EFFECT OF COPPER AND ZINC ON THE ISOLATED OPERCULAR EPITHELIUM OF Fundulus heteroclitus.

J. I. Scheide and J. A. Zadunaisky, Department of Physiology and Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016.

The opercular epithelium of seawater adapted killifish, Fundulus heteroclitus, is rich in chloride cells and secretes chloride ions from blood to the seawater side. Thus, the opercular epithelium provides a model for the study of gill chloride secretion (Zadunaisky, Fish Physiology, Vol Xb:129-176, 1984). In this in vitro preparation of Fundulus heteroclitus, an inhibition of the chloride current has been observed with symmetrical addition of copper or zinc (Crespo and Karnaky, J. Exp. Biol. 102:337-341, 1983) or with copper added to the mucosal side (Degnan, J. Exp. Zool. 236:19-25, 1984). In this report, we confirm the effect of adding copper or zinc to the opercular epithelial serosa or mucosa and investigate the interaction of these effects on the chloride current by isoproterenol, a beta adrenergic agonist that stimulates chloride secretion (Degnan and Zadunaisky, J. Physiol. 294:483-495, 1979).

Killifish, Fundulus heteroclitus, were collected locally near Mount Desert Island Biological Laboratory and were maintained in running seawater aquaria for at least one week prior to experimentation. The inner opercular epithelium was dissected and mounted in Ussing chambers with 4 ml teleost Ringer on each side (Degnan et al., J. Physiol. 271:155-199, 1977). Solubility difficulties required $CuSO_4$ and $ZnSO_4$ be diluted in deionized water prior to addition in the Ringer solution. The concentrated stock solutions, of $CuSO_4$ and $ZnSO_4$, were mixed such that a 10 ul aliquot, when added to 4 ml Ringers, would result in the final concentration and not modify Ringer pH. Control DW additions had no effect on the I_{sc} or G_t (data not shown). Values are reported as the mean $\pm$ standard error of the mean and significant values were ascertained by paired student 't' test.

Serosal addition of $ZnSO_4$ inhibits the chloride current 42% at 10^{-5} M (Table 1). Maximal effect was observed at 5 X 10^{-5} M $ZnSO_4$ with the current being inhibited 74%. Opercular conductance did not significantly change with $ZnSO_4$ treatment. The inhibition of the opercular epithelium with serosal 10^{-4} M $ZnSO_4$ (Figure 1) could be reversed with the addition of 10^{-6} M isoproterenol with the current increasing nearly 5X from 18.8 $\pm$ 4.4 to 91.7 $\pm$ 13.2 uA/cm^2 ($P<0.01$, n=4). Mucosal addition of $ZnSO_4$ did not significantly alter the spontaneous opercular I_{sc} or G_t (Figure 1).

The addition of $ZnSO_4$ affects the chloride current in the opercular epithelium only when added to the serosal side, indicating a specific side/site of action. The presence of an isoproterenol response after serosal zinc addition indicates that the inhibition of the opercular current observed with zinc may not be attributed to a zinc effect on Na/K ATPase or the Na/Cl cotransporter, which if inhibited would not permit the isoproterenol response.

Mucosal and serosal addition of $CuSO_4$ were equally effective in significantly inhibiting ($P<0.05$) the opercular epithelium at 10^{-5} M (Tables 2 and 3). Adding 5 x 10^{-5} M $CuSO_4$ to the mucosa or serosa effectively inhibited the spontaneous opercular epithelium short circuit current (Figure 2). Maximal inhibition occurs at about 5 X 10^{-5} M, inhibiting the chloride current approximately 70%. The opercular epithelial conductance did not

Table 1. Effects of serosal $ZnSO_4$ addition on the opercular epithelium.

$ZnSO_4$ X 10^{-5} (M/l)	n	Control I_{sc} uA/cm^2	Zinc I_{sc} uA/cm^2	Control G_t mS/cm^2	Zinc G_t mS/cm^2
1	13	83.3 ± 10.3	48.2 ± 8.2*	13.3 ± 1.6	13.4 ± 1.8
2	5	103.9 ± 38.1	36.8 ± 16.0*	11.8 ± 2.8	9.0 ± 2.1
5	6	98.7 ± 10.7	26.0 ± 6.9*	14.2 ± 2.4	10.3 ± 1.3
10	6	80.0 ± 16.3	19.8 ± 3.4*	10.7 ± 2.3	10.6 ± 2.6

* Significantly different from paired control, $P<0.05$.

significantly change with the copper inhibition of the chloride current. The increase in $CuSO_4$ dose decreased the time required for the opercular chloride current to reach a new steady state value (inhibited value, data not shown). Opercular epithelia, pretreated with serosal $CuSO_4$ (in a dose sufficient to inhibit the I_{sc}, 2 X 10^{-5} to 10^{-4} M), were stimulated with serosal isoproterenol (10^{-6} M) from 46.7 ± 8.0 to 78.2 ± 10.4 uA/cm^2 ($P<0.05$,n=5), although the increase in the I_{sc} (a 50% increase) was less than expected. Pretreatment of opercular epithelia with a mucosal dose of $CuSO_4$ (in a concentration that significantly inhibits the I_{sc}, 2 X 10^{-5} to 10^{-4} M) also reduced the increase resulting from the serosal addition of isoproterenol (10^{-6} M) increasing the I_{sc} from 43.5 ± 9.0 to 92.2 ± 13.3 uA/cm^2,(difference not significant, n=4).

