EFFECTS OF HEAVY METAL IONS ON THE ELECTRICAL PROPERTIES OF MUCOUS EPITHELIAL CELLS IN THE NEWT STOMACH

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Abstract---Zinc ion, Cu$^2+$ and Cd$^2+$ at 10$^{-4}$M or lower concentrations caused a marked decrease in the membrane potential of mucous epithelial cells in the isolated newt stomach without appreciable change in the effective membrane resistance and electrical coupling ratio. Cysteine and acetylpenicillamine antagonized the action of these ions. These ions as well as ouabain blocked the hyperpolarization of membrane due to an increase in intracellular Na$^+$. The effect of the ions on the relationship between external K$^+$-concentration and membrane potential was similar to that of ouabain. Discussion is made on the possibility that the ions inhibit active ion transport by combining with a SH-group of the cell membrane and, thus, bring about a decrease in membrane potential.

INTRODUCTION

During the last ten years many studies have been made of the effects of heavy metal ions on the electrical properties of cell membrane. Almost all of these studies have been carried out on excitable cell membranes such as muscle fiber and nerve fiber membranes (Hagiwara and Takahashi, 1967; Kleinfeld and Stein, 1968; Ito et al., 1970; Begenisich and Lynch, 1974). In contrast, few studies have been done on nonexcitable cell membrane. However, one should also attach importance to studies on nonexcitable cell membrane such as digestive epithelial cell membranes because a heavy metal ion which enters the mouth would act first on these epithelial cell membranes to exert its toxicity.

The present experiments were undertaken to investigate the effects of several divalent heavy metal ions on the electrical properties of mucous epithelial cells in the isolated newt stomach. The mucous epithelial cells of the newt stomach offer a favorable material for electrophysiological work on nonexcitable cell membrane. The cells are readily impaled with microelectrodes because they are large in size (about 30μm in diameter), visible under the microscope without staining and arranged in a single layer (Kanno et al., 1971). The results obtained suggest that some heavy metal ions inhibit active ion transport which is considered to be involved in maintaining the membrane potential of the cells (Kanno et al., 1977).
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Methods

The isolated stomach of the Japanese newt (Triturus pyrrhogaster) was used throughout the experiments. The membrane potential, effective membrane resistance, and electrical coupling ratio of its mucous epithelial cells were measured in the conventional way using glass capillary microelectrodes. Unless otherwise stated, the methods of dissecting the stomach, mounting in the experimental chamber (3 ml) and recording from the mucous epithelial cells were the same as those described in a previous paper (Kanno et al., 1977).

The physiological solution had the following composition: 111.2 mM NaCl, 1.3 mM KCl and 1.4 mM CaCl₂. Solutions with higher K⁺-concentrations were obtained by replacing an equivalent amount of NaCl with KCl and solutions with lower K⁺-concentrations were obtained by replacing KCl with NaCl. In some experiments isotonic NaCl solution containing 2 mM EGTA was used. All of the solutions were adjusted to pH 7.2 by 10 mM Tris-HCl.

Heavy metal compounds and drugs used were as follows: ZnCl₂ (Katayama Chemicals), CuCl₂ (Katayama Chemicals), CdCl₂ (Katayama Chemicals), NiCl₂ (Katayama Chemicals), CoCl₂ (Katayama Chemicals), Uranyl acetate (Mitsuwa Chemicals), L-cysteine (Nakarai Chemicals), N-acetyl-DL-penicillamine (Sigma), mersalyl (Sigma) and ouabain (Merck). These agents were used diluted in the appropriate solution from stock solutions (10⁻¹ or 10⁻² M of each agent in 0.5 M Tris-HCl solution, pH 7.2).

The test solutions were applied to the epithelium by filling the whole chamber except when the membrane potential were continuously recorded from an epithelial cell as shown in Fig. 8. When the continuous recording was made, the test solutions were applied by perfusing the chamber at a rate of 1–2 ml/min.

All experiments were carried out at a temperature of about 7°C, being maintained with a Komatsu-Seisakusho Co. CTE-120 thermoregulator (Kanno et al., 1977).

