THE EFFECT OF MERCURY ON CHLORIDE SECRETION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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In the mammalian kidney, inorganic and organic mercurials produce a natriuresis and diuresis. The predominant site of action of mercurials is the thick ascending limb of Henle (TAL) (Burg and Green, Kid. International 4:245, 1973), where their effect is exerted from the lumenal side of the cell, is pH dependent, and is attenuated by thiols (Weiner et al., J. Pharm. Exp. Ther. 138:96, 1962). The unique characteristic of the TAL is that chloride is the ion actively reabsorbed. The rectal gland of the shark also transports chloride actively. Although the direction of transport is reversed (secretory rather than reabsorptive), similar membrane transport proteins appear to be involved including a 2Cl-Na-K cotransporter, Na^+-K^+ ATPase, and Cl and K conductive pathways. We have previously studied the effects of inorganic and organic mercurials in the shark rectal gland and found that, in contrast to the mammalian kidney, only inorganic mercury but not organic mercurials was able to inhibit chloride transport. In this report, we describe the effects of the aryl organic mercurial, phenyl mercuric acetate, and inorganic mercury with cysteine on chloride secretion by the rectal gland and the effects of mercuric chloride on Na^+-K^+ ATPase and adenylate cyclase activity in isolated rectal gland membranes.

Isolated shark rectal glands were perfused as previously described (Solomon et al., Amer. J. Physiol. 1992). Theophylline 2.5 x 10^{-4}M and dibutyryl cAMP 5 x 10^{-5}M was added throughout to stimulate chloride secretion. All glands were perfused for a 30 minute control period after which the experimental drug was added for 30 minutes. All experimental periods were followed by a final 30 minute recovery period without the experimental drug. Mean chloride secretion for each 30 minute period was reported as $\mu Eq \cdot h^{-1} \cdot gww^{-1} \pm$ SEM.

Membranes of shark rectal gland were prepared by homogenization in (mM): Tris 50, EDTA 1, dithiothreitol 1, sucrose 250, pH 8.0 and differential centrifugation. For the adenylate cyclase assay, 50 µl of supernatant from the homogenate was added to 50 µl of assay solution containing in final concentration : Tris 50 mM, pH 7.6, phosphocreatine 15 mM, creatine phosphokinase 26.35 µg/ml, theophylline 10 mM, and IBMX 2 mM. The reaction was started by adding 10 µl of ATP 10 mM and $MgCl_2$ 40 mM at 10 second intervals. The reaction was stopped by the addition of 900 µl of sodium acetate 50 mM, pH 4.0, boiled for three minutes, centrifuged at 5000 rpm for 15 minutes and the supernatant stored frozen for cAMP assay. cAMP generated was measured using a commercial radioimmunoassay kit (Amersham). Results were expressed as picomoles of cAMP generated·mg protein $^{-1} \cdot h^{-1}$.

For Na^+-K^+ ATPase measurements, rectal gland membranes were prepared as above by homogenization of two rectal glands in (mM): sucrose 250, HEPES 20, pH 6.8. Membranes were separated by differential centrifugation and Na^+-K^+ ATPase was measured using the method of Barnett (Barnett, Biochem. 9: 4644, 1970).

Inorganic mercuric chloride inhibited chloride secretion in a dose dependent manner as previously described (Table 1). The monothiol, cysteine (10 mM), was added to the perfusate during control, experimental, and recovery periods. Cysteine attenuated the effect of $HgCl_2$ although this did not reached statistical significance. The effects of dithiothreitol, DTT, a dithiol is also presented for comparison.

Table 1. The effect of inorganic and organic mercurials on chloride secretion in the isolated perfused shark rectal gland. Glands were perfused for 30 minutes at each concentration after which an additional 30 minutes recovery period in the absence of the mercurial was performed.

	BASAL	10^{-6} M	10^{-5} M	10^{-4} M	RECOVERY
$HgCl_2$ [7]	1235±159 (100)	867±162* (70)	796±85* (64)	349±33* (28)	141±20* (11)
$HgCl_2$+ cysteine [7]	1345±173 (100)	1057±202 (79)	1201±262 (89)		749±143* (56)
$HgCl_2$+ DTT [8]	990±129 (100)	1233±372 (125)	761±164 (77)	599±110** (61)	1207±175 (122)
Phenyl mercuric acetate [6]	1744±258 (100)	1773±183 (102)	1292±298 (74)	760±279* (44)	575±79* (33)

Data = mean ($\mu Eq \cdot h^{-1} \cdot gww^{-1}$) ± SEM; [] = number of gland perfused; () = percentage of BASAL value; * = significant compared to BASAL; ** = significant compared to $HgCl_2$

The aryl organic mercurial, phenyl mercuric acetate, which is acid stable and has no diuretic action in the mammalian nephron (Weiner et al, J. Pharm. Exp. Ther. 138:96, 1962) surprisingly inhibited active chloride secretion in a dose dependent manner and with a potency similar to that of $HgCl_2$ (Table 1). There was no evidence of recovery following its elimination from the perfusate.

