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EFFECT OF ANTIHISTAMINES AND CHLORPROMAZINE ON THE CALCIUM-INDUCED HYPERPOLARIZATION OF THE *AMPHIUMA* RED CELL MEMBRANE

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SUMMARY

1. It has previously been demonstrated that an increase in extracellular Ca^{2+} concentration induces a transient increase in K^+ permeability and associated hyperpolarization of the red cell membrane of the giant salamander, *Amphiuma means*. This phenomenon is analogous to the Ca^{2+}-induced KCl loss observed in ATP-depleted human red cells and red cell ghosts.

2. Histamine, which enhances the Ca^{2+}-induced K^+ loss from depleted human red cells, is without effect on this Ca^{2+}-induced hyperpolarization of *Amphiuma* red cells.

3. Promethazine (10 μM) and mepyramine (1 mM), which inhibit the Ca^{2+}-induced K^+ loss in depleted human red cells, also block the Ca^{2+}-related hyperpolarization of *Amphiuma* erythrocytes.

4. Chlorpromazine (25 μM), despite being a weak antihistamine, is equally effective in blocking the Ca^{2+}-induced hyperpolarization of *Amphiuma* red cells.

5. Ionophore A23187 causes a large and sustained Ca^{2+}/K^+-dependent hyperpolarization even in the presence of normal (1.8 mM) concentrations of Ca^{2+}. This hyperpolarization is relatively insensitive to chlorpromazine and promethazine.

6. The inhibition of the Ca^{2+}-induced hyperpolarization of the *Amphiuma* red cell membrane by chlorpromazine and promethazine may be related to their properties as local anaesthetics.

INTRODUCTION

Following the initial observation of Gárdos [1, 2] that calcium induces a net K^+ loss from ATP-depleted human red cells, an extensive literature dealing with this phenomenon has developed [3]. In an extension of these studies Gárdos and Szász [4] reported that histamine potentiated the effect of calcium whereas antihistamines as well as the tranquilizer chlorpromazine [5] blocked the effect.

Abbreviation: MOPS, morpholinopropane sulphonate.

In the presence of Ca^{2+}, $^{42}K^+$ was rapidly taken up by ATP-depleted (Blum and Hoffman [6], Gárdos et al. [7]) and propranolol-treated (Manninen [8]) erythrocytes and temporarily reached a cellular concentration higher than the medium. Glynn and Warner [9] have shown, based on computations, that a temporary hyperpolarization could give rise to a similar accumulation of $^{42}K^+$.

It has been shown that giant red cells from the salamander *Amphiuma means* are transiently hyperpolarized following an increase in the extracellular Ca^{2+} concentration (Lassen et al. [10]). This hyperpolarization is caused by an increase in K^+ permeability of the membrane. Thus the Ca^{2+}-dependent K^+ permeability change in *Amphiuma* red cells is analogous to that seen in human red cells. Therefore, these cells provide a suitable experimental system for investigating the effect of drugs on the electrical properties of red cell membranes. This study describes the effects of histamine, antihistamines, and chlorpromazine on the Ca^{2+}-induced hyperpolarization of the *Amphiuma* red cell membrane.

MATERIALS AND METHODS

Red cells were obtained from mature specimens of *Amphiuma means* by cardiac puncture. The blood samples were immediately washed in approx. 50 volumes of cold Ringer's solution containing 118 mM Na^+, 2.5 mM K^+, 124 mM Cl^-, 1.8 mM Ca^{2+} and 10 mM morpholinopropane sulphonate buffer (MOPS) titrated to pH 7.2 ("normal Ringer's solution"). The cells were then incubated in the same medium until used for potential measurements. In order to bring the cells into the Ca^{2+} induced hyperpolarized state, they were transferred rapidly to a Ringer's solution with the following composition: 95 mM Na^+, 2.5 mM K^+, 128 mM Cl^-, 15 mM Ca^{2+} and 10 mM MOPS, pH 7.2 ("15 mM Ca^{2+} Ringer's solution"). For measurement of the effect of various drugs, cells were transferred from normal Ringer's solution to a solution with an increased Ca^{2+} concentration and the appropriate drug. Membrane potentials were compared to those obtained in Ringer's solution containing the same Ca^{2+} concentration without the drug.

