Finally, if the cell transports equal amounts of Na and Cl into the space, then, in the steady-state, Na and Cl effluxes out of the space must also be equal, i.e.,

$$ {}_oJ_m^{Na} + {}_oJ_s^{Na} = {}_oJ_s^{Cl} + {}_oJ_s^{Cl} \quad . \tag{3} $$

For $J_{net}^{Na} = 2.0$, $J_{net}^{Cl} = 5.5$, $J_{sm}^{Na} = 10.4$ and $J_{sm}^{Cl} = 3.0$, the values shown in Figure 1A for ΔC, $\Delta\Psi$, p_j^i and J_j^i can be obtained. If short-circuiting doesn't change the cellular output of NaCl, then relevant values for the open-circuited state can also be predicted (see Figure 1B).

Although more complex and realistic analyses could probably be made, the present one serves to illustrate the potential influence of the paracellular pathway on transepithelial ion transport. In general, if lateral space resistances to ion movements are significant relative to junctional resistances, it becomes hazardous to extrapolate from transmural PD and flux measurements to transport events at the cellular level. A serosa-negative PD has also been noted in the thick ascending limb of Henle's loop in rat kidney (Burg and Green, Am. J. Physiol., 224:659, 1973). It may be worthwhile to consider the interpretation offered here as an alternative to an electrogenic Cl pump at that site. The role of lateral space diffusion potentials in modifying transepithelial ion transport has previously been pointed out by Machen and Diamond (J. Membrane Biol., 1:194, 1969) and by Armstrong (in Intestinal Ion Transport, MTP press, 1976).

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CHARACTERIZATION OF CYCLIC AMP-MEDIATED CHLORIDE PERMEABILITY CHANGE IN THE INTESTINAL EPITHELIUM OF THE FLOUNDER, *Pseudopleuronectes americanus*

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We previously reported (Field and Smith, MDIBL Bull., Vol. 15) that cyclic 3',5'-AMP (cAMP) increases the unidirectional serosa (s)-to-mucosa (m) Cl flux across short-circuited flounder intestinal mucosa ($_oJ_{sm}^{Cl}$) without producing appreciable changes in net Cl flux ($_oJ_{net}^{Cl}$) or in unidirectional or net Na fluxes, suggesting a specific effect on passive Cl permeability. In the present study we have further characterized this effect. Experiments were designed to answer the following three questions: (1) In the absence of an increase in intracellular cAMP concentration, does $_oJ_{sm}^{Cl}$ result exclusively from diffusion over the paracellular shunt pathway or does it contain a transcellular component? (2) Does the increase in $_oJ_{sm}^{Cl}$ caused by cyclic AMP result from an alteration of Cl permeability in the paracellular pathway or in a transcellular pathway? (3) Finally, since stimulation by cAMP of active Cl secretion in mammalian intestine appears to require both exogenous Na and a functioning Na pump, does active Na transport also play an essential role in the cyclic AMP-induced Cl permeability change in flounder intestine?

In order to evaluate the transcellular and paracellular components of $_oJ_{sm}^{Cl}$ that are normally present, we measured this flux at different extracellular Cl concentrations and at different applied transmural PDs. Figure 1 shows the effect of [Cl] on $_oJ_{sm}^{Cl}$ and on short-circuit current (Isc). Over the range 27 to 120 mM, $_oJ_{sm}^{Cl}$ varies linearly with [Cl], although Isc, which should be proportional to J_{net}^{Cl}, approaches saturation. Thus, in the absence of cAMP, $_oJ_{sm}^{Cl}$ does not appear to traverse the saturable, presumably transcellular limb of the pathway for $_oJ_{net}^{Cl}$.