Results

After immersing the stomach in the physiological solution of 7°C, the membrane potential of mucous epithelial cells was measured by the method of random penetration. The potential decreased gradually with time and reached a steady level in 30 min, though during this period the electrical coupling ratio due to permeable junctional membrane between two contiguous cells and the effective membrane resistance were maintained almost constant. The potentials which reached a steady level averaged −23±2 mV (S.D., N=7). Average values of the effective membrane resistance and electrical coupling ratio were 340±42 kΩ (S.D., N=7) and 0.64±0.03 (S.D., N=7), respectively. Due to this reason the test solutions were applied to the stomach after immersing it in physiological solution for 30–60 min and confirming that the membrane potential had reached a steady level. Unless otherwise stated, the electrical properties of cell membrane in the test solutions were measured about 30 min after the application of solutions to the stomach.
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Figure 1 shows the effect of Zn$^{2+}$ on the electrical properties of mucous epithelial cells. Zinc ion was the most effective among the heavy metal ions tested. It markedly decreased the membrane potential. This effect was increased with increase in Zn$^{2+}$-concentration from 10$^{-7}$M to 10$^{-4}$M. At 10$^{-4}$M Zn$^{2+}$ decreased the potential to 50% of the control value. On the other hand, the coupling ratio was not changed appreciably at any Zn$^{2+}$-concentration tested and the effective membrane resistance was changed only when 10$^{-4}$M Zn$^{2+}$ was applied. At this concentration Zn$^{2+}$ decreased the resistance to 81% of the control value.

The membrane potential was decreased also by Cu$^{2+}$. This effect of the ion was increased by increasing its concentration from 10$^{-7}$M to 10$^{-4}$M as shown in Fig. 2. At 10$^{-4}$M Cu$^{2+}$ decreased the potential to 67% of the control value. On the contrary, the changes in the effective membrane resistance and electrical coupling ratio were slight even when high concentrations of Cu$^{2+}$ were applied.

At concentrations of 10$^{-7}$M to 10$^{-5}$M, Cd$^{2+}$ did not affect any of the electrical properties. At 10$^{-4}$M, however, it decreased the membrane potential to 66% of the control value (Fig. 3), though the effective membrane resistance and electrical coupling ratio were not affected appreciably.

Both Ni$^{2+}$ and Co$^{2+}$ at concentrations of 10$^{-7}$M to 10$^{-4}$M did not affect the electrical properties of the cells, except that 10$^{-4}$M Ni$^{2+}$ slightly decreased the membrane potential. At this concentration of Ni$^{2+}$, the potential was decreased to 82% of the control value.

Uranyl ion can react with membrane ligand and disturb the membrane function (Rothstein, 1962). The ion potentiates twitch contraction of the frog skeletal muscle by prolonging the action potential of the muscle fiber. In this respect, the action of UO$_2$$^{2+}$ is the same as that of Zn$^{2+}$ (Sandow and Isaacsion, 1966). In the present study, the effect of UO$_2$$^{2+}$ on the electrical properties of mucous epithelial cells was examined for comparison with that of Zn$^{2+}$. The result obtained is shown in Fig. 4. Uranyl ion had almost no effect on the electrical properties at concentrations of 10$^{-6}$M to 10$^{-4}$M.

Uranly ion does not combine with SH-groups (Rothstein, 1962), though Zn$^{2+}$ can bind with them (Gurd and Wilcox, 1956). Therefore, the effect of mersalyl on the electrical properties of mucous epithelial cells was examined. Mersalyl is an organic mercurial and is known to be a powerful SH-reagent. At 5×10$^{-4}$M the reagent decreased the membrane potential of the cells but did not change the effective membrane resistance and electrical coupling ratio. In this respect, the action of mersalyl is quite similar to that of the heavy metal ions mentioned above. The decrease in potential 30min after application of mersalyl was 41% of the control membrane potential. However, when mersalyl was applied to the cells pretreated with cysteine, the decrease in potential 30min after administration was only 13%: pretreatment with cysteine antagonized the action of mersalyl (Fig. 5). In this experiment, the cells were pretreated with 5mM cysteine for 15min and then washed out for 10min before mersalyl was applied. Cysteine itself did not affect the electrical properties.

The effect of pretreatment of the mucous epithelial cells with 5mM cysteine on the
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**Fig. 1.** Effect of Zn$^{2+}$ on the electrical properties of mucous epithelial cells. ●: membrane potential, ○: effective membrane resistance, ▲: electrical coupling ratio. Vertical: percent of control values. Each point shows the mean (N=28-49, from 7-9 animals).

