EFFECTS OF MERCURIALS AND ARSENICALS ON SODIUM-TYROSINE COTRANSPORT IN BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM FLOUNDER (Pseudopleuronectes americanus) INTESTINE.

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In the intact flounder intestine tyrosine flux across the epithelium has been shown to be inhibited by mercuric chloride ($HgCl_2$), p-chloromercuri phenylsulfonic acid (PCMBS), phenylmercuric acetate (PMA) and oxophenylarsine (OPA) (Chauncey et al., Bull. MDIBL 26:98, 1986). The means, however, by which these compounds exert their inhibitory action remain unclear. Potential targets include the sodium-tyrosine cotransport system in the mucosal membrane, the sodium permeability of the membranes or the Na-K-ATPase in the serosal plasma membranes. In order to elucidate the action of these inhibitors on the sodium-tyrosine cotransport independent of effects of the heavy metals on other cellular targets, flux studies using isolated brush border membrane vesicles were performed.

Flounders were kept in a holding tank for 2 to 7 days without food before use. 8 to 10 flounders were killed by spinal transsection, the intestines were removed, rinsed in mannitol buffer (300mM mannitol, 12mM Tris-HCl, pH 7.1), everted and the mucosa was scraped off. 4-7g of mucosa were mixed with 12ml mannitol buffer and diluted 6 times with ice water. Brush border membrane vesicles were isolated by differential precipitation as described by Eveloff et al. (J. Comp. Physiol. 135:175-182, 1980). Isolated brush border vesicles were taken up in vesicle buffer containing: 50mM mannitol, 2mM $CaCl_2$, 20mM Hepes-Tris (pH 7.0). The vesicles were then preincubated for 30-60 min in vesicle buffer with a heavy metal, final concentration of: 0.1mM $HgCl_2$, 0.5mM PCMBS, 0.1mM PMA or 0.25mM OPA. Control vesicles were preincubated in vesicle buffer without heavy metal. L-tyrosine uptake was determined at 15°C in transport media containing vesicle buffer with 10 uCi ^{3}H-L-tyrosine, 0.05mM L-tyrosine and 75mM NaCl or 75mM mannitol, respectively. Uptake was terminated as described previously (Kinne-Saffran et al., Bull. MDIBL 26:18, 1986) except that the 'stop solution' also contained 0.1mM $HgCl_2$. Mercury was included on the stop solution since in pilot experiments it was observed that it inhibited tyrosine efflux from the vesicles during the dilution, filtration and rinsing process used to stop transport and to separate the vesicles from the extravesicular medium.

$HgCl_2$ increased the initial uptake of tyrosine in sodium-free medium as well as the equilibrium uptake in both sodium-containing and sodium-free media. The sodium-dependent uptake of tyrosine calculated as the difference between the uptake observed in the presence and absence of sodium was inhibited 84% after 15 sec but no difference was observed at equilibrium. PCMBS inhibited uptake both in sodium containing and sodium-free media, the inhibition increased with time reaching 45-70% at the equilibrium time point. PMA stimulated initial uptake in the absence of sodium while the equilibrium values remained unchanged. Sodium-dependent tyrosine uptake was

inhibited by 70%. The arsenical OPA had no effect on any of the parameters measured.

These results can be interpreted as follows. $HgCl_2$ apparently has two effects on the brush border vesicles. It increases the amount of tyrosine found in the vesicles, either by increasing the number of functionally tight vesicles or by inhibiting tyrosine efflux during the termination of the transport by rapid dilution and filtration. In addition $HgCl_2$ inhibits sodium-tyrosine cotransport. The effect of PCMBS can be explained by a time-dependent decrease in the number of tight vesicles or by an increase of membrane leakiness. Sodium-tyrosine cotransport seems not to be affected by this hydrophilic and relatively slowly permeating organic mercury. The lipophilic PMA seems to combine effects of $HgCl_2$ and PCMBS. Initially sodium-tyrosine cotransport seems to be inhibited and the number of tight vesicles increased, later the number of functionally tight vesicles decreases leading to equilibrium values similar to those in the controls.

The exact nature of the inhibition of sodium-tyrosine cotransport by $HgCl_2$ and PMA remains to be determined. If one assumes, however, that these two compounds inhibit the transport system directly and not by dissipation of the sodium gradient, the following mechanism of action on tyrosine transport in the intact epithelium can be hypothesized. $HgCl_2$ and PMA inhibit the sodium-tyrosine cotransporter by interacting with a cytoplasmic region of the transporter. This assumption could explain the lack of inhibition of the transporter in vesicles by the slowly permeating PCMBS. PCMBS and OPA would act mainly by inhibiting the Na-K-ATPase, thereby dissipating the sodium gradient necessary for transepithelial tyrosine transport. Their serosal action and some data in the literature on the interaction of these compounds with the Na-K-ATPase in isolated membranes support this speculation. PCMBS could additionally also increase the nonspecific permeability of the plasma membranes, which would also lead to a dissipation of the electrochemical potential difference for sodium across the membranes.

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