EFFECTS OF INHIBITORS OF Na/K/2Cl COTRANSPORT ON CYCLIC GMP CONTENT OF THE INTESTINE OF *PSEUDOPLEURONECTES AMERICANUS.*

Mrinalini C. Rao*, Nancy T. Nash *, Mark W. Musch ¥ and Michael Field †,
*Dept. of Physiology and Biophysics, University of Illinois at Chicago; ¥ Dept. of Medicine, The University of Chicago, Chicago, IL; †Dept. of Medicine, Columbia University, New York, NY.

The intestinal epithelium of the winter flounder actively absorbs Na and Cl via an Na/K/2Cl cotransport mechanism on its luminal surface and is highly cation-selective (Musch et al., Nature 300:351-353, 1982 ; O'Grady et al., J. Membr. Biol. 91:33-41, 1986). Furosemide and bumetanide completely inhibit this cotransport mechanism (Frizzell et al., J. Membr. Biol. 46:27-39, 1979; Musch et al., ibid; O'Grady et al., ibid.). Ouabain appears to inhibit salt absorption via Na/K/2Cl cotransport in two ways: directly, by inhibiting the cotransporter and indirectly, by its action on Na/K-ATPase (O'Grady et al., ibid.). The mechanism by which ouabain directly inhibits cotransport is not known. The phosphodiesterase-resistant nucleotide 8-Br-cyclic GMP is also a potent inhibitor of Na/K/2Cl cotransport and its effects are indistinguishable from those of bumetanide (Rao, et al., Am. J. Physiol. 246:C167-C171, 1984). However, the various cAMP analogues had differing effects. All the cAMP analogues tested increase tissue Cl permeability (Frizzell et al.,1979 ibid; Field et al., J. Membr. Biol. 55:157-163, 1980; Rao et al., 1984 ibid., Krasny et al., Fed. Proceed., 42:1100, 1983). However, while dibutyryl cAMP or cAMP in the presence of theophylline, partially inhibit the cotransport process, 8-Br-cAMP, does not inhibit it (Rao and Nash, 25:36-38, MDIBL Bull., 1985). As cGMP is a potent inhbitor of cotransport in this tissue, we determined if the effects of theophylline, a known non-specific phosphodiesterase inhibitor, and ouabain on Na/K/2Cl cotransport may be explained by their effects on tissue cGMP content.

Flounder maintenance, tissue preparation and mounting onto modified Ussing chambers was as previoulsy described (Rao and Nash 1985 ibid). The P.D. was allowed to stabilize and tissues were then exposed to serosal addition of 200μM ouabain or 5mM theophylline on the serosal surface or to 400μM of furosemide on the mucosal surface. The electrical parameters were again allowed to stabilize (45 min) and the tissues rapidly (<10 sec) punched out and transferred to ice-cold trichloroacetic acid and frozen until assay. The tissues were processed and cGMP content measured by radioimmunoassay as previously described (Rao et al., BBA 632:35-46, 1980).

As shown in the Table ouabain caused a 2-fold increase and theophylline a 5-fold increase in tissue cGMP content whereas furosemide did not have any affect.

EFFECTS OF VARIOUS INHIBITORS ON FLOUNDER INTESTINAL cGMP CONTENT

Condition	(n)	pmoles cGMP/mg protein (Mean ± S.E.M.)
Control	(5)	0.153 ± 0.031
Ouabain 200μM	(5)	0.322 ± 0.092*
Furosemide 400μM	(5)	0.138 ± 0.027
Theophylline 5mM	(5)	0.798 ± 0.159**

*difference from control $p < 0.1$ and ** different from control $p < 0.05$; paired analyses.

We conclude that the inhibition of Na/K/2Cl cotransport either by cAMP analogues in the presence of theophylline or by ouabain may be due to stimulation of cGMP content by the latter two agents.

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