BETAINE AND CHOLINE FLUXES IN RECTAL GLAND OF SHARK (SQUALUS ACANTHIAS)

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Betaine and other trimethylamines participate in cell volume maintenance in some tissues, e.g. mammalian renal medulla (Bagnasco et al., J. Biol. Chem. 261:5872, 1986) and in whole organisms, e.g. elasmobranchs (Yancey et al., Science 217:1214, 1982). Additionally, methylamines protect cellular macromolecules form the perturbing effects of high urea concentrations. Cellular betaine may originate from active uptake coupled to slow efflux (Nakanishi et al., Kidney Int. 35:499, 1989) and/or from enzymatic oxidation of choline (Grossman & Hebert, Am. J. Physiol. 256:F107, 1988). In elasmobranch skeletal muscle, betaine concentration is approximately 100 mM (Robertson, Biol. Bull. 148:303, 1975); levels in other tissues are estimated to be in the millimolar range. In this study, we began an investigation to characterize betaine and choline transport in shark rectal gland cells (RGC). Fluxes of labeled osmolytes were measured in tissue slices as described (Ziyadeh et al., Biochim. Biophys. Acta 943:43, 1988).

^{14}C-betaine uptake was slow, and nearly linear during 5 h. At 4 h, the apparent intracellular concentration (Si) of labeled betaine exceeded that of the medium; e.g. Si = 35 ± 3 μM (mean ± SE, n=5) in Ringer's containing 20 μM betaine, and 133 ± 5 in 100 μM. Thus, uptake proceeded against an apparent concentration gradient. Furthermore, the profile of uptake as a function of media betaine concentration in the low range (20 μM-2mM) exhibited a saturable, carrier-mediated pathway (Km = 222 ± 15 μM, Vmax = 140 ± 17 μmol/h · kg wet weight). At higher media concentrations (5-40 mM), betaine uptake was slow and non-saturable. The saturable component of betaine uptake was not dependent on external Na: addition of 0.5 mM ouabain, 1 mM amiloride or 0.2 mM bumetanide, or Na substitution with N-methyl-glucamine (NMG) did not inhibit betaine uptake. While Cl substitution with gluconate or propionate inhibited uptake by 70 ± 6% and 30 ± 4%, respectively, Cl-requirement for betaine uptake remains to be confirmed; it is likely that gluconate (and to a lesser extent, propionate) acted as competitive inhibitors at the carboxylate site of the betaine uptake carrier. Choline, which differs from betaine by a hydroxyl instead of a carboxylate group, did not competitively inhibit betaine uptake. Hypertonicity (1300 mosM, high NaCl) increased betaine uptake by only 28 ± 4%.

Betaine efflux from ^{14}C-betaine-loaded RGC was also relatively slow; more than 85% of the label was retained in cell H_2O after 2h of washout in standard shark Ringer's. Thus, betaine may function as an intracellular osmolyte in RGC. In contrast, efflux was markedly accelerated in hypotonic Ringer's (600 mosM, low NaCl); only 10% of the label remaining in cells after 2 h. Comparable hypotonicity produced by omission of 350 mM urea from the medium, or isotonic salines in which the omitted Na was replaced by NMG, did not accelerate betaine efflux. As RGC exhibited regulatory volume decrease in the hypotonic low-NaCl Ringer's (cf. Ziyadeh et al., this Bulletin), the efflux of betaine likely contributed to this response.

In contrast to betaine transport, ^{3}H-choline fluxes were much faster; more than 65% of the label in loaded RGC was washed out in 2h. In uptake studies, Si exceeded media choline concentration within 30 min: Si = 44 ± 5 μM (n=5) in Ringer's containing 20 μM, and 114 ± 6 in 100 μM. By 4 h, Si exceeded the lower and higher medium concentration by 13- and 6-fold, respectively. Kinetic analysis revealed two saturable uptake processes, with Km's of 54 ± 2 μM and 480 ± 7 μM. Choline uptake was not dependent on either external Na or Cl (no effect of ouabain, bumetanide, DIDS; Na substitution with Li or NMG; Cl substitution with gluconate, propionate or methysulfate). However, high-K salines inhibited net choline uptake by approximately 50%, whether cell swelling occurred (KCl media) or not (K gluconate). Choline uptake in RGC was not inhibited by betaine, and was only slightly inhibited by hemicholinium-3, an inhibitor of a distinct Na-choline carrier in synaptosomes (Guyenet et al., Mol. Pharmacol. 9:630, 1973).

Thus, the uptake of betaine and choline in RGC are via distinct pathways. The choline uptake system is also different from that of neurons. The slow efflux of betaine is consistent with an osmolyte function. Intracellular betaine accumulation may require a process additional to that of a slow uptake. It is possible that the rapid uptake of choline and the subsequent oxidation to betaine may contribute to betaine accumulation. However, the requisite enzyme(s) remains to be demonstrated in RGC. [Supported by a Lucille P. Markey Fellowship to F.N.Z.]