PRESENCE OF A Na^+/H^+ EXCHANGER IN BRUSH BORDER MEMBRANES ISOLATED FROM DOGFISH (SQUALUS ACANTHIAS) KIDNEY

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The renal acid secretion in the dogfish, in contrast to acid secretion in mammals, is independent of carbonic anhydrase and involves intracellular protolysis not involving CO_2 buffering of OH^- (Swenson and Maren, Am. J. Physiol. 250:F288-F293, 1986). This significant difference prompted us to investigate the question whether the proton translocating mechanisms in shark and mammalian kidney are identical. In order to elucidate this question brush border membrane vesicles were isolated from the dorsal part of dogfish kidneys by a differential calcium precipitation method described earlier (Kinne-Saffran et al., Bull. MDIBL 24:61-63, 1984). The vesicles were enriched 20 fold in the brush border membrane marker enzyme alkaline phosphatase and insignificantly contaminated with basal lateral plasma membranes or intracellular membranes.

Activity of the Na^+/H^+ exchanger was monitored in two ways. Using the pH sensitive fluorescent dye acridine orange the effect of sodium on the intravesicular pH was monitored as described previously (Shetlar and Towle, Bull. MDIBL 26:125-127, 1986). Sodium stimulated in a dose dependent manner proton translocation across the vesicle membrane with an apparent affinity of 82 mM/l. Sodium dependent proton transport was almost completely inhibited by $5x10^{-4}$ M amiloride. The apparent stoichiometry of the Na^+/H^+ exchanger derived from the sodium dependence was 1 sodium/1 proton.

As further test for the presence of a Na^+/H^+ exchanger in the vesicles ^{22}Na uptake was investigated (Zadunaisky et al., Exp. Eye Res., in press). At an inside pH of 6.1 and an outside pH of 8.1, initial rate of sodium uptake was 2 fold higher than in the absence of a proton gradient. Proton gradient dependent sodium uptake exhibited a slight (40%) overshoot and was completely abolished by 0.1 mM amiloride. In the absence of a pH gradient amiloride had no effect on sodium uptake.

These results demonstrate that it is possible to isolate functionally intact plasma membrane vesicles from shark kidney which probably are derived from the brush border of proximal tubular segments. The data also suggest that these membranes possess a Na^+/H^+ exchange system with properties similar to that of the Na^+/H^+ exchanger found in mammalian kidney.

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