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SHORT COMMUNICATIONS

COPPER AND ZINC INHIBIT CHLORIDE TRANSPORT ACROSS THE OPERCULAR EPITHELIUM OF SEAWATER-ADAPTED KILLIFISH *FUNDULUS HETEROCLITUS*

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Effects of heavy metals on osmoregulation in aquatic organisms have recently been reviewed by Bouquegneau & Gilles (1979). Whereas most of the data in the literature deal with the toxic effects of mercurials, only a few reports can be found on the toxicity of essential trace elements such as Cu and Zn. Rainbow trout exposed to lethal (Skidmore, 1970) and sublethal (Watson & Beamish, 1980) concentrations of Zn maintain a relatively constant internal ionic environment. On the other hand Lewis & Lewis (1971) showed, in the channel catfish, a decreased osmolarity of blood serum after treatment with either Cu or Zn, and Katz (1979) reported an increased Na efflux in freshwater teleosts exposed to heavy metals. An increased gill Na, K-ATPase activity was described in the Zn-treated rainbow trout (Watson & Beamish, 1980). Shephard & Simkiss (1978) showed, in the same species, an increase in the gill protein content of fish exposed to Cu and Zn. These data agree with reports from Cu-treated winter flounder (Baker, 1969) and from Zn-treated dogfish (Crespo, Soriano, Sampera & Balasch, 1981; Crespo, 1982) each of which shows an increase in the number of the gill chloride cells.

The opercular epithelium of the killifish, *Fundulus heteroclitus*, is a flat epithelium with a high density of chloride cells (Karnaky & Kinter, 1977) identical in ultrastructure to that of the gill chloride cells (Karnaky, Kinter, Kinter & Stirling, 1976). This preparation has been studied under short-circuit current conditions in a lucite chamber and proposed as an *in vitro* model for gill osmoregulatory function (Karnaky, Degnan & Zadunaisky, 1977; Karnaky, 1980). The short-circuit current (I_{sc}) across the epithelium is attributed uniquely to the active transport of chloride ions from the blood to the seawater side of the epithelium (Karnaky *et al.* 1977; Degnan, Karnaky & Zadunaisky, 1977). There is a direct correlation between the number of chloride cells and the I_{sc}, demonstrating that these cells are responsible for chloride secretion (Karnaky *et al.* 1979).

To investigate the effects of Cu and Zn on chloride transport across the opercular

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epithelium of 100% seawater-adapted *Fundulus heteroclitus*, electrical parameters were recorded following the addition of small aliquots (20–200 μl), of Cu ($CuSO_4 \cdot 5H_2O$) or Zn ($ZnSO_4 \cdot 7H_2O$) in Ringer's solution either to both mucosal and serosal sides of the preparation (M + S) or to one side only. Procedures for the dissecting and mounting of the operculum, and descriptions of the Lucite chambers and Ringer's solution are presented in detail elsewhere (Degnan *et al.* 1977). The epithelia used for the present study reached a steady-state within 30 min and thereafter exhibited an average spontaneous decay of 11%/30 min.

Table 1 shows that the addition of either Cu or Zn (4×10^{-5} M) to both sides of the preparation caused a statistically significant decrease in both short-circuit current (I_{sc}) and transepithelial potential difference (P.d.). Transmural resistance (R), however, remained unaltered following exposure to either heavy metal. Concentrations as low

Table 1. *The effects of Cu and Zn on the electrical properties of the opercular epithelium of seawater-adapted* Fundulus heteroclitus

	I_{sc} (μA/cm^2)	P.d. (mV)	R ($\Omega \cdot$ cm^2)
Control (8)	136·5 ± 27·9	13·4 ± 1·5	118·7 ± 14·0
Cu	74·2 ± 14·1*	9·1 ± 1·2**	119·1 ± 20·6
% change	45·6%	32·1%	0·4%
P	< 0·005	< 0·005	N.S.
Control (9)	126·1 ± 23·3	15·1 ± 1·1	164·9 ± 33·5
Zn	25·6 ± 5·4*	4·1 ± 1·0**	159·2 ± 18·2
% change	81·5%	79·0%	3·5%
P	< 0·001	< 0·001	N.S.

