COPPER MODIFIES THE ISOPROTERENOL STIMULATION OF THE CORNEAL CHLORIDE CURRENT IN THE BULLFROG, Rana catesbeiana.

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The corneal epithelium of the bullfrog secretes chloride with the net chloride transport approximating the short circuit current when the cornea is isolated, placed in an Ussing chamber and voltage clamped (Zadunaisky, J. A., Am. J. Physiol. 211:506-512, 1966). Chloride secretion is the result of a basolateral Na/K ATPase responsible for a Na gradient favoring the inward movement of chloride by the NaCl cotransporter. Cellular chloride transport is regulated by cAMP (Chalfie, Neufeld and Zadunaisky, Invest. Ophthal. 11:644-650, 1972), with most agents that elevate cell cAMP causing an increase in I_{sc}.

We previously detailed the effect of stromal copper on the corneal transport properties. The addition of $CuSO_4$ initially produced an increase in the I_{sc} and G_t, then an inhibition of both to a level below the original steady state. After the copper response, the tissue was refractive to any additional copper. The copper inhibited corneas still responded to 10^{-6} M isoproterenol, a beta-adrenergic agonist that stimulates the I_{sc} and increases G_t (Scheide and Zadunaisky, Am. J. Physiol. 254:C519-C525, 1988).

In this report, we describe the effect of copper on the stimulation of the I_{sc} by isoproterenol, noting that the effect of copper on the corneal epithelium may be due to copper induced changes on the mechanism regulating chloride transport.

Corneas from the bullfrog, Rana catesbeiana (West Jersey Biological Supply), were isolated form the eye as previously described (Zadunaisky, J. A., Am. J. Physiol. 211:506-512, 1966) and mounted in a modified Ussing chamber. Both sides of the cornea was bathed in frog Ringer gassed with 95/5 Air/CO_2 for a final pH of 7.4. Chloride-free Ringer was substituted gluconate salts for the chloride salts and had the same pH. Copper concentrations in the bath were measured with a Perkin Elmer Atomic Absorption Spectrophotometer. The short circuit current (I_{sc}) was continuously monitored with a Voltage clamp unit (University of Iowa, Bioengineering) and the corneal conductance discontinuously monitored with a 5 mV bidirectional pulse every 50 sec. $CuSO_4$ was initially solubilized in deionized water, then added to Ringer with the dilution never exceeding 5%. This method of copper addition was successful for adding the initial copper concentration, however after 30 min the copper concentration in the chamber fell approximately 50% with or without the cornea present, indicating the loss of bath copper to the walls of the lucite chamber. The corneal chambers were washed thoroughly with water and dilute acetic acid daily, to remove the copper contamination. In addition, copper from the tap water supply (5×10^{-6} M) was found to affect the bullfrog corneas, thus the frogs were maintained in deionized water with 1% sea water.

Changing the bathing solution from a chloride Ringer to a chloride -free Ringer (Cl^--free Ringer) solution decreased the corneal I_{sc} to

18.5 ± 3.7% and the G_t to 57.8 ± 10.6% control values (control values were 15.3 ± 3.8 $\mu A/cm^2$ for I_{sc} and 0.70 ± 0.21 mS/cm^2 for G_t, n=5), indicating the chloride dependence of these two properties. The addition of 5 X 10^{-5} M $CuSO_4$ to corneas in the Cl^--free Ringer did not significantly change the I_{sc} or G_t. The I_{sc} and G_t values after copper addition to corneas in Cl-free Ringer were 12.9 ± 4.4% and 55.6 ± 10.7% (respectively, normalized to control values, n=5). The effect of copper was dependent on chloride transport.

Paired corneas from the same bullfrog were used to determine isoproterenol sensitivity with one cornea not treated with copper, thus used as control. The other cornea was treated with 5 X 10^{-5} M $CuSO_4$ and displayed the typical copper response. The isoproterenol response of the corneas was investigated by sequential addition of increasing isoproterenol concentrations. In the control corneas, isoproterenol was effective in stimulating the I_{sc} above control values at 10^{-8} M with maximal response at 10^{-7} M. In the copper-treated corneas, isoproterenol stimulated at 10^{-6} and maximal stimulation occurred at 10^{-5} M. The copper treatment resulted in a shift of corneal sensitivity to isoproterenol to the right. The maximal isoproterenol response was similar between the two groups (11.9 ± 1.9 and 10.2 ± 2.0 $\mu A/cm^2$, increases for control (10^{-7} M) and copper treated corneas (10^{-5} M), respectively, n=7). Tissue conductance followed the trend of the I_{sc}, isoproterenol sensitivity of the G_t in copper-treated corneas was 100 fold less than control.

The biphasic copper effect observed in the cornea is dependent on chloride indicating that $CuSO_4$ is affecting chloride secretion. The steady state current of the cornea is an equilibrium state reached after the tissue is mounted in an Ussing chamber and represents a balance of remaining regulatory processes. The reduced isoproterenol sensitivity due to the copper treatment indicates copper may be inhibiting some of the regulatory mechanisms that normally increase chloride secretion.

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