THE EFFECT OF MERCURY ON CHLORIDE SECRETION IN THE EURYHALINE FISH *FUNDULUS HETEROCLITUS*

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It is well known that mercurials inhibit chloride transport in kidney tubules (Burg, M.& N.Green, *Kidney Int.*4, 245-251,1973) and in the shark rectal gland (Silva, P et al. *Comp.Biochem. Physiol.*Vol. 103 C, No.3, 569-575, 1992). The objective of the present study was to investigate the sensitivity of the chloride secretion of the fish gill to mercuric chloride.

When an euryhaline fish moves into seawater with higher salinity there is a rapid signal that induces an increase in the Cl^- secretion of the gill chloride-secretory epithelium. Previous studies using the killifish (*Fundulus heteroclitus*) have demonstrated that there is a rapid increase in the plasma osmolarity when a fish is transferred from fresh to salt water and that this increase in plasma osmolarity is responsible for the activation of the Cl^- secretion (see Zadunaisky, in *Ionic regulation in Animals*, Hazon, Eddy & Fink, eds., pp.87-105, Springer.N.York 1997). Previous results have moreover demonstrated that stimulation of the Cl^- current by increased plasma osmolarity or by stimulation of β-receptors involves phosphorylation processes by protein kinases (Hoffmann et al. *MDIBL Bulletin* vol. 37, 1998). It is thus likely that stimulation of the chloride secretion could have a different sensitivity to mercurials than has the basic Cl^- secretion. In the present study therefore, we specifically wanted to investigate the effect of $HgCl_2$ on the chloride secretion after an increase in osmolarity or after agonist stimulation.

The isolated opercular epithelium of the killifish (*Fundulus heteroclitus*) is a useful model for the study of chloride secretion in fish (Zadunaisky, J. *Fish* Physiology, XB, eds.Hoar & Randall, Academic Press, 1984). In the present work the opercular epithelium was used to investigate the effect of $HgCl_2$ on the basic chloride secretion and on the signals to activate this secretion. An increase in chloride secretion was achieved by increasing the osmolarity of the medium or by addition of the β-receptor agonist isoproterenol. Adult killifish were acclimated to circulating seawater (32 ppt) and the dissected opercular epithelium was mounted in a modified Ussing chamber in regular teleost Ringer's solution on both sides as described by Zadunaisky et al. *J.Membrane Biol.*143, 207-217, 1995. The short circuit current was used as a measure of the chloride current.

After 30 min of an initial control period, mercuric chloride, a soluble inorganic salt of mercury was added in the wanted concentration to the Ussing chamber at both sides, and the short circuit-current was followed for an additional 2 hours. Mercury inhibited the short circuit current in a dose dependent way with half maximal inhibition at about (1.5-2.0) microM $HgCl_2$. After 2 h exposure to 4 microM Hg, short circuit current was reduced to 68% of controls.

The osmolarity was increased by 100 mOsm by adding either hypertonic Ringer's or manitol to both sides of the epithelium. In 13 independent experiments addition of 100 mOsm manitol to both sides increased the short circuit current with 103 ± 10 μAmp/cm^2, which was a doubling compared to the steady state short circuit current. This activation was completely abolished when the tissue was pre-incubated for 2 hours with 4 microM $HgCl_2$ (figure 1).

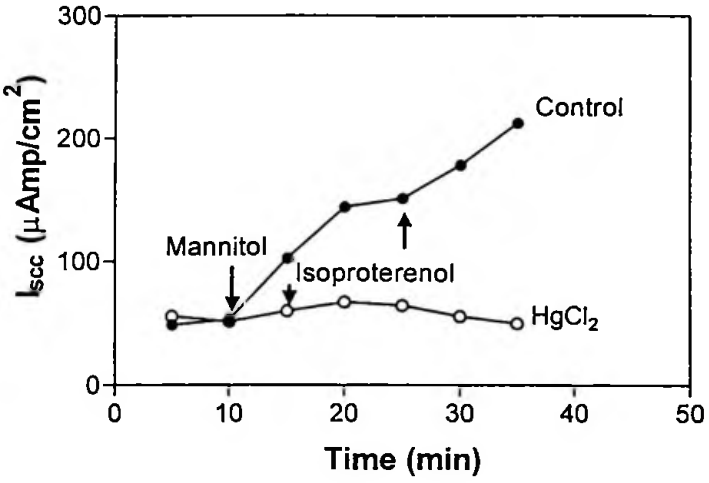

Figure 1. Effect of $HgCl_2$ (4 microM) on the activation of the short-circuit current by addition of manitol (100 mOsm) and isoproterenol (10^{-5} M). The epithelium was pre-incubated 2 hours with 4 microM $HgCl_2$.

