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RAPID COMMUNICATION ZIPGRAM

PROSTAGLANDIN E_2 INHIBITION OF SODIUM TRANSPORT IN THE FRESHWATER MUSSEL (1)

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ABSTRACT Ligumia subrostrata (Say) will maintain a steady state for Na when acclimated to an artificial pondwater. Prostaglandin E_2 (0.6 µM/g dry tissue) injected into the blood cause an 80% reduction of Na influx and the animals experience a net loss of Na. Injections of indomethacin (0.1 µM/g dry tissue), a prostaglandin synthetase inhibitor, cause a doubling of Na influx relative to control animals. Prostaglandin-like material can be extracted from the blood of L. subrostrata and may be a component of the endocrine control of ion regulation in freshwater bivalves.

Freshwater bivalves maintain their body fluids hyperosmotic to the environment with Na being the principal cation. Blood Na concentration ranges between 15-25 mM/l in the various freshwater mussels which have been studied (Dietz, '77, '79). Recently, we observed that freshwater mussels display a diurnal rhythm in blood Na concentration (Yeider and Dietz, '78). We noted that the changes in blood ion concentration correlated with changes in rates of Na transport suggesting an endogenous control mechanism. In addition, a transient stimulation of Na transport has been observed in the mussel Margaritifera hembeli in response to handling (Dietz, '79). These studies suggest a hormonal control mechanism is functioning in Na regulation. Since prostaglandins have been reported in marine bivalves (Freas, '78) we have examined the effects of prostaglandins on Na transport in freshwater mussels. In this report, we present evidence that prostaglandins are capable

of inhibiting Na transport in the freshwater unionid Ligumia subrostrata.

MATERIALS AND METHODS Ligumia subrostrata were obtained from local ponds and acclimated to an artificial pondwater (0.5 or NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.05 KCl in mM/l). The mussels were rinsed for 30 min. in distilled water before injection of the drugs. All drugs were injected into the anterior foot tissue, and the animals were returned to distilled water for equilibration. Those animals that opened within 1 hour after the injection were used for the transport studies. For the flux studies, each animal was placed in a [illegible] container of 0.5 mM $^{22}Na_2SO_4$. Bath samples were taken on an hourly basis. Sodium concentrations were determined by flame photometry and radioactivity was determined with a liquid scintillation counter (Triton-X 100, p-terphenyl, toluene counting fluid). The animals were shucked, dried 90-100° C and weighed. The drugs used were indomethacin (Sigma), arachidonic acid (Sigma), and prostaglandin E_2 (Upjohn).

The net flux (J_n) for sodium was determined by the changes in the sodium concentration of the bath. The unidirectional influx (J_i) was determined from the disappearance of ^{22}Na from the bathing media (Dietz and Branton, [illegible]) the efflux (J_o) was estimated by the difference:

$$J_n = J_i - J_o.$$

Blood was collected from animals by pericardial puncture (Fyhn and Costlow, '75). Samples were centrifuged at 8000 X g for 10 minutes at 2-3° to prepare them for prostaglandin extraction (Unger et al., '71). The resulting extract was separated and visualized on chromatograms (Woods and Jocoy, [illegible]). The standards used to identify the unknown extraction products of the blood were arachidonic acid, PGE_2, PGE_1, PGF_{2a} (Upjohn).

The accuracy of the extraction procedure was checked by the percent recovery of tritium in blood samples spiked with ^3H-arachidonic acid (American

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TABLE 1

effect of drug treatments on the flux rates of sodium from 0.5 mM NA_2SO_4

Injection (µl/g dry tissue; N)	µEq/g dry tissue-hr ($\bar{X}$ ± SE)		
	J_n	J_i	J_o
ETOH[a] (.2 ± 0.02; 29)	0.32 ± 0.25	1.53 ± 0.17	1.21 ± 0.18
domethacin (.0 ± 0.01; 12)	2.49 ± 0.32**	3.34 ± 0.15**	0.85 ± 0.18
PGE_2 (.3 ± 0.04; 5)	-1.16 ± 0.73*	0.29 ± 0.17**	1.45 ± 0.72
chidonic Acid (.5 ± 0.41; 15)	-1.34 ± 0.37**	1.45 ± 0.31	2.79 ± 0.53**

ontrols for the different treatments were not significantly different
ed were pooled.
<0.05
<0.01

extract of spiked blood was separated and visualized on a chromatogram.
ximately 4 mm sections of the 80 mm chromatogram strip were scraped into
idual counting vials and counted to determine the radioactive R_f values.
Differences between groups were determined using the student "t" test
ere considered significant if $P<0.05$.

RESULTS Injections of indomethacin caused a significant stimulation
e net flux of sodium compared to control animals injected with either
nol or distilled water vehicles (fig. 1). The indomethacin treated animals
erienced a net uptake of Na from the pondwater bath for at least 9 hrs.
er the injection. The control animals (DW and ETOH) were not significantly
fferent and remained essentially in a steady state. All of the animals
ained in a Cl steady state.