Electrical parameters were recorded 30 min after the addition of 4×10^{-5} M-Cu or Zn to both the mucosal and serosal sides. Results are expressed as the mean ± S.E.M. and the number of observations is given in parentheses. *P* values are given according to the paired t-test except ** ($P < 0{\cdot}01$) and * ($P < 0{\cdot}02$) according to the Student's t-test.

as 10^{-5} M (M + S) caused a significant inhibition of the I_{sc} ($P < 0{\cdot}05$, n = 3). Initial I_{sc} values were recovered after washing (×3) with Ringer's solution. When heavy metals were added to the mucosal side only, no inhibition of I_{sc} was detected, even at higher doses (5×10^{-4} M; n = 6). After addition of 4×10^{-5} M-Cu or Zn to the serosal side only, the pattern for I_{sc} inhibition was the same as the one recorded for M + S exposure (Fig. 1). Zn caused a greater inhibition of the I_{sc} ($P < 0{\cdot}02$) and P.d. ($P < 0{\cdot}01$) than did Cu (Table 1).

From our results it is apparent that low concentrations of Cu or Zn can affect chloride transport across the opercular epithelium of the seawater-adapted *Fundulus heteroclitus*. In contrast to our findings for Cu and Zn, Hg inhibits I_{sc} across the killifish opercular epithelium after mucosal as well as serosal exposure (Degnan & Miller, 1980). The absence of effects from the mucosal side argues against the direct action of these heavy metals on the chloride cell apical membrane. Likewise, these data suggest that these heavy metals do not rapidly penetrate the opercular epithelium.

The fact that Cu and Zn are effective only when added to the serosal side suggests that these metals might interact with Na,K-ATPase located on the basolateral membrane of the chloride cell (Karnaky *et al.* 1976).

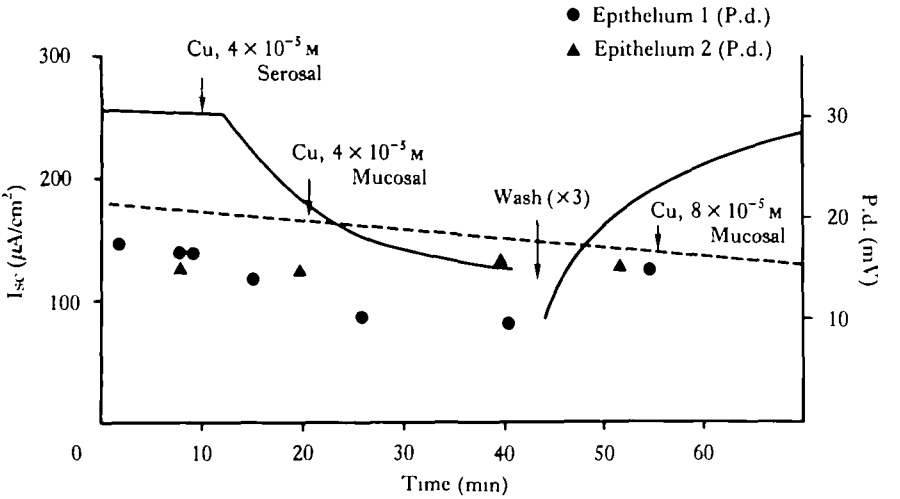

Fig. 1. The effect of Cu on the electrical properties of the short-circuited opercular epithelium of seawater-adapted *Fundulus heteroclitus*. A 4×10^{-5} M addition to the serosal side only (epithelium 1) results in marked inhibitions of the I_{sc} (continuous-line) and the P.d. (dots), both of which recover to control values after washing (× 3) with Ringer's solution. This inhibition is absent from mucosal only additions (epithelium 2). The inhibition by Zn (not shown) is also restricted to the serosal side, but is greater than that from equivalent concentrations of Cu.

