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Highly selective blockade of the frog skin sodium channels by monovalent copper cations

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0.5 mM Cu^+ added to the mucosal side of frog skin caused rapid reversible inhibition of short-circuit current while no effect of Cu^+ could be observed at the serosal side. In both cases Cu^{2+} was reduced to Cu^+ by adding 10 mM ascorbic acid. Cu^+ being similar to Na^+ both in charge and crystal radius (0.096 and 0.095 nm, respectively) appears to block Na^+ channels in the apical membrane. Cu^{2+} being of a smaller size (crystal radius 0.072 nm) was ineffective at the mucosal side causing only a rather slow irreversible inhibition of Na^+ transport when added to the serosal bathing solution.

Introduction

There is a vast literature concerning biological effect of Cu^{2+}, whereas the action of Cu^+ has not received much attention [1–4]. We supposed that in some cases Cu^+ may specifically interact with Na^+-dependent systems due to the identity of Cu^+ and Na^+ both in charge and crystal radius (0.096 nm and 0.095 nm, respectively). The data presented support this hypothesis. It has been shown that Cu^+ but not Cu^{2+} (crystal radius 0.072) inhibits transport of Na^+ through the apical membrane of the frog skin.

Materials and Methods

Abdominal skin of the frog species *Rana temporaria* was mounted in an Ussing-type chamber (1.2 cm^2) designed to allow rapid addition of various ingredients to bathing solutions while studying Na^+ transport. The skin was kept in open-circuit state, the short-circuit current being measured from time to time for 30–60 s. In all the experiments the, bathing solutions were nitrate Ringer-type media of the composition (mM): Na 113; K 3.35; Ca 2.8; glucose 10; Tris-buffer 10; pH 7.4–7.5. Cl^- was substituted by NO_3^- in order to prevent possible precipitation of CuCl. Complete reduction of Cu^{2+} to Cu^+ was achieved with 10 mM ascorbic acid. The concentration of Cu^+ was determined by using disodium 2,2-bicinchoninate (Sigma, St. Louis, MO) as a chromogen [5].

Results and Discussion

Figs. 1–4 demonstrate the typical results obtained in 5–7 similar experiments. Fig. 1 shows that incubation of frog skin in Ringer-NO_3^- containing 0.5 mM mucosal Cu^{2+} did not affect the short-circuit current (SCC). Adding 10 mM ascorbic acid to the mucosal medium caused fast inhibition of SCC presumably due to the reduction of Cu^{2+} to Cu^+, while ascorbic acid itself had no effect (not shown). The inhibition of SCC

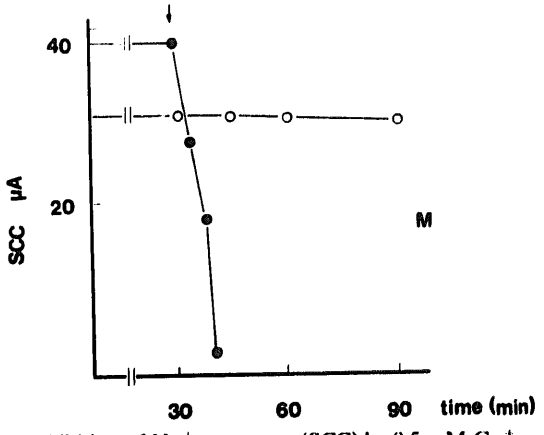

Fig. 1. Inhibition of Na^+ transport (SCC) by 0.5 mM Cu^+ produced in the mucosal bathing solution by reduction of Cu^{2+} with 10 mM ascorbic acid (↓). ●, Cu^+; ○, Cu^{2+}.

