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Resting plasma membrane potential of rat peritoneal mast cells is set predominantly by the sodium pump

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Changes in the plasma membrane potential of two histamine-releasing cells, rat peritoneal mast cells and basophilic leukemia cells 2H3 (RBL 2H3), were recorded with the potential-sensitive dye *bis*-oxonol. For mast cells, the presence of ouabain or the absence of K^+ increased the fluorescence intensity of *bis*-oxonol; gramicidin had no effect. For RBL 2H3 cells, the presence of ouabain and the absence of K^+ also increased *bis*-oxonol fluorescence but gramicidin also increased it. These results show that the plasma membrane potential of RBL 2H3 cells is set, in part, by the acitivity of the Na^+ pump and in part by the K^+ conductance, while that of rat mast cells is set predominantly by the Na^+ pump.

Mast cell; Basophil; Membrane potential; Oxonol, *bis*-; Potassium; Sodium pump

1. INTRODUCTION

Histaminocytes include mast cells and basophilic leucocytes. These cells secrete inflammatory mediators upon aggregation of cell surface receptors for immunoglobulin E (IgE). However, it is not yet fully understood how the activation of the IgE receptor initiates the exocytotic process. It has been reported that the IgE-dependent stimulation of mast cells does not require the opening of ion channels [1], whereas Ca^{2+} influx might occur [2]. No significant changes in the plasma membrane conductance have been observed following IgE-dependent stimulation of mast cells whereas 2 types of ion channel that are normally inactive have been detected [3]. The first type was a voltage-independent and Ca^{2+}-dependent cation-selective channel. The second type was also Ca^{2+}-dependent but was slightly selective for Cl^-. Lindau and Fernandez [3] suggested that these channels might rather be involved in the osmotic regulation of the cells. More recently, however, it was proposed that the Cl^- channel might be involved in non-immunological stimulation of mast cells, i.e. by compound 48/80 or substance P [4]. It is not yet clear how the plasma membrane potential is regulated in mast cells. Using the patch-clamp technique, Lindau and Fernandez [3] showed that the resting potential of mast cells fluctuated between 0 and -30 mV, with a very small whole-cell conductance (10–30 pS), and an input resistance of 30–100 GΩ. It was postulated that these values were due mainly to the imperfect pipette membrane seal. However, the authors could not exclude a resting potential caused by the electrogenic activity of the Na^+ pump or by the Na^+/Ca^{2+} exchanger which might have been short-circuited by the leakage conductance of the seal. We therefore studied the role of the Na^+ pump in maintaining the resting membrane potential of rat peritoneal mast cells using the potential-sensitive dye *bis*-oxonol. Rat peritoneal mast cells were compared to rat basophilic leukemia cells, subline 2H3 (RBL 2H3), whose electrophysiologic properties have been widely studied [5,6].

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2. MATERIALS AND METHODS

Crude cell suspensions were obtained from lavages of the peritoneal cavities of male Wistar rats (300–350 g), with a buffered salt solution containing (mM): NaCl, 137; KCl, 2.7; $MgCl_2$, 1; NaH_2PO_4, 0.4; glucose, 5.6; $CaCl_2$, 1; Hepes, 10; NaOH, pH 7.4. Mast cells were purified by centrifugation in discontinuous gradients of bovine serum albumin as described previously [7]. Experiments with rat basophilic leukemia cells were performed with a secreting subline (2H3) maintained in monolayer culture [8]. Purified mast cells (>97%) or RBL 2H3 cells were washed twice in the buffer without K^+ and Ca^{2+} but supplemented with 0.05% gelatin (w/v). Then, 2.5 ml of the mast cell suspension (5×10^5 cells/ml) or of RBL 2H3 cells (10^6 cells/ml) were placed in 1-cm quartz cuvettes. The cuvettes were pre-incubated for 2 min at 37°C in the thermostatted cuvette holder of a Jobin-Yvon spectrofluorimeter under gentle stirring. Changes in the plasma membrane potential of mast cells and RBL 2H3 cells were monitored using *bis*-oxonol a potential-sensitive dye taking advantage of the fact that it does not accumulate in mitochondria and so does not translate their changes in membrane potential [6]. To evaluate the plasma membrane potential of the cells from fluorescence intensities, the valinomycin K^+ null point method is usually recommended [9]. However, this is not possible with oxonol dyes since they bind to valinomycin. The fluorescence of *bis*-oxonol has been correlated to the plasma membrane potential [10]. 100 nM *bis*-oxonol (*bis*-(1,3-diethylthiobarbiturate)trimethine oxonol, Molecular Probes, Eugene, OR) were added to the cell suspension. Fluorescence was monitored at 540 and 580 nm. Reagents were added at a volume of 25 μl from 100-fold concentrated stock solutions. Trypan blue exclusion was used to monitor cell viability. None of the drugs was cytotoxic and had no effect on *bis*-oxonol fluorescence at the concentrations used in this study.

