THE EFFECT OF COPPER AND ZINC ON THE ISOLATED CORNEA OF THE BULLFROG, Rana catesbiana.

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Exposure of the eye to copper produces deposits in the cornea (Wilson's disease), uveitis, retinal damage, and changes in electrolyte concentrations of the ocular fluids. The reaction of ocular tissues to copper provides ideal models for the study of the toxicity of this divalent heavy metal that may be found in the environment in abnormal concentrations. The corneal epithelium secretes chloride to maintain corneal transparency by dehydrating the stroma (Zadunaisky, J.A., Lande, M.A., Am. J. Physiol. 221:1837-1844, 1971). The short circuit current of the cornea equals the net chloride secretion (Zadunaisky, J.A., Am J. Physiol. 211:506-512, 1966). Chloride secretion in the corneal epithelium is the result of basolateral chloride entry by way of a NaCl cotransporter system with Na/K ATPase responsible for maintaining the basolateral Na/K gradient, while chloride exits the corneal epithelial cell passively by chloride channels. Copper has been reported to block chloride channels and zinc is toxic in many tissues. In this study on an ocular tissue, in vitro, we tested the effects of $CuSO_4$ and $ZnSO_4$ on the metabolic transport of the corneal epithelium.

Bullfrogs, Rana catesbiana, were purchased from West Jersey Biological Supply. The corneal button was dissected from the eye and mounted in a corneal lucite chamber (described in Zadunaisky, J.A., Am. J. Physiol. 211:506-512, 1966) with an aperature of 0.333 cm^2. Frog Ringers was gassed with air for a final pH=8.3. Recordings of short-circuit current and tissue conductance were obtained using an Iowa Voltage-Current Clamp 710C-1. Solubility difficulties required $CuSO_4$ and $ZnSO_4$ be mixed up in deionized water, then added to the Ringer solution. All chemicals were purchased from Sigma. Drug additions to the bathing solutions were never allowed to increase the chamber volume by more than 5% and the addition of the various compounds did not alter the bath pH.

The addition of 10^{-5} M $CuSO_4$ to the serosal side of the cornea resulted in a biphasic response (Figure 1), however the mucosal addition of $CuSO_4$ (10^{-5} M) was without effect (Table 1). The serosal $CuSO_4$ addition resulted in a transient 9% elevation of the short-circuit current accompanied with an 8% increase in tissue conductance, the maximal response occurring approximately 20-30 minutes after $CuSO_4$ addition. After the transient I_{sc} increase, the I_{sc} decreased for the next 120 minutes to a significantly lower I_{sc} ($P<0.01$). Serosal $CuSO_4$ decreased the I_{sc} 64% and tissue conductance was reduced 24%. At the final steady state ($CuSO_4$ inhibited), the corneal electrical parameters were refractive to any further copper effect. In addition, the observed biphasic $CuSO_4$ response was a threshold type, all or none, making a dose response curve impossible.
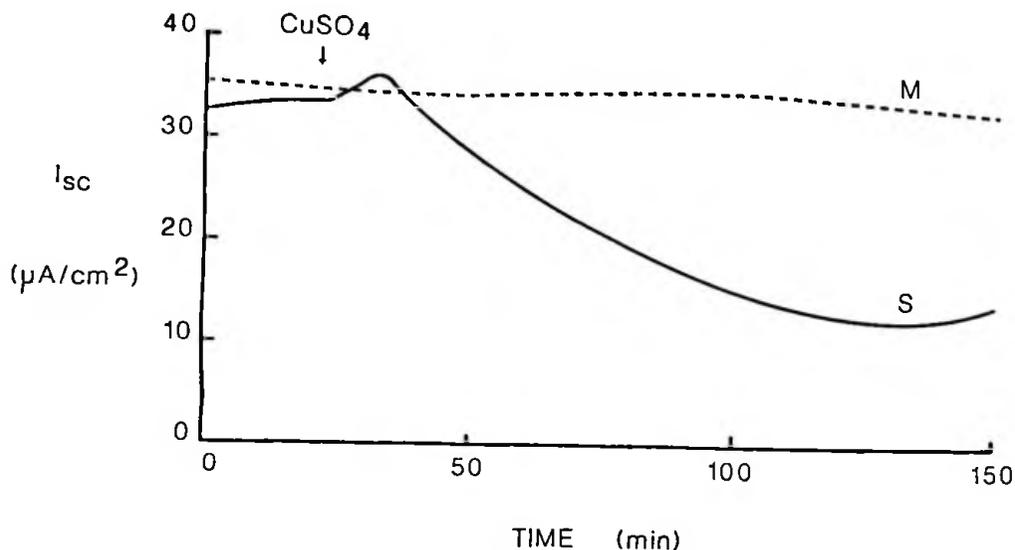
Figure 1. The I_{sc} response of the isolated cornea to the serosal (solid line) and mucosal (dashed line) addition of 10^{-5} M $CuSO_4$.

Table 1. Effects of 10^{-5} M $CuSO_4$ addition on either side of the isolated cornea. The biphasic effect of $CuSO_4$ is noted with the maximal current reached (max, a non-steady state peak) and the minimal current reached (min, a steady state level).

Side of Addition	n	Control I_{sc} ($\mu A/cm^2$)	Change in I_{sc} ($\mu A/cm^2$)	Control G_t (mS/cm^2)	Change in G_t (mS/cm^2)
Mucosa	3	24.6 ± 5.8	1.8 ± 0.6	0.85 ± 0.28	0.03 ± 0.06
Serosa max	7	25.9 ± 2.9	2.3 ± 0.5**	0.93 ± 0.15	0.07 ± 0.03*
min		28.2 ± 3.0	−18.0 ± 3.5**	0.99 ± 0.16	−0.24 ± 0.07*

Significantly different from paired control or previous value, * $P<0.05$; ** $P<0.01$.