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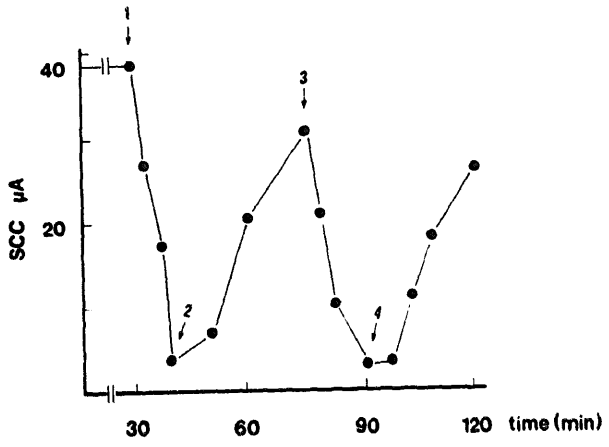

Fig. 2. Reversibility of the inhibitory effect of Cu^+. 1, Inhibition of SCC by mucosal Cu^+; 2, restoration of SCC by Cu^+ free solution; 3, inhibition of SCC by mucosal Cu^+; 4, restoration of SCC by complexation of Cu^+ with 2,2-bicinchoninic acid.

by Cu^+ was fully reversible (Fig. 2). Replacement of the Cu^+ containing mucosal solution by a fresh Cu-free solution or removal of Cu^+ by non-toxic complex formation with 2,2-bicinchoninate [5], restored SCC up to initial level. No effect could be observed when Cu^+ was applied at the serosal side. Exposing the serosal membrane to Cu^{2+} led to a slow irreversible inhibition of SCC (not shown). An increase of the concentration

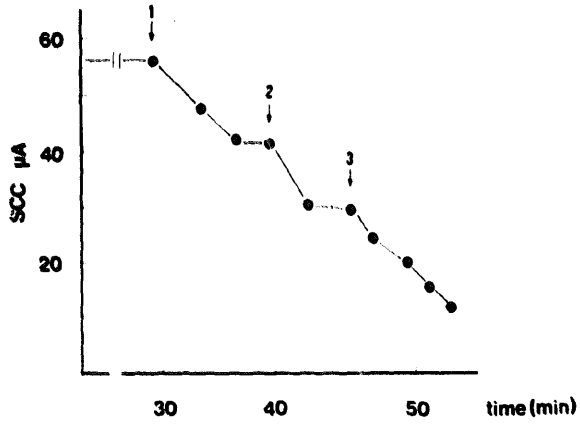

Fig. 3. Dependence of the inhibitory effect of Cu^+ on concentration in the mucosal solution. 1, 0.1 mM; 2, 0.2 mM; 3, 0.3 mM.

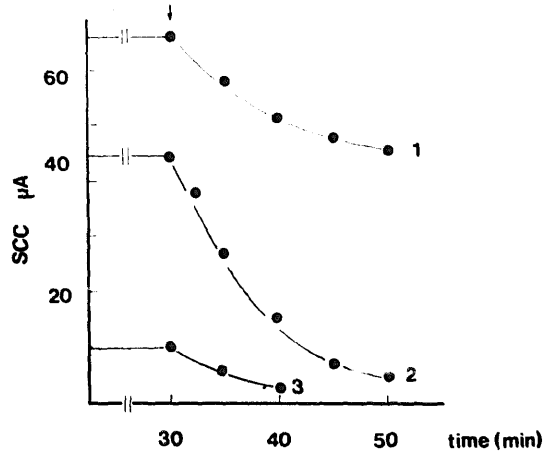

Fig. 4. Inhibition of SCC by 0.5 mM Cu^+ in the presence of various concentration of Na^+. 1, 113 mM; 2, 5 mM; 3, 2 mM.

of mucosal Cu^+ led to increased inhibition of SCC (Fig. 3). The inhibition of SCC by Cu^+ diminished with increase of the mucosal Na^+ concentration (Fig. 4). Cu^+ seems to compete with Na^+. Being equal to Na^+ both in charge and size, Cu^+ can occupy the entry to the Na^+-selective channel, thereby blocking SCC. Chemically, it could be expected that binding of Cu^+ with various membrane ligands is stronger compared with Na^+. To our knowledge Cu^+ is the only inorganic cation shown specifically inhibit of Na^+ transport across the apical membrane of the frog skin. The effects of Cu^+ seem to depend on the system studied, for instance, there was no interaction of Cu^+ with Na^+/H^+ antiport in rat liver mitochondria [3].

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