Table 1. Percentage of initial cardiac output by isolated hearts after 30 min of perfusion.

Additions to perfusate	sea raven	skate
none	84 ± 14.6 (N=8)	86 ± 8.6 (N=9)
IAA*	41 ± 12.1 (N=6)	12 ± 9.3 (N=6)
IAA + LACTATE (2 mM)	88 ± 5.2 (N=4)	72 ± 11.1 (N=6)
IAA + ACETOACETATE (0.5 mM)	76 ± 8.1 (N=8)	94 ± 3.6 (N=7)
PALMITATE (0.5 mM)	90 ± 8.9 (N=5)	76 ± 8.7 (N=6)
PALMITATE (1.0 mM)	99 ± 4.8 (N=5)	59 ± 10.5 (N=6)
IAA + PALMITATE (1.0 mM)	95 ± 5.2 (N=6)	–

* IAA - iodoacetic acid (0.5 mM)

All data are expressed as mean ± S.E.M.

The findings of this study are in keeping with the previously recognized enzyme distribution and catabolic activities of teleost and elasmobranch hearts in that the former can utilize a variety of metabolic fuels whereas the latter are best supported by ketone bodies. Research supported by operating grants from N.B. Heart Foundation and N.S.E.R.C. of Canada to William R. Driedzic.

TAURINE TRANSPORT BY FLOUNDER (Pseudopleuronectes americanus) RENAL BRUSH BORDER MEMBRANE VESICLES

Patricia King, Philip Newsholme, Rolf Kinne and Leon Goldstein, Division of Biology and Medicine, Brown University, Providence, R.I., and Departments of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, N.Y.

INTRODUCTION--Taurine (2-aminoethane sulfonic acid) is one of the major amino acids participating in the cell volume regulation of a variety of marine fish as well as marine invertebrates and other vertebrate species. In the marine fish, taurine is a relatively inert compound metabolically and its plasma levels appear to be regulated by excretion. In vivo investigations of the renal handling of taurine in the little skate, flounder, and dogfish revealed that there is a net secretion of this amino acid by the kidneys of these fish (Schrock et al, Am. J. Physiol. 242:R64-R69, 1982). Recently, we have been studying the mechanism of taurine secretion by the renal epithelium of the flounder. Previous studies indicate that taurine movement across the basolateral membrane into the cell is an uphill concentrating step dependent on the presence of Na^+ and Cl^-, and inhibited by ouabain, as well as other β-amino acids and GABA (King et al, J. Exp. Zool., 223(2):103-114, 1982). Taurine movement from the cell to the tubular lumen then occurs down its chemical concentration gradient (0.1 mM in plasma, 30 mM in cell, 0.5 mM in urine). The purpose of the present study was to investigate taurine transport across the apical membrane by assaying taurine uptake into flounder renal brush border membrane vesicles. In particular, we were interested in determining whether a carrier system facilitating efflux of taurine from the cell was present at the apical surface.

METHODS--Brush border membrane vesicles were prepared from freshly dissected flounder kidneys by methods previously described (Eveloff et al, Am. J. Physiol. 237(4):F291-F298, 1979). The final preparation of vesicles was suspended in vesicle buffer (mannitol 100 mM; $CaCl_2$ 2 mM; Tris-HEPES, 20 mM; pH 8.) at a protein concentration of approximately 10 mg/ml and stored at -80°C until use (up to 5 days). An aliquot of the vesicles as well as the whole renal homogenate and discarded fractions were assayed for Na^+-K^+-ATPase and alkaline phosphatase activities. Alkaline phosphatase activity in the brush border membrane vesicles averaged 10.5 ± 2.2 μmoles/mg protein-hr (mean ± S.E., n=8) and was enriched 10-fold compared to activity in the whole homogenate. Brush border Na^+-K^+-ATPase activity was 3.0 ± 1.3 μmoles/mg protein-hr (mean ± S.E., n=8); the ratio of vesicle/homogenate activity for

Na^+-K^+-ATPase was 0.4. Total recovery of enzyme activity averaged 97.6% ± 4.9 for alkaline phosphatase and 83.3% ± 14.5 for Na^+-K^+-ATPase (mean ± S.E., n=8).

