COPPER INHIBITS cAMP PRODUCTION STIMULATED BY ISOPROTERENOL IN THE OPERCULAR EPITHELIUM OF Fundulus heteroclitus.

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The opercular epithelium of the killifish, Fundulus heteroclitus, when isolated and mounted in an Ussing chamber secretes chloride equivalent to the short-circuit current (I_{sc}) (Degnan, et al., J. Physiol. 271:155-191, 1977). The opercular epithelium provides a model for ion transport in the seawater-adapted teleosts due to the presence of chloride cells and similar ion transport properties for the opercular and gill epithelia (Zadunaisky, Fish Physiology, Vol XB, pp 129-176, 1984). Chloride transport in the opercular epithelium is regulated by agents that affect cellular cAMP (adenosine 3'5' cyclic monophosphate; Mendelsohn et al., J. Comp. Physiol. B 145:29-35, 1981; May and Degnan, Amer. J. Physiol. 256:R741-R746, 1984). Copper inhibits the chloride current in the opercular epithelium (Crespo and Karnaky, J. Exp. Biol. 337-341, 1983; Degnan, J. Exp. Zool. 236:19-25, 1985; Scheide and Zadunaisky, Bull. MDIBL 26:153-155, 1986). In this report, we detail the effect of copper on the ability of the operculum to increase tissue cAMP resulting from the addition of isoproterenol, a beta agonist that stimulates chloride transport (Degnan and Zadunaisky, J. Physiol. 294:483-495, 1979) and elevates opercular cAMP levels (May and Degnan, Amer. J. Physiol. 246:R741-R746, 1984).

Fundulus heteroclitus were collected locally near Salsbury Cove, Maine. The killifish were maintained in running seawater aquaria and fed daily for at least 2 weeks prior to experimentation. Killifish were pithed and the opercular flap removed. The inner lining of the opercular epithelium was gently removed and incubated for at least 30 min. in gassed (95/5 Air/CO_2) teleost saline, pH 7.3 (as previously described Scheide and Zadunaisky, Amer. J. Physiol. 254:R27-R32, 1988). Incubation tubes were prepared with 4 ml teleost saline plus 4 mM theophylline and fully gassed just prior to being aliquoted. Isoproterenol (at the given concentration) was mixed in saline. Copper sulfate in deionized water was added to the incubation saline at a final concentration of 5×10^{-5} M. The control tube received deionized water. All chemicals were purchased from Sigma Chemical Company.

Incubation was initiated with the introduction of opercular tissue into the incubation tube. Incubation was terminated after 5 min by removing the opercular tissue and immediate tissue homogenation in 0.4 ml acidified ethanol (0.01 M HCl) using a ground glass tissue homogenizer. The homogenate was placed in a 1.5 ml vial, the homogenizer rinsed and this wash added to the vial. Vials were dried to completion and resolubilized in a solution containing 50 mM tris, 1.2 mM EDTA, pH=7.6. The solubilized material was assayed for cAMP content with a cAMP assay kit (Amersham). Protein content determined by the Lowry method with BSA used as a standard (Lowry et al., J. Biol. Chem. 193:265-275, 1951). Opercular cAMP content was measured as pMol cAMP produced/mg protein and normalized to control values.

Under control conditions 10^{-7} M isoproterenol elevated tissue cAMP content above control and was maximal at 10^{-6} M (figure 1). The presence of copper (5 X 10^{-5} M) in the incubation media did not effect control levels of opercular cAMP, however inhibited the production of cAMP stimulated by isoproterenol. Elevation of opercular cAMP content by 10^{-6} M isoproterenol was not evident in operculi incubated with copper present even though this concentration of isoproterenol normally increased the tissue cAMP content 3.5 fold in control operculi.

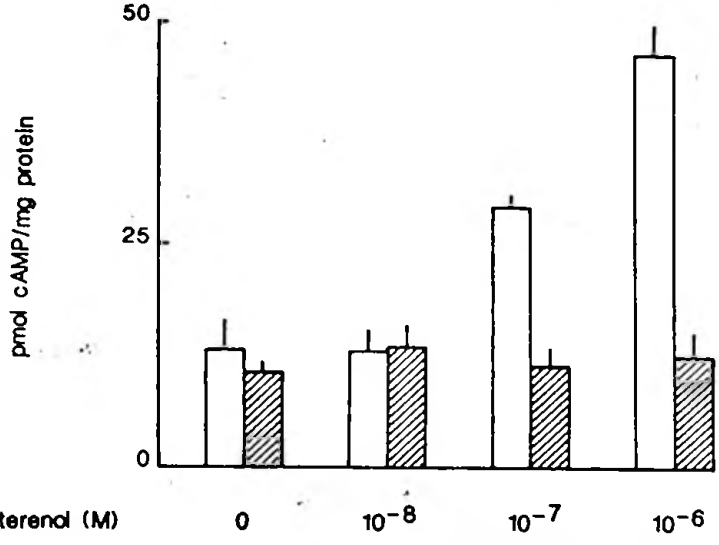

Figure 1. Changes in opercular cAMP content following a 5 min incubation under the noted conditions. Values were normalized to control with no copper or control with copper present in the incubation medium. Control values are represented with the open histogram. The slashed line histogram denotes incubation with 5 X 10^{-5} M $CuSO_4$. * Significantly different from paired control. ** Significantly different from zero control value.

Chloride transport in the opercular epithelium is dependent upon a cAMP system, with factors that increase opercular tissue cAMP, such as forskolin and isoproterenol, stimulating the I_{sc} (Mendelsohn et al., J. Comp. Physiol. B 145:29-35, 1981; May and Degnan, Amer. J. Physiol. 256:R741-R746, 1984). Copper inhibits the short-circuit current of the opercular epithelium (Crespo and Karnaky, J. Exp. Biol. 337-341, 1983; Degnan, J. Exp. Zool. 236:19-25, 1985; Scheide and Zadunaisky, Bull. MDIBL 26:153-155, 1986). Data presented here indicates that copper inhibition of the I_{sc} may be the result of an interaction between copper and the cAMP regulatory system responsible for chloride transport. We are currently investigating the interaction between copper and the opercular cAMP regulatory mechanism.

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