rectal gland [MI] = 17.9 ± 0.9 mmol/kg wet wt., N=19, serum [MI] = 0.44 ± 0.03 mM, N=16). Sorbitol has little apparent osmolyte function. Rectal gland sorbitol is less than 0.2 mmol/kg wet wt., measured enzymatically (MacGregor et al., J. Biol. Chem. 261:4046-4051, 1986).

Shortterm maintenance of intracellular MI is not apparently Na^+ dependent. [MI] in slices of rectal gland was unaltered after 60, 120, or 180 min incubations in Na^+-free lithium saline (Li^+=293 mM). Stimulation of tissue secretory activity for 120 min with 0.05 mM dibutyryl cAMP and 0.25 mM theophylline, in Na^+-containing or Na^+-free medium, also had no effect on tissue MI content.

Increasing membrane permeability with high K^+ medium or exposure to mercurials (e.g., PCMBS) resulted in decreased MI content in rectal gland slices. Incubation of rectal gland slices in K^+-saline for 120 min caused a 25% decrease in tissue [MI]. PCMBS incubation (120 min, 1 mM) resulted in similar MI loss.

Uptake of ^3H-MI by rectal gland slices is very slow. Slices accumulated radioactive MI to 6% of the medium specific activity by 60 min, and to 9% by 180 min. Neither MI uptake nor incorporation of label into inositol phosphates was affected by the absence of sodium.

Efflux of ^3H-MI from preloaded rectal gland slices was extremely slow, following an initial rapid equilibration of extracellular space label. Inclusion of 1 mM PCMBS caused a 7.5 fold increase in second phase efflux rate as compared to control, presumably due to increased membrane permeability. Replacement of medium sodium by lithium had no effect on MI efflux.

The primary role of intracellular osmolytes is to osmotically balance the high extracellular NaCl. Presumably, tissue content of organic solutes with osmolyte function would decrease if extracellular osmolarity were lowered. To test the response of rectal gland MI under such conditions, we maintained several sharks for 24 hr in 100% (control, N=3) or in 70% (hypotonic, N=7) sea water. Serum and rectal gland, but not muscle, [MI] fell significantly in sharks maintained in hypotonic sea water. Serum [MI] was 0.59 ± 0.07 mM in controls and 0.39 ± 0.04 mM in hypotonicity-exposed sharks. For control and experimental groups, muscle [MI] was respectively 4.1 ± 0.5 and 3.8 ± 0.4, and rectal gland [MI] was respectively 20.5 ± 0.6 and 14.6 ± 1.2 mmol/kg wet wt.

We conclude that MI serves as an intracellular osmolyte in shark rectal gland. The rectal gland maintains a high tissue:serum MI gradient, with slow uptake and efflux, presumably due to relative membrane impermeability to MI.

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