3. RESULTS

Addition of *bis*-oxonol to mast cells suspended in a balanced salt medium led to an immediate increase in the fluorescence intensity which stabilized rapidly (fig.1a). Addition of gramicidin, which forms non-selective cation channels in the membrane and causes total depolarization of mast cells, induced an increase in the fluorescence of the probe. When mast cells were suspended in a medium without K^+ and Ca^{2+} (fig.1b) the fluorescence intensity of *bis*-oxonol was higher than in the presence of K^+ and Ca^{2+}. Gramicidin did not further increase the fluorescence, but rather reduced it transiently, showing that K^+ and Ca^{2+} deprivation completely depolarized mast cells. The addition of 2.7 mM K^+ (fig.1c), which stimulates the Na^+ pump, decreased the fluorescence intensity, i.e. repolarized the cells. This effect was dose-dependent (fig.1h) and was inhibited by ouabain

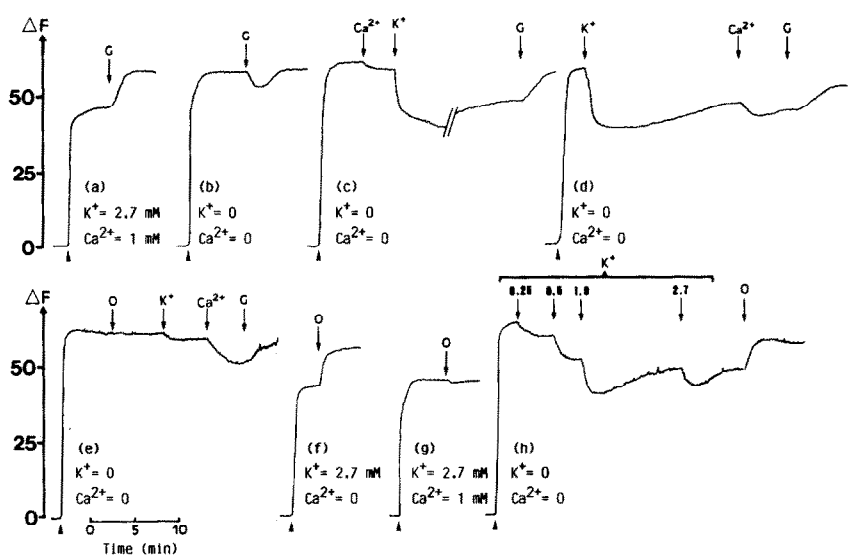

Fig.1. Effects of potassium (K^+), calcium (Ca^{2+}), ouabain (O) and gramicidin (G) on the plasma membrane potential of purified rat peritoneal mast cells under various conditions. These conditions were determined by the initial K^+ and Ca^{2+} concentrations, given under traces. *bis*-Oxonol (100 nM) was added as indicated by an arrow. K^+ and Ca^{2+} were added at 2.7 and 1 mM, respectively (final concentrations) except when stated otherwise. Ouabain and gramicidin were added at 1 mM and 1 μg/ml, respectively (final concentrations). In fig.1c, gramicidin was added 23 min after K^+. Fluorescence intensities were recorded continuously and have been expressed as arbitrary units. Traces are representative of at least 7 experiments.