Measurements of membrane potentials of single cells were performed with conventional microelectrodes (filled with 3 M KCl) mounted in a piezoelectric transducer which advanced the electrode tip 1 μm within 50 μs for penetration of the membrane. This technique as well as temperature control of the measuring chamber has been described in detail previously [11].

Stock solutions of ionophore were prepared by dissolving the ionophore in a mixture of 1 : 9 acetone/ethanol (99 %). The final concentration of acetone/ethanol in cell suspensions to which the ionophore was added was usually 0.5 % and never more than 1.0 %.

RESULTS

The distribution of measured potentials obtained from a representative group of *Amphiuma* red cells suspended in normal Ringer's solution is shown in Fig. 1A. The mean value of the potentials is -15 mV. When these cells are transferred to solutions with increased concentrations of Ca^{2+} they undergo a transient hyperpolarization. The distribution of measured potentials recorded within the first three

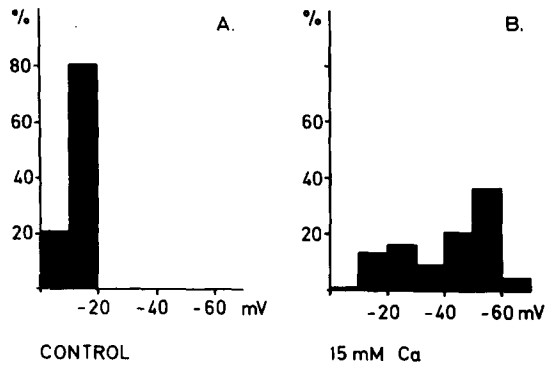

Fig. 1. The effect of an increase in extracellular concentration of Ca^{2+} on the distribution of measured membrane potentials in single *Amphiuma* red cells. A, Potential distribution in control Ringer's solution containing 1.8 mM Ca^{2+}. B, Potential distribution after transferring cells from control Ringer's solution to Ringer's solution containing 15 mM Ca^{2+}. Data was obtained within the first three minutes after transfer to 15 mM Ca^{2+} Ringer's solution. Ordinate: fraction of cells having a potential within a 10 mV interval (indicated by the abscissa). 17 °C, pH 7.2.

min after cells are transferred to 15 mM Ca^{2+} Ringer's solution is shown in Fig. 1B. As can be clearly seen, the distribution is bimodal with the measured potentials being grouped around −20 and −40 to −70 mV. This distribution pattern has been discussed by Lassen et al. [10] in terms of an all-or-none effect of Ca^{2+} on K^+ permeability of the *Amphiuma* red cell membrane. In addition it has been shown that the threshold concentration of Ca^{2+} required to induce hyperpolarization of these cells at pH 7.2 is 6 mM.

As reported by Gárdos and Szász [4] histamine enhances the Ca^{2+}-induced K^+ loss from depleted human red cells. The equivalent effect of histamine in *Amphiuma* red cells would be an increase in the fraction of hyperpolarized cells under the influence of increased external Ca^{2+} concentration. Such an effect would be most clearly demonstrated in the presence of a threshold concentration of Ca^{2+} (6 mM). Individual potential recordings obtained within the first three min after suspending cells in 6 mM Ca^{2+} Ringer's solution with histamine were plotted as a function of histamine concentration in Fig. 2. No increase in the fraction of cells being hyperpolarized can be demonstrated with rising histamine concentrations in the range 0.05–2 mM.

Although histamine is without apparent effect in the present system the effect of antihistamines was investigated. Cells were transferred from normal Ringer's solution to 15 mM Ca^{2+} Ringer's solution plus 10 μM promethazine and potentials were measured within the first three min. Fig. 3 shows the relative distribution of the obtained potential values. Comparison of this figure with Fig. 1 indicates that there is almost complete blockage of the hyperpolarization associated with 15 mM Ca^{2+}. In the light of the above results membrane potentials were measured in 15 mM Ca^{2+} Ringer's solution containing both 10 μM promethazine and 5 mM histamine. The results are shown in Fig. 4 and indicate that histamine is unable to prevent promethazine from blocking hyperpolarization. The typical antihistamines are substituted ethylamines of which promethazine is an example. Another drug of this type is mepyramine which is chemically very different from promethazine (see e.g. Goodman and Gilman [12]). 1.0 mM mepyramine employed under similar conditions to those

