PHYSIOLOGICAL AND BIOCHEMICAL EVIDENCE FOR H^+/K^+ ATPase MEDIATED RENAL ACID SECRETION IN THE ELASMOBRANCH

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The kidneys of marine elasmobranchs lack carbonic anhydrase (CA) (Hodler et al., Am J Physiol, 183:155,1955). Despite this, they consistently acidify the urine under a variety of conditions (Swenson and Maren, Am J Physiol. 250:F288,1986). During high rates of stimulated acid secretion and HCO3- reabsorption, less than 10% of H^+ formation or OH^- dissipation can be accounted for by the known uncatalyzed reaction rates of CO_2 in the renal tubule (Swenson and Maren, ibid). It has been our goal to elucidate the mechanisms of CA and CO_2 independent H^+ secretion and HCO_3^- reabsorption in marine fish. Insights into these processes may lead to a better understanding of acid-base transport in the mammalian kidney under conditions such as CA inhibition and in segments of the nephron which lack the enzyme.

Little is known about the actual membrane events involved in elasmobranch renal acid secretion. Recently Bevan et al. (J Comp Physiol B. 159:339,1989) demonstrated that renal proximal brush border membrane vesicles of the dogfish are capable of Na^+/H^+ exchange. This process is saturable and inhibited by amiloride. However, direct evidence supporting a role for a luminal membrane Na^+/H^+ antiporter in acid secretion and HCO_3^- reabsorption is lacking. Amiloride did not alter luminal pH in micropuncture studies (Sibernagl et al.,Bull MDIBL 26:156,1986) or reduce urinary acid output in whole animal studies (Swenson, unpublished data). These lack of effects may, however, simply represent the weak inhibitory effect of amiloride in the presence of very high physiologic $[Na^+]$.

Another membrane transporter of possible importance is an apical membrane associated proton translocating ATPase. An attractive candidate is H^+/K^+ ATPase once thought only limited to the gastric mucosa, but now demonstrated in many acid secreting epithelia; mammalian kidney (Wingo, J Clin Invest. 84:361,1989), colon (Perrone and McBride, Pflugers Arch. 416:632, 1990), and lung (Boyd et al., J Physiol. 325:46P,1991), amphibian kidney (Planelles et al., Am J Physiol. 260:F806,1991), and jejunum (White, Am J Physiol. 248:G256,1985) and turtle urinary bladder (Sabatini et al., Clin Res 38:984A,1991). In these tissues, the enzyme is inhibited by SCH 28080, a highly specific inhibitor of mammalian gastric H^+/K^+ ATPase. Therefore, we set out to determine whether renal acid secretion in the elasmobranch involves an H^+/K^+ ATPase.

Spiny dogfish, Squalus acanthias (males, weight range 1.8 - 2.2 kg) were studied 12 to 16 hours after transfer into small plexiglass tanks and placement of caudal artery and urinary papilla catheters. The effects of SCH 28080 were studied under two conditions: a) unstimulated basal acid secretion and b) stimulated acid secretion. In the first group, a continuous infusion of elasmobranch Ringer's solution was begun at 15 ml/h-kg. Thereafter hourly urine samples were collected and measured for pH, volume and titratable acid (TA) concentration. Arterial blood gas samples were also analyzed for pH and PO_2 and total CO_2 content at two hour intervals to document stable oxygenation and acid base status. After two hours, the fish were either given SCH 28080 (62 mg/kg) disolved in 5 ml of ethanol (n=5) or 5 ml of ethanol (n=4) as a control. These were given over 30 minutes by a constant infusion pump and measurements were continued

over the next 3 hours. In the second group, renal acid secretion was stimulated by a constant infusion of 225 mM imidazole in Ringer's at 7.5 ml/h-kg, which we have shown previously to increase acid output 5-10 fold (Swenson and Maren,ibid). After three hours, either 62 mg/kg SCH 28080 dissolved in ethanol (n=5) or ethanol alone (n=5) were given as described above. The dose of SCH 28080 was calculated to achieve a concentration of roughly 0.5 - 1.0 mM if distributed into total body water.