**Fig. 2.** Effect of Cu$^{2+}$ on the electrical properties of mucous epithelial cells. ●: membrane potential, ○: effective membrane resistance, ▲: electrical coupling ratio. Vertical: percent of control values. Each point shows the mean (N=14-70, from 3-11 animals).

actions of $10^{-5}$ M Zn$^{2+}$, $10^{-6}$ M Cu$^{2+}$, and $10^{-4}$ M Cd$^{2+}$ was also examined by the same procedure as that used in the mersalyl-experiment, and it was found that pretreatment antagonized the actions of the ions. Figure 6 shows the effect of pretreatment on the action of $10^{-5}$ M Zn$^{2+}$. When Zn$^{2+}$ was applied to the cells not pretreated with cysteine, the decrease in membrane potential 30 min after application of the ion was 36%, but when it was applied to pretreated cells, the decrease was only 12%.

The effect of pretreatment with 1 mM acetylpenicillamine, a SH-compound, on the
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actions of $5 \times 10^{-4}$ M mersalyl, $10^{-5}$ M Zn$^{2+}$, $10^{-6}$ M Cu$^{2+}$, and $10^{-7}$ M Cd$^{2+}$ was also examined by the same procedure used in the cysteine-experiment, and it was found that the action of acetylpenicillamine was quite similar to that of cysteine. Acetylpenicillamine itself did not affect the membrane potential.

The recovery of membrane potential of mucous epithelial cells after washing out $5 \times 10^{-4}$ M mersalyl, $10^{-5}$ M Zn$^{2+}$, $10^{-6}$ M Cu$^{2+}$, or $10^{-7}$ M Cd$^{2+}$ applied to the cells for 60 min was

Fig. 3. Effect of Cd$^{2+}$ on the electrical properties of mucous epithelial cells. ●: membrane potential. ○: effective membrane resistance. △: electrical coupling ratio. Vertical: percent of control values. Each point shows the mean ($N=21-70$, from 4-10 animals).

Fig. 4. Effect of UO$_2$$^{2+}$ on the electrical properties of mucous epithelial cells. ●: membrane potential. ○: effective membrane resistance. △: electrical coupling ratio. Vertical: percent of control values. Each point shows the mean ($N=13-23$, from 4-6 animals).
very slow. However, recovery was markedly accelerated by adding 1 mM acetylpenicillamine to the washing solution. Figure 7 shows the effect of acetylpenicillamine on the recovery of membrane potential of the cells exposed to $10^{-5}$ M Zn$^{2+}$ for 60 min. Acceleration was also observed when 5 mM cysteine was added to the washing solution, but its effect was not so marked as that of 1 mM acetylpenicillamine.

Figure 8 shows the effect of $10^{-5}$ M Zn$^{2+}$ on the membrane potential of a mucous epithelial cell and the effect of 1 mM acetylpenicillamine on the recovery of the potential after washing out Zn$^{2+}$. In this experiment, a mucous cell was impaled with two micro-electrodes, one for recording membrane potential and the other for passing rectangular pulses of current, and the membrane potential and effective membrane resistance were simultaneously and continuously recorded from the impaled cell. The record clearly indicated that 1 mM acetylpenicillamine accelerated the recovery of membrane potential after washing out Zn$^{2+}$. The record also indicated that these potential changes were caused without any appreciable change in the effective membrane resistance.

In a previous paper (Kanno et al., 1977), it has been shown that the membrane potential of mucous epithelial cells in the newt stomach increases transiently beyond the control level when the stomach is returned to a normal solution after being exposed to isotonic NaCl solution with 2 mM EGTA and that the transient potential increase is blocked by $10^{-5}$ M ouabain. In the present study, these results were confirmed and the effect of heavy metal ions ($10^{-5}$ M Zn$^{2+}$, $10^{-5}$ M Cu$^{2+}$, and $10^{-4}$ M Cd$^{2+}$) on the transient
potential increase was examined. All heavy metal ions tested like ouabain blocked the transient potential increase. One of the results is shown in Fig. 9. It was also observed that $5 \times 10^{-4}$ M mersalyl blocked the transient potential increase.

This previous paper has also shown that the membrane potential of mucous epithelial cells decreases in solutions of both higher $K^+$-concentrations and lower $K^+$-concentrations than normal one and that the decreased membrane potential in $K^+$-free solution is not further changed by adding $10^{-5}$ M ouabain to the solution. In the present study, these results were confirmed and the effect of heavy metal ions ($10^{-5}$ M Zn$^{2+}$, $10^{-8}$ M Cu$^{2+}$ and $10^{-4}$ M Cd$^{2+}$) on the relationship between membrane potential and external $K^+$-concentration was examined. One of the results is shown in Fig. 10. The effect of heavy metal ions was similar to that of ouabain; the membrane potential in $K^+$-free solution was not further changed by applying heavy metal ions. The membrane potential of the cells in $K^+$-free solution was not changed also by $5 \times 10^{-4}$ M mersalyl.