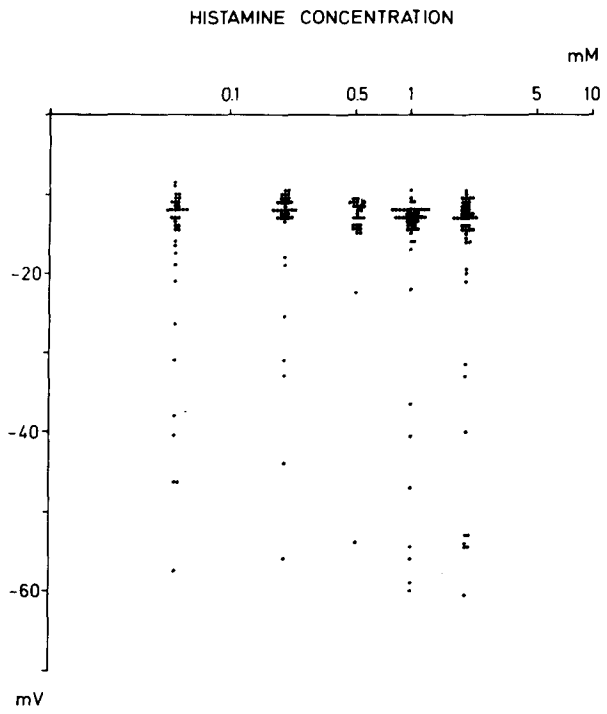

Fig. 2. Measured membrane potentials of single cells suspended in 6 mM Ca^{2+} Ringer's solution as a function of extracellular histamine concentration. Potentials were measured within the first three minutes after transfer to the higher Ca^{2+} concentration. Abscissa: histamine concentration in mM (log scale). Ordinate: membrane potential in mV. 17 °C, pH 7.2.

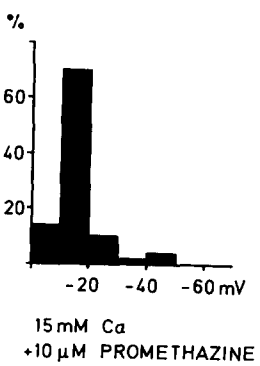

Fig. 3. Distribution of measured membrane potentials of single cells suspended in 15 mM Ca^{2+} Ringer's solution with 10 μM promethazine. Data from the initial three minutes of exposure. Ordinate: fraction of cells with potentials in a 10 mV interval (indicated by the abscissa). 17 °C, pH 7.2.

mentioned above resulted in total blockage of the Ca^{2+} induced hyperpolarization, whereas 0.1 mM mepyramine was without significant effect.

Promethazine shares its phenothiazine structure with a number of other pharmacologic agents, one of which is the antipsychotic drug, chlorpromazine. Although chlorpromazine has at best a weak antihistamine action, it has a number of pharmacologic effects in common with promethazine and other local anaesthetic amines [12, 13]. The distribution of measured potentials in the presence of 10 μM chlorpromazine is shown in Fig. 5. It can readily be seen that the action of chlorpromazine in blocking the Ca^{2+}-induced hyperpolarization of the *Amphiuma* red cell membrane is similar to that of promethazine. Fig. 6 is a plot of individual measured potentials as a function of chlorpromazine concentration of the medium (log scale). When the concentration of chlorpromazine exceeds 10 μM there is a total inhibition

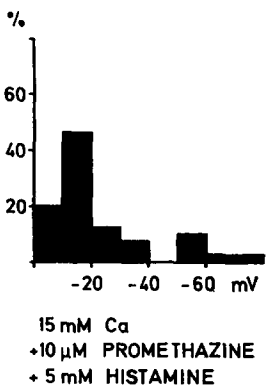

Fig. 4. Distribution of measured membrane potentials of single cells suspended in 15 mM Ca^{2+} Ringer's solution containing both promethazine (10 μM) and histamine (5 mM). Data from the initial three minutes of exposure. Ordinate: fraction of cells with potentials in a 10 mV interval (indicated by the abscissa). 17 °C, pH 7.2.