The net uptake of Na in the indomethacin injected animals is due to a

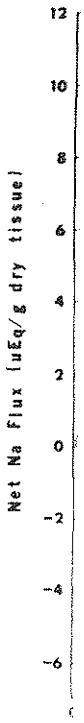
significantly increased influx of Na with no change in the efflux when compared to ethanol injected controls (table 1). Injections of PGE_2 and its precursor arachidonic acid, caused depressions of the net flux when compared to the ethanol controls. However, the losses of sodium apparently involved two different mechanisms. PGE_2 injections caused a significant decrease of the influx rate with the efflux not changed from the controls. Arachidonic acid injections caused the loss of sodium by significantly increasing the efflux with the influx remaining constant.

To determine if these freshwater mussels have endogenous prostaglandin like material, we extracted blood from animals acclimated to pondwater. The blood extracts were chromatographically identified as arachidonic acid (Rf 70) and PGE_2 (Rf 28). We frequently observed substantial spots at Rf 38 and 48 which may have been keto derivatives of prostaglandins. When arachidonic acid was added to control blood samples, the recovery of tritium labeled arachidonic acid was 86%.

DISCUSSION Sodium transport in freshwater mussels is apparently regulated in part, by prostaglandins. Indomethacin is an effective inhibitor of prostaglandin synthetase and would prevent the endogenous synthesis of prostaglandins from cellular aracidonic acid (Hansen, '74). At the dosage of indomethacin injected (about 0.1 µM/10 g wet tissue), the animals experience a positive balance for several hours. The effect of indomethacin is primarily a stimulation of the influx of Na. The action of indomethacin is specific for

FIGURE LEGENDS

1 The effect of injections of indomethacin (0.15 ± 0.02 µM/g dry tissue), 95%ETOH (10 µl/animal), distilled water (10 µl/animal) on net flux of Na. Each point represents 4 animals and the vertical line is 1 SEM.



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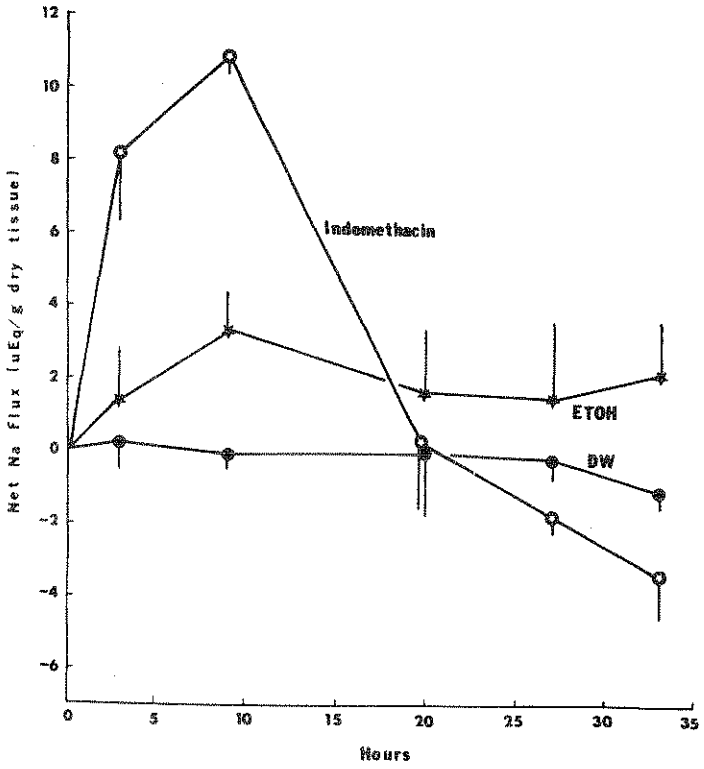

± 0.02 µM/g dry

l/animal) on net

vertical line is

Na transport since the animals simultaneously remained in a Cl steady state.

When the animals were injected with prostaglandin E_2, they experienced a net loss of Na. The PGE_2 significantly depressed the influx of Na with no effect on the efflux. These data suggest PGE_2 interacts with the epithelial cells responsible for Na accumulation from the bath. The drug apparently had no effect on the renal tissue since the urinary Na loss was unchanged.

Arachidonic acid, a precursor of prostaglandins, apparently acts indirectly by increasing the efflux. These data suggest the arachidonic acid primarily affects the renal tissue at the dose injected. Because of the rapid metabolism of arachidonic acid in bivalves, there may not have been sufficient PGE_2 synthesis in pondwater acclimated mussels to inhibit the Na influx. However, in preliminary studies using salt depleted mussels, we have observed arachidonic acid to cause a significant reduction of Na influx.

Sodium transport in freshwater bivalves is controlled by endogenous mechanisms. Previous reports demonstrated that salt depletion leads to enhanced Na influx (Dietz and Branton, '75; Dietz, '78). The data reported here suggest that prostaglandins are responsible for suppressing epithelial Na influx. In addition, there may be separate controls for the renal tissue. Prostaglandins have been noted to influence Na transport in a variety of tissues (Zins, '75; Declusin et al., '74; Lee, '74). The synthesis of prostaglandins has been reported in marine bivalves (Freas, '78) and we have extracted prostaglandin-like material from the blood of freshwater mussels. However, this is the first report of prostaglandin control of Na transport in freshwater bivalves.

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