DITHIOTHREITOL REDUCES THE EFFECT OF COPPER ON THE ISOLATED CORNEA OF THE BULLFROG, Rana catesbiana

Jose A. Zadunaisky[1], Ben Lowenstein[2], and John I. Scheide[3], [1] Dept. Physiology and Biophysics, New York University Medical Center, NY, NY 10016, [2] Bates College, Lewiston, ME 04240 [3] Dept. Biology, Central Michigan University, Mt. Pleasant, MI 48859.

The short circuit current (I_{sc}) of the bullfrog corneal epithelium approximates net chloride transport when the cornea is mounted in an Ussing chamber (Zadunaisky, Amer. J. Physiol. 211:506-512, 1966). We have previously observed that the addition of $CuSO_4$ to the stromal side of the cornea produced a biphasic change in the I_{sc} and tissue conductance (G_t) with both the I_{sc} and G_t first increasing, then falling below the original steady state (Zadunaisky, Reing and Scheide, Bull. MDIBL 26:159-162, 1986). We have also observed that copper inhibits the response of the corneal epithelium to isoproterenol (Zadunaisky, Reing and Scheide, Bull. MDIBL 27:86-87, 1987), a beta-adrenergic agonist that normally stimulates chloride transport. In this report, we show that corneas pretreated with dithiothreitol (DTT), a sulfhydryl protecting agent, do not show copper sensitivity.

Corneas were isolated from bullfrogs, Rana catesbiana, as previously described and mounted in a modified Ussing chamber with amphibian saline on both sides (Scheide and Zadunaisky, Amer. J. Physiol. 254:C519-C524, 1988). Both sides of the chamber were gassed with 95/5 % air/CO_2 for a final pH=7.3. Dithiothreitol in amphibian saline was added for a final concentration of 10^{-4} M. Copper sulfate was solubilized in deionized water was then added to the Ussing chamber (20 µl) for a final concentration of 5 X 10^{-5} M. All additions were made on the stromal side of the cornea. All chemicals were purchased from commercial sources.

The stromal addition of 10^{-4} DTT to the cornea would sometimes affect the I_{sc} (Figure 1). Two tracings are shown (dashed lines) to note that sometimes an overt effect was observed and other times none occurred. This consistent decreasing trend was significant ($P<0.05$, $n=5$) and may be real or skewed due to the consistent decrease in I_{sc} observed in the steady state condition. The effect of DTT at pH=7.3 warrants further investigation. Addition of 5 X 10^{-5} M $CuSO_4$ to the stromal side of the DTT-treated corneas did not produce the "classic copper response" (dashed lines) as observed in the control tracing without DTT (solid line). In addition, the DTT-treated corneas responded to 10^{-7} M isoproterenol whereas the nonDTT-treated cornea did not. While no significant change was observed with copper addition in the DTT-treated corneas, the addition of isoproterenol increased both the I_{sc} 100 $\pm$ 15% and G_t 24 $\pm$ 6% ($P<0.05$ for both, $n=5$).

These results suggest that the sulfhydryl protecting agent dithiothreitol, was effective in reducing or totally eliminating the effect of copper on the corneal epithelium. From our previous observations, we have noted that the copper effect appears to occur at the corneal apical membrane, suggesting that the chloride channels may be affected. These studies also showed that copper treatment inhibits the presumed regulatory response observed with isoproterenol. The data presented in this

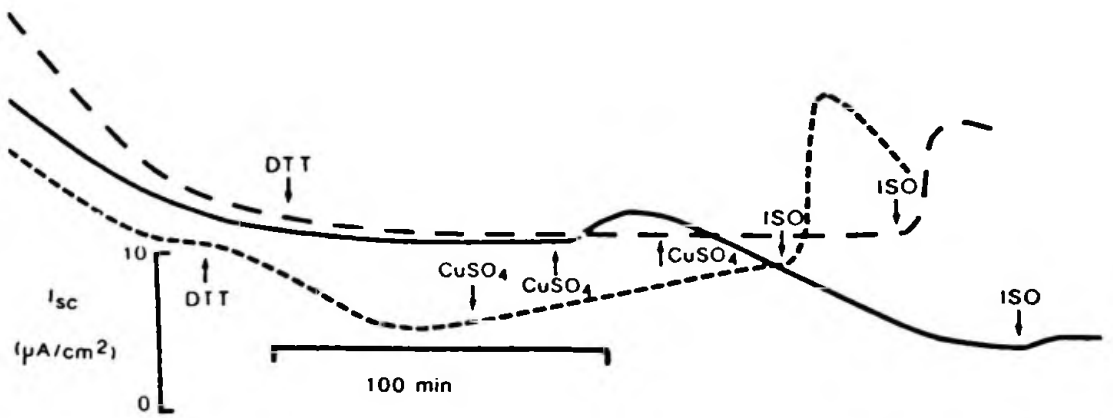

Figure 1. Tracings of corneas with (dashed lines) or without (solid line) 10^{-4} M dithiotreitol (DTT), showing the lack of copper ($CuSO_4$, 5 X 10^{-5} M) interaction with the DTT-treated corneas and the response of these corneas to 10^{-7} M isoproterenol (ISO).

communication and previous communications (Zadunaisky et al. Bull. MDIBL 26:159-162, 1986; Bull MDIBL 27:86-87, 1987) indicates that copper may be affecting the regulatory mechanism that dictates chloride channel function, perhaps at the receptor or at the adenylate cyclase level. In summary, the addition of dithiothreitol eliminated the copper response in the bullfrog corneal epithelium and permitted a "normal" response to isoproterenol.

This work was supported by NIEHS 1 P50 ES 03828-01 S to the Center for Membrane Toxicity Studies.