INHIBITION OF MYOCARDIAL SODIUM-POTASSIUM PUMP ACTIVITY IN THE SEA-WATER ADAPTED GOLDFISH (CARASSIUS AURATUS)

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The goldfish (Carassius auratus), a fresh water stenohaline teleost, readily survives transfer to dilute sea water. In making this adaptation, plasma sodium concentration and total exchangeable sodium content are increased; increased sodium intake via gill transfer and intestinal absorption of ingested sea water is offset by increased gill sodium efflux and by increased renal excretion of sodium (Lahlou et al, Comp Biochem Physiol. 28:1427-1433, 1969). Increased renal sodium excretion is attributable to decreased fractional reabsorption of sodium since glomerular filtration rate and the filtered load of sodium are decreased (Elger and Hentschel , Cell Tissue Res 229: 73-85, 1981).

We have examined the effects of sodium loading during sea-water adaptation on vascular and cardiac muscle sodium-potassium pump activity. Sodium-potassium pump activity was assessed by measurement of ouabain-sensitive ^{86}Rb uptake (Bernstein and Israel, J Pharm Exp Therap 174: 323, 1970). Carassius auratus (10-20 grams) were maintained in fresh water aquaria (Na^+=9.8 mEq/l, K^+=0.5 mEq/l). Sea-water adaptation was achieved by transfer directly to 1/3 sea water (Na^+=137 mEq/l, K^+=3.3 mEq/l); this transfer was not associated with any mortality or apparent morbidity. Fish were studied after 1-8 days of sea-water adaptation. After pithing, the suprabulbar aorta and heart were removed. The aorta was cleared of blood and divided into two fragments. Two adjacent slices of ventricle were sectioned with a sharp scalpel. Tissues were incubated in teleost Ringer's solution (Na^+=150 mEq/l) containing 10 nmole carrier-free ^{86}Rb Rubidium chloride with and without added ouabain (10^{-4}M), gassed with 95% O_2-5% CO_2 at room temperature, for 15 minutes. After incubation, the tissues were blotted dry and digested overnight in concentrated nitric acid, diluted with distilled water, and aliqots taken for liquid scintillation counting and protein determination by a modification of the method of Lowry exploying protein standars prepared by nitric acid digestion of crystalline albumin. Ouabain-sensitive pump activity (Na-K ATPase) was taken as the difference between ^{86}Rb uptake in the presence and absence of ouabain and was expressed as pmol ^{86}Rb/mg protein/15 min.

Results: (Table 1) Na-K ATPase activity in cardiac muscle was inversely proportional to the duration of sea-water adaptation (r= .923). Pooling data obtained in fish adapted to sea water for 1-3 days and those adapted for more than 3 days ouabain-sensitive ^{86}Rb uptake was significantly reduced (1.65±.37) after 3 days as compared with that in fresh water fish

TABLE I

	FRESH WATER	SEA WATER 1 DAY	SEA WATER 2-3 DAYS	SEA WATER 5-6 DAYS	SEA WATER 7-8 DAYS
CARDIAC MUSCLE		OUABAIN-SENSITIVE ^{86} Rb UPTAKE (pmol/mg/15 min)			
Mean	28.4	29.8	21.0	20.2	12.1
S.D.	15.1	19.7	9.7	11.2	15.5
		OUABAIN-INSENSITIVE ^{86} Rb UPTAKE (pmol/mg/15 min)			
Mean	23.0	29.1	22.6	23.3	30.1
S.D.	7.4	7.8	3.3	4.7	10.9
n	16	9	11	7	6
AORTA		OUABAIN-SENSITIVE ^{86}Rb UPTAKE (pmol/mg/15 min)			
Mean	16.4	6.3	12.8	19.1	23.0
S.D.	13.5	15.4	21.4	16.1	20.7
		OUABAIN-INSENSITIVE ^{86}RB UPTAKE (pmol/mg/15 min)			
Mean	24.2	28.9	26.6	30.8	29.6
S.D.	8.3	15.1	17.0	11.0	5.1
n	12	8	8	6	5

(2.84±.38, $p<.03$). Ouabain-sensitive ^{86}Rb uptake, reflecting the passive entry of rubidium into the tissue, was not different in cardiac slices from fresh water and sea water adapted fish. No significant changes were found in aortic Na-K ATPase activity in sea-water adapted goldfish. Plasma sodium concentration increased from 149 mEq/l in fresh water, to 179 mEq/l after 1-3 days and 189 mEq/l after 4-8 days of sea-water adaptation.

The finding that sodium loading during sea-water adaptation results in inhibition of sodium-potassium pump activity (Na-K ATPase activity) in cardiac muscle but not in aortic smooth muscle suggests that pump inhibtion is not a non-specific response to sea-water transfer. Studies by Haddy and others (Hypertension 3: Suppl II 96-101, 1981) have provided evidence that salt loading in the rat and dog is associated with inhibition of vascular and myocardial Na-K ATPase activity mediated by a circulating inhibitor of Na-K ATPase ("endoxin"). It has been suggested that this inhibitor of Na-K ATPase might mediate the decrease in renal tubular sodium reabsorption which serves to maintain sodium homeostasis in the salt loaded animal. While active gill secretion of sodium, by a transport system which is dependent on Na-K ATPase and is inhibitable by ouabain, appears to play an important role in the adaptation of euryhaline marine teleosts to sea water, the importance of such an adaptive gill mechanism in euryhaline or stenohaline freshwater teleosts has been questioned (Evans in Fish Physiology Vol XB, Hoar and Randall, Editors Academic Press, 1984). The data of Lahlou et al (Comp Biochem Physiol 28:1427-1433, 1969) suggest that in the goldfish, inhibition of renal tubular sodium reabsorption may be the major mechanism for sea-water adaptation. Further studies will be required to establish whether pump inhibition in the sea-water adapted goldfish is mediated by a circulating factor, whether there are tissue-specific recpetors for such a putative inhibitor of Na-K ATPase, and whether renal sodium reabsorption is modulated by a circulating inhibitor of the sodium pump.

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