EFFECT OF COPPER AND ZINC ON INTESTINAL CHLORIDE ABSORPTION IN THE WINTER FLOUNDER (Pseudopleurnectes americanus)

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Both copper and zinc are normally present in the marine environment at low concentrations. Increased concentrations of these heavy metals may be found when coastal waters are polluted, the winter flounder is at risk because of the pollution of its habitat. Over the past couple summers, we have investigated the effect of pH on the flounder small intestine chloride absorption (Charney et al., Bull MDIBL 25:111-113, 1985; Charney et al., Bull MDIBL 26:64-66, 1986). This year, we examined th effects of copper and zinc on intestinal chloride absorption directly and determined the effect of copper on pH-mediated changes on chloride absorption.

Winter flounder (Pseudopleuronectes americanus) small intestine was stripped of its muscle layers and mounted in modified Ussing chambers as described in Charney et al., Bull. MDIBL 25:111-113, 1985. Tissues were bathed in a teleost Ringer's solution at 15 C containing 168.5 mM Na, 5 mM K, 1.2 mM Mg, 1.3 mM Ca, 154 mM Cl, 2.7 mM PO_4, 1.2 mM SO_4, 20 mM HCO_3 and 10 mM mannitol (mucosal) and 10 mM glucose (serosal). Solutions were gassed with either 5% CO_2/95% O_2 (pH 7.11) or 1% CO_2/99% O_2 (pH 7.74) during 2 consecutive 20 min periods. Each change in metal concentration or pH was followed by a 15 min equilibration period. Unidirectional chloride fluxes (J) using ^{36}Cl were measured across paired, short-circuited tissues. Conductance (G_t) was measured by intermittent voltage pulses throughout the experiment and the I_{sc} was expressed as the equivalent ionic flux.

As shown in Table 1, in the absence of detectable levels of either copper or zinc by atomic absorption spectrophotometry, increasing the bathing solution pH increased chloride absorption. The increase in J_{net} was due to an increase in J_{ms} and to a small decrease in J_{sm}, and was accompanied by an increase in I_{sc} and G_t.

The effect of $CuSO_4$ was examined by adding increasing concentrations to either the mucosal or serosal side of the bathing solution gassed with 5% CO_2 (pH 7.11). Table 2 shows that as the mucosal concentration was increased from 10 μM to 100 μM, net chloride absorption progressively decreased. At 100 μM $CuSO_4$, net chloride secretion was observed. These transport changes were due to both a reduction in J_{ms} and an increase in J_{sm} and were accompanied by decrements in I_{sc} and G_t. The addition of 100 μM dithioethylcarbamate, to chelate the copper in solution, did not reverse these transport changes. Serosal addition of $CuSO_4$ had a much smaller and somewhat different effect on chloride absorption. Only when the serosal concentration was raised to 100 μM did net chloride absorption decrease and this decrease was due entirely to a reduction in J_{ms}. As noted in the mucosal studies, reductions in I_{sc} and G_t accompanied the changes in net chloride absorption.

We then tested the effect of increasing bathing solution pH from 7.11 to 7.74 in the presence of 100 uM $CuSO_4$. Net chloride absoption increased as pH was raised, but the increase was smaller than in the absence of $CuSO_4$ (1.28 ± 0.96 vs. 2.20 ± 0.46 μeq/cm^2*hr), and was due entirely to a decrease in J_{sm}.

In a separate series of experiments, $ZnSO_4$ was added to either the

mucosal or serosal bathing solution at pH 7.11. As shown in Table 3, mucosal concentrations of 10 μM and 50 μM (but not 100 μM) appeared to stimulate chloride absorption by increasing J_{ms}. Serosal $ZnSO_4$ did not have this effect, but at 100 μM net chloride absorption was inhibited due to a reduction in J_{ms}.

We conclude from these findings that 1. $CuSO_4$ inhibits intestinal chloride absorption and the effect of increasing pH to augment chloride absorption in this tissue. Inhibition is predominantly a mucosal effect where concentrations as low as 50 μM are effective, and inhibition is not reversed by the addition of a copper chelator. 2. $ZnSO_4$ stimulates chloride absorption at mucosal concentrations between 10 μM and 50 μM, but serosal addition of 100 μM inhibits absorption.

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Table 1. Effect of pH on the flounder intestinal chloride flux, with 20 mM HCO_3 Ringer either pH 7.11 with 36 mm PCO_2 or Ringer pH 7.74 with 7 mm PCO_2 (n=7).

pH	J_{ms} μeq/cm²*hr	J_{sm} μeq/cm²*hr	J_{net} μeq/cm²*hr	I_{sc} μeq/cm²*hr	G_t (mS/cm²)
7.11	7.09 ± 0.55	3.46 ± 0.81	3.63 ± 0.64	1.50 ± 0.19	17.0 ± 1.1
7.74	8.97 ± 0.44*	3.15 ± 0.31	5.82 ± 0.42*	2.06 ± 0.24*	19.4 ± 1.4*

Values represent the mean ± the standard error of the mean, * $P < 0.001$.

Table 2. Effect of $CuSO_4$ on the flounder intestine chloride flux, with Ringer pH 7.11 and 36 mm PCO_2 .

(μM)	n	J_{ms} μeq/cm²*hr	J_{sm} μeq/cm²*hr	J_{net} μeq/cm²*hr	I_{sc} μeq/cm²*hr	G_t (mS/cm²)
			MUCOSAL ADDITION			
0	7	7.09 ± 0.55	3.46 ± 0.81	3.63 ± 0.64	1.50 ± .19	17.0 ± 1.1
10	3	6.96 ± 0.36	4.34 ± 1.43	2.62 ± 1.53	1.12 ± .27	16.1 ± 2.3
50	6	6.88 ± 0.88	6.44 ± 0.77*	0.44 ± 0.84**	0.24 ± .27**	15.3 ± 1.2
100	7	6.17 ± 0.91	7.19 ± 1.43*	-1.02 ± 0.95**	0.24 ± .25**	14.6 ± 1.3
			SEROSAL ADDITION			
100	2	4.42 ± 0.27	2.43 ± 0.58	1.99 ± .84	0.56 ± .02	11.9 ± 0.3

A Values represent the mean ± the standard error of the mean, * = $P < 0.05$ and ** = $P < 0.01$.

Table 3. Effect of $ZnSO_4$ on the flounder intestine chloride flux, with Ringer pH 7.11 and 36 mm PCO_2.

(μM)	n	J_{ms} μeq/cm²*hr	J_{sm} μeq/cm²*hr	J_{net} μeq/cm²*hr	I_{sc} μeq/cm²*hr	G_t (mS/cm²)
			MUCOSAL ADDITION			
0	7	7.09 ± 0.55	3.46 ± 0.81	3.63 ± 0.64	1.50 ± .19	17.0 ± 1.1
10	2	9.30 ± 1.08	3.45 ± 0.33	5.85 ± 0.75	0.45 ± .33	17.3 ± 1.7
50	2	9.65 ± 1.79	4.13 ± 0.88	5.52 ± 0.91	-0.05 ± .56	17.8 ± 2.5
100	4	6.59 ± 1.17	3.29 ± 1.05	3.30 ± 0.71	0.36 ± .37	15.6 ± 2.0
			SEROSAL ADDITION			
100	3	4.85 ± 0.41	2.39 ± 0.38	2.46 ± .31	0.87 ± .09	12.5 ± 0.6

Values represent the mean ± the standard error of the mean.