**DISCUSSION**

At appropriate concentrations some heavy metal ions, such as Zn$^{2+}$, Cu$^{2+}$, and Cd$^{2+}$, decrease the membrane potential of mucous epithelial cells in the newt stomach without any appreciable change in the effective membrane resistance and electrical coupling ratio. These facts suggest that heavy metal ions may inhibit active ion transport, whose contribution to the maintenance of the membrane potential of mucous epithelial cells in the newt stomach has been suggested by Kanno et al. (1977), and, thus, bring about a de-
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![Graph showing the effect of N-acetyl-DL-penicillamine (NAPA) on the recovery of membrane potential of mucous epithelial cells after treatment with Zn^{2+}.](image)

**Fig. 7.** Effect of N-acetyl-DL-penicillamine (NAPA) on the recovery of membrane potential of mucous epithelial cells after treatment with Zn^{2+}. ○: membrane potential of the cells in NAPA after treatment with Zn^{2+}; the cells were treated with 10^{-5} M Zn^{2+} for 60 min and then washed for 10 min before 1 mM NAPA was applied. ●: membrane potential of the cells in normal solution after treatment with Zn^{2+}; the cells were treated with 10^{-5} M Zn^{2+} for 60 min and then washed with normal solution. At time 0, Zn^{2+} was applied. Vertical: percent of control values. Each point shows the mean with standard deviation (N=7-10, from 2 animals).

![Graph showing the effects of Zn^{2+} and N-acetyl-DL-penicillamine (NAPA) on membrane potential and effective membrane resistance of a mucous epithelial cell.](image)

**Fig. 8.** Effects of Zn^{2+} and N-acetyl-DL-penicillamine (NAPA) on membrane potential and effective membrane resistance of a mucous epithelial cell. Zn^{2+}: 10^{-5} M; W: washing Zn^{2+} with normal solution. NAPA: 1 mM. Rectangular pulses of current (5×10^{-9} Amp., 200 msec, inward) were applied at 1/10 sec.

It has been reported that increased intracellular Na^{+} stimulates a Na^{+}-pump (Kerkut and Thomas, 1965; Thomas, 1969). Kanno et al. (1977) observed that the membrane of mucous epithelial cell in the newt stomach hyperpolarizes transiently when the stomach is returned to a normal solution after being exposed to isotonic NaCl solution with EGTA. They have assumed that hyperpolarization is brought about by an increase in intracellular...
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Na⁺ and the consequent activation of an electrogenic Na⁺-pump because hyperpolarization can be completely blocked by ouabain. Similar results and conclusion were also reported by Yamaguchi (1975) on the anterior byssal retractor muscle of Mytilus, a common sea mussel. In the present study, it has been observed that hyperpolarization is blocked not

![Graph](image1)

**Fig. 9.** Block by Zn²⁺ of the transient hyperpolarization of membrane due to increase in intracellular Na⁺ in mucous epithelial cells. The cells were exposed to K⁺-free solution (0 K⁺ solution) for 20 min and then to isotonic NaCl solution with 2 mM EGTA (0 K⁺, 0 Ca²⁺ solution) for 10 min. After this period, the cells were restored to 0 K⁺ solution for another 20 min and normal solution was readmitted. Zn²⁺ (10⁻⁵ M) was introduced 10 min before the readmission of normal solution. Each point shows the mean with standard deviation (N=4-12, from one animal).

![Graph](image2)

**Fig. 10.** Effect of Zn²⁺ on the relationship between membrane potential and external K⁺-concentration in mucous epithelial cells. ○: in the presence of 10⁻⁵ M Zn²⁺. ●: in the absence of Zn²⁺. External K⁺-concentrations are represented relatively to the normal concentration (1.3 mM). At each K⁺-concentration, the membrane potentials were measured first in the absence of Zn²⁺, then the cells were exposed to Zn²⁺ for 30 min and the membrane potentials in it were measured. Each point shows the mean with standard deviation (N=9-18, from 3 animals). Each paired points were obtained from each of 3 animals.
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only by ouabain but also by Zn²⁺, Cu²⁺, Cd²⁺, and mersalyl. It is well known that the Na⁺-pump is inhibited in K⁺-free solution. The membrane potential of mucous epithelial cells decreases in K⁺-free solution and the decreased membrane potential level is not further changed by the application of ouabain (Kanno et al., 1977). Heavy metal ions and mersalyl like ouabain do not change the membrane potential of the cells in K⁺-free solution. From these facts, it seems probable that heavy metal ions inhibit Na⁺-pump and, thus, bring about a decrease in membrane potential without any appreciable change in the effective membrane resistance and electrical coupling ratio.

