KINETICS AND REGULATION OF TAURINE TRANSPORT IN THE FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*) INTESTINE

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Taurine is found in relatively high concentrations in the tissues of a variety of marine fishes where it makes a significant contribution to the intracellular osmolyte pool and is important in the regulation of cell volume. While there is little or no evidence for the synthesis of taurine in fish tissues, the dietary content of taurine is high and it is likely that the diet is the major source of taurine for these species. Therefore, taurine transport across the fish intestine is important to the overall regulation of taurine balance in these species. In a previous investigation (King et al., MDIBL Bull. 23:31, 1983) taurine uptake across both the mucosal and serosal membranes of the flounder intestine was found to be completely Na^+-dependent. In addition, when assayed at low taurine concentrations (0.01 mM) the rates of uptake across the two membranes were approximately equal. The present study continues this investigation and attempts to identify the factors responsible for net uptake of taurine across the flounder intestinal epithelium as well as the effects of potential regulators.

Transport was studied by following taurine uptake into the intestinal cell across the luminal or antiluminal membrane. We had previously found that taurine transport is better studied in this way rather than by measuring transepithelial flux, since the slow rate of transport across both membranes together with a large cellular taurine pool ($\sim$30 mM) do not allow for equilibration of labeled taurine with the cellular taurine within a reasonable experimental time.

Flounder were killed by transection of the spinal cord, the intestine removed and immediately stripped of the muscle layers. The epithelia were mounted in modified Ussing chambers and allowed to equilibrate for 30-60 minutes, at which time the TEP had stabilized and was between -3 and -9 mV lumen positive. The standard Ringers was 148 mM NaCl, 5.0 mM KCl, 2.7 mM NaH_2PO_4, 1.26 mM $CaCl_2$, 1.24 mM $MgSO_4$, 11.0 mM $NaHCO_3$, bubbled with 99% O_2/1% CO_2 and maintained at 15°C. In ion replacement studies Na^+ was replaced with N-methyl-D-glucamine. Following the equilibration period, the uptake assay was started by adding taurine (to a final conc. of 0.1 mM) to both sides of the epithelium and ^{14}C-taurine to either the luminal or antiluminal side of the membrane. After 1 hr. of incubation, samples of the bathing medium were taken from the luminal and antiluminal sides of the membrane. The chamber was then opened and the tissue spanning the aperture of the chamber cut out, blotted, weighed and digested in Protosol at 37°C overnight. The samples (medium and tissue) were then counted for radioactivity by standard LS procedures. Extracellular space was measured by similar procedures using ^{14}C-PEG (cold PEG 0.1 g/100 ml).

Taurine uptake across the mucosal and serosal membranes was measured as a function of taurine concentration. The kinetic constants as determined by Lineweaver-Burk plots are shown in Table 1.

Table 1. Kinetic parameters of taurine uptake across the mucosal and serosal membranes of the flounder intestine.

	mucosa	serosa
[TAU]	3.89 mM*	0.11
K_m	3.27 + 0.49 mM	0.78 + 0.09 mM
V_{max}	6071 + 179 nmols/g-hr	1817 + 289 nmols/g-hr
$v = \frac{V_{max}[TAU]}{K_m + [TAU]}$	3,298 nmols/g-hr	224 nmols/g-hr

*Amino Acid analysis of intestinal contents of freshly caught (fed) fish

The Km for taurine uptake across the mucosal membrane was 4-fold higher than across the serosal membrane, indicating a greater affinity for transport at the serosal membrane. V_{max} rates of transport, on the other hand, were greater for taurine uptake across the mucosal membrane. If the rate of transport is calculated for the *in vivo* plasma and intestinal lumen taurine concentrations, transport across the mucosal membrane is 10 times greater than uptake across the serosal membrane. Assuming similar permeabilities for taurine leak out of the cell at both membranes, the greater rate of taurine accumulation across the mucosal membrane may provide the mechanism for net taurine uptake across the epithelium from lumen to serosal side.

Possible modulation of taurine transport across the mucosal and serosal membranes by cyclic nucleotides (cAMP and cGMP) was also investigated. When 1.0 mM cGMP was added to the serosal medium, taurine (0.1 mM) uptake was increased across the mucosal membrane 2-times control (163 ± 14.4 to 320 ± 38.3 nmols/g-hr. n=4) and across the serosal membrane 1.8 times control (98.2 ± 14.2 to 173 ± 49.2 nmols/g-hr, n=4). cAMP (1.0 mM on serosal side) increased uptake 1.5-times across the mucosal side (147 ± 20.7 to 227 ± 28.1 nmols/g-hr, n=4) but had no effect on the serosal side (98.8 ± 18.5 versus 93.5 ± 8.86 nmols/g-hr). cGMP has been shown to cause a hyperpolarization of the apical membrane potential (Halm et al., MDIBL Bull. 22:80, 1982). If Na^+-dependent taurine transport is electrogenic, the effect of cGMP could increase taurine transport by hyperpolarizing the intestinal cell thereby increasing the driving force for Na^+-dependent taurine uptake. This cGMP-induced change in membrane potential would also affect other Na^+-solute coupled transport systems that were electrogenic. cAMP has no reported effect on flounder intestinal cell membrane potential.