The vesicles were quickly thawed (37°C) before use. Taurine uptake into the vesicles was measured by a rapid filtration technique (Eveloff et al, Am. J. Physiol. 237(4):F291-F298, 1979). The final taurine concentration in the incubation medium was 0.1 mM with 10 μCl ^{3}H-taurine. In some experiments glucose or alanine uptake was measured; identical solute concentrations and specific activity were used for the incubations. All experiments were run at 15°C.

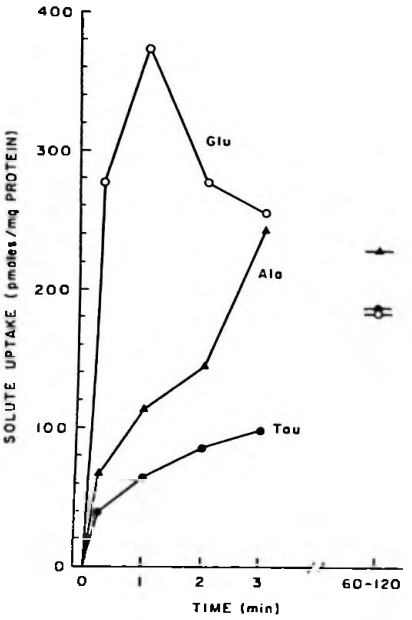

Figure 1.--Uptake of D-glucose, L-alanine, and taurine into flounder renal brush border membrane vesicles. The vesicles were prepared in 200 mM mannitol, 20 mM Tris-HEPES, pH 8.2, and 2 mM $CaCl_2$. The incubation media contained 75 mM NaCl, 50 mM mannitol, 20 mM Tris-HEPES, ph 8.2, 2 mM $CaCl_2$, and 0.1 mM [^{3}H]-glucose, [^{3}H]-taurine, or [^{14}C]-alanine. The values are the means of 2 experiments.

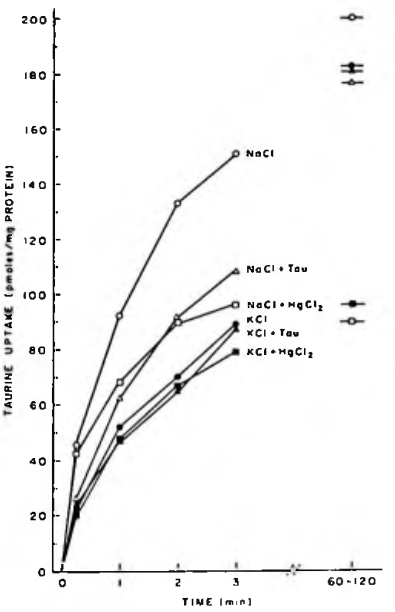

Figure 2.--Uptake of taurine into flounder renal brush border membrane vesicles. Vesicles were prepared as indicated in Figure 1. Incubation media contained 20 mM Tris-HEPES, pH 8.2, 50 mM mannitol, 2 mM $CaCl_2$, 0.1 mM [^{3}H]-taurine and 75 mM NaCl (○), 75 mM NaCl + 10 mM taurine (△), 75 mM NaCl + 0.1 mM $HgCl_2$ (□), 75 mM KCl (○), 75 mM KCl + 10 mM taurine (△), 75 mM KCl + 0.1 mM $HgCl_2$ (□). The values are the means, n=6.

RESULTS AND DISCUSSION--The time courses for taurine, glucose, and alanine uptake into renal brush border membrane vesicles are shown in Figure 1. In the presence of a 75 mM NaCl gradient directed into the vesicle, the uptake of glucose displayed a large overshoot, while alanine showed a slight overshoot and taurine displayed none. The equilibrium values for uptake of taurine, glucose, and alanine were similar indicating that each of the solutes enters the same intravesicular space.

Accumulation of taurine in the brush border membrane vesicles was tested as a function of incubation medium osmolarity. When the osmolarity of the incubation medium was increased by the addition of sucrose, the equilibrium concentration of taurine decreased indicating that taurine is taken up into an osmotically reactive space (not shown). These data demonstrate that taurine uptake reflects transport into the vesicles with binding to the membrane making a minimal contribution.

