EFFECT OF CHLORIDE TRANSPORT INHIBITORS ON INTRACELLULAR CHLORIDE CONCENTRATION IN THE INTESTINE OF THE WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS

Michael Field, Stephanie A. Orellana and Mark W. Musch, Departments of Medicine and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois

Prior studies have identified 3 components of the process for active Cl absorption in flounder intestine: (1) NaCl cotransport across the brush border (Field et al., J. Memb. Biol., 41:265, 1978; Frizzell et al., J. Memb. Biol. 46:17, 1979); (2) Cl accumulation intracellularly above Nernst equilibrium (Duffy et al., J. Memb. Biol. 50:331, 1979); and (3) Cl movement across the basolateral border by non-conductive pathways, probably mainly KCl co-transport (Stewart et al., Bull. MDIBL. 20: 1980). Elsewhere in this volume, Stewart et al and Smith et al., report intracellular activities and electrochemical potentials of Cl in flounder intestine, determined with Cl-selective and reference microelectrodes. As these electrodes are not absolutely specific for Cl, we wished to also measure intracellular Cl by another technique. We did so by equilibrating intestinal mucosa with ^{36}Cl and then, after correcting for the extracellular space, calculating the concentration of exchangeable Cl in cell water. The results differ in certain respects from those obtained with Cl-selective microelectrodes.

METHODS

The method for measuring intracellular Cl using ^{36}Cl has been previously reported in this bulletin (Smith et al., Bull. MDIBL. 19:24, 1979). Briefly, mucosa was stripped of muscle and mounted in multiport chambers, which permitted perfusion of both sides with oxygenated Ringer solution, temperature control (15°) and continuous monitoring of transmural PD and short-circuit current (I_{sc}). Tissues were incubated for 1 h with ^{36}Cl and [^{3}H] PEG (equal concentrations on both mucosal and serosal sides) prior to removal and extraction of radioactivity. The PEG "space", presumably a measure of the extracellular space, was on the average about one-third of total tissue water. For incubations, the standard 20 mM HCO_3 - Flounder Ringer (see Field et al., J. Memb. Biol. 41:265, 1978), was employed and was bubbled with either 1% or 5% CO_2 in O_2.

RESULTS AND DISCUSSION

Effects on cell Cl of (1) decreasing medium pH, (2) adding furosemide to the mucosal side medium, and (3) adding 8-Bromo-cAMP and theophylline are shown in Table 1. As indicated by the I_{sc} values, each of these maneuvers

Table 1.--Effects of Cl transport inhibitors on intracellular Cl concentration

	intracellular Cl (mM)	I_{sc} ($\mu Eq \cdot h^{-1} \cdot cm^{-2}$)
A. Effect of pH (n = 8)		
pH 8.0	61.5 ± 5.3	-3.69 ± .40
pH 7.3	52.3 ± 3.6	-1.08 ± .25
	$p < .05$	$p < .001$
B. Effect of furosemide (n = 3)		
control	62.5 ± 3.9	-2.84 ± .43
furosemide	37.5 ± 4.2	-0.11 ± .33
	$.05 < p < .10$	$.05 < p < .10$
C. Effect of Cyclic AMP (n = 7)		
control	57.8 ± 4.1	-3.53 ± .43
cAMP	46.2 ± 6.1	0.04 ± .19
	$.05 < p < .01$	$p < .001$

Means ± 1 SE for (n) fish. $[HCO_3]$ = 20 mM in all instances; solutions were bubbled with 1% CO_2 in O_2

in all instances except for the pH 7.3 solution, which was bubbled with 5% CO_2 in O_2. Furosemide was placed on the mucosal side at 1 mM. Cyclic AMP tissues were exposed on their serosal side to 0.2 mM 8-Br-cAMP and 2.5 mM theophylline. All tissues were pre-equilibrated for one hour and then equilibrated with ^{36}Cl and [^{3}H] PEG on both sides for another hour.

inhibited transepithelial Cl transport. Both furosemide and cAMP inhibit NaCl cotransport across the brush border and the pH effect is most likely exerted on the Na pump (see Smith et al in this volume).

Control $[Cl]_i$ was about 60 mM which, after correcting for the Ringer solution activity coefficient for Cl (0.75), is twice as high as that measured with Cl-selective microelectrodes (See Smith et al in this volume). Decreasing medium pH from 8.0 to 7.3 slightly decreased $[Cl]_i$, which contrasts with the increase in $[Cl]_i$ determined under the same conditions with microelectrodes. Furosemide and cAMP, as might be anticipated, both decreased the average value for $[Cl]_i$, although the changes are not quite significant at the 95% level.

The reasons for the differences between ^{36}Cl and Cl-selective microelectrode measurements are not clear. The presence of competing anions in the cell should increase rather than decrease $[Cl]_i$ as determined with microelectrodes. A tissue extracellular [Cl] higher than bulk medium [Cl] could explain some of the discrepancy but it would have to be about 60 mM higher than the bulk medium concentration to account for all of it. Possibly, some intracellular Cl is bound to membranes or sequestered in organelles. The other major discrepancy between these and the microelectrode results is in the effect of pH. In the microelectrode study, the same decrease in medium pH caused a rise in intracellular Cl activity from 22 to 35 mM (Smith et al, this volume), whereas the comparable change in activity noted in this study was a decrease from 46 to 39 mM. Since inhibition of transepithelial Cl transport should result in a return of tissue extracellular Cl concentration toward bulk medium concentration, this may account for a part of the observed discrepancy. This work was supported by NIH grant AM 21345 and a student research fellowship to M.M. from the American Gastroenterological Association.

EVIDENCE FOR A COUPLED NaCl CARRIER IN HERRING GULL (LARUS argentatus) NASAL SALT GLAND

David S. Miller and Franklin H. Epstein, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine and Department of Medicine Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts

The nasal salt gland is the major osmoregulatory organ of marine birds. Like elasmobranch rectal gland and marine teleost gill, this organ secretes a concentrated NaCl solution in response to osmotic loads. In vivo and in vitro studies have shown that nasal gland transport function is dependent on the enzyme, Na,K-ATPase and is stimulated by cyclic GMP, acetyl choline and cholinomimetrics (Stewart et al., Amer. J. Physiol., 237:C200-204, 1979; Peaker and Linzell, Salt Glands in Birds and Reptiles, Cambridge, 1975). Data on mechanisms of nasal gland salt transport have been difficult to obtain, because no convenient in vitro preparation in which secretion can be measured directly, e.g., perfused gland, is available. Secretory transport can be measured indirectly in slices by monitoring O_2 consumption and we use that technique here to show that, as in elasmobranch rectal gland, secretory transport in avian nasal gland involves a coupled NaCl carrier.

Young Herring Gulls, weighing 700-1000 g, were collected on nearby islands and transported to the laboratory, where they were maintained in metal sheds and given herring to eat and sea water to drink. Birds were kept in captivity for at least 1 week, at which time nasal salt gland, Na,K-ATPase activities had reached maximal values of about 40 μmoles P_i/mg protein/h. For experiments, gulls were decapitated and the paired nasal glands excised rapidly and placed in oxygenated (95% O_2/5% CO_2) Krebs-Henseleit buffer (KHB with the following composition in mM: 118 NaCl, 9.4 KCl, 1.25 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$ at pH 7.4) at 4°C. The glands were