# CHARACTERIZATION OF A $Na^+/H^+$ EXCHANGER IN RENAL BRUSH BORDER MEMBRANES OF SQUALUS ACANTHIAS

C. Bevan*, R. Kinne*, R.E. Shetlar*, T.H. Maren+, and E. Kinne-Saffran*
* Max-Planck-Institut fuer Systemphysiologie, 4600 Dortmund, F.R.G.
+ Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainsville, Fl 32610

The urinary pH of the spiny dogfish, Squalus acanthias, is fixed at a pH of 5.7 - 5.8 even during infusions of high concentrations of $NaHCO_3$ (W.W. Smith, J. Cell Comp. Physiol. 14:95, 1939). This constant urinary pH is thought to be maintained by a powerful acid secreting mechanism which can far exceed the rate of $HCO_3^-$ reabsorption. The present study was undertaken to define the mechanism underlying this renal acidification on a membrane molecular level by the use of isolated brush border membrane vesicles.

Membrane vesicles from shark kidney were prepared by a modification of the calcium precipitation method as previously described (Kinne-Saffran et al., Bull. MDIBL 24:61, 1984). Alkaline phosphatase, the enzyme marker for brush border membranes, was enriched 12.4±2.6 fold (mean ± S.D., n = 7); whereas Na-K-ATPase activity, the enzyme marker for basolateral membranes, was reduced to 0.66±0.32 (mean S.D., n = 6). D-glucose uptake in the presence of a NaCl or KCl gradient, as determined by a rapid filtration technique, was sodium-dependent, confirming that these vesicles are derived from the proximal tubular segment.

Sodium-proton exchange activity was monitored by a rapid filtration technique. In the presence of an outwardly-directed $H^+$ gradient, a marked stimulation in $^{22}Na^+$ uptake was observed. The concentration of amiloride found to inhibit $Na^+$ uptake at 1 mM $Na^+$ half-maximally was $1.7 \times 10^{-5}$ M. A plot of $1/Na^+$ uptake vs. amiloride concentration yielded a straight line indicating a single amiloride inhibitory site. The kinetics of the $Na^+/H^+$ exchanger was determined by measuring the initial rate at 5 sec of the amiloride-sensitive $Na^+$ uptake. The $K_m$ and $V_{max}$ of the $Na^+/H^+$ exchanger calculated from three experiments was 9.1±0.8 mM $Na^+$ (mean ± S.D.) and 48.0±0.2 nmol·mg protein$^{-1}$·min$^{-1}$, respectively. The stoichiometry of the $Na^+/H^+$ exchanger was determined by examining the effect of the membrane potential difference on the $Na^+/H^+$ exchange system. Replacement of nitrate with gluconate in the extravesicular medium had no effect on the amiloride-sensitive sodium uptake. When D-glucose uptake was measured under similar conditions, Na-D-glucose cotransport was stimulated 4.8 fold by nitrate as compared to gluconate. This suggests that the $Na^+/H^+$ exchange under these experimental conditions is electroneutral.

We conclude that an electroneutral $Na^+/H^+$ exchanger with properties similar to that found in mammalian kidney is also present in the spiny dogfish. Its contribution to the urinary acidification of this marine animal remains to be established.

---

supported by DFG grant Ki 333/2-1