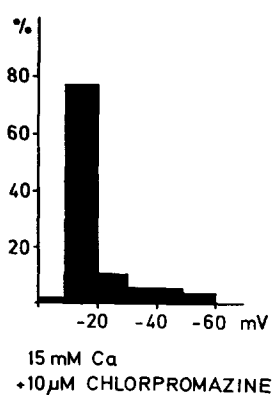

Fig. 5. Distribution of measured membrane potentials of single cells suspended in 15 mM Ca^{2+} Ringer's solution with 10 μM chlorpromazine. Data from the initial three minutes of exposure. Ordinate: fraction of cells with potentials in a 10 mV interval (indicated by the abscissa). 17 °C, pH 7.2.

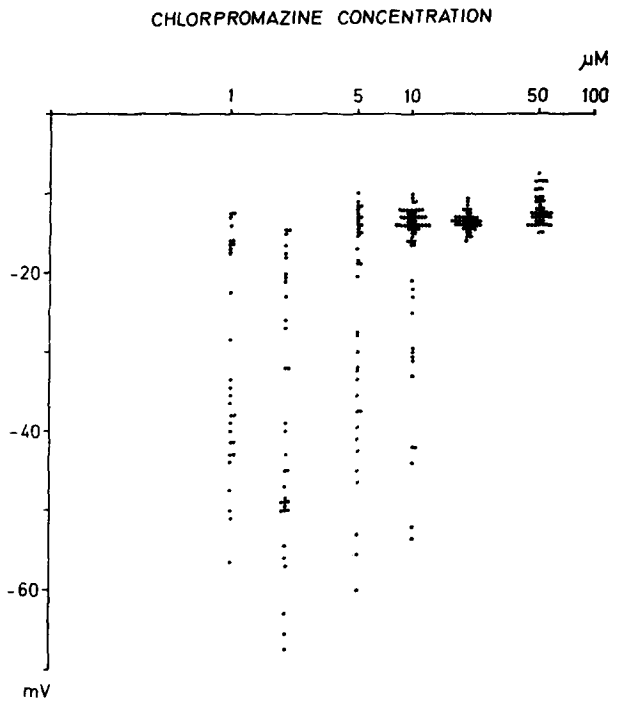

Fig. 6. Measured membrane potentials of single cells suspended in 15 mM Ca^{2+} Ringer's solution as a function of extracellular chlorpromazine concentration. Abscissa: chlorpromazine concentration in μM (log scale). Ordinate: membrane potential in mV. 17 °C, pH 7.2.

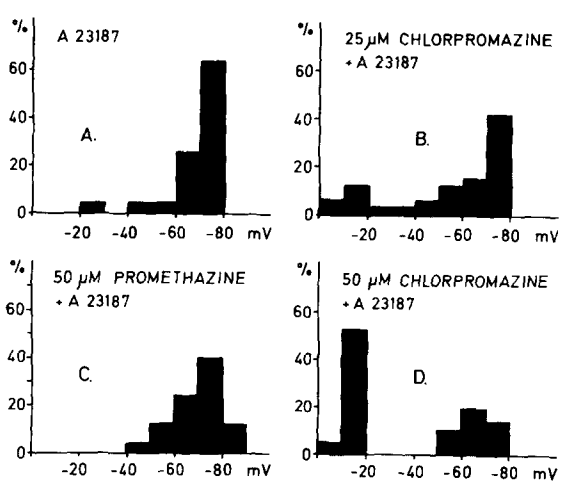

Fig. 7. Distribution of measured membrane potentials of single cells suspended in Ringer's solution containing control concentration of Ca^{2+} (1.8 mM) and: A, $1.9 \cdot 10^{-5}$ M divalent cation ionophore A23187; B, $1.9 \cdot 10^{-5}$ M A23187 plus 25 μM chlorpromazine; C, $1.9 \cdot 10^{-5}$ M A23187 plus 50 μM promethazine; D, $1.9 \cdot 10^{-5}$ M A23187 plus 50 μM chlorpromazine. Ordinates: fraction of cells with potentials in a 10 mV interval (indicated by the abscissae). 17 °C, pH 7.2.

of the hyperpolarization normally seen upon suspension of cells in Ringer's solution with 15 mM Ca^{2+}.

