RELATIVE EFFECTS OF pH, pCO_2 AND HCO_3 ON INTESTINAL CHLORIDE ABSORPTION IN THE FLOUNDER (Pseudopleuronectes americanus)

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Several years ago Field et al. (J. Membrane Biol. 41:265-293, 1978) observed that chloride absorption by the isolated small intestine of the winter flounder (Pseudopleuronectes americanus) was influenced by pH and/or bicarbonate concentration of the medium. This finding was particularly intriguing given the apparent importance of a Na-K-2Cl contransport process (Musch et al. Nature 300:351-353, 1982) in mediating chloride absorption across the apical border of the transporting epithelial cells. We have examined the relative effects of the acid-base variables pH, pCO_2 and HCO_3 on chloride absorption in this tissue in an attempt to define the site and mechanism of action of this effect.

Flounder (Pseudopleuronectes americanus) small intestine was stripped of its muscle layers and mounted in modified Ussing chambers as described by Field, et al. (J. Membrane Biol. 41:265-293, 1978). The tissue was exposed to a teleost Ringer's solution containing 150.5 mM Na, 5 mM K, 1.2 mM Mg, 1.3 mM Ca, 154 mM Cl, 2.7 mM PO_4, 1.3 mM SO_4, and either 5 mM or 20 mM $NaHCO_3$ at 15°C. Ringer's solutions were gassed with either 1% CO_2/99% O_2, 5% CO_2/95% O_2 or room air in random order during consecutive one hour flux periods. A 15 minute equilibrium period was observed with each change on gas mixture, during which both the current and conductance would reach a new steady state. Unidirectional chloride fluxes (J) were measured on paired tissues under short-circuited conditions using ^{36}Cl. Tissue conductance (G) was measured by voltage pulse throughout the experiment. Tissue short circuit current (I_{sc}) is expressed as equivalent ionic flux.

TABLE 1. Chloride fluxes across the short-circuited intestinal mucosa of the flounder.

pH	CO_2	J_{ms}	J_{sm}	J_{net}	I_{sc}	G
	mmHg	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$mmho/cm^2$
6.81[A]	36	4.90 ± 1.34	3.57 ± 1.02	1.32 ± 0.95	0.45 ± 0.12	12.5 ± 0.6
7.10[A]	7	5.77 ± 0.49	2.17 ± 0.38	3.60 ± 0.51	1.09 ± 0.27	15.1 ± 0.5
7.11[B]	37	6.98 ± 1.95	3.73 ± 1.63	3.25 ± 0.66	1.27 ± 0.09	20.6 ± 1.7
7.74[B]	8	9.88 ± 0.98	2.95 ± 0.76	6.94 ± 1.12	2.32 ± 0.62	23.9 ± 1.8
7.85[A]	1	6.68 ± 0.77	2.15 ± 0.68	4.53 ± 0.09	2.13 ± 0.01	15.8 ± 0.5

Values are the mean ± the standard error of the mean (n=4 for all tissue values except pH 7.85, n=2). A = 5 mM HCO_3 Ringers and B = 20 mM HCO_3 Ringers.

Alterations in the medium pH, pCO_2 and HCO_3 concentration markedly affected net chloride flux (J_{net}) and I_{sc} (Table 1). The effect on the net chloride flux appeared to be primarily due to changes in mucosal to serosal chloride flux. The acid-base variable responsible for these changes was pH rather than pCO_2 or HCO_3 concentration. Thus, when media with similar pH values (7.10, 7.11), but with differing values for pCO_2 (7 and 37 mmHg) and HCO_3 concentration (5 and 20 mM) were compared, the mucosal to serosal and net chloride flux values were similar. In addition, the two experiments performed at pH 7.85 suggest that the effect of pH is maximal near pH 7.74.

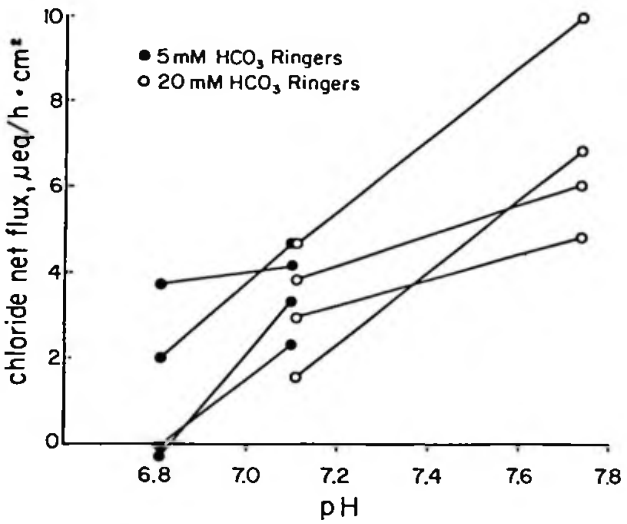

Figure 1. Relationship between pH and chloride net flux in the winter flounder intestine.

The relative importance of pH may also be seen when the values for net chloride flux are plotted as a function of medium pH (Figure 1). As shown by the connected data points, tissues exposed to two pH conditions always responded by increasing net chloride absorption in response to increases in medium pH regardless of the prevailing pCO_2 and HCO_3 concentration ($p<0.05$). In addition, a linear relationship was found between medium pH and net chloride flux when the individual values in Figure 1 were considered: $r = 0.80$, slope = 5.9, y intercept = -39, $n = 16$, $p<0.001$.

To examine whether the effect of medium pH was mediated through an effect on cell membrane potassium conductance, chloride flux was measured in the presence of 1 mM barium chloride mucosally added (Halm et al., Bull. MDIBL 21:88, 1981; Halm et al., Bull. MDLBL 22:80, 1982). A similar increase in net chloride flux was observed in response to increases in pH in the presence of barium chloride as in its absence. Net chloride flux (expressed in $\mu eq/h \cdot cm^2$) was 1.58 $\pm$ 0.70 at pH 6.81 (5 mM HCO_3), 3.39 $\pm$ 0.54 at pH 7.10 (5 mM HCO_3), 3.35 $\pm$ 1.35 at pH 7.11 (20 mM HCO_3), and 7.89 $\pm$ 0.10 at pH 7.74 (20 mM HCO_3) (n=2) (compare to Table 1). The slope (6.8) and y intercept (-95) of this relation between medium pH and net chloride flux was similar to that observed in the absence of barium (Figure 1). The I_{sc} also increased with increasing pH although the absolute values were lower: 0.11 $\mu eq/h \cdot cm^2$ at pH 6.81 and 1.55 $\mu eq/h \cdot cm^2$ at pH 7.74.

These results delineate the specific acid-base variable (pH) that affects net chloride absorption in the isolated flounder small intestine. In addition, the effect of increased medium pH to increase net chloride absorption was observed in the presense of barium chloride, a substance that blocks potassium conductance channels through a change in cell membrane potassium conductance. The nature of the pH-chloride flux interaction remains uncertain and is currently under investigation.

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