

EFFECTS OF pH, BARIUM AND COPPER ON INTESTINAL CHLORIDE TRANSPORT IN THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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Decreases in extracellular pH (pHe) reduce the rate of Na and Cl absorption in the small intestine of the winter flounder. The major target of pH action is a Na-K-2Cl absorptive process located along the luminal membrane of intestinal epithelial cells. In addition, a poorly characterized Cl secretory process is stimulated by reductions in pHe. In a previous study, we found that amiloride at 1 mM also inhibited Na and Cl absorption suggesting that changes in pHi may mediate the effects of pHe. In the present study, we examined the possibility that pHe (or pHi) affects Na-K-2Cl co-transport indirectly by altering luminal membrane K conductance. In this scheme, reductions in pHi decrease K conductance and the resulting increase in intracellular K inhibits the Na-K-2Cl absorptive process. Such a mechanism has been observed in other epithelia with transport pathways similar to the flounder small intestine. To examine this possibility, we studied the effects of barium on Cl flux at concentrations known to inhibit K conductance in the flounder intestine. We also examined the link between the Cl absorptive and secretory processes by studying the effects of copper on Cl flux. In previous studies we found that this metal also inhibits Cl absorption.

Winter flounder were housed for 1-3 days before use. Small intestinal segments were stripped of serosa and studied under short circuit conditions in the Ussing chamber. Bathing solution pH was maintained at 7.8 by gassing a 20 mM bicarbonate teleost Ringers solution with 1% CO₂/99% O₂ (PCO₂ 7 mmHg). Barium chloride was added to the mucosal or serosal bathing solution at a final concentration of 5 mM. In other experiments, cupric chloride was added to the mucosal or serosal solution at 20 or 100 uM.

	n	pHe	JClms	JClsm	JClnet	Isc
Control	9	7.8	9.3±0.9	4.8±0.9	4.5±0.7	-1.8±0.3
Barium 5mM (m)	9	7.8	8.6±1.0	5.8±1.1	2.8±0.5	-0.9±0.1
Copper 20uM (m)	4	7.8	8.1±2.7	5.2±2.0	2.9±1.7	-0.1±0.7
Copper 100uM (m)	10	7.8	6.4±0.7	5.2±1.0	1.2±0.9	0.3±0.3

(Results expressed as means±SE in uEq/cm².h)

In control tissues, net Cl absorption was present associated with a serosa negative PD. Mucosal barium inhibited Cl absorption by decreasing Jms and increasing Jsm. Furthermore, the presence

of mucosal barium prevented the effect of a reduction in pHe on Cl flux (data not shown). Mucosal copper at concentrations between 20 uM and 100 uM also inhibited Cl absorption by decreasing Jms and increasing Jsm. The serosal addition of barium or copper did not affect Cl flux.

The results of the barium studies the possibility that pHe affects Cl absorption through an effect on luminal membrane K conductance. The similar effects of copper and pHe suggest that the Cl absorptive and secretory processes may be linked or coordinated in some way.

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