Taurine uptake by the vesicles was dependent on the NaCl gradient; at 15 seconds uptake in 75 mM NaCl was

approximately 2 times that observed in a KCl gradient (Figure 2). In addition NaCl-dependent taurine uptake was inhibited by $HgCl_2$ and showed tracer replacement as nonlabeled taurine (10 mM) decreased the rate of labeled taurine transport. There was no effect of nonlabeled taurine or $HgCl_2$ on taurine uptake in KCl medium. It should be noted that in both NaCl and KCl medium the presence of $HgCl_2$ resulted in a decrease in taurine equilibrium values; this is possibly due to a destabilizing effect of $HgCl_2$ on the vesicle membrane. Taurine uptake was also investigated in vesicles pre-equilibrated with NaCl or KCl thus eliminating the salt gradients. Taurine transport displayed a Na^+-dependence even in the absence of salt gradients; tracer replacement decreased Na^+-dependent uptake but had no effect on taurine uptake in KCl medium (not shown).

The influence of electrical potential on taurine uptake into the renal brush border vesicles was tested. Two methods of generating an inside positive potential were used and included an inward-directed potassium diffusion potential in the presence of valinomycin and a Na^+ conductive pathway generated by sodium glucose co-transport. Both approaches resulted in a decrease in taurine uptake by the vesicles (Table 1). Since the electrogenic system

Table 1. The effect of membrane potential on sodium dependent flux of taurine into flounder renal brush border vesicles.

Incubation medium	Taurine uptake (pmoles/mg protein-min)	
A. 75 mM NaCl	61.8 ± 1.0	
75 mM NaCl + 0.1 mM glucose	52.8 ± 1.5	$p<.05$
75 mM NaCl + 0.1 mM glucose + 0.1 mM phlorizin	67.5 ± 4.1	
B. 75 mM NaCl + 50 mM K^+-gluconate	43.0 ± 1.7	
75 mM NaCl + 50 mM K^+-gluconate + valinomycin	34.0 ± 1.5	$p<.05$

The vesicles were prepared as in Figure 1 and the uptake of 0.1 mM taurine studied. An inside-positive potential was generated by (A) Na^+ and glucose cotransport or (B) a K^+ diffusion potential. In addition to the conditions indicated in the table, each incubation included 20 mM Tris-HEPES, pH 8.2, 2 mM $CaCl_2$, and 50 mM mannitol. For the K^+ diffusion potential, valinomycin (dissolved in ethanol) was present at a concentration of 90 μM. The final incubating concentration of ethanol was less than 1.0% of the reaction volume and controls were run in the presence of 1.0% ethanol. The values are the means± S.E. (n=4). Data for groups A and B were analyzed within the groups using one-way analysis of variance and Student-Neuman-Keuls test for A and Student t-test for B. Significant differences in uptake are indicated by p values.

for Na^+-glucose cotransport is not present on the basolateral membrane, a glucose-generated potential would only occur in brush border membrane vesicles. Therefore, these data also corroborate the enzyme enrichment values identifying the vesicles as brush border membranes. In addition, anion substitution experiments revealed that Na^+-dependent taurine transport requires an accompanying anion, as substitution of Cl^- with gluconate (a less permeable anion) depressed uptake (not shown). Substitution of SCN^- for Cl^- resulted in no change in the rate of taurine accumulation. This characteristic differs from taurine transport at the basolateral membrane where taurine uptake exhibits a specific requirement for chloride.

The results of the present study indicate the presence of a Na^+-dependent electrogenic facilitated transport system for taurine in the brush border membrane of the flounder kidney. No evidence for a Na^+-independent facilitated diffusion system was obtained. It should be noted that in addition to the taurine uptake studies reported here a number of efflux experiments were performed to test for assymetric transport systems that would facilitate exit of taurine from the cell but would be undetectable by uptake measurements. The results of these experiments

did not support the presence of a carrier-mediated efflux across the apical surface.

