EFFECTS OF H^+/K^+ ATPASE INHIBITION ON RENAL AND BRANCHIAL ACID-BASE METABOLISM IN THE DOGFISH SHARK, SQUALUS ACANTHIAS

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The kidneys of marine elasmobranchs lack carbonic anhydrase (CA) (Maren, Physiol. Rev. 47: 595-781,1967) and yet consistently acidify the urine under a variety of conditions (Swenson and Maren, Am. J. Physiol. 250:F288,1986). In particular, bicarbonate reabsorption can rise to many times its basal rate when fish are given an acute intravenous bicarbonate load. We showed last year (Swenson et al., Bull. MDIBL 31:105-107,1992) that basal and stimulated renal titratable acid secretion could be suppressed 80-90% with SCH-28080, a specific inhibitor of mammalian H^+/K^+ ATPase. The role of an apical membrane H^+/K^+ ATPase was further confirmed by immunocytochemistry and by the presence of a potassium-stimulated ouabain-insensitive Mg^{++} ATPase activity in renal brush border membranes. We sought to determine whether renal bicarbonate reabsorption is mediated also by this same mechanism.

The mechanisms of acid-base transfer in the gills of elasmobranchs are largely unknown although, in contrast to the kidneys, CA is present and its inhibition impairs some aspects of acid base regulation (Swenson and Maren, Am. J. Physiol. 253:R450-R458,1987). In conjunction with our renal experiments, we investigated whether SCH 28080 affects the rate at which the gills correct metabolic alkalosis and metabolic acidosis to determine whether this proton translocating ATPase is present in the gills.

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 as described previously (Swenson and Maren, 1986 and 1987).

The effects of SCH 28080 on bicarbonate reabsorption and correction of metabolic alkalosis were studied at two doses; 5 mg/kg and 62 mg/kg, both of which suppress stimulated renal acid secretion in the dogfish (Swenson et al., 1992, ibid) and gastric acid secretion in mammals. In basal conditions, 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) and total CO_2 concentrations. Arterial blood gas samples were also analyzed for pH and PO_2 and total CO_2 concentration at one hour intervals to document stable oxygenation and acid base status. Urine pH and titratable acid were measured by a glass electrode and total CO_2 by a microgasometer (Kopp-Natelson). Arterial PO_2 and pH were measured on a blood gas analyzer (Cameron Instruments) and total CO_2 by microgasometer. After two hours, the fish were either given SCH 28080; 62 mg/kg (in 5 ml of ethanol) or 5 mg/kg (in 1 ml of ethanol). These were given over 30 minutes by a constant infusion pump and measurements were obtained over the next 3 hours. In a second group, metabolic alkalosis was induced and renal bicarbonate reabsorption was augmented by a one hour constant infusion of 1 M sodium bicarbonate (9 mEq/kg), which we have shown previously to increase bicarbonate reabsorption by 12 fold (Swenson and Maren, 1986). At the end of the bicarbonate infusion either 5 or 62 mg/kg SCH 28080 dissolved in ethanol were given as described above. We did not use an ethanol placebo because we showed recently (Swenson et al.,1992 ibid) that an equivalent 5 ml dose of ethanol given over 30 minutes did not affect blood pressure or arterial oxygenation or acid-base status.

Urinary and blood acid base parameters were obtained for the next three hours. Lastly, in a third group of fish, metabolic acidosis was induced by a one hour infusion of 1 mEq/kg HCl and SCH 28080 was given in a dose range of 2-125 mg/kg to study its effects on rate of normalization of plasma HCO_3^-.

TABLE 1

Effects of SCH 28080 on plasma and urine acid-base values at 2 hours after drug administration in either saline or bicarbonate loaded fish

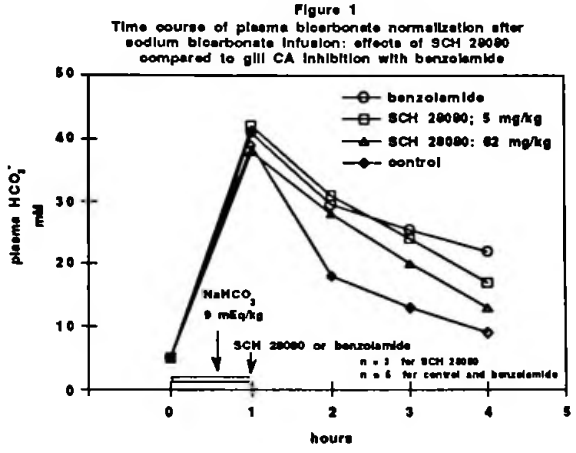
	plasma HCO_3^-	urine pH	urine T_{CO2}	urine V_{TA}
	mM		mM	uEq/kg·hr
Control				
saline (n=5)	6.1 (0.3)	5.80 (0.04)	<1	27 (8)
bicarbonate (n=5)	13 (1.2)*	5.83 (0.03)	<1	36 (10)
SCH 28080 (5 mg/kg)				
saline (n=3) **	6.4 (0.3)	5.84 (0.04)	<1	25 (10)
bicarbonate (n=5) **	24 (1.3)*	6.32 (0.06)*	5 (0.5)*	10 (4)*
SCH 28080 (62 mg/kg)				
saline (n=4)	6.2 (0.3)	6.29 (0.04)	2 (0.5)	5 (10)
bicarbonate (n=4) **	20 (1.5)*	6.42 (0.07)*	4 (0.5)	17 (10)

values are means (± SD)
* $p < 0.05$ by t test
** present data, other data are taken from Swenson and Maren, 1986 and 1987; and Swenson et al., 1992 (see text)
T_{CO2} = total CO_2, V_{TA} = titratable acid output