Table 2. Effects of mucosal $CuSO_4$ addition on the opercular epithelium.

$CuSO_4$ X 10^{-5} (M/l)	n	Control I_{sc} uA/cm^2	$CuSO_4$ I_{sc} uA/cm^2	Control G_t mS/cm^2	$CuSO_4$ G_t mS/cm^2
1	4	60.4 ± 12.6	48.9 ± 12.7*	9.6 ± 0.6	9.1 ± 1.5
2	5	61.5 ± 9.2	38.5 ± 6.2*	8.9 ± 1.2	7.4 ± 0.9
5	7	130.6 ± 29.3	42.4 ± 18.2*	15.3 ± 2.5	18.1 ± 3.6
10	4	92.4 ± 19.4	44.8 ± 13.1*	12.0 ± 1.4	13.3 ± 4.3

* Significantly different from paired control, $P<0.05$.

The $CuSO_4$ inhibition of the opercular chloride current was equally effective on both the serosa and mucosa. The site of action is not clear. There appears to be a reduction in the isoproterenol response which could be the result of a number of different transport processes in the operculum, all of which are necessary for chloride secretion. Copper added to either side may be acting at different sites to inhibit chloride secretion, with the some final result, inhibition of the opercular chloride current.

Table 3. Effects of serosal $CuSO_4$ addition on the opercular epithelium.

$CuSO_4$ X 10^{-5} (M/l)	n	Control I_{sc} uA/cm^2	$CuSO_4$ I_{sc} uA/cm^2	Control G_t mS/cm^2	$CuSO_4$ G_t mS/cm^2
1	5	73.3 ± 15.8	56.4 ± 11.3*	12.8 ± 3.4	15.1 ± 4.5
2	3	92.2 ± 22.8	50.2 ± 11.0*	10.6 ± 1.4	20.0 ± 3.9
5	6	178.9 ± 35.9	58.7 ± 12.0*	15.4 ± 2.9	21.8 ± 5.1
10	6	143.4 ± 23.1	38.2 ± 5.6*	9.4 ± 2.3	11.2 ± 3.1

* Significantly different from paired control, $P<0.05$.

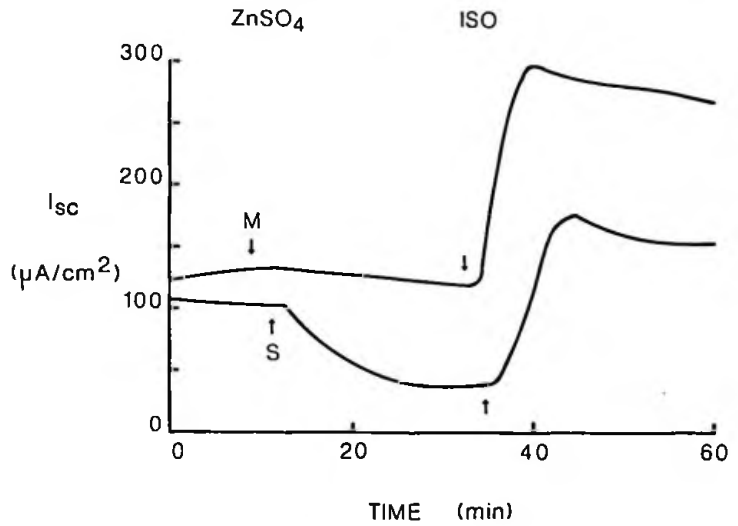

Figure 1. The effect of mucosal (M) or serosal (S) $ZnSO_4$ (10^{-4} M) to the spontaneous opercular epithelial short circuit current. Following pretreatment with $ZnSO_4$, both tissues were stimulated with isoproterenol (10^{-6} M, serosally added).

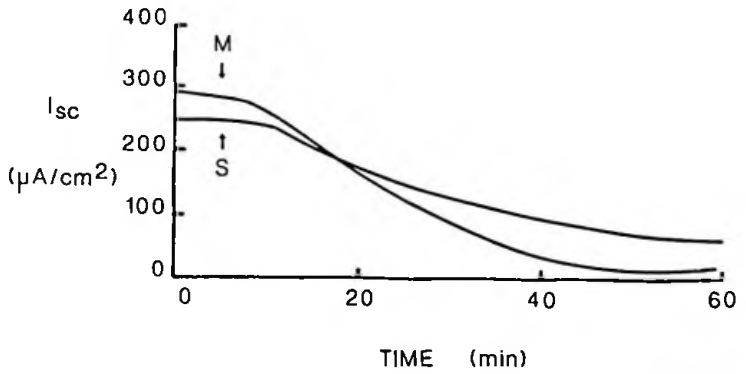

Figure 2. Effect of mucosal (M) and serosal (S) addition of 5 X 10^{-5} M $CuSO_4$ on the opercular epithelial chloride current.