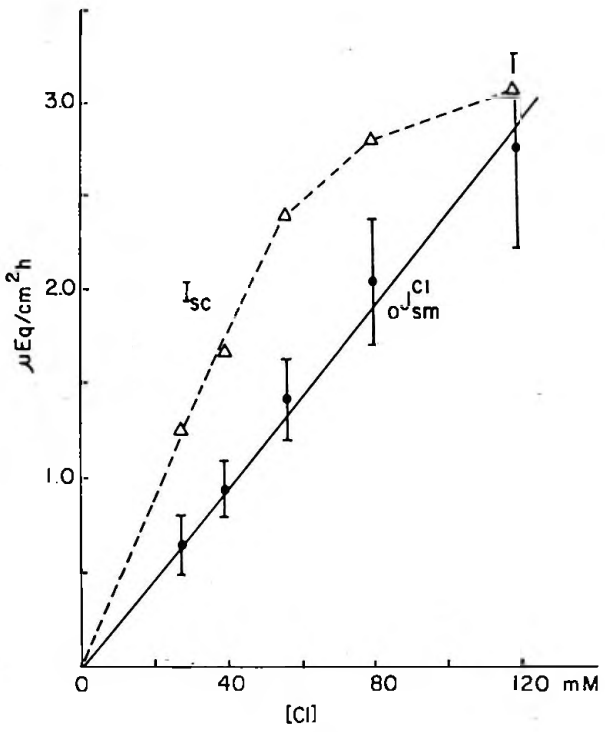

Figure 1. Variation of $_oJ^{Cl}_{sm}$ and Isc with [Cl]. Equimolar amounts of SO_4 and mannitol were substituted for Cl. Results are means ± 1 SE for 7 experiments.

The effects of imposed PDs on J^{Cl}_{sm} were determined in the presence and absence of cAMP. Each tissue was clamped at 0 mV (short-circuited) and also at either +10 or -15 mV (mucosal reference), the clamping order being randomized. To reduce the effect of tissue variability, fluxes measured at +10 and -15 mV were normalized against fluxes measured at 0 mV as follows:

$$_kJ^{Cl}_{sm}(i)_n = {}_kJ^{Cl}_{sm}(i) \times \overline{{}_oJ^{Cl}_{sm}} / {}_oJ^{Cl}_{sm}(i) \qquad (1)$$

where k = applied PD, n refers to the normalized value, i to the particular experiment, and $\overline{{}_oJ^{Cl}_{sm}}$ to the group mean flux at 0 mV. In Figure 2, J^{Cl}_{sm} has been plotted against $-\mu/(1 - e^{u})$ where $\mu = (F/RT) \times \Psi_{ms}$. A linear relationship would result from diffusion across a single membrane and a positive Y-intercept would suggest a PH-independent, and therefore presumably transcellular, component of the flux (since nearly all the clamping current traverses the paracellular route). The straight lines have been drawn by the method of least squares. The curvilinear relationship shown is that predicted for diffusion over two membranes in series. From a compartmental analysis of the paracellular pathway that we have described elsewhere in this bulletin and ignoring the contribution of the lateral space salt concentration gradient,

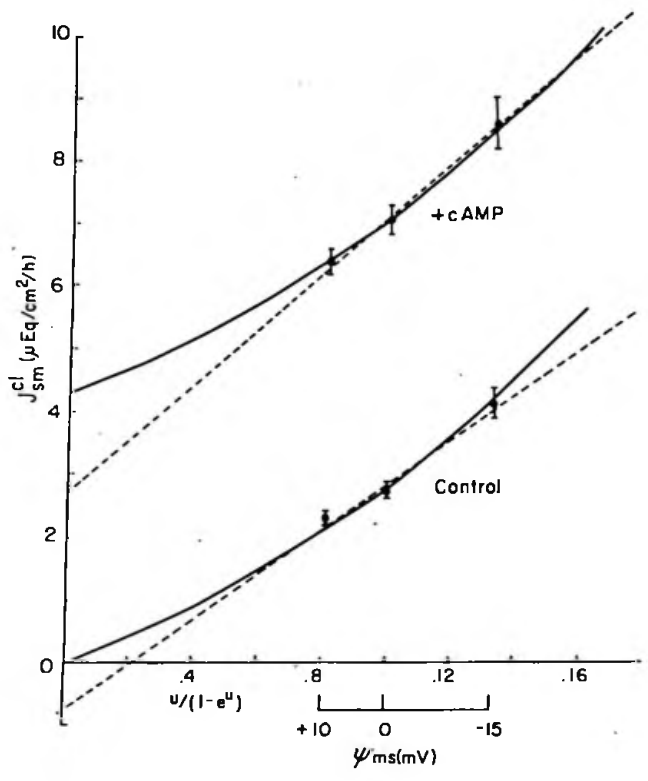

Figure 2. Effect of PD on J^{Cl}_{sm} in cAMP-treated and control tissues. $\mu = (F/RT) \times \Psi_{ms}$. Results are means ± 1 SE for 23 to 29 experiments at 0 mV and 13 to 15 experiments at +10 and -15 mV. See text for details.

