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PHARMACOLOGIC INTERFERENCE WITH OSMOREGULATION IN THE EEL (Anguilla rostrata): OBSERVATIONS ON ETHACRYNIC ACID, ACETAZOLAMIDE, AND OUABAIN

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Osmoregulation in fresh water teleosts is accomplished by renal conservation of sodium with excretion of dilute urine and net absorption of sodium and chloride by the gill if these ions are present in the environment. In saltwater, teleosts swallow large amounts of seawater, sodium and water are reabsorbed by the gut, and the excess sodium is actively extruded by the gill. Renal function in saltwater is characterized by marked reduction in GFR and excretion of a low volume isotonic urine. Since characterization of the specific ion pumps involved in osmoregulation is incomplete, preliminary studies were performed using three agents known to inhibit ion transport under certain conditions.

ETHACRYNIC ACID (EA)

Ethacrynic acid (2 mg/ 150 gm eel) was injected into saltwater and freshwater eels after obtaining a baseline plasma sodium in each animal. A second plasma sodium was obtained four hours after ethacrynic acid. In a second experiment, ethacrynic acid (same dose) was injected every twelve hours x four doses in seven saltwater and six freshwater eels, and plasma sodiums were obtained four hours after the last dose. Plasma sodium concentrations were measured in control saltwater and freshwater eels. The results (Table 1) indicate that one dose of ethacrynic acid in saltwater eels increased mean

TABLE 1

EFFECT OF ETHACRYNIC ACID (SINGLE AND MULTIPLE DOSES) ON PLASMA SODIUM IN SW AND FW EELS

GROUP	CONTROL		AFTER ETHACRYNIC ACID*			
	n	*Plasma* Na^+ *(meq/L)*	*n*	*Plasma* Na^+ *(meq/L)*	Δ *Plasma* Na^+ *(meq/L)*	*t test*** *p*
SW eels—1 dose E.A.	3	151 ± 2.6	3	163 ± 0.8	+ 12	< .005
SW eels—4 doses E.A.	20	157 ± 2	7	174 ± 6.0	+ 17	< .01
FW eels—1 dose E.A.	3	145 ± 4.9	3	139 ± 2.4	- 6	ND
FW eels—4 doses E.A.	9	148 ± 3.0	6	114 ± 7.0	- 35	< .001

* All samples drawn 4 hours after last ethacrynic acid injection (2 mg/150 gm eel, IM) given once or every 12 hours X 4.

** t test on control plasma Na^+ vs plasma Na^+ following E.A.

plasma sodium by 12 mEq/l (151 to 163 mEq/L) and four doses resulted in a further slight increase (mean 174 mEq/L). Eels given four doses of ethacrynic acid were observed to have watery diarrhea and on sacrifice most had distended, fluid-filled intestinal cavities. A single dose of ethacrynic acid to freshwater eels had little effect on plasma sodium, but prolonged administration produced severe hyponatremia (mean 114 mEq/L). The intestines of freshwater eels appeared grossly normal.

METHAZOLAMIDE

Plasma sodium was measured before and after methazolamide in four saltwater-adapted eels. Two animals received methazolamide 15 mg/Kg, and two received 150 mg/Kg in 24 hours. No change from baseline plasma sodium was noted. In other experiments, gill sodium efflux was measured with Na^{22} in five saltwater eels given 45-60 mg/Kg methazolamide one hour before flux measurement (Table 2). Sodium efflux in all experimental animals (mean 604 $\pm$ 31.7 mEq/100 gm/1

TABLE 2

GILL SODIUM EFFLUX IN SW EELS RECEIVING
45-60 mg/Kg METHAZOLAMIDE ONE HOUR BEFORE FLUX

EEL #	CONDITIONS	PLASMA CLEARANCE Na^{22}	PLASMA Na^+ mEq/L	Na^+ EFFLUX uEq Na^+/100 gm/hour
	Methazolamide			
42	60 mg/Kg	3.30	105	544
45A	60 mg/Kg	3.42	151	516
61	45 mg/Kg	3.74	167	625
68	50 mg/Kg	4.25	158	671
84	50 mg/Kg	4.14	101	666
		3.77 ± 0.19	160 ± 2.8	604 ± 31.7
41	same day control	8.64	173	1495
46A	same day control	5.96	153	911
62	same day control	5.39	155	835
67	same day control	4.90	156	764
		6.22 ± 0.83	159 ± 4.6	1001 ± 167
	other salt water controls (n=8)			929 ± 50
p	(Methazolamide vs. same day control)	< .025	ND	< .025

hour) was lower than both simultaneous saltwater controls and other saltwater eels studied on different days.

OUABAIN

Gill sodium efflux was measured 7 hours after im. injection of ouabain (200 $\mu g/Kg$) in two saltwater eels and in two untreated eels from the same stock (Table 3). Hypernatremia and marked reduction in gill sodium efflux occurred in both eels receiving ouabain.

