duration of the experiment (~ 6 hours). In one instance when the experiment was not terminated, the tubule continued to secrete fluid until the following morning, nearly 18 hours after the experiment had been started. With temperatures higher than 18-20°C rates of fluid secretion usually declined paralleling the morphological deterioration of the tubule. Experiments conducted in collaboration with P. King and L. Goldstein on taurine secretion (this bulletin) further attest to the usefulness of the preparation shown in Figure 2 in studies of tubular secretory transport. (I thank Dr. B. Schmidt-Nielsen for the use of the Clifton Nanoliter Osmometer and Mr. H. Church for his technical assistance. Supported in part by NIH AM 26633.)

## 25 TAURINE TRANSPORT BY TEASED RENAL TUBULES OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS.

Patricia King, Susanne Goldstein and Leon Goldstein, Divisions of Biology and Medicine, Brown University, Providence, Rhoide Island

Taurine (2-amino ethane sulfonic acid) is an amino acid found in abundance in the cells of many marine vertebrates and invertebrate animals. For many of these species, taurine is an important intracellular osmolyte which participates in cell volume regulation. Recent studies on the in vivo handling of taurine in the dogfish, skate and flounder have revealed that taurine is actively secreted by the renal tubules of these fishes (Schrock et al., Am. J. Physiol., in press). On the other hand, neither oxidation nor synthesis of this amino acid could be demonstrated when investigated in a number of skate tissues including muscle, brain, liver, erythrocyte, and kidney (King et al., J. Exp. Zool., 212:69, 1977). Thus it appears that the renal excretion of taurine is important for maintaining the balance of this amino acid especially in the face of osmotic stresses and dietary changes. The present study makes use of the teased tubule preparation of the flounder kidney to study the mechanism and control of taurine secretion by the kidney epithelium.

Our laboratory had previously studied the characteristics of taurine uptake by dogfish kidney slices. The first part of this investigation extends these studies to the flounder renal tubule in order to verify similar characteristics of taurine transport. Employment of flounder renal tubules permits further investigation of the taurine transport mechanism not possible with the dogfish kidney, including the use of perfused renal tubules (see companion report) and brush border membrane vessicles. The second part of the study is a preliminary investigation of possible hormonal control of taurine secretion by the flounder kidney, initiated as a result of the variability of taurine secretion found both in vivo and in vitro. We performed extended incubations of the tubules taking advantage of the longterm viability of this tissue in vitro (Maack and Kinter, Am. J. Physiol. 216(5):1034, 1969). This allowed us to examine the effects of the more slowly acting steroid hormones.

Methods. Taurine transport across the peritubular side of the tubule was measured by uptake of $^{14}$C-taurine by teased flounder renal tubules. Flounder were killed by transection of the spinal cord and the kidney carefully dissected out. Teased tubules were prepared as described by Forster (Science, 108:65, 1948) using teleost Ringers composed of NaCl 148 mM, KCl 2.6 mM, $NaH_2PO_4$, 2.7 mM, $CaCl_2$ 1.26 mM, $MgSO_4$ 1.24 mM, and $NaHCO_3$ 11 mM, and gassed with 99% $O_2$/1% $CO_2$ to a final pH of 7.8. Approximately 20-30 mg of tissue was placed in a 25 ml Erlenmeyer flask containing 3 ml of teleost Ringers which was 0.1 mM in taurine and included 0.1 $\mu$Ci/ml $^{14}$C-taurine (56 mCi/mmol, 1, 2-$^{14}$C-taurine). The flasks were gassed with 99% $O_2$/1% $CO_2$ and incubated at 15° C for 1 hour. Taurine uptake was linear over this time period. At the end of the incubation, the renal tubules were removed, blotted and weighed. The tissue was digested in 1 ml of Protosol (NEN) overnight and then analyzed for taurine uptake by liquid scintillation counting. An aliquot of the bath medium was also counted and tissue:medium taurine ratios were calculated. The effects of ion substitutions, competitors, and inhibitors were tested by altering the incubating medium (concentrations as indicated in Table 1). Na-free medium