Luminal membrane fractions from dogfish kidney were prepared by a modified calcium precipitation method (Kinne-Saffran et al., Bull MDIBL 24:61,1984). Alkaline phosphatase, a brush border membranemarker, was enriched approximately 12 fold whereas Na^+/K^+ ATPase, a basolateral membrane marker was reduced 0.66. H^+/K^+ ATPase activity in these membranes was measured as the rate of K^+ stimulated ATP hydrolysis in the presence of ouabain. The assay conditions were as follows: 20 mM HEPES, 3 mM Tris-ATP, 2 mM ouabain at pH 7.0 and varying amounts of KCl from 10 -100 mM. ATP hydrolysis was determined by the release of inorganic phosphate (Pi). Incubation time was 30 minutes at 25 degrees C. Several cations were tested to examine the specificity of K^+ in stimulating this ATPase activity. The inhibitory effects of SCH 28080 on the stimulation by 75 mM KCl were studied at 0.5 mM.

In unstimulated fish, SCH 28080 increased urinary pH from 5.81 to 6.25; reduced urine flow from 0.9 to 0.3 ml/h-kg and reduced urinary [TA] from 34 to 16 mEq/l. Urinary pH, flow and [TA] in the control (ethanol alone) fish were not altered. Figures 1 and 2 show equally marked reductions (87% and 77%, respectively) in the rate of titratable acid secretion with SCH 28080 in unstimulated and imidazole stimulated fish.

In the absence of K^+, the renal plasma membrane fractions had an average ATPase activity in the presence of Mg^{++} of 6.8 $\pm$ 1.2 umol Pi/h-mg protein. Potassium at: 10 mM increased this activity by 13.4 $\pm$ 6.3%, at 50 mM by 27.9 $\pm$ 5.5%, at 75 mM by 32.0 $\pm$ 8.3 % and at 100 mM by 38.6 $\pm$ 8.3% (means $\pm$ SD). This concentration dependent response yields an apparent Km of roughly 25 mM. At 75 mM, RbCl caused a 38% increase, CsCl a 33% increase and LiCl a 26% increase in ATPase activity. SCH 28080 at 0.5 mM caused a 55% inhibition of the ATPase activity stimulated by 75 mM KCl.