(fig.1e), an inhibitor of the Na^+ pump, demonstrating that the repolarization of the cells induced by K^+ was due to the activation of the Na^+ pump. Furthermore, high doses of K^+, from 10 to 50 mM, did not affect the fluorescence of *bis*-oxonol (results not shown) showing that the plasma membrane potential of mast cells is not determined by the transmembrane gradients of K^+. Ouabain increased the fluorescence intensity of *bis*-oxonol only in the absence of extracellular Ca^{2+} (fig.1f, g and h). The addition of Ca^{2+} before (fig.1c) or after K^+ (fig.1d) slightly decreased the fluorescence, suggesting that beside the sodium pump another ion exchanger, e.g. Na^+/Ca^{2+}, might be involved in maintaining the resting membrane potential of mast cells. Fig.2 shows that when RBL 2H3 cells were suspended in a Ca^{2+}- and K^+-free medium (fig.2b), the fluorescence of *bis*-oxonol was higher than in a Ca^{2+}-free medium (fig.2a). The effect of K^+ was dose-dependent up to 2.7 mM (fig.2c) and was inhibited by ouabain (results not shown). Unlike for the mast cells, gramicidin further increased the fluorescence intensity in the absence of K^+ (fig.2b), indicating that under these conditions RBL 2H3 cells were not fully depolarized. They were almost completely depolarized when the extracellular K^+ concentration reached 30 mM (fig.2c) and were completely depolarized in the presence of 140 mM of K^+ [6]. Fig.2c shows that the fluorescence intensity after the addition of gramicidin was lower than initially because of the dye dilution after adding K^+ from a 150 mM stock solution. Ouabain only increased the fluorescence of *bis*-oxonol in the absence of added Ca^{2+} (fig.2d,e). The effect of K^+ was dependent upon the intracellular ATP content since pretreatment of the cells with dinitrophenol and deoxyglucose hinders the decrease in K^+-induced fluorescence (fig.2f). Ouabain also had no effect under these conditions, indicating that its effect is dependent on the activity of the pump. Ca^{2+} also appears to regulate the resting membrane potential (fig.2c).

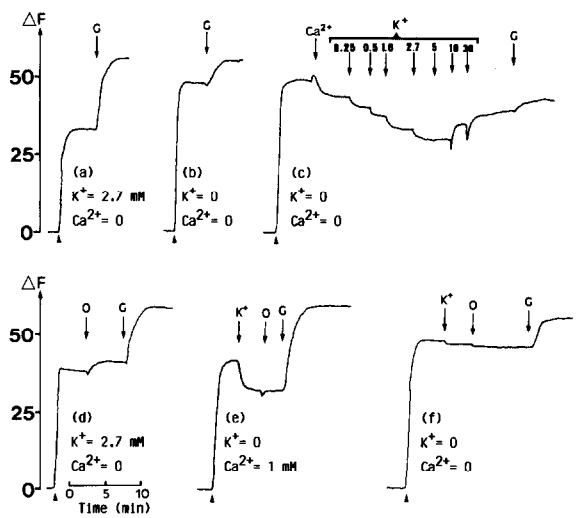

Fig.2. Effects of potassium, calcium, ouabain and gramicidin on the plasma membrane potential of rat basophilic leukemia cells under various conditions. These conditions were determined by the initial K^+ and Ca^{2+} concentrations, given under traces. Ouabain (O) and gramicidin (G) were added at 1 mM and 1 μg/ml (final concentrations), respectively. Potassium (K^+) and calcium (Ca^{2+}) were added at 2.7 and 1.8 mM (final concentrations), respectively except when stated otherwise. In fig.2f RBL 2H3 cells were pretreated for 45 min with 2×10^{-4} M dinitrophenol and 5.6 mM deoxyglucose. Fluorescence intensities were recorded continuously and have been expressed as arbitrary units. Traces are representative of at least 5 experiments.

4. DISCUSSION

The resting plasma membrane potential of RBL 2H3 cells [6], like that of other cells, is established by the control of ionic conductance operated by channels or electrogenic carriers. However, in certain cells, including human neutrophils [12] and Lettré cells [9] (review in [11]), the resting plasma membrane potential is controlled primarily by the electrogenic activity of the Na^+ pump and not by the K^+ permeability. Indeed, the inhibition of the Na^+ pump results in a rapid depolarization of these cells whereas increasing the extracellular K^+ has no effect. The present data demonstrate that rat peritoneal mast cells belong to the group of cells whose plasma membrane potential is set predominantly by the sodium pump and not by the outward diffusion of K^+. Our results are in full agreement with the hypothesis of Lindau and Fernandez [3] who suggested, but did not demonstrate, a possible contribution of the Na^+ pump as well as that of an Na^+/Ca^{2+} exchanger in maintaining the resting membrane potential of rat mast cells. The role of Ca^{2+} in maintaining the membrane potential shown here might be due to the Na^+/Ca^{2+} exchanger. Mast cells do not