Table 1. General characteristics of taurine uptake by teased flounder renal tubules

A. Specificity of taurine uptake

| incubation conditions | | control T/M | experimental T/M | % of control |
|---|---|---|---|---|
| B-amino acids: | B-alanine (1 mM) | 10.54 | 4.95 | 47 |
| | B-alanine (2 mM) | 10.54 | 4.32 | 41 |
| | hypotaurine (1 mM) | 13.48 | 6.27 | 47 |
| Y-amino acids: | Y ABA (1 mM) | 12.72 | 7.07 | 57 |
| a-amino acids: | αAIBA (1 mM) | 10.54 | 10.21 | 97 |
| | glycine (1 mM) | 10.54 | 8.72 | 83 |
| organic acids: | PAH (1 mM) | 10.54 | 8.79 | 83 |
| | probenicid (1mM) | 12.90 | 11.52 | 89 |

B. Ion dependency

| incubation conditions | control T/M | experimental T/M | % of control |
|---|---|---|---|
| $Na^+$-free (choline substitution) | 19.32 | .98 | 5 |
| $Cl^-$-free (gluconate substitution) | 19.32 | 5.58 | 29 |
| (thiocyanate " ) | 15.73 | 6.16 | 39 |
| ouabain* (0.1 mM) | 12.22 | 1.04 | 8 |
| furosemide (0.1 - 0.2 mM) | 18.60 | 14.52 | 78 |
| bumetanide (0.2 mM) | 15.64 | 14.66 | 94 |
| SITS (50 uM) | 15.64 | 14.11 | 90 |

T/M = tissue:medium ratio for taurine uptake by the flounder renal tubules. The values are the averages of 2 or 3 experiments.
*The tissue was preincubated with ouabain for 30 minutes prior to the addition of $^{14}$C-taurine to the incubation medium.

was prepared by replacement of sodium with choline. Cl-free medium was prepared using gluconate salts or sodium thiocyanate.

In the second part of the study we investigated the effects of hormones and second messengers on taurine uptake by flounder renal tubules. Three types of experiments were performed. In the first, the renal tissue was incubated for 1 hour with the effector to be tested and taurine uptake was measured simultaneously. In similar experiments, the tissue was pre-incubated with the effector for 5 hours and taurine uptake measured during the sixth hour. In the second group of experiments, tissues were preincubated with the hormones and cAMP for 24-48 hours at 5° C (Maack and Kinter, Am. J. Physiol., 216(5):1034, 1969). Taurine uptake was then measured at 15° C as previously described. The third set of experiments tested the effects of dexamethasone *in vivo*. Fish were given a dose of 10 mg dexamethasone/kg-day by intramuscular injection for 5 days. At the end of this period, the fish were sacrificed, the tubules removed and taurine uptake measured during a 1 hour incubation period as before.

For each of the experiments above, tubule viability was verified by measurement of $^{14}$C-PAH uptake (final incubating PAH 10 μM and 0.1 μCi $^{14}$C-PAH/ml). Extracellular space was determined by measuring uptake of $^{14}$C-PEG (PEG 0.1 g/100 ml) by the tubules. Tissue: medium (T/M) ratios for PAH and PEG were calculated.

Results and discussion. The T/M ratios for taurine uptake by flounder renal tubules averaged 15.04 ± .99 (mean ± S.E., n=31) and ranged from 6.3 to 25.16. As noted above this variability was observed for *in vivo* taurine secretion as well. In terms of μmoles taurine/g-hr, uptake averaged 1.50 and ranged from 0.63 to 2.56. Kinetic analysis of taurine uptake revealed a Km of 1.19 mM taurine and a Vmax equal to 19.80 μmoles taurine/g-hour. The *in vivo* flounder plasma taurine concentration has been found to be 0.11 mM (Schrock et al., Am. J. Physiol., in press).