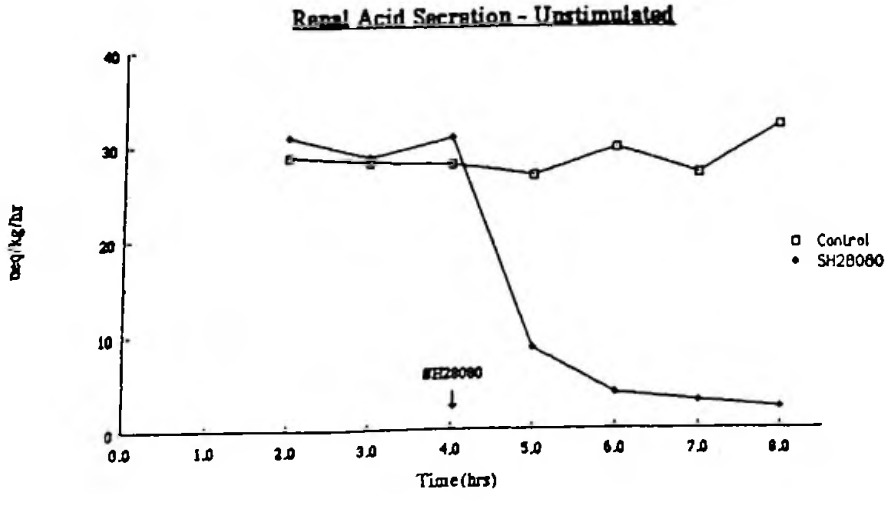

FIGURE 1

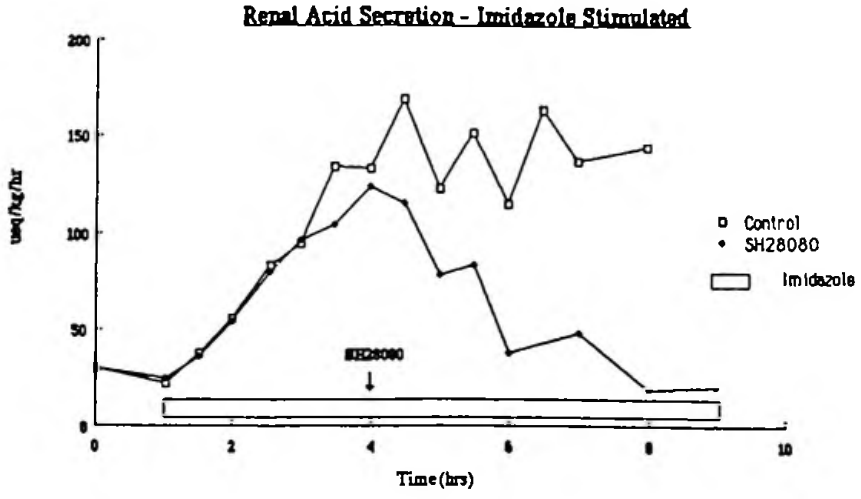

FIGURE 2

For the first time, it is possible to inhibit acid secretion in the marine fish. All previous attempts have failed including those known to decrease mammalian renal acid output such as metabolic alkalosis, CA inhibitors and amiloride. Reduction of total titratable acid output occurs as a consequence of raised pH (lower [TA]) and reduced flow. We did not measure urinary phosphate, so it is not possible to determine whether inhibition of acid output in the unstimulated state is solely an effect of reduced phosphate secretion. Under other conditions (such as phosphate loading) it appears tightly linked to acid output and could be conceived to be primary with H^+ following secondarily. However, the increase in urinary pH and reduction in stimulated H^+ output with imidazole (when it becomes the predominant urinary buffer) are more consistent with a direct SCH 28080 effect on H^+ secretion. These physiological data are supported by our biochemical studies revealing a brush border membrane K^+ stimulated, ouabain insensitive, ATPase activity reduced by a known inhibitor of H^+/K^+ ATPase. It is clear, however, that this putative enzyme differs from that in the mammal and amphibian. H^+/K^+ ATPases from these vertebrate classes, have a lower Km for K^+ (0.5-1.0 mM) and are more sensitive to K^+ than other univalent cations.

Our findings point to a major role of renal H^+/K^+ ATPase in acid secretion in marine elasmobranchs. It is conceivable that the H^+/K^+ ATPase we have demonstrated in the shark developed early in vertebrate evolution and that selectivity and sensitivity for K^+ arose later. Inhibition by SCH 28080 and preliminary evidence that multiple segments of the skate nephron stain specifically along the apical (luminal) membrane with antibodies against two portions of the alpha chain of hog gastric H^+/K^+ ATPase (Swenson et al, FASEB J 1992, in press) suggest that despite these differences some features of this proton translocating ATPase have been preserved over time. Several aspects of elasmobranch renal function and acid-base regulation are possibly explained by H^+/K^+ ATPase mediated acid secretion. The first is that the enzyme generates a proton for transport directly in the hydrolysis of ATP, thus eliminating any need for CA and CO_2. Furthermore, since we have discovered a means to inhibit renal H^+ secretion, it will be possible to test directly whether bicarbonate reabsorption is mediated via H^+ secretion or by some independent mechanism of direct ionic transport. Lastly, H^+/K^+ ATPase mediated acid secretion may explain the tight linkage of acid and phosphate excretion and provide a mechanism for potassium reabsorption in the elasmobranch kidney, a process surprisingly neglected in fish renal physiology.

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