The data on the effects of SCH 28080 on plasma HCO_3^- and urinary acid base values are shown in Table 1. Also included are appropriate control data when necessary from our earlier studies (Swenson and Maren,1986 and 1987) utilizing equivalent protocols. Under basal conditions (no bicarbonate loading), SCH 28080 raised urinary pH and decreased titratable acid excretion only at the higher dose. With bicarbonate loading and generation of a metabolic alkalosis, SCH 28080 at both doses resulted in a slower rate of plasma bicarbonate normalization than compared with the normal rate.

Figure 1 shows the time course of plasma HCO_3^- normalization with 2 doses of SCH 28080 and compares them to the rapid normal (control) rate and to the suppressed rate of normalization by gill CA inhibition with benzolamide (Swenson and Maren,1987). There is no statistically significant difference between the two SCH 28080 doses and at least at two hours their effect is equivalent to benzolamide. These data are consistent with an effect of SCH 28080 on branchial HCO_3^- clearance since the kidneys are not capable of any quantitative excretion of bicarbonate (Swenson and Maren,1986).

It would appear from the urinary data in Table 1 that SCH 28080 also inhibited renal bicarbonate reabsorption since both doses caused urine pH to rise and in the case of the higher dose titratable acid excretion also fell. However, due to the greater sustained plasma HCO_3^- elevation with SCH 28080 (Figure 1), the filtered load of bicarbonate likely exceeded even the normal capacity of the kidneys to achieve total reclamation. We have previously shown that bicarbonaturia develops whenever HCO_3^- filtration rates surpass 80 uEq/hr·kg in the normal fish (Swenson and Maren,1986). Assuming normal GFR in the SCH 28080 treated fish, these HCO_3^- filtration rates were exceeded long enough to cause some HCO_3^- to appear in the urine as we have shown with gill CA inhibition after a bicarbonate load (Swenson and Maren,1986). It is important to realize that the magnitude of HCO_3^- appearance in the urine with SCH 28080 during bicarbonate loading is trivial since it represents only a 2-4 % excretion of the filtered load. Thus we interpret these data to indicate no role for H^+/K^+ ATPase in renal bicarbonate reabsorption.



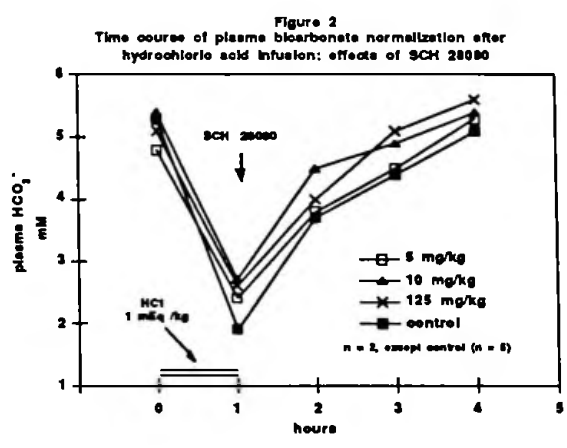


Figure 2 shows the time course of plasma HCO_3^- normalization after generation of a metabolic acidosis with HCl over a broad dose range of SCH 28080. No dose was capable of altering the very rapid branchial disposal of acid. Because we found an effect of SCH 28080 on branchial bicarbonate excretion in metabolic alkalosis suggesting the presence of H^+/K^+ ATPase, it was somewhat surprising to find that the gill does not appear to utilize this very powerful mechanism to excrete acid as it does in the kidney. Since we did not measure acid or base excretion into seawater directly, we can not rule out an inhibition by SCH 28080 on intracellular buffering mechanisms in metabolic alkalosis.

In conclusion, our findings demonstrate a role of H^+/K^+ ATPase in renal acid secretion but not bicarbonate reabsorption in marine elasmobranchs. The mechanism of bicarbonate reabsorption remains to be discovered. It may be very likely dependent upon apical membrane Na^+/H^+ exchange since Bevan et al. (J. Comp. Physiol. B 159:339-347,1989) have shown in vitro evidence for this antiporter in shark renal brush border membranes.

The effects of SCH 28080 on branchial correction of metabolic alkalosis and lack of effect in metabolic acidosis are most consistent with a basolateral localization of H^+/K^+ ATPase in the gill. If true, this is a novel finding in acid base transporting epithelia since H^+/K^+ ATPase is an apical membrane protein in all other tissues studied.

This work was supported by NIH grant # HL 45571 to ERS, NIH student training grant # 2-T35-HL07489 to ADR, and University of Florida research grant to THM.