The characteristics identified for taurine transport in the flounder brush border membrane vesicle are similar to those that have been found in mammalian renal brush border membranes studied. In the latter species, however, the kidney epithelium displays a net reabsorption of taurine. It is likely that the difference between net secretion in fish and net reabsorption in mammals results in part from a low rate of taurine uptake across the apical membrane in fish. In this light, it is important to reemphasize the difference in the rates of uptake by the vesicles for glucose and alanine, both known to be almost completely reabsorped by the fish kidney, versus the rate of taurine accumulation (Figure 1). The fish renal epithelium is characterized by a high rate of taurine transport across on the basolateral membrane, a low rate for its accumulation across the brush border membrane, and concentration gradients favoring net efflux from both "sides" of the cell. As a result, there is a bidirectional flux of taurine across both membranes which results in a net influx of taurine at the basolateral membrane and a net efflux at the apical membrane. The relatively high cellular concentration of taurine, therefore, is due to uptake across the basolateral membrane. This in turn provides the large concentration gradient favoring the exit of taurine from the cell into the lumen by simple diffusion.

MICROELECTRODE STUDIES OF THE SKATE GASTRIC MUCOSA

George W. Kidder III and Elizabeth L. Kidder, Department of Physiology, University of Maryland School of Dentistry, Baltimore, Maryland, and Department of Biology, University of Maryland Baltimore County, Cantonsville, Maryland

Microelectrode studies require a free air-fluid interface, thus precluding an "air lift" circulating system, and requiring a pump to recirculate fluid. The PVC tubing used in peristaltic pumps emits some substance which inhibits gastric acid secretion in frog, skate and dogfish; silicon tubing is satisfactory. However, dogfish mucosae seem contaminated by some microorganism which rapidly proliferates in the tubing, inhibiting secretion in the tissue from which derived, as well as other tissues. We could never produce satisfactory secretion in dogfish using the pump system.

The gastric mucosa of the little skate (Raja erinacea) will secrete acid, if care is taken to maintain clean and uncontaminated tubing. Of 21 tissues, 18 produced satisfactory secretory rates, averaging 2.7 ± 0.20 $\mu Eq/cm^2 \cdot hr$ (mean $\pm$ SE), which is somewhat higher than expected in dogfish at 1 atm total pressure (Kidder, Am. J. Physiol. 231:1240,). Transepithelial resistance (Rms) was 152 ± 12 ohm$\cdot cm^2$; short circuit current (Isc) was -1.0 ± 2.1 $\mu A/cm^2$, or indistinguishable from zero. Thiocyanate (SCN, 10 mM, serosal) inhibits secretion and raises Rms, as in other species.

Microelectrode studies were performed to determine the intracellular potential (Vmc) for this tissue, monitoring Vmc, fractional resistance (fr - $\Delta Vmc/\Delta Vms$ for a current or voltage pulse) and the tip resistance of the electrode. When these were steady for 10 seconds and of reasonable value, the data were extracted and used to produce Figure 1.

Two clusters of values are observed, which are identified in Figure 1 and summarized in Table 1. The "O" cluster (oxyntic cells?) has its Vmc but not fr reduced by SCN; the "S" cluster (surface epithelial cells?) has its fr increased with no change in Vmc.

The current-voltage plot was explored by voltage clamping to different potentials for 1 sec, recording the current required to maintain this potential. In all cases, the steady state clamp potential was zero, and the potential was alternated between positive and negative displacements. The current-voltage plots consist of straight lines intersecting at breakpoints, as previously found for dogfish (Kidder, Bull. MDIBL 18:4, 1978). In 37 such experiments performed under secreting conditions in 18 tissues, a breakpoint was found at -8.2 ± 0.5 mV, or 38.8 ± 4.1 $\mu A/cm^2$. In 25 of these experiments, a second breakpoint was found at 43.0 ± 1.4 mV, 186 ± 6.9 $\mu A/cm^2$. Although it is conventional to refer to breakpoint voltages, there is no indication from these data that the breakpoint voltage is more stable than the breakpoint current. Supported by NIH AM 27229 to George W. Kidder, III.