undergo changes in ionic conductance upon IgE-dependent stimulation [1] but this may be due to the limits of the patch-clamp technique which cannot detect ionic currents below 1 pA. Currents below 1 pA can be expected [3] since no information on the density of the Na^+ pump in mast cell membrane is yet available. In contrast to mast cells, the resting membrane potential of RBL 2H3 cells is maintained, in part, by the electrogenic activity of the Na^+ pump (present results) and in part to the transmembrane K^+ gradient (present results and [6]). The presence of ouabain or the absence of K^+ depolarizes the cells, whereas Mohr and Fewtrell [6] found no effect of ouabain on the membrane potential of RBL 2H3 cells. This discrepancy might be due to the presence of Ca^{2+} in the experiments of Mohr and Fewtrell [6]. Indeed, in previous studies we have observed that the effect of ouabain on histamine release only emerged in the absence of Ca^{2+} (see references in [13]). This was recently confirmed by Knudsen and Johansen [14], who showed that extracellular Ca^{2+} strongly inhibited the ouabain-sensitive K^+ fluxes in mast cells. Thus, the activity of the Na^+ pump appears to contribute in generating the negative resting membrane potential in RBL 2H3 cells ranging from -10 to about -90 mV which were observed by different groups using different techniques [3,15–18]. Mohr and Fewtrell [5] proposed that the IgE-dependent activation of RBL 2H3 cells involved the opening of a non-selective cation channel which might be responsible for the Ca^{2+} entry and was thought to be governed by the membrane potential, i.e., by the K^+ conductance. We suggest that the activity of the Na^+ pump might also modulate such Ca^{2+} influx since the presence of ouabain (personal observations) and the absence of K^+ [5] inhibit both ionophore- and IgE-dependent stimulation of RBL 2H3 cells.

The present results confirm that the plasma membrane potential of mast cells and RBL 2H3 cells are set differently. The role of ionic pumps in controlling the exocytotic process remains to be defined in histaminocytes.

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REFERENCES

[1] Lindau, M. and Fernandez, J.M. (1986) Nature 319, 150–153.
[2] Ishizaka, T., Foreman, J.C., Sterk, A.R. and Ishizaka, K. (1979) Proc. Natl. Acad. Sci. USA 76, 5858–5862.
[3] Lindau, M. and Fernandez, J.M. (1986) J. Gen. Pharmacol. 88, 349–368.
[4] Penner, R., Matthews, G. and Neher, E. (1988) Nature 334, 499–504.
[5] Mohr, F.C. and Fewtrell, C. (1987) J. Cell. Biol. 104, 783–792.
[6] Mohr, F.C. and Fewtrell, C. (1987) J. Immunol. 138, 1564–1570.
[7] Bronner, C., Landry, Y., Fontenau, P. and Kuhry, J.G. (1986) Biochemistry 25, 2149–2154.
[8] Barsumian, E.L., Isersky, C., Petrino, M.G. and Siraganian, R.P. (1981) J. Immunol. 11, 317–323.
[9] Bashford, C.L. and Pasternak, C.A. (1984) J. Membrane Biol. 79, 275–284.
[10] Wiloson, H.A. and Chused, T.M. (1985) J. Cell Physiol. 125, 72–81.
[11] Bashford, C.L. and Pasternak, C.A. (1986) Trends Biochem. Sci. 11, 113–116.
[12] Bashford, C.L. and Pasternak, C.A. (1985) Biochim. Biophys. Acta 817, 174–181.
[13] Bronner, C., Gies, J.P., Vallé, A. and Landry, Y. (1987) Life Sci. 41, 2555–2562.
[14] Knudsen, T. and Johansen, T. (1989) Br. J. Pharmacol. 96, 773–778.
[15] Kanner, B.I. and Metzger, H. (1983) Proc. Natl. Acad. Sci. USA 80, 5744–5748.
[16] Sagi-Eisenberg, R. and Pecht, I. