EFFECTS OF MERCURIAL AND ARSENICAL COMPOUNDS ON SEROSAL INFLUX OF RUBIDIUM IN WINTER FLOUNDER INTESTINE (PSEUDOPLEURONECTES AMERICANUS)

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Heavy metals, including mercury and arsenite, affect cellular transport processes through interaction with protein sulfhydryl groups. We recently demonstrated that tyrosine absorption can be inhibited by mercurial and arsenical compounds (Chauncey et al., Toxicol. Env. Health 23:257-265, 1988). Additionally, we demonstrated that tyrosine uptake in winter flounder intestine brush border membrane vesicles (BBMV) could be inhibited by some, but not all of these compounds (Chauncey et al., MDIBL Bulletin 27:92-93, 1988). Oxophenylarsine (OPA) did not inhibit BBMV tyrosine uptake nor the equilibrium uptake of tyrosine; mercuric chloride ($HgCl_2$) increased the Na-independent initial rate of uptake and inhibited the Na-dependent portion of initial (15 sec) uptake by 84%; parachloromercuric benzene sulfonate (PCMBS) decreased the initial rate in both Na-free and Na-containing medium, however, the Na-dependent portion of uptake was not significantly inhibited. Thus, the inhibition of tyrosine absorption by $HgCl_2$ but not by PCMBS or arsenicals can be explained by inhibition of the BBM Na-tyrosine cotransporter. Dissipation of the BBM Na gradient could also explain the inhibition of tyrosine absorption as measured by the mucosa (m)-to-serosa (s) flux. Both mercurial and arsenicals are known to inhibit Na-K-ATPase activity. Therefore, the initial rate of uptake of ^{86}Rb into stripped small intestine (Musch et al., Nature 300:351-353, 1982) from the serosal bathing medium was measured in the presence and absence of ouabain and the effects of $HgCl_2$, PCMBS, and OPA on influx were determined.

Uptake was linear for at least 2 min, during which time there was negligible appearance of ^{86}Rb in the mucosal medium. Preincubation with ouabain (500 μM for 45 min) inhibited the initial rat (2 min) of ^{86}Rb uptake by 65%.

Thirty minutes preincubation with 0.1 mM $HgCl_2$, 1 mM PCMBS, or 0.25 mM OPA inhibited serosal ^{86}Rb uptake to varying degrees. These concentrations have previously been determined to maximally inhibit intestinal tyrosine absorption. $HgCl_2$ was the most effective, inhibiting 61±4% (3) of the influx, whereas PCMBS and OPA inhibited the influx 24±11% (3) and 38±6% (3), respectively. Ouabain inhibited ^{86}Rb uptake by about 60%. In the presence of ouabain none of the compounds showed an additional inhibitory action. All three agents caused a decrease in intracellular [K] albeit to varying extents, consistent with their inhibition of the Na-K-ATPase.

To determine whether membrane penetration might explain the differential inhibition of serosal Rb influx by these agents, the time of preincubation was varied. Influxes were measured after 30 and 60 minutes preincubation (Table 1). Cell [K] was also measured for each sample. The water-soluble, highly permeable inorganic $HgCl_2$ rapidly inhibited both serosal Rb influx and lowered cell K, although much more slowly. PCMBS, an organic mercurial which penetrates less readily, as well as OPA, took appreciably longer to inhibit RB influx and lower cell K. In general, inhibition of serosal Rb influx paralleled changes in cell K, despite the fact that ouabain-inhibitable influx represents the activity of one enzyme, the Na-K-ATPase, whereas cell K is complex and is influenced by many other permeability properties of the basolateral membrane.

TABLE 1

Effect of $HgCl_2$, PCMBS, OPA, and ouabain on serosal ^{86}Rb influx and K content in flounder intestine

		Rb influx (% control)		K content (% control)	
preincubation	n	30 min	60 min	30 min	60 min
0.1 mM $HgCl_2$	2	34±2	37±4	67±5	37±2
1 mM PCMBS	3	88±15	53±3	103±5	73±4
0.25 mM OPA	2	74±7	50±12	95±9	62±10
0.5 mM ouabain	5	38±2	30±2	40±5	32±5

Values are means ±S.E.. ^{86}Rb influx in control tissue averaged 3081±474 after 30 min and 2898±312 nmols/g tissue/2 min after 60 min of preincubation. K content was 121±4 mM and 127±5 mM, respectively.

In conclusion, the effects of mercurial and arsenical agents on tyrosine absorption are complex, and may be due to interactions with more than one transport system. For $HgCl_2$, both Na-coupled tyrosine transporter and the Na-K-ATPase are target proteins. It directly inhibits uptake at the brush border, but also diminishes uptake by raising intracellular Na, thus decreasing the driving force for sodium gradient driven tyrosine uptake. The inhibition of the Na-K-ATPase appears to account for the entire effect of OPA and PCMBS. OPA did not decrease sodium-dependent tyrosine uptake in isolated BBMV, yet inhibited transmural m-to-s flux by 60% at 60 minutes. This is quite similar to the inhibition of serosal Rb influx after 60 minutes incubation. The correlation between inhibition of serosal Rb influx and m-to-s tyrosine flux (at 60 minutes) also holds well for $HgCl_2$ and PCMBS. Their respective inhibitions of serosal Rb influx and m-to-s tyrosine fluxes were 63 and 64% for $HgCl_2$ and 47 and 44% for PCMBS. In conclusion, inhibition of Na-K-ATPase activity appears to be a major mechanism for heavy metal inhibition of tyrosine absorption and may inhibit the absorption of many other nutrients whose uptake is facilitated by the Na-gradient.

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