Figure 2 gives the dose-response curve for the effect of $HgCl_2$ on the increase in short circuit current after hypertonic stimulation in 4 independent experiments. The IC_{50} value is estimated at 2.5 microM $HgCl_2$.

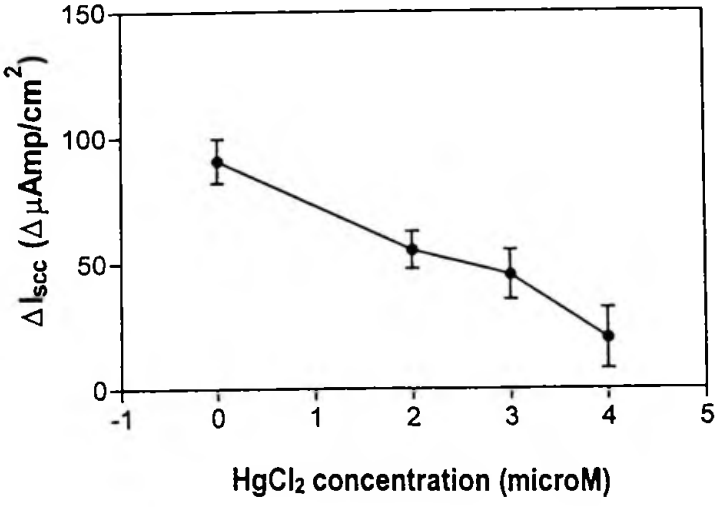

Figure 2. Dose-response curve for the effect of $HgCl_2$ on the increase in the short circuit current after addition of 100 mOsm manitol. The experiments were performed as described in figure 1.

Addition of the β-receptor agonist isoproterenol (10^{-5} M) increased the short circuit current with 129±19 μAmp/cm² in 12 independent experiments. The isoproterenol stimulation was in 4 independent experiments completely blocked by 4 microM $HgCl_2$. Figure 3 gives the dose-response curve for $HgCl_2$ on the stimulation after addition of isoproterenol. The IC_{50} values is estimated at (2.0-2.5) micro M $HgCl_2$.

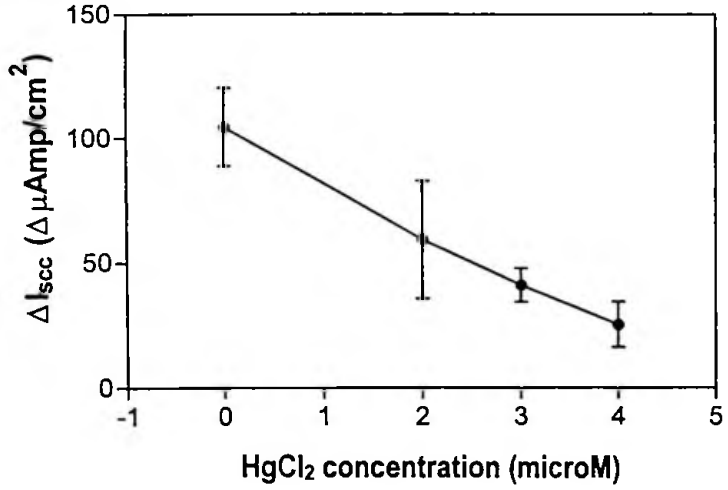

Figure 3. Dose-response curve for the effect of $HgCl_2$ on the increase in the short circuit current after addition of 100 mOsm manitol and 10^{-5} M isoproterenol. The experiments were performed as described in figure 1

When the epithelium was stimulated with manitol as well as isoproterenol the current increased with 133±21 μAmp/cm². This stimulation was also completely blocked by 4 microM $HgCl_2$ as seen on figure 1.

In conclusion, stimulation of the Cl^- secretion by increase in plasma salinity or by stimulation of β-receptors involves processes with an extremely high sensitivity to $HgCl_2$. Whether the mechanism underlying the $HgCl_2$ inhibitory effect is the consequence of binding of Hg^{2+} to sulfhydryl groups on the apical Cl^- channel and/or on the basolateral Na^+, K^+, $2Cl^-$ cotransporter is the subject for future investigations. Since euryhaline fish species are critically dependent on their ability to a rapid acclimation to high salinities it follows from the above results that this group of fish are likely to be particularly sensitive to mercurials.

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