PATHWAYS FOR PASSIVE NA AND CL TRANSPORT ACROSS THE URINARY BLADDER OF THE WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS

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The urinary bladder of the winter flounder has previously been demonstrated to absorb Na and Cl by a mechanism which involves an interrelationship between the two species. This cotransport system is not dependent on K and therefore does not represent a Na/K/Cl cotransport system. Furthermore, the process does not appear to involve Na/HCl/OH parallel countertransport systems. The sensitivity to thiazide-type diuretics and the relative insensitivity to furosemide and bumetanide provide a clear pharmacological delineation of the system (Stokes, J.B.: J. Clin. Invest. 74:7-16, 1984).

The present experiments were designed to examine the passive pathways of Na and Cl transport across the intact urinary bladder. Tracer movement of Na and Cl from serosa to mucosa was examined using standard techniques with bladders mounted in Ussing chambers. Transepithelial conductance was monitored by an automatic voltage clamp device whereby transepithelial voltage was maintained at 0 and, at one to two minute intervals, a voltage of 10 mV applied for 0.5 seconds. The conductance of the epithelium is calculated from the magnitude of the current passed by this voltage.

Previous experiments have demonstrated that exposure of the mucosa to hydrochlorothiazide (HCTZ) caused a consistent increase in the transepithelial conductance. To test the possibility that this increase in conductance was owing to an increase in an apical K conductive pathway (Dawson, D.C., and Andrew, D.: Bull. MDIBL 20:89-92, 1980), the effect of prior treatment with barium was examined. In the presence of mucosal barium, HCTZ did not produce any change in transepithelial conductance. The interpretation of these results is that HCTZ induces an increase in the cellular conductance which can be blocked by barium. There does not appear to be paracellular component for this increase in conductance.

A second series of experiments revealed that exposure of the mucosa to both barium and HCTZ reduced not only the transepithelial conductance but also the serosa-to-mucosa flux of Na and Cl. There was a 60% reduction in the Na flux and an 80% reduction in the Cl flux. In untreated bladders the sum of the partial ionic conductances of Na and Cl exceeded that of the measured transepithelial conductance indicating a substantial component of electroneutral transport. With HCTZ and barium treatment, the electroneutral component was strikingly reduced although not completely eliminated. In order to examine the possibility that the residual Na and Cl flux traversed a paracellular pathway, additional studies were conducted in the presence of HCTZ and barium. The NaCl dilution voltages imposed either from the mucosal solution or the serosal solution gave small but symmetrical voltage deflections indicating that the permeability ratio of Na:Cl was approximately 0.71±.02. In other experiments the ratio of these permeabilities measured with tracers indicated a ratio of 0.74±0.03, values which are not different from each other. A third series of experiments indicated that the residual tracer flow was influenced by imposition of +30 mV or -50 mV across the epithelium. The magnitude of the alteration in Na and Cl flux was close to that predicted by

considerations of simple ionic diffusion across a single membrane. Thus, within experimental error, it appears that almost all of Na and Cl permeation under conditions of HCTZ and barium exposure is by way of the paracellular pathway. This pathway has a high resistance (1-3 Kohm.cm^2) and has a Na:Cl permselectivity to close to that of free solution.

The observation that mucosally applied HCTZ inhibited the serosa-to-mucosa flux of both Na and Cl together with the observations regarding the nature of the paracellular transport pathway suggested that the thiazidesensitive component traversed a cellular pathway. To examine this possibility further, experiments were conducted whereby Na and Cl serosa-to-mucosa fluxes were examined under conditions of varying mucosa Na and Cl concentrations. Removal of Na from the mucosal medium reduced both the Na and Cl S-to-M fluxes to values not appreciably different from the thiazide-inhibited values. Reduction of the Cl concentration in the mucosal solution likewise produced similar findings. These results provide additional evidence that the Na and Cl backfluxes traverse a cellular pathway with one of the transport processes being the NaCl cotransporter presumably located on the apical membrane. Thus, it appears that the thiazide-sensitive transporter is the only mechanism whereby Na and Cl can traverse the apical membrane.

In order to evaluate the mechanism whereby Na and Cl permeate the basolateral membrane (from the serosa), various inhibitors were applied to the serosal solution. Furosemide (1 mM), amiloride (1 mM), furosemide plus barium (4 mM), ouabain (0.1 mM), and diphenylamine carboxylate (0.1 mM) all had no effect on the permeation of Na and Cl. Thus, we were unable to demonstrate that pathway through which these ions permeated the basolateral membrane.

These results demonstrate the paracellular and cellular pathways for Na and Cl transport. In addition, they also demonstrate that Na-Na and Cl-Cl exchange diffusion can occur via this NaCl cotransporter.