MANIPULATION OF RENAL INORGANIC PHOSPHATE HANDLING BY WINTER FLOUNDER (*PLEURONECTES AMERICANUS*)

Rolf K.H. Kinne[1], Cicely A. Jette[2], Eva Kinne-Saffran[1], Chet Patel[3], Jason Mills[4] and J. Larry Renfro[5]

[1]Max-Planck-Institut fuer molekulare Physiologie, Dortmund, FRG, [2]University of Maine, Orono, ME, [3]Univ. Florida Hlth. Sci. Center, Gainsville, FL, [4]Harvard University, Cambridge, MA, and [5]Department of Physiology and Neurobiology, Univ. Connecticut, Storrs, CT

Preliminary kinetic comparisons of the sodium-phosphate cotransport system (Na^+/ P_i) in winter flounder renal brush border membrane (BBM) vesicles and the system cloned from the winter flounder kidney and expressed in oocytes (Werner et al., AJP 267:F311-F317, 1994) revealed differences which may result from the influence of expression in oocytes or may be indicative of more than one Na^+/ P_i transport system in flounder renal tubule (Kinne and Kinne-Saffran, Bulletin MDIBL 34:58-59, 1995). Different systems are possible since studies on primary cultures of flounder renal epithelium show that P_i can be either reabsorbed or secreted (Renfro and Gupta, Comp. Physiol. 7:216-240, 1990). The latter have suggested that increased capacity of a Na^+/ P_i transport process in the basolateral membranes (BLM) could be part of a P_i secretory pathway. Thus, in reabsorbing epithelia P_i may enter the cell by Na^+/ P_i cotransport at the BBM; however, in secreting epithelia Na^+/ P_i cotransport may occur at the BLM. Because these transporters may differ we are seeking ways to stimulate or suppress each of the systems to provide better characterization. In addition, to determine the physiological role of the cloned Na^+/ P_i cotransporter in the flounder nephron in vivo, its cellular location and function must be determined in the intact system. To facilitate this characterization, we have begun to establish conditions which provide tissues in the reabsorptive or secretory state in vivo.

Renal phosphate clearance studies in several fishes have revealed both net reabsorption and net secretion (Hickman and Trump, Fish Physiology 1:91-239, 1969) as well as inducible net secretion (Kaune and Hentschel, Comp. Biochem. Physiol. 87A:359-362, 1987). Several studies (Renfro, Fish Physiology, 14:147-171, 1995) indicate that pharmacological activation of protein kinase C or protein kinase A reciprocally activate net secretion and net reabsorption of P_i, respectively, in primary monolayer cultures of flounder renal epithelium. In vitro screening of two teleost hormones with this system shows that salmon stanniocalcin and somatolactin, at physiological concentrations, stimulate net transepithelial P_i absorptive flux (Lu et al., AJP 267:R1356-R1362, 1994; Lu et al., AJP 268:R577-R582, 1995). The following studies are preliminary attempts to assess the effects of these hormones on renal phosphate excretion in winter flounder.

The procedures for determination of renal clearances in winter flounder have been reported (Renfro, AJP 238:F92-F98, 1980). Following anesthetization with MS-222, heparinized polyethylene tubing (PE10) was inserted into the hemal vein near the caudal peduncle. A polyethylene tube (PE90) was inserted into the urinary bladder. Urine was collected from the urinary bladder at varying times with a syringe attached to a PE50 tube inserted through the PE90 catheter. Inulin was injected (150 mg/kg body weight) intramuscularly 24 hours prior to clearance measurements. Deproteinated plasma and urine inulin and phosphate concentrations were determined by the indole acetic acid colorimetric method (Benyajati and Dantzler AJP:R712-R720, 1986) and a modification of the Fiske and SubbaRow (J. Biol. Chem. 66:375-400, 1925) method, respectively. Stanniocalcin (STC) was isolated from salmon corpuscles of Stannius and was graciously supplied by Dr. Graham Wagner, University of Western Ontario. The hormone was given intramuscularly (~12 µg/kg body weight). Urine was collected between 8 and 24 hours following hormone injection. The values in Table 1 for controls were determined on samples collected immediately before hormone injection or calcium infusion.

Table 1
Renal phosphate clearance in winter flounder together with the effects of sSTC and intravenous infusion of calcium chloride, sufficient to raise the extracellular concentration by the amount indicated.

	Control	sSTC	Control	Δ 0.6 mM Ca
$[P_i]_{Plasma}$ (mM)	2.4 ± 0.41	2.2 ± 0.24	1.3 ± 0.22	1.6 ± 0.29
GFR (ml/kg/h)	2.2 ± 0.89	1.9 ± 0.79	0.7 ± 0.31	0.7 ± 0.19
Urine Flow Rate (ml/kg/h)	0.7 ± 0.24	0.9 ± 0.37	0.6 ± 0.25	0.6 ± 0.16
$[P_i]_{Urine}$ (mM)	4.7 ± 1.12	5.1 ± 1.07	3.9 ± 0.90	3.3 ± 0.59
Q_{Pi} Excretion (μmoles/kg/h)	3.0 ± 0.83	4.2 ± 2.03	1.7 ± 0.37	1.9 ± 0.53
Fractional Excretion	0.7 ± 0.10	1.2 ± 0.29	3.4 ± 1.05	1.8 ± 0.41

Table 1 shows the phosphate renal clearance data in control and treated flounder. Plasma $[P_i]$ varied somewhat in the control groups but was unaffected by either sSTC or calcium infusion. Average glomerular filtration rate (GFR) was similar to values reported for a variety of other marine teleost species and was consistently greater than urine flow rate; thus no net fluid secretion was apparent. Urine phosphate concentration was about 4 mM in controls and was not changed by the treatments. The net phosphate loss indicates that fasted fish are in negative phosphate balance; however, fractional P_i excretion was highly variable, ranging from 0.7 in one control group to 3.4 in the other. This variation was explained by the variability in control GFR, i.e., when filtration rates were low as in the second control group (Table 1), net P_i secretion was apparent and vice versa.

Calcium infusion had no significant effect on fractional phosphate excretion (Table 1); however, this lack of significance may be due to the very large variance. When sufficient urine volume was obtained in a shorter time interval, as shown in Figure 1, calcium infusion appeared to decrease fractional phosphate excretion.

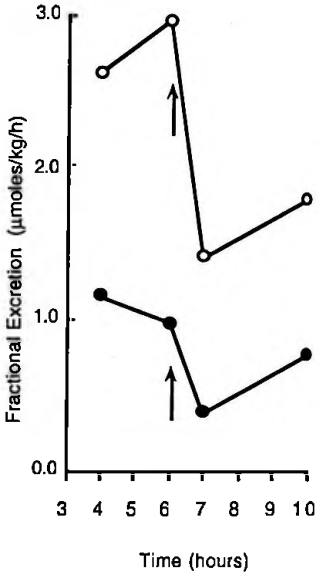

Figure 1. Two examples of the effect of infusion of $CaCl_2$ on fractional P_i excretion. The arrows indicate the time at which a bolus infusion of $CaCl_2$ (1 ml/Kg of isosmotic $CaCl_2$) was administered. The average increase in extracellular space calcium concentration was 0.6 mM.

Intramuscular infusion of sSTC clearly had no effect on relative phosphate clearance in the flounder, and further studies will be necessary to determine ways to stabilize GFR which may allow more accurate determination of fractional P_i excretion.

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