Mercuric chloride inhibited Na^+-K^+ ATPase activity in a dose dependent manner between 10^{-6} and 10^{-4}M (Figure 1). Exposure of rectal gland membranes to $HgCl_2$ in this assay was for a duration of 15 minutes.

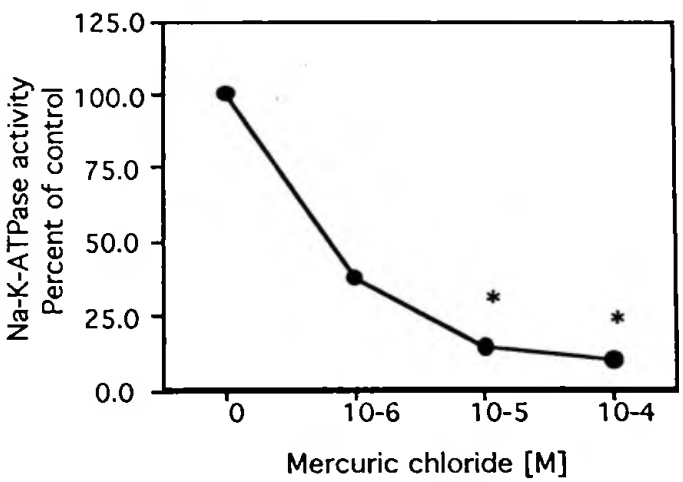

Figure 1. Inhibition of Na^+-K^+ ATPase by mercuric chloride. Each point represents the mean of 5 cell preparations. * indicates that the mean is significantly different from the "0" concentration.

In three separate experiments, mercuric chloride produced inhibition of adenylate cyclase in rectal gland membranes at 10^{-5} and 10^{-4}M but not at 10^{-6}M (Figure 2).

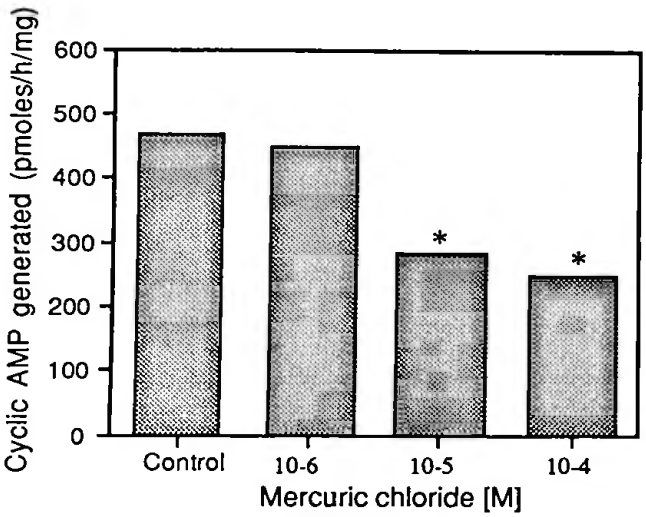

Figure 2.

Figure 2. Inhibition of adenylate cyclase activity by mercuric chloride. Each bar represents the mean of 3 cell preparations. * indicates that the mean is significantly different from the control mean.

The present experiments confirm and extend our previous observations of significant differences between the reported effects of mercurials in the mammalian nephron and those we observed in the shark rectal gland. With regard to the organic mercurials, which are potent inhibitors of chloride reabsorption in the mammalian nephron, the explanation for their lack of effect in the rectal gland remains unknown. These agents inhibit chloride transport from the lumenal side of the mammalian nephron. We have exposed the rectal gland to these agents on the basolateral (blood) side. However, because of different directions of transepithelial transport in these two tissues, similar membrane transport protein are present at the site of highest exposure to the organic mercurial. In addition, oganic mercurials are highly lipid soluble, more so than inorganic mercurials, and therefore entry into the cell is an unlikely explanation for the differential effects seen with inorganic and organic mercurials in the shark rectal gland (Webb in Enzyme and Metabolic Inhibitors, Academic Press, 1966, p742).

We used another organic mercurial, phenyl mercuric acetate, an aryl compound without diuretic potency in the mammalian nephron (Weiner et al., J. Pharm. Exp. Ther. 138:96, 1962) to further investigate the mechanism of action of these compounds. We found that this compound effectively inhibited chloride transport in the shark rectal gland model. The compound may behave more like an inorganic mercurial (Winship, Adv. Drug React. Ac. Pois. Rev.3:141, 1986). Its lack of effect in the mammalian nephron may be attributed to its poor urinary excretion .

The effect of cysteine on the inhibitory effect of inorganic mercury again supports the role of SH-groups as ligands for inorganic mercury. The addition of cysteine competes with the target SH-groups on the cell membrane reducing the effective concentration of mercury.

Finally, we have demonstrated that inorganic mercury inhibits two enzymes involved in mediating transepithelial transport in this tissue. The inhibition of Na+K ATPase is consistent with a variety of previous observations on the membrane effects of mercury. The ability to inhibit adenylate cyclase activity suggests that this enzyme also possesses critical SH-groups necessary for its function.

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