To test the effects of Cu and Zn on the Na pump we studied the *in vitro* inhibition of the specific activity of Na,K-ATPase. 25 µl crude gill homogenates (2–3 mg protein/ml) treated with 0·001 % Na deoxycholate were initially preincubated for 10 min at 37 °C in a final volume of 0·5 ml [0·4 mM-EGTA (pH 8·1), 10 mM-Na azide, 2·5 mM-$MgCl_2$, 104 mM-NaCl, 16 mM-KCl, 40 mM-Tris-HCl (pH 7·2)] with or without heavy metals. The reaction was started by the addition of Mg-Tris-ATP (3 mM-ATP, pH 7·5) and stopped with 2% trichloroacetic acid. The inorganic phosphate liberated by ATP hydrolysis was determined according to the method of Fiske & Subarrow (1925). Na,K-ATPase activity was calculated as the difference between ATPase activity in the presence and absence of 0·3 mM-ouabain. Protein content of the homogenate was determined according to the method of Lowry, Rosebrough, Farr & Randall (1951).

Fig. 2 shows the dose response curves for Zn and Cu. A statistically significant ($P < 0{\cdot}02$) decrease in Na,K-ATPase specific activity was first detected following exposure to 10^{-5} M-Zn and 10^{-4} M-Cu, which caused inhibitions of 7 % and 20 %, respectively. These results suggest that the inhibition of chloride secretion (I_{sc}) across the opercular epithelium of seawater-adapted *Fundulus heteroclitus* following exposure to 4×10^{-5} M-Cu or Zn is only partially due to an inhibition of the Na pump. Moreover, Cu and Zn dose response curves for the inhibition of enzyme activity do not differ statistically from each other, yet Zn causes a greater inhibition of the I_{sc} and P.d. than does Cu. The striking difference in responses to these heavy metals suggests that the Na pump is not the only mechanism involved in their toxicity.

According to recent models of chloride cell function (Silva, Solomon, Spokes & Epstein, 1977; Ernst, Dodson & Karnaky, 1980) chloride is transported initially into chloride cells from the blood side by a Na-facilitated, neutral-coupled carrier at the basolateral interface. This 'secondary' active transport is driven by the low

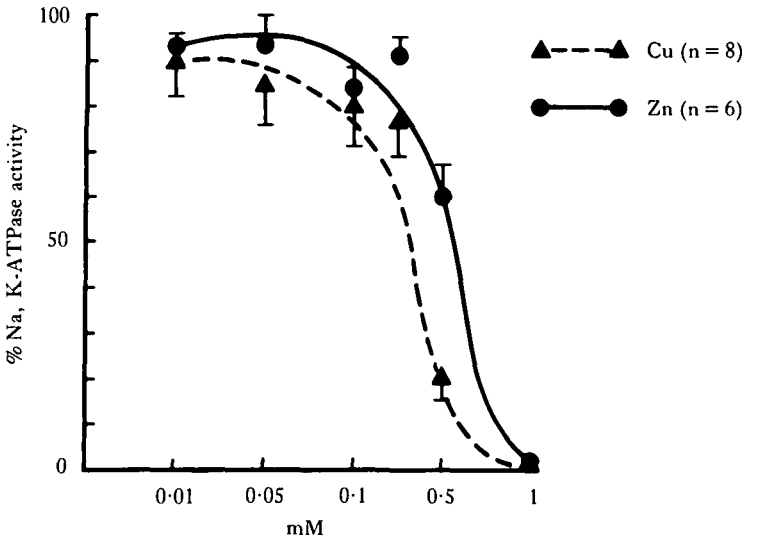

Fig. 2. The influence of Cu and Zn on Na,K-ATPase from crude whole gill homogenates of seawater-adapted *Fundulus heteroclitus*. Specific activity is expressed as mean ± S.E.M. as a percentage of the control.

intracellular Na gradient established by the primary active transport of Na out of the cell by basolateral, plasmalemmal Na,K-ATPase. Clearly, Cu and Zn are potent inhibitors of chloride transport across the opercular epithelium. They inhibit Na,K-ATPase and may interact as well with the coupled NaCl carrier. Since the I_{sc} across the killifish opercular epithelium is inhibited by α-adrenergic agents (Degnan *et al.* 1977; Mendelsohn, Cherksey & Degnan, 1981) Cu and Zn may also act directly on α-adrenergic receptors. Additionally, heavy metals are thought to alter permeability of the cell membrane (Rothstein, 1959; Kinter & Pritchard, 1977). Further work on this *in vitro* preparation may help elucidate the underlying mechanisms of Cu and Zn toxicity to electrolyte transport processes.

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