$$J^{Cl}_{sm} = \frac{-1/2\,\mu}{1 - e^{1/2\,\mu}} \quad \frac{C_o\, P^{Cl}_m\, P^{Cl}_s}{P^{Cl}_m + P^{Cl}_s\, e^{1/2\,\mu}} \tag{2}$$

where $\mu = (F/RT) \times \Psi_{ms}$, $C_o = 150$ mM and P^{Cl}_m and P^{Cl}_s are the Cl permeabilities of mucosal and serosal boundaries of the lateral space. The fraction 1/2 results from the fact that the predicted resistances of the m and s boundaries are almost exactly equal and therefore the lateral space PD will be one-half Ψ_{ms}. The values for P^{Cl}_m and P^{Cl}_s are those predicted by,

$${}_oJ^{Cl}_{sm} = \frac{P^{Cl}_m\, P^{Cl}_s}{P^{Cl}_m + P^{Cl}_s} C_o \frac{{}_o\mu_L}{1 - e^{-{}_o\mu_L}} \tag{3}$$

where ${}_o\mu_L$ refers to the lateral space PDx(F/RT) under short-circuit condition, and

$$P^{Cl}_s = 6.25\, P^{Cl}_m, \tag{4}$$

relationships which we have described elsewhere in this bulletin. The curves, which provide a somewhat better fit of the data than do the straight lines, assume that ${}_oJ^{Cl}_{sm}$ in control tissues (2.78 μEq/hr cm²) is entirely diffusional and that the values for p^{Cl}_m and p^{Cl}_s are not affected by cAMP. Both methods of analysis result in positive Y-intercepts for cAMP-treated tissues and zero or negative Y-intercepts for control tissues. The straight line slopes do not differ significantly for cAMP-treated and control tissues. Thus the voltage clamp data supports the diffusional nature of ${}_oJ^{Cl}_{sm}$ in control tissues and suggests that the increase in ${}_oJ^{Cl}_{sm}$ caused by cAMP is not affected by imposed PDs in the range tested.

The influence of active Na transport on the cAMP-mediated increase in ${}_oJ^{Cl}_{sm}$ was examined in ouabain-treated tissues (see Table 1). Ouabain greatly increased ${}_oJ^{Cl}_{sm}$ in the absence of cAMP, possibly by eliminating the intracellular PD. An additional increase in ${}_oJ^{Cl}_{sm}$ was produced by cAMP, indicating that the two agents have independent effects on Cl permeability and that the cAMP-mediated effect does not require active Na transport.

TABLE 1

Effect of cAMP and/or Ouabain on ${}_oJ^{Cl}_{sm}$

	-ouabain	+ouabain	P
-cAMP	4.72 ± .551 (8)	11.6 ± .682 (9)	< .001 (8)
+cAMP	10.3 ± .625 (6)	14.6 ± 1.06 (9)	< .05 (6)
P	< .005 (5)	< .02 (8)	

Values are means ± 1 SE for (n) experiments; probabilities (p) are for paired differences in (n) experiments. Unpaired experiments were excluded from the probability calculations. Ouabain (0.5 μmol/ml) and cAMP (2 μmol/ml) together with theophylline (5 μmol/ml) were added to the serosal reservoir and flux measurements begun 50-60 min thereafter.

We conclude (1) that flounder intestinal epithelial cells do not normally permit significant transcellular diffusion of free Cl and therefore that, normally, all passive transepithelial Cl movement is through the paracellular pathway and (2) that cAMP opens up an ouabain-insensitive pathway for transcellular diffusion of free Cl and does not affect paracellular Cl permeability. It remains to be determined whether this effect of cAMP is exerted on the brush border or basolateral cell membrane. Since these epithelial cells, in contrast to mammalian intestinal cells, do not appear to possess a secretory pump, the cAMP-mediated increase in Cl permeability does not result in active secretion. It is interesting to speculate that cAMP causes a similar increase in Cl permeability in secretory cells (mammalian intestine and pancreas, shark rectal gland, etc.) that in the presence of a secretory driving force, is then translated into secretion.

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