EFFECT OF HCO_3 AND BUMETANIDE ON pH-SENSITIVE INTESTINAL CHLORIDE ABSORPTION IN THE FLOUNDER (Pseudopleuronectes americanus).

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Last summer we reported the net chloride flux across short-circuited flounder intestine was related to the bathing solution pH (Charney et al., Bull. MDIBL 25:111-113, 1985). Increases in pH, between 6.8 and 7.7, increased net chloride absorption approximately three-fold. This effect was associated with smaller increases in I_{sc}, but apparently was not influenced by the prevailing bathing solution HCO_3 concentration or pCO_2. In addition, the mucosal addition of 1.0 mM barium (a K channel blocker) did not inhibit the increase in chloride absorption suggesting that the effect of pH was not mediated by a change in epithelial potassium conductance. In the current study, we examined whether the bathing solution HCO_3 was required and whether bumetanide could inhibit the effect of pH on the net chloride flux.

Winter flounder, Pseudopleuronectes americanus, small intestine was stripped of its muscle layers and mounted in modified Ussing chambers as previously described. Tissues were exposed to teleost Ringer's solutions 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 HCO_3, 11 mM HCO_3 or 11 mM HEPES (N-2- hydroxyethylpiperazine-N'-2-ethanesulfonic acid). In addition, 10 mM mannitol and 10 mM glucose were added to the mucosal and serosal solutions respectively. Ringer's-HCO_3 solutions were gassed with either 5% CO_2/95% O_2, 1% CO_2/99% O_2 or room air during 2 consecutive 45 min flux periods. The HEPES-Ringers solutions were gassed with 100% O_2 and the pH adjusted with additions of 1 M HCl or 1 M NaOH. Each change of pH or gas mixture was followed by a 15 min equilibration period. Unidirectional chloride fluxes (J) using ^{36}Cl were measured across paired, short circuited tissues. Tissue conductance (G_t) was measured by intermittent voltage pulses throughout the experiment. Tissue short circuit current (I_{sc}) was expressed as the equivalent ionic flux.

As shown in Table 1, increases in bathing solution pH increased net chloride absorption. This effect was observed between pH levels of 6.56 and 7.86 in the presence or absence of bathing solution HCO_3 and CO_2. That is, when 11 mM HCO_3 was replaced with 11 mM HEPES and the pH was increased by addition of NaOH, a marked increase in the net chloride flux occurred. This increase was similar to that caused by a change in pCO_2 from 36 to 7 mm Hg in a Ringers solution containing 11 mM HCO_3. In addition, all increases in net chloride absorption were accompanied by somewhat smaller increases in I_{sc} suggesting that at least part of the stimulated chloride flux was electroneutral.

The relation of net chloride absorption to bathing solution pH is shown in figure 1. A linear relationship ($P<0.001$)was observed between the bathing solution pH and net chloride flux over the pH range of pH 6.56 to 7.60 ($r = 0.98$, slope = 3.0, y intercept = -19, n=5). Above pH 7.60, net chloride flux decreased, although the I_{sc} continued to increase. Figure 1 also indicates the effect of pH when the mucosal solution contains 0.1mM bumetanide. Net chloride flux was diminished at pH 6.93 and failed to increase when the pH was raised to 7.60. As shown in Table 2, increases in pH also did not increase I_{sc} in the presence of mucosal bumetanide.

These results and our previous findings suggest: a) that the pH range effective in stimulating chloride absorption extends from 6.56 to 7.74, b) the effect of pH does not require the presence of either HCO_3 or CO_2 in the bathing solution, and c) that the Cl absorptive process stimulated by pH is inhibited by bumetanide and is therefore most likely the Na-K-2Cl absorptive process described in flounder small intestine. The mechanism of this pH effect remains uncertain and is currently under investigation.