The T/M ratios for $^{14}$C-PEG uptake, used as an index of extracellular space, averaged 0.39 ± 0.03 (mean ± S.E., n=8). These ratios did not change when incubations included Na-free medium (0.38), Cl-free medium (0.40), 0.1 mM furosemide (0.40), or 0.1 mM ouabain (0.37). The T/M ratios for $C^{14}$-PAH uptake averaged 14.62 ± 1.87 and ranged from 8.00 to 23.54. There was no correlation between PAH and taurine T/M ratios.

The general characteristics of taurine transport across the peritubular side of the renal tubule are shown in Table 1. Taurine uptake was inhibited by B-alanine, GABA, and hypotaurine, compounds all closely related in structure to taurine. αAIBA, glycine, PAH, and probenecid did not appear to affect taurine uptake in the flounder renal tubule. These results are similar to those characteristics of taurine uptake in dogfish renal slices, except for the inhibitory effect of GABA.

Tests for ion dependency of taurine uptake are shown in the second half of Table 1. In $Na^+$-free Ringers, T/M ratios fell to 5% of control values and 0.1 mM ouabain reduced taurine T/M ratios to 8% of control levels. In Cl-free Ringers containing gluconate salts, T/M ratios were 29% of control values and taurine uptake remained inhibited (39% of control ratios) when thiocyanate was used as the accompanying anion. Taurine uptake was slightly inhibited by furosemide (74% control T/M ratios) but not affected by 0.2 mM bumetanide or 50 μM SITS. $Na_2SO_4$ (10 mM), $NaS_2O_3$ (10mM), and $SO_4$-free medium had no effect on taurine transport (not shown). Thus taurine uptake across the peritubular membrane is dependent on the presence of both $Na^+$ and $Cl^-$ for full activity. $Na^+$ and $Cl^-$ dependent transport has also been found for glycine entry into pigeon red blood cells (Imler and Vidaver, Biochem. Biophys. Acta. 288:153, 1972).

Both in vivo taurine secretion and in vitro taurine uptake by flounder renal tubules show great variability from fish to fish. In addition, dogfish tubular secretion of taurine increases when fish are acclimated to 70% seawater, but when dogfish or flounder renal tissues are incubated in diluted Ringers, there is no change in taurine T/M ratios. This variability among fish and in vivo response to osmotic stress suggested that taurine secretion by the tubule may be under hormonal control. Demonstration of hormonal effects in vitro was difficult. There was no consistent change in taurine uptake when renal tubules were incubated for 1 hour and 5 hours with any of the hormones tested (serotonin, $10^{-4}$M; thyrocalcitonin, $10^{-8}$M; epinephrine, $5 \times 10^{-6}$M; prolactin, $10^{-8}$M; and dexamethasone, $10^{-6}$M). In addition, incubations of tubules with cAMP (0.2 mM 8-bromo-cAMP), cGMP (0.2 mM 8-bromo-cGMP), or adenosine ($10^{-5}$M) produced no change in taurine uptake as compared to control teased tubules. When $Ca^{++}$ ionophore (A12384, 50 μg/ml) was present in the medium, the taurine uptake was 66% of control values. However, under the same incubation conditions PAH uptake was reduced to 48% of control indicating a general loss of viability by tissue exposed to the ionophore. The absence of a response to second messengers suggested that the regulating hormone may be a steroid requiring longer incubations to have a measureable effect. When tubules were preincubated 24 and 48 hours with dexamethasone ($10^{-6}$M), there was a stimulation of taurine uptake, but the response was not consistent. An increase in taurine T/M ratios was observed in 4 out of 7 dexamethasone incubations with the maximum responses after 24 and 48 hour preincubations equalling 126% and 148% of the control, respectively. The effect of dexamethasone in vivo was then investigated (Table 2). When fish were given repeated daily injections of the steroid, the taurine uptake by isolated teased renal tubules was significantly increased over uptake by tubules of untreated flounder. The T/M ratios for dexamethasone treated flounder averaged 20.18 while control fish ratios for tauring uptake were 13.60. These results indicate that the adrenocorticosteroids could be important modulators of taurine secretion by the flounder renal tubule.