If this is the case, the action sites of heavy metal ions in inhibiting the Na⁺-pump might be SH-groups of the cell membrane, because it is well known that heavy metal ions can combine with SH-groups (Gurd and Wilcox, 1956) and that some organic mercurials inhibit (Na⁺, K⁺)-ATPase by combining with SH-groups which are located on the surface of cell membrane (Ohta et al., 1971). The fact that some divalent heavy metal ions, such as Zn²⁺ and Cu²⁺ inhibit brain microsomal (Na⁺, K⁺)-ATPase activity (Donaldson et al., 1971) supports this idea. The idea is also supported by the following facts: UO₂²⁺, which does not combine with SH-groups (Rothstein, 1962), can not change the membrane potential, while mersalyl, a SH-reagent, can decrease the membrane potential without any appreciable change in the effective membrane resistance and electrical coupling ratio; the action of mersalyl is also quite similar to those of heavy metal ions and ouabain in that the transient hyperpolarization due to increase in intracellular Na⁺ is blocked by mersalyl and that the membrane potential of the cells in K⁺-free solution is not further changed by adding the agent to the solution; the action of heavy metal ions on the membrane potential is, as well as that of mersalyl, antagonized with SH-compounds, cysteine and acetylpenicillamine.

Nickel ion and Co²⁺ are not or less effective on the membrane potential of the cells, as compared with Zn²⁺, Cu²⁺, or Cd²⁺. The binding affinities of Ni²⁺ and Co²⁺ to SH-groups of the cell membrane may be lower than those of Zn²⁺, Cu²⁺, and Cd²⁺. Begenisich and Lynch (1974) reported that the affinity of Co²⁺ to SH-group is far lower than that of Zn²⁺ or Cd²⁺.

In summary, the present experimental results suggest that heavy metal ions (Zn²⁺, Cu²⁺, and Cd²⁺) may inhibit active ion transport mechanism, probably the Na⁺-pump, in the mucous epithelial cells of the newt stomach and thus bring about a decrease in the membrane potential. The ion might combine with a SH-group of the cell membrane and inhibit the transport mechanism, though it can not be ruled out the possibility that the ions act on a process of energy supply for the active transport mechanism and inhibit it.

SUMMARY

Effect of heavy metal ions (Zn²⁺, Cu²⁺, Cd²⁺, Ni²⁺, and Co²⁺) on the electrical properties (membrane potential, effective membrane resistance, and electrical coupling ratio) of mucous epithelial cells in the isolated newt stomach was studied and the effect was compared with that of UO₂²⁺, which does not combine with SH-groups, and of
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Mersalyl, which is a powerful SH-reagent. Zinc ion, Cu²⁺ and Cd²⁺ at 10⁻⁴ M or lower concentrations caused a marked decrease in the membrane potential without any appreciable change in the effective membrane resistance and electrical coupling ratio. The effect of other two ions, Ni²⁺ and Co²⁺, was slight even at 10⁻³ M. Mersalyl (5 × 10⁻⁴ M) also decreased the membrane potential without any appreciable change in the effective membrane resistance and electrical coupling ratio but UO₂²⁺ (10⁻⁵–10⁻⁴ M) did not affect any of the electrical properties. Cysteine and acetylpenicillamine, both of which are SH-compounds, antagonized the action of Zn²⁺, Cu²⁺, Cd²⁺, and mersalyl. Ions, as well as mersalyl and ouabain, blocked the hyperpolarization of the membrane due to an increase in intracellular Na⁺. The effect of ions on the relationship between external K⁺–concentration and membrane potential was similar to that of ouabain and mersalyl: the decreased membrane potential level in K⁺-free solution was not changed by adding those agents to the solution. These results suggest that Zn²⁺, Cu²⁺, and Cd²⁺ inhibit active ion transport mechanism, probably the Na⁺-pump, in the mucous epithelial cell by combining with a SH-group of the membrane and, thus, bring about a decrease in the membrane potential.

**Key words:** Stomach, epithelial cell, heavy metal ion, membrane potential, ion transport, sodium pump, sulphydryl group.

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