The results described above, while clearly indicating that chlorpromazine blocks the Ca^{2+}-induced hyperpolarization, do not make it possible to determine whether the drug acts to prevent entry of Ca^{2+} or whether it blocks the internal action of Ca^{2+} on the K^+ permeability mechanism. In an effort to distinguish between these modes of action, cells were exposed to the ionophore A23187 which has been shown to transport Ca^{2+} across the plasma membrane [14]. The distribution of membrane potentials in *Amphiuma* red cells measured in normal Ringer's solution (1.8 mM Ca^{2+}) containing $1.9 \cdot 10^{-5}$ M A23187 is shown in Fig. 7A. It can be seen that practically all of the cells are hyperpolarized. This hyperpolarization which requires Ca^{2+} in the suspending medium and is blocked by high external K^+ concentrations (unpublished data) closely resembles the hyperpolarization induced by an increase in extracellular Ca^{2+} concentration (see Fig. 1B). The corresponding distribution of membrane potentials measured in cells suspended in Ringer's solution containing chlorpromazine or promethazine after having been first washed in A23187 Ringer's solution can be seen in Figs. 7B, C and D. These results indicate that chlorpromazine at a concentration of 50 μM resulted in a partial blockage of the ionophore induced hyperpolarization. This partial blockage may be due to inhibition by chlorpromazine of Ca^{2+} transport via the ionophore. Promethazine at a concentration (50 μM) capable of totally blocking hyperpolarization elicited by 15 mM Ca^{2+} left the hyperpolarization induced by A23187 unaffected.

DISCUSSION

The Ca^{2+}-induced increase in K^+ permeability and associated hyperpolarization of the *Amphiuma* red cell membrane is analogous to the Ca^{2+}-dependent K^+ loss in depleted human red cells (Lassen et al. [10]). Gárdos and Szász [4] demonstrated that low concentrations of histamine (0.5 mM) stimulated, and higher concentrations of histamine inhibited the Ca^{2+}-induced net K^+ loss from depleted human erythrocytes. Neither of these effects could be demonstrated in the *Amphiuma* red cell under the present experimental conditions. Szász [15] has reported that the Ca^{2+} influx rate is not increased by histamine in ATP-depleted human red cells. In the case of the *Amphiuma* cells used in the present study the intracellular concentrations of ATP are 3–5 times higher than that of non-depleted human red cells (unpublished observations). Therefore inadequate ATP levels cannot be the reason for the lack of a histamine effect in *Amphiuma* red cells. In non-depleted human red cells a Ca^{2+}-dependent K^+ loss can be provoked by the β-blocking agent propranolol. Like histamine, this drug is also without effect on the membrane potential of the *Amphiuma* red cells (unpublished observations).

There is however a marked inhibitory effect of antihistamines on the Ca^{2+} induced hyperpolarization of the *Amphiuma* red cell membrane. The fact that promethazine is capable of inhibiting the hyperpolarization of these cell membranes, while histamine is without effect, raises the question as to whether this drug is acting as an "electrical membrane stabilizer" [13] (local anaesthetic) rather than solely as an antihistamine. Chlorpromazine which has a weak antihistamine effect is as effective as promethazine in inhibiting hyperpolarization. Both promethazine and chlorpromazine

are phenothiazine derivatives and act as local anaesthetics [13].

Chlorpromazine is capable of blocking the Ca^{2+}-induced hyperpolarizations and at high concentrations partially abolishes the hyperpolarization caused by the ionophore A23187. This observation is consistent with the work of Kwant and Seeman [16], as well as others [17], who have shown that chlorpromazine causes a displacement of membrane bound Ca^{2+} from the fixed negative sites. It is of interest to note that the concentrations of chlorpromazine to prevent Ca^{2+}-induced hyperpolarization in *Amphiuma* red cells are essentially the same as those required to block nerve conduction [13]. For the purposes of comparison, if one assumes a uniform distribution in the extracellular fluid of an average person, intramuscular injection of the lowest effective dose of 100 mg chlorpromazine [12] results in a plasma concentration of about 20 μM.

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