TAURINE UPTAKE AND EFFLUX IN RECTAL GLAND CELLS OF SHARK (SQUALUS ACANTHIAS)

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The osmolyte taurine is present in relatively high concentrations in the tissues of a variety of marine fishes (King and Goldstein, Molec. Physiol. 4:53, 1983). The characteristics of taurine accumulation have previously been examined in a variety of tissues of several species. Information on taurine transport in the shark rectal gland is lacking, although in this tissue taurine appears to be a major intracellular osmolyte.

The preparation of slices of rectal gland and incubation media was as detailed previously (Kleinzeller and J. Goldstein, J. Comp. Physiol B 154:561, 1984). [^{14}C] taurine was used as the label. Radioactivity of tissue extracts (0.04 N HNO_3 for 24 h) and of media was counted by liquid scintillation for the calculation of taurine uptake, expressed in μmol/g wet weight (WW).

The uptake of taurine (0.1 μCi/ml) at a medium concentration of 0.2 mM (close to plasma levels) was nearly linear over 30-270 min. The accummulation ratio Si/So -- the ratio of apparent intra- and extracellular concentrations-- was 2.0 ± 0.19 (mean ± SE, n=9) at 180 min incubation. Given the high endogenous intracellular taurine concentration in rectal gland cells (50 mM; Goldstein and Kleinzeller, Curr. Topics Membr. Transp. 30:181, 1987), at this uptake rate the steady-state value would be reached within several days. Total substitution of media Na with choline, Li or K resulted in 85% inhibition of taurine uptake; the Si/So ratios did not exceed 0.3 at 180 min of incubation. This suggests that taurine uptake is largely Na-dependent, consistent with the involvement of a Na-taurine cotransport mechanism as decribed in other epithelia including mammalian kidney (Chesney et al., Biochim. Biophys. Acta 812:702, 1985) and intestine of winter flounder (King et al., J. Exp. Zool. 238:11, 1986). Inhibition of taurine uptake by 0.5 mM ouabain in Na media (26% at 180 min incubation) was lower than that seen in Na-free media, reflecting the slow action of this inhibitor on the intracellular ionic distribution (Kleinzeller and J. Goldstein, loc. cit.); however, the results are indicative of an involvement of the Na-K-ATPase in the accummulation process.

Substitution of Cl with gluconate or propionate resulted in a moderate (33%) inhibition of taurine uptake. Thus, taurine uptake into rectal gland cells is in part dependent on extracellular Cl, consistent with similar observations in rat kidney (Chesney et al., loc. cit.) and in kidney of aglomerular fish (Wolff et al., MDIBL Bull. 25:90, 1985). Hence, extracellular Cl may not be an absolute requirement for taurine uptake as is the case with extracellular Na. It remains to be determined whether Cl is additionally cotransported with Na and taurine via the putative 2Na/Cl/taurine symport as has been suggested for flounder RBC (cf. Fincham et al., J. Membr. Biol. 96:45, 1987). "Loop-type" diuretics (bumetanide, furosemide or piretanide) at 10^{-4}M had no inhibitory effect on taurine uptake at 180 min. Thus, inhibition of Na/K/2Cl cotransport in rectal gland does not disminish taurine uptake, nor does taurine influx seem to utilize the Na/K/2Cl cotransport system. We also did not observe any inhibition of taurine uptake with

10^{-4}M hydrochlorothiazide (an inhibitor of Na/Cl cotransport) or with DIDS (a stilbenedisulfonate which inhibits the anion-exchange carrier).

The structurally related β-alanine (5 mM) inhibited taurine uptake (at S_o = 0.2 mM) to the same extent as 5 mM taurine (self inhibition): S_i/S_o values were 2.26 in the control, vs. 0.42 with β-alanine and 0.36 with taurine 5 mM. Isethionate produced only a 10% inhibition, while glutamate and aspartate had no inhibitory effect. Thus, β-alanine and taurine share the rather specific carrier for the Na/β-amino acid cotransport system.

Kinetic parameters of taurine uptake at 180 min revealed two saturable kinetic systems: a high-affinity system (Km for taurine of 40 μM and a Vmax of 0.074 μmol/h·g WW) and a low-affinity system (Km of 10.5 mM and a Vmax of 1.83 μmol/h·g WW (Eadie-Hofstee plots). The physiologically - relevant high - affinity Km for taurine is identical with that obtained in brush border membrane vesicles of rat kidney (40 μM) (Chesney et al., Kidney Int. 24:588, 1983) and comparable to the value in flounder erythrocytes (80 μM) (Fincham et al., loc. cit.). The affinity of taurine uptake for Na was tested by varying the media Na concentration using iso-osmotic Li substitution while media taurine was kept at 0.2 mM. The Km for Na was 31 mM.

The efflux of taurine from rectal gland slices was determined (cf. Kleinzeller and J. Goldstein, loc. cit.). Slices were first preloaded with [^{14}C] taurine (0.2 mM, 0.2 μCi/ml) for 180 min in standard Ringer; washout was then carried out in media of different composition for 120 min. From the plotted data (log % activity remaining in the tissue vs. time) the efflux rate constants were determined. The efflux of taurine is described by a three-compartment model. The initial rapid efflux at all experimental conditions in the first 4-7 min corresponds to the flux of the label from the extracellular space (22% of tissue WW). The efflux from the cellular compartment showed a rapid and a slow component. In control media, the rate constant could be determined accurately only for the slow component (k' = 0.00041 ± 0.00009 min^{-1}, relative pool size 65 ± 3% of total tissue taurine, n=3). This demonstrates that rectal gland cells are effectively impermeable to taurine (cf. urea and H_2O; Kleinzeller and J. Goldstein, loc. cit.), consistent with the role of taurine as an osmolyte. From the difference between the size of the extracellular pool and that coresponding to the slow kinetic component, a fast efflux component from the cellular pool can be deduced, corresponding to 13 ± 3% of tissue taurine. In high K media (KCl replacing NaCl) taurine eflux was enhanced 5.7 fold (k' = 0.0023 ± 0.0003, n=3). Here, the fast cellular component could be documented (k" = 0.0856 ± 0.004). In K-gluconate media (replacent for NaCl), k' was increased 4.4 fold (k' = 0.0018 ± 0.0003, n=3). The potassium-induced increase in taurine efflux cannot be related to cell swelling (i.e. membrane stretching) since, as compared with controls, cell water increases only in KCl media while actually a slight cell shrinkage occurs in K-gluconate (see Kleinzeller et al., J. Comp. Physiol. B 155:145, 1985). Furthermore, in Li media (Li substituting for Na), taurine efflux was retarded by 28% compared with controls despite a modest 10% increase in tissue water content. Since under these conditions the cell depolarizes to a level close to that found in K media (Kleinzeller et al., MDIBL Bull. 26:163, 1986), the data also exclude changes in cell polarity as a cause for the enhanced efflux in K media. Similarly, cell swelling in propionate media (Feldman et al., this bulletin) did not affect taurine efflux. It is concluded that enhanced taurine efflux in K media reflects a specific effect of external K on membrane permeability.

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