TABLE 3

EFFECT OF OUABAIN (200 $\mu g/Kg$) ON GILL Na^+ EFFLUX IN SALTWATER EELS

EEL #	CONDITIONS	PLASMA CLEARANCE Na^{22} ml/100 gm/Hr	PLASMA Na^+ mEq/L	Na^+ EFFLUX $\mu Eq/100$ gm/Hr
106	SW adapted + ouabain	1.50	170	255
108	SW adapted + ouabain	2.39	164	391
104	SW adapted, control	4.11	144	591
105	SW adapted, control	5.01	151	756

The first experiments indicate that ethacrynic acid is capable of interrupting osmoregulation in both saltwater and freshwater eels. In saltwater there is an adaptive increase in the activity of Na-K-ATPase in both gill and gut (Epstein, Bull. MDIBL 8:32, 1968) in association with the increased sodium transport by these tissues. Ethacrynic acid has been shown to inhibit sodium transport across numerous epithelial tissues including the rat, rabbit, and hamster small intestine and isolated toad urinary bladder. The mechanism of this inhibition is presently unclear. The findings of hypernatremia and diarrhea in the saltwater eel receiving ethacrynic acid suggest interference by this agent on both gut and gill sodium transport. Suppression of gill efflux will require confirmation by flux measurements. The profound hyponatremia resulting from ethacrynic acid in freshwater eels suggests an inhibition of renal sodium reabsorption with a resulting naturesis. Ethacrynic acid may therefore interrupt saltwater and freshwater osmoregulation by inhibition of sodium transport in both environments.

Maetz has reported (Phil. Trans. Roy. Soc. Lond. B 262:209, 1971) a higher activity for carbonic anhydrase in the saltwater eel gill compared to freshwater and Kunau (Clin. Res. 19:536, 1971) has recently demonstrated inhibition of NaCl reabsorption in the rat proximal tubule following carbonic anhydrase inhibition by benzolamide. The dose of methazolamide used in our experiment was sufficient to inhibit completely red cell carbonic anhydrase (Maren, Bull. J.H.H. 95:244, 1954). The reduction in gill sodium efflux observed in these preliminary experiments suggests that carbonic anhydrase activity may be necessary for maximum sodium efflux in saltwater.

Our demonstration of ouabain inhibition of gill sodium efflux is consistent with the action of ouabain in inhibiting both Na-K-ATPase and sodium transport in many tissues. Zangg (Comp. Biochem. Physiol. 388:501, 1971) has recently demonstrated ouabain inhibition of Na-K-ATPase activity by 40-50% in microsomes of freshwater Coho salmon and by 80-90% in saltwater salmon, using serial sampling of gills from each fish as controls. Kamiya (Biochem. Physiol. 26:675, 1968) had previously demonstrated that isolated gills from ouabain-injected saltwater eels lost their capacity to extrude sodium when incubated in saltwater in comparison to control saltwater gills. The present experiments demonstrate for the first time that ouabain may inhibit sodium efflux across the gill *in vivo*, suggesting that Na-K-ATPase may be rate limiting for gill sodium transport.

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ECOLOGICAL SIGNIFICANCE OF SHELL AVAILABILITY TO SHELL SELECTION IN HERMIT CRABS

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Different species of hermit crabs show preferences for different types of mollusc shells (Reese, E.S. 1963 Behavior 21:78-126). In the Frenchman Bay area immature *Pagurus acadianus* and *P. pubescens* may compete for shells of *Littorina*, *Thais* and smaller *Buccinum*. The larger size of mature *P. acadianus* which occupy large *Buccinum*, *Polinices* and *Neptunea* shells (Lindsey and Grant 1970 MDIBL Bulletin) probably precludes inter-specific competition with adults of the smaller *P. pubescens* species.

The results of laboratory investigations designed to test the influence of shell availability on preference are given in Table I. The ratio of *Littorina/Thais* shells varied with each test. In only two

Table I. Shell availability studies: results of twelve hour selections by individual crabs presented with empty shells in different species ratios. All animals small intermediates.

	P. acadianus			*P. pubescens*		
Ratio Littorina/Thais	No. Trials	Littorina	Thais	No. Trials	Littorina	Thais
5/2	12	11	1	10	7	3
2/5	12	9	3 P<0.05	12	2	10
4/4	13	11	2 P<0.02	15	9	8

Table II. Selection of shells with colonies of Hydractinia. Each trial represents a choice made by a crab in its home shell presented with a hydractinia covered shell of appropriate size. L = Littorina; T = Thais; B = Buccinum. Shell types. H = Hydractinia

P. acadianus					*P. pubescens*				
No. trials	Test: home shell/	hydractinia shell	Select home shell	Select H shell	No. trials	Test: home shell/	hydractinia shell	Select home shell	Select H shell
15	B	B	2	13	6	B	B	6	0
10	L	L	0	10	12	L	L	11	1
3	L	P	0	3	4	T	L	4	0
					4	L	T	4	0

test situations both involving *P. acadianus* was the ratio of *Littorina/Thais* occupied, significantly different from the null hypothesis that no preference occurs. Another series of tests (Table II) showed that *P. acadianus* has a strong tendency to leave home shells if presented with shells in the appropriate