Table 2. Effects of dexamethasone treatment in vivo on taurine uptake by teased flounder renal tubules.

| | control fish | dexamethasone-treated fish | |
|---|---|---|---|
| taurine T/M | 13.60 ± 1.03 (n=10) | 20.18 ± 1.83 (n=10) | p<.01 |

T/M = tissue:medium ratios for taurine uptake by the isolated flounder renal tubules. The values are the means ± S.E. The number of fish in each group is indicated in parenthesis. The dexamethasone-treated fish were given intramuscular injections of dexamethasone (10 mg/kg-day) for 5 days. Significant difference between means is indicated by p values.

## 26 TAURINE SECRETION BY ISOLATED RENAL TUBULES OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

Patricia A. King, Klaus Beyenbach and Leon Goldstein, Divisions of Biology and Medicine, Brown University, Providence, Rhode Island and Division of Biological Science, Cornell University, Ithaca, New York

Taurine (2-aminoethane sulfonic acid) is an amino acid found in high concentrations in the tissues of many marine invertebrate and vertebrate species and is an important intracellular osmolyte for cell volume regulation. An investigation of the renal handling of taurine in vivo has indicated that there is a net secretion of taurine by the kidneys of flounder, dogfish and the little skate (Schrock et al., Am. J. Physiol., in press). The present study, along with the companion report (see King et al., this Bulletin), makes use of the isolated flounder renal tubule to examine the magnitude and mechanism of taurine secretion by the renal epithelium.

Renal tubules from the winter flounder were dissected and prepared for collections of in vitro secreted fluid as described by Beyenbach (this Bulletin). The Ringer's composition of the bath in which the tubules were suspended was NaCl 148 mM, KCl 2.6 mM, $NaH_2PO_4$ 2.7 mM, $Cacl_2$, 1.26 mM, $MgSO_4$ 1.24 mM, $NaHCO_3$ 11.0 mM, gassed with 99% $O_2$/1% $CO_2$ to a pH of 7.8. After a control period to verify fluid secretion by the isolated tubule, $^{14}$C-taurine was added to the bath (approximately 10 $\mu$Ci/ml bath; specific activity 56.08 $\mu$Ci/$\mu$mol). Tubular taurine secretion was measured from the appearance of the label in the collected secreted samples. Bath samples were taken to determine $^{14}$C-taurine activity and taurine concentration calculated. At the end of the experiment, the tubules length and inner and outer diameters were measured and the tubule itself was harvested for the measurement of total $C^{14}$ activity. Epithelia cell taurine concentration was then calculated as the difference of total $C^{14}$-activity and the lumen $C^{14}$-activity.

Results and discussion. The time course of taurine secretion for a representative tubule is shown in Table 1.

Table 1. TIME COURSE OF TAURINE SECRETION FROM A REPRESENTATIVE RENAL TUBULE EXPERIMENT

| sample time (min) | $\dot{V}_s$ pl/min·mm | bath [TAU] mM | lumen [TAU] mM | L/B | TAU secretion rate fmole/min·mm |
|---|---|---|---|---|---|
| t=10 | 21.57 | 0.317 | 0.20 | 0.64 | 4.4 |
| t=40 | 66.83 | 0.317 | 1.69 | 5.34 | 113 |
| t=68 | 14.61 | 0.317 | 12.11 | 38.13 | 177 |
| t=100 | 19.40 | 0.317 | 18.46 | 58.26 | 358 |
| t=132 | 16.04 | 0.317 | 23.98 | 75.56 | 385 |
| t=166 | 16.28 | 0.317 | 26.42 | 83.28 | 430 |
| t=198 | 23.06 | 0.317 | 25.04 | 78.90 | 577 |

The sample times indicate the minutes elapsed after adding $^{14}$C-taurine to the bath. At t=198 min., the last sample of secreted fluid was collected; the cell taurine concentration at this time was 84.96 mM.

$\dot{V}_s$ represents fluid secretion rates. L/B = lumen/bath taurine concentration ratios.