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

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We have previously examined the effect of organic and inorganic mercurials on chloride secretion by the rectal gland. We found that mercuric chloride inhibits chloride secretion in a dose dependent and irreversible way. Mersalyl, an organic mercurial had no effect. This findings are interesting because they are contrary to those observed in the mammalian kidney where their site of action, the thick ascending limb, is thought to be the mammalian counterpart to the rectal gland. In this report we explore the effect of additional organic mercurials and also investigate the effects of dithiotreitol (DTT) a protector of sulfhydryl groups. We also examined the effect of cadmium on the inhibitory effect of mercuric chloride because cadmium prevents the effect of mercury in some tissues (Webb et al. Chem Biol Interact 14:357-69, 1976).

Isolated shark rectal glands were perfused using a technique developed in our laboratory. Dogfish were pithed and the rectal glands removed by an abdominal incision. The rectal gland artery, vein and duct were catheterized and the glands placed in a glass perfusion chamber maintained at a temperature of 15° C with running sea water. The glands were perfused by gravity at a pressure of 40 mm Hg. The composition of the perfusate was (in mM): Na, 280; Cl, 280; K, 5; bicarbonate, 8; phosphate, 1; Ca, 2.5; Mg, 1; sulfate, 0.5; urea, 350; glucose, 5; ph, 7.6 when gassed with 99% O_2/ 1% CO_2. Rectal gland secretion was collected in tared 1.5 ml centrifuge tubes over 10 minute intervals. Chloride concentration in the rectal gland secretion was measured by amperometric titration. Glands were stimulated to secrete chloride with dibutyryl cyclic AMP 5 x 10^{-5}M and theophylline 2.5 x 10^{-4}M.

Effect of organic mercurials on chloride secretion

Figure 1 shows that meralluride at a concentration of 10^{-4}M had no effect on chloride secretion. In the initial experiments meralluride was dissolved in DMSO. Because of the possibility that the drug was not adequately dissolved we repeated the experiments with meralluride dissolved in glacial acetic acid and the perfusate solution titrated back to 7.6 after the addition of the meralluride. Again there was no effect. The results of both series of experiments were pooled together.

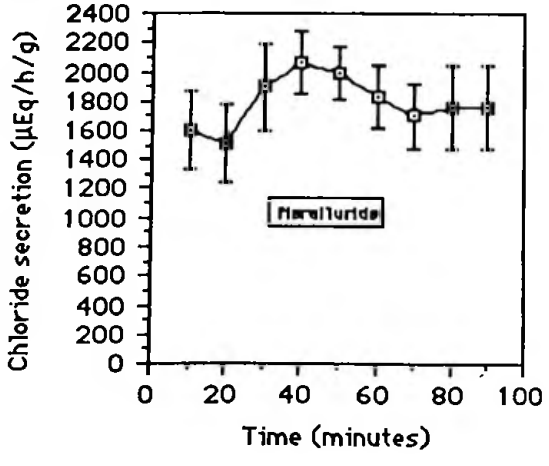

Figure 1. Effect of meralluride 10^{-4}M on chloride secretion. Meralluride was added to the perfusate during the time indicated by the box. There was no effect of meralluride on the secretion of chloride. Values are mean ± SEM, n=6.

Another organic mercurial, PCMBS was then tested. Figure 2 shows a dose response curve for PCMBS. There was no effect on chloride secretion. A small but statistically not significant effect was discernible at 10^{-4}M.

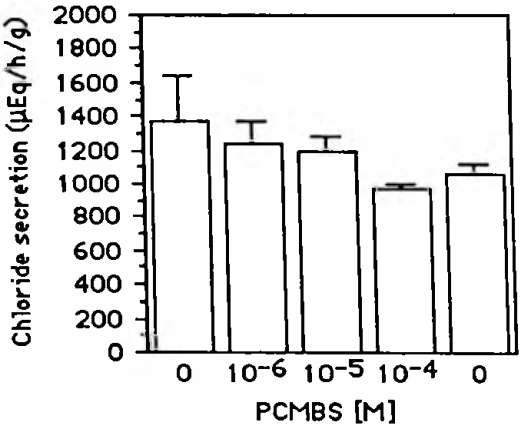

Figure 2. Effect of PCMBS on chloride secretion. Each column represents the sequential addition of increasing concentrations of PCMBS after an initial control period and ended with another control period. All periods were 30 minutes in duration There was no effect of PCMBS on the secretion of chloride by the rectal gland. Values are mean ± SEM, n=6.

Effect of DTT

DTT is known to prevent the toxic effect of mercuric chloride in many cell systems. We therefore examined the effect of DTT on the inhibitory effect of mercuric chloride. Figure 3 shows that DTT reduced the effect of mercuric chloride. In addition, the effect of mercuric chloride was no longer irreversible in the presence of DTT.