Addition of 10^{-6} M isoproterenol to the serosal side of the cornea following inhibition of the chloride current by serosal 10^{-5} M $CuSO_4$ resulted in the typical secretagogue response, elevated I_{sc} and increased G_t (Table 2). The $CuSO_4$ inhibited cornea increased the I_{sc} to the control level, the isoproterenol increase in the $CuSO_4$ inhibited corneas was significantly greater ($P<0.01$) than control (2 fold greater).

Table 2. The isoproterenol (10^{-6} M) response with control and $CuSO_4$-inhibited corneas.

	n	Control I_{sc} ($\mu A/cm^2$)	Change in I_{sc} ($\mu A/cm^2$)	Control G_t (mS/cm^2)	Change in G_t (mS/cm^2)
Control	4	23.0 ± 4.2	$15.9 \pm 2.4^*$	0.74 ± 0.16	$0.47 \pm 0.09^*$
$CuSO_4$	7	15.2 ± 2.9	$29.7 \pm 2.7^*$	1.08 ± 0.33	$0.59 \pm 0.06^*$

* Significantly different from paired values, * P<0.01.

Corneas stimulated with serosal isoproterenol (10^{-6} M) were observed to have a diminished initial increase (the non-steady state, max) in the I_{sc} associated with the initial phase of the biphasic $CuSO_4$ response (Table 3). The increase in I_{sc} with serosal 10^{-5} M $CuSO_4$ following pretreatment with isoproterenol was 30% that observed with $CuSO_4$ (see Table 1).

Corneas pretreated with serosal 5 X 10^{-7} M ouabain did not have the typical biphasic response associated with 10^{-5} M $CuSO_4$ (table 3). The values decreased after $CuSO_4$ addition, however the decrease in I_{sc} was a reflection of the ouabain treatment, there were no changes in the I_{sc} slope as a result of $CuSO_4$ treatment and $CuSO_4$ did not cause any significant change in corneal conductance.

Pretreatment of the cornea with serosal 5 X 10^{-6} M furosemide did not alter the $CuSO_4$ response (table 3). The chloride current of the furosemide-inhibited corneas was initially inhibited 31% by furosemide (from 25.4 ± 2.6 to 17.5 ± 3.2 uA/cm^2, n=4, P<0.01). The changes due to $CuSO_4$ and furosemide were not significant due to a small sample size. However, qualitatively all of the characteristics of the biphasic $CuSO_4$ response were retained despite furosemide inhibition.

The addition of 10-5 M $CuSO_4$, mucosally, was without any significant effect. This addition (mucosal) may serve as a control for the serosal addition of $CuSO_4$, which produced a biphasic corneal response and indicates a specific site of action, $CuSO_4$ being effective only at the serosa.

The biphasic effect of serosal copper follows the predicted trends in the bullfrog cornea. The increase in the chloride current was associated with an increase in G_t and the decrease in the I_{sc} was associated with a decrease in G_t. Serosal copper may be acting at several areas affecting chloride secretion. It is unlikely that copper is affecting metabolism, Na/K ATPase, or Na/Cl cotransport, since the isoproterenol response was present in corneas pretreated with copper and equal to the control isoproterenol response. The serosal addition of copper may be affecting the chloride channels.

Table 3. Interaction of 10^{-5} M serosal $CuSO_4$ in corneas pretreated with 10^{-6} M isoproterenol (ISO), 5×10^{-7} M ouabain (OUA), or 5×10^{-6} M furosemide (FURO), each added serosally.

$CuSO_4$ added after		n	Control I_{sc} ($\mu A/cm^2$)	Change in I_{sc} ($\mu A/cm^2$)	Control G_t (mS/cm^2)	Change in G_t (mS/cm^2)
ISO	Max	4	25.7 ± 4.0	0.8 ± 0.3	1.03 ± 0.26	0.05 ± 0.02
	Min		26.5 ± 4.0	-15.6 ± 5.4	1.07 ± 0.27	-0.24 ± 0.13
OUA	Max	6	8.6 ± 0.6	NP	1.03 ± 0.27	NP
	Min		8.6 ± 0.6	-4.2 ± 1.1*	1.03 ± 0.27	0.02 ± 0.02
FURO	Max	4	18.1 ± 2.9	1.5 ± 0.7	1.06 ± 0.26	0.05 ± 0.04
	Min		19.6 ± 3.4	-9.1 ± 2.6*	1.11 ± 0.26	0.28 ± 0.15

* Significantly different from paired controls, * P<0.05.
NP Not present.

The addition of $ZnSO_4$ to the mucosal and serosal bathing solutions in concentrations ranging up to 10^{-4} M did not result in any significant changes in the cornea chloride current or the cornea conductance (Table 4).

Table 4. Effects of 10^{-4} M $ZnSO_4$ addition to either the mucosa or serosa of the isolated cornea.

Side of Addition	n	Control I_{sc} ($\mu A/cm^2$)	Change in I_{sc} ($\mu A/cm^2$)	Control G_t (mS/cm^2)	Change in G_t (mS/cm^2)
Serosa	5	25.4 ± 5.2*	-1.0 ± 1.8	1.21 ± 0.34	-0.04 ± 0.07
Mucosa	4	21.6 ± 3.6	2.1 ± 0.9	0.74 ± 0.22	0.04 ± 0.02

* Values represent the mean $\pm$ the standard error of the mean.

The addition of 10^{-4} M $ZnSO_4$ to either side of the bullfrog cornea did not result in any acute response of the corneal tissue.