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Table 1. Effect of pH on net chloride flux across short-circuited intestinal mucosa of the flounder.

pH	n	HCO_3 mM	pCO_2 mmHg	J_{net} μeq/h·cm^2	I_{sc} μeq/h·cm^2	G_t mS/cm^2
6.56	5	5	36	1.24 ± 0.46*	0.24 ± 0.09	18.3 ± 2.4
6.93	3	11	36	1.81 ± 0.44	0.46 ± 0.52	17.3 ± 0.5
7.07	5	0**	0	2.75 ± 1.43	1.81 ± 0.20	14.9 ± 2.2
7.60	3	11	7	4.78 ± 1.14	1.41 ± 0.30	21.4 ± 2.0
7.86	5	0**	0	4.69 ± 1.54	1.48 ± 0.27	16.5 ± 1.8
7.88	5	5	0	2.35 ± 0.88	1.74 ± 0.33	19.4 ± 1.8

* Values represent the mean ± the standard error of the mean.
** The 11 mM HEPES-Ringers was gassed with 100% O_2.

Table 2. Effect of pH on net chloride flux across short-circuited intestinal mucosa in the presence of 0.1 mM bumetanide.

pH	HCO_3 mM	pCO_2 mmHg	J_{net} μeq/h·cm^2	I_{sc} μeq/h·cm^2	G_t mS/cm^2
6.93	11	36	0.91 ± 0.67*	0.36 ± 0.12	14.5 ± 1.4
7.60	11	7	1.18 ± 0.56	0.42 ± 0.07	15.9 ± 1.6

* Values represent the mean ± the standard error of the mean (n=6).

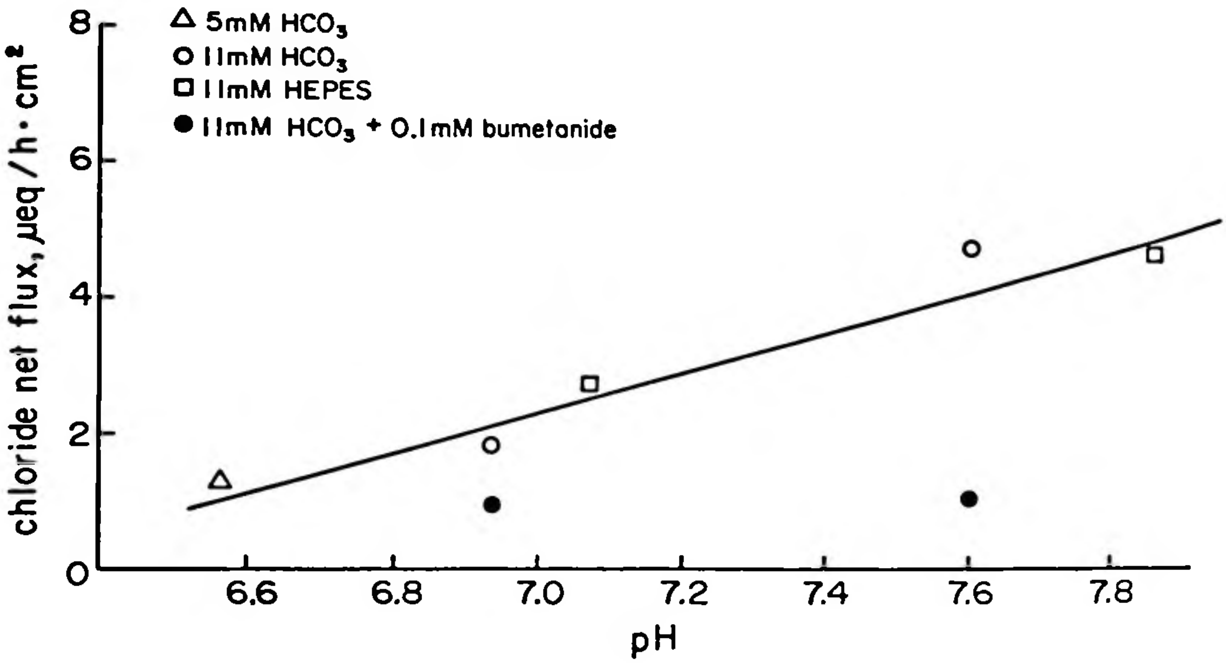

Figure 1. The relationship between pH and chloride flux. The line represents the best fit from the linear regression (all nonbumetanide data, values in the text). The symbols represent; (△) 5 mM HCO_3 Ringers, (○) 11 mM HCO_3 Ringers, (□) 11 mM HEPES Ringers and (●) 11 mM HCO_3 Ringers with 0.1 mM bumetanide.