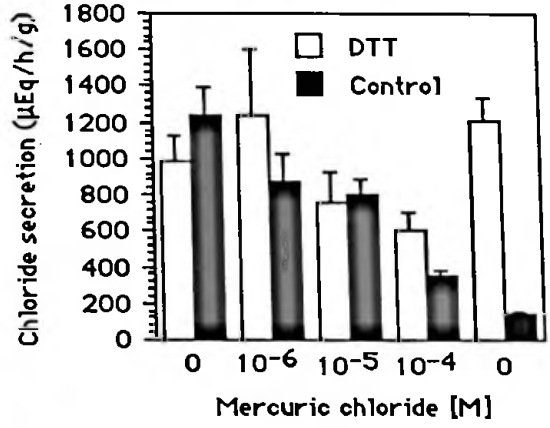

Figure 3. Effect of DTT on the toxic effect of mercuric chloride. DTT reduced the effect of mercuric chloride seen at 10^{-6}M and also at 10^{-4}M. In the presence of DTT the effect of mercuric chloride was no longer irreversible, compare DTT with control in the last control period labeled 0. Values are mean ± SEM, n=11 for DTT experiments and n=8 for the control.

Effect of cadmium

Because cadmium has been found to antagonize the effects of inorganic mercury we tested the effect of cadmium chloride on the toxic effect of mercuric chloride. Figure 4 summarizes the findings. Cadmium chloride did not prevent the effect of mercuric chloride.

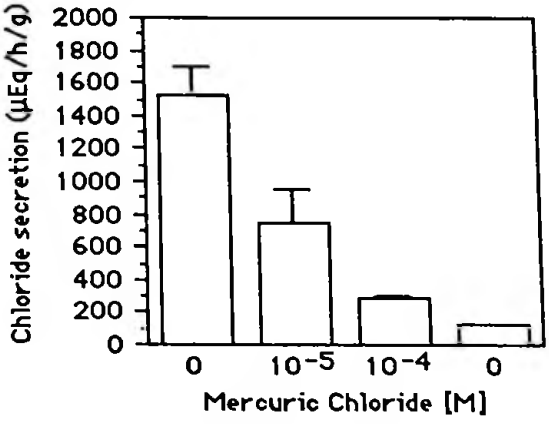

Figure 4. Effect of cadmium chloride on the toxic effect of mercuric chloride. Cadmium at a concentration 250 mM did not prevent the toxic effect of mercury. Values are mean ± SEM, n=3.

These experiments complement and corroborate the results we had previously obtained. Inorganic mercury has a toxic effect on the secretion of chloride by the rectal gland. The effect of mercury appears to be linked to its ability to bind to sulfhydryl groups inasmuch as it is prevented by DTT that protects these groups, although it is also possible that DTT may be acting as a sink and preventing mercury from reaching the tissue. A consistent and puzzling result is the lack of effect of organic mercurials. Neither mersalyl, previously reported to be without effect, nor meralluride or PCMBS had an inhibitory effect on chloride secretion at concentration as high as 10^{-4}M. Of note is the observation by Kleinzeller et al. (Biochim. Biophys. Acta 1025:21-31, 1990) that PCMBS and other organic mercurials induce swelling of slices of shark rectal gland. Our results are particularly interesting because they are opposite to those observed in the mammalian kidney. In the kidney mercurials exert their effect on the thick ascending limb of the loop of Henle, a segment of the nephron that is considered in general terms to be homologous to the rectal gland. In the kidney, organic mercurials like mersalyl and meralluride have a powerful effect while inorganic mercurials are not nearly as effective. Moreover, in isolated perfused thick ascending limbs of the loop of Henle, mersalyl inhibits chloride reabsorption. Interestingly, this effect is prevented by PCMB. We have no explanation for these differences. The effect of organic mercurials on the kidney is thought to depend on their ability to release inorganic mercury. Both mersalyl and meralluride are very efficient diuretics in the kidney whereas they have no effect in the rectal gland. Another explanation for the differential effect of inorganic versus organic mercurials is that their effect depends on pH. We tested for this in previous experiments and found that reducing the pH did not evoke an inhibitory effect of mersalyl. On the other hand, inorganic mercury that is not nearly as efficient in the kidney has a clear effect in the rectal gland. We conclude from this experiments that the site of action of rials in the thick ascending limb of the mammalian kidney is not present or not accessible in the rectal gland. Alternatively, if the release of inorganic mercury from the organic compounds is the necessary step for an effect of organic mercurials, the rectal glands may lack the capacity to do so thus rendering these compounds completely ineffective.

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