INTERACTION OF THE (Na+K+2Cl) COTRANSPORTER WITH THE Na - TYROSINE COTRANSPORTER IN THE FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) INTESTINE.

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Serosally applied cyclic GMP (cGMP) has been shown to increase the uptake of taurine across the flounder intestine two-fold (2). It also inhibits the (Na+K+2Cl) cotransport process, abolishing transepithelial potential differences, in the same tissue (3). The aim of the present study was to investigate a possible association between the effects of cGMP on the ion cotransport and on amino acid transport.

Since the cellular pool of taurine in the flounder intestine is relatively large (30mM) equilibration of labeled taurine in the medium with unlabeled cellular taurine is too long (>100h) to be practical for transepithelial flux experiments. Therefore, tyrosine was chosen as a representative nutrient amino acid with a relatively rapid rate of transepithelial transport. The possible interaction between the (Na+K+2Cl) and tyrosine transport systems was tested by monitoring the uptake of tyrosine, whilst applying to the tissue compounds known to inhibit the ion co-transporter. One of the effects of inhibiting the (Na+K+2CL) transporter is hyperpolarization of the apical cell membrane, a physical change which could have its own effect on the Na-tyrosine cotransporter. With this in mind, in some experiments the polarity of the apical membrane was altered using other means : addition of barium (5mM) or extra potassium (45mM) to the mucosal medium, both of which depolarize, and voltage clamping of the tissue in the presence of mucosal barium.

The intestines of freshly killed flounder were removed and stripped of their muscle layers, before being mounted in modified Ussing chambers. The standard Ringer solution contained 198 mM NaCl, 5mM KCl, 2.7mM NaH_2PO_4, 1.3mM $CaCl_2$, 1.2mM $MgSO_4$, and 11mM $NaHCO_3$, with 5mM glucose and 0.1mM tyrosine; it was bubbled with 1% CO_2 in O_2, yielding a pH of 7.4. Measurement of the transepithelial movement of tyrosine was performed as described by King et al. (2). To determine the rate of transfer of tyrosine across the apical surface of the mucosal cells, initial rates of ^{14}-C tyrosine absorbtion were determined using influx chambers designed for the purpose (see 3). Preliminary trials showed that the linear period of ^{14}C-tyrosine uptake was represented most reproducibly in tissues exposed to the isotope for 4 min; all values for apical tyrosine transfer have been derived from 4-min fluxes. Similar experiments with ^{3}H-PEG were used to measure extracellular space in tissue samples. The 4-min PEG uptake proved negligible and was therefore ignored in calculating tyrosine influx. Ouabain (10^{-4}M) and 8-bromo derivatives of cyclic nucleotides (cGMP &cAMP- both 10^{-4}M) were added to the serosal medium. Other agents were introduced into the mucosal bath: bumetanide (10^{-5}M), barium ($5x10^{-3}$M), or modified Ringer (50mM KCL & 103mM NaCL; Na-free, N-methyl-D-glucamine being substituted for Na; and Cl-free, gluconate being substituted for Cl). All voltage-clamped tissues were pre-treated with $5x10^{-3}$M Ba; it has been shown by Halm et al (1) that the change in p.d. in tissues so clamped occurs largely at the apical membrane. Tissues were clamped to +30mv or -30mv.

The average rate of tyrosine influx for all tissues tested was 23 nmol g^{-1} tissue min^{-1}. The SE with an n of 18 was ± 0.3. Significant reductions in transport rate were effected by the application of 10^{-4}M ouabain (decreases the lumen-cell Na gradient and abolishes transmural p.d.) or Na-free medium : the rates were 6 ± 1 and 9 ± 1, respectively.

Table I summarizes the effects on tyrosine influx in the flounder intestine of known inhibitors of the (Na+K+Cl) cotransporter: bumetanide, cGMP and Cl^--free medium.

Table I Tyrosine uptake in tissues with inhibitors of the (Na+K+2Cl) cotransporter.

	cGMP (10^{-4}M)		Bumetanide (10^{-5}M)		Cl-free medium	
	Control	Treatment	Control	Treatment	Control	Treatment
Tyrosine Uptake (nmol g^{-1} min^{-1}	25±6	71±13	25±6	61±10	23±3	46±9
P		<0.05		<0.01		0.1
P.D. (mv)	4.0±0.2	1.0±0.6	4.0±0.2	0.7±0.3	4.4±0.7	11.2±1.6
P		<0.01		<0.01		<0.02

Values are means ±S.E.(n=5)

All three treatments significantly altered transepithelial p.d., whilst the amino acid transport rates were enhanced by all three treatments, although only the former two were statistically significant. In contrast to cGMP, cAMP had no effect on tyrosine transport (30± 3 nmol g^{-1} min^{-1} in its presence; 31± 2 nmol g^{-1} min^{-1} in controls).

Maneuvers affecting membrane potential difference (50mM KCl mucosal medium, Ba treatment. and p.d. clamping following Ba treatment) caused no statistically significant change in tyrosine uptake. The 50mM KCl treatment was repeated using a Ringer with a lowered tyrosine concentration (0.01mM) to approximate more closely the normal physiological lumen-cell gradient of the amino acid; control and experimental uptakes remained similar.

The results of the transmural tyrosine flux determinations, shown in Table II, demonstrate that cGMP and bumetanide increase the mucosal to serosal transepithelial transport. Transport in the reverse direction was not affected by either treatment. Thus, both treatments produced a net increase in tyrosine transport in the absorptive direction.

Table II Transmural tryosine fluxes.

	Control	cGMP	Bumetanide
M --> S Tyrosine flux nmol cm^{-2} h^{-1}	33±4	47±2	41±1
n=8			
*P		<0.02	<0.1 †
S --> M Tyrosine flux	5±3	7±2	5±2
n=3			

Values are means ± S.E. * from comparison of means by group data analysis †paired data analysis gives a P value of <0.05.

The results of this study are consistent with a functional association between the (Na+K+2Cl) cotransporter and the Na-dependent tyrosine transporter, although the nature of the association remains ill-defined. cGMP has a profound influence on both systems. Membrane p.d. does not appear to be the decisive factor in that influence since changes in membrane p.d. had little or no effect on tyrosine influx. Extreme p.d. changes, such as those anticipated with ouabain, may have an effect, however, since ouabain inhibited tyrosine influx. Possibly, both (Na+K+2Cl) and (Na+Tyrosine) cotransports utilize a common Na-translocating subunit, which is rate-limiting. This would result in the two transports being inversely related. That cGMP produces a membrane phosphorylation which enhances tyrosine is unlikely, since bumetanide and Cl-replacement had the same stimulatory effect. The common feature of these 3 maneuvers is inhibition of (Na+K+2Cl) cotransport.

1. Halm et al. J. Gen. Physiol. 85: 843-883, 1985 2. King et al MDIBL Bull. 24: 50-51, 1984 3. Rao et al Am. J. Physiol. 246: C167-171, 1984.