HEAVY METAL EFFECTS ON THE FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) INTESTINE.

Beth Chauncey, Elizabeth C. Schmid, and Leon Goldstein.
Division of Biology and Medicine, Brown University, Providence, RI 02912

In this study we examined the effects of a variety of heavy metals on intestinal amino acid absorption using the model tyrosine transport system found in the gut of the winter flounder. This Na-dependent tyrosine cotransport system is found in the luminal membrane of the epithelial cells lining the gut. We monitored tyrosine absorptive activity in isolated intestinal strips using ^{14}C-tyrosine to determine the mucosal to serosal fluxes and tissue accumulation. Heavy metals were added to mucosal solutions, as they would reach the gut from the environment, and also serosally, to determine effects of metals that had entered the blood stream.

Flounder were kept in a holding tank for 2 to 7 days (without food) before being killed by transection of the spinal cord. The intestine was removed, stripped of the muscle layers, and mounted onto Ussing chambers. The bathing media in the Ussing chamber was a teleost Ringers containing: 148mM NaCl, 5mM KCl, 2.7mM NaH_2PO_4, 1.6mM $CaCl_2$, 1.24mM $MgSO_4$, 11.0mM $NaHCO_3$, 1mM glucose, and 0.1mM tyrosine; this was bubbled with 1%CO_2 in O_2 (pH 7.4) and maintained at 15°C. After the equilibration period of 30 to 60 min, ^{14}C-tyrosine (0.1uCi/ml) was added to the mucosal side and samples were taken from both mucosal and serosal media initially and after a control period of 60 min. Then the heavy metal was added to the mucosal (or serosal) medium and samples were taken again at 120 min. At the end of the second hour (experimental period) the chambers were opened and the tissue cut out, blotted, weighed, and digested with Protosol. All samples were assayed for radioactivity by standard liquid scintillation procedures. The transmural fluxes (M->S) were calculated for the initial hour (control period) and for the experimental period (second hour). Tissue content was also calculated.

Mercurial Effects

The effects of heavy metals on membrane transport is dependent upon the chemical affinities of the metals for membrane ligands and their ability to reach sensitive sites. Three mercurials, chosen for their different chemical properties, were studied: mercuric chloride is a water soluble, highly permeable, inorganic molecule; p-chloromercuriphenyl sulfonic acid (PCMBS) is a small water soluble, organic compound; and phenylmercuric acetate (PMA) is an organic, lipid soluble mercurial. Furthermore, in $HgCl_2$, mercury is in the Hg^{++} form, whereas in the organic mercurials it exists as Hg^{+}. As shown in Table 1, mucosal 0.1mM $HgCl_2$ markedly inhibited tyrosine flux (64% inhibition) and reduced the tyrosine tissue content by 68%. A 10-fold lower concentration (0.01mM) of mercuric chloride had no significant effect on tyrosine movement in the flounder intestine. Mucosal PCMBS at 1.0mM had a 41% inhibition on tyrosine M->S flux and a 42% inhibition on tyrosine accumulation in the tissue (Table 1). A 10-fold lower concentration (0.1mM), did not affect either tyrosine M->S flux or tissue content. Mucosal PMA (0.1mM) had no significant effect on tyrosine transport in flounder intestine (Table 1). However, two of the five tissues treated with PMA showed a marked decrease in tyrosine flux (a 42-55%

Table 1 Effect of mercurials on tyrosine absorption by isolated flounder intestine

Treatment (Mucosal)	Transmural Flux (M->S) (nmol/cm^2·h) Control ($t_{0'}$-$t_{60'}$)	Experimental ($t_{60'}$-$t_{120'}$)	n	Tissue conc. (nmol/g)	n
None	19±5	17±6	3	153±25	3
$HgCl_2$ (0.01mM)	28±3	24±3	4	163±24	4
None	33±4	37±6	7	197±25	5
$HgCl_2$ (0.10mM)	25±3	9±2†	7	64±10§	5
None	30±3	33±7	4	213±26	4
PCMBS (0.1mM)	34±4	36±3	4	176±29	4
None	35±2	23±6	3	227±14	3
PCMBS (1.0mM)	32±3	19±1*	4	133±15§	4
None	36±4	36±7	5	191±19	5
PMA (0.1mM)	31±6	21±3	5	163±16	5

Values are means ±S.E. *$p<0.02$ (experimental vs. control period); † $p<0.01$ (experimental vs. control period); § $p<0.05$ (treatment vs. no treatment tissues), PCMBS = p-chloromercuriphenyl sulfonic acid, PMA = phenylmercuric acetate.

inhibition), but the overall decrease in flux was not significant.

Serosal addition of $HgCl_2$ (0.1mM) had no significant effect on transmural tyrosine flux or tissue content. However, serosal PCMBS (1.0mM) inhibited tyrosine flux by 45% (40 ± 7 nmol/cm^2·h control period vs. 18 ± 5 nmol/cm^2·h experimental period, n=4, $p< 0.05$) but not tissue content.

Mucosal addition of 0.1mM $HgCl_2$ had a significant effect on transepithial potential (TEP). The ΔTEP in the control period ($t_{0'}$-$t_{60'}$) was -0.37 ± 0.33mV vs. 0.96 ± 0.59mV in the experimental period ($t_{60'}$-$t_{120'}$); $p< 0.05$. Mucosal addition of PCMBS (0.1mM, 1.0mM) and PMA (0.1mM) did not alter ΔTEP significantly. Serosal application of $HgCl_2$ (0.1mM) and PCMBS (1.0mM) showed significant effects on the ΔTEP ($HgCl_2$: ΔTEP = -0.30 ± 0.12mV control period vs. 1.88 ± 0.24mV experimental period, $p< 0.01$; PCMBS: ΔTEP = -0.08 ± 0.13mV control period vs. 2.85 ± 0.82mV experimental period, $p< 0.05$).

PCMBS was not as potent as $HgCl_2$ in inhibiting tyrosine absorption; 0.1mM $HgCl_2$ and 1.0mM PCMBS produced comparable inhibition. The difference may be due to poor penetration of lipid barriers in the cell membrane by hydrophilic PCMBS or to modification of the chemical properties of the Hg atom by attachment to a benzene ring and Cl atom. The latter possibility is supported by the fact that PMA, in which Hg is attached to a benzene ring and an acetate group, is no more potent than PCMBS, even though PMA is more lipid soluble.

Arsenical Effects

The arsenicals tested were oxophenylarsine (phenylarsine oxide) and arsenamide (dithioglycolyl p-arsenobenzamide). Oxophenlyarsine at 0.25mM added mucosally produced a 62% inhibition of tyrosine tissue content and a

Table 2 Effects of arsenicals on tyrosine absorption by isolated flounder intestine

Treatment (Mucosal)	Transmural Flux (M->S) ($nmol/cm^2 \cdot h$) Control ($t_{0'}-t_{60'}$)	Experimental ($t_{60'}-t_{120'}$)	n	Tissue conc. (nmol/g)	n
None	34±7	37±11	3	162±19	3
Oxophenylarsine (0.05mM)	38±5	27±2	4	165±34	4
None	28±3	29±6	6	176±38	4
Oxophenylarsine (0.25mM)	33±5	17±2*	7	67± 9†	5
None	32±5	26±10	4	229±42	4
Arsenamide (0.10mM)	36±3	27±5	4	196±22	4

Values are means ±S.E. * $p<0.01$ (experimental vs. control period), † $p<0.05$ (treatment vs. no treatment tissue, group data analysis).

48% inhibition of tyrosine M->S flux (Table 2). No significant effect on tyrosine absorption was seen at 0.05mM oxophenylarsine. Arsenamide at 0.25mM had no significant effect on tyrosine uptake in flounder intestine. Serosal addition of oxophenylarsine (0.25mM) did not significantly affect the tyrosine M->S flux or tissue content. It should be noted that although the M->S flux was not significantly affected ($p< 0.1$, due to high S.E.), there was a 61% inhibition evoked by the serosal addition of 0.25mM oxophenylarsine. The ΔTEP was significantly increased by all additions of oxophenylarsine: mucosally at 0.25mM, $\Delta TEP = -0.43 \pm 0.20$mV control period vs. 1.86 ± 0.40mV experimental period, $p< 0.01$; at 0.05mM, $\Delta TEP = -0.30 \pm 0.11$mV control period vs. 0.48 ± 0.16mV experimental period, $p< 0.05$; and serosally at 0.25mM, $\Delta TEP = -0.05 \pm 0.15$mV control period vs. 1.72 ± 0.20mV experimental period, $p< 0.02$.

Oxophenylarsine and arsenamide are both trivalent organic arsenicals. However, the side chains on the As atom in the arsenamide is much more complex (2 thioglycols, where oxophenylarsine has an oxygen atom), which may account for its lack of effect. The side chains of arsenamide might have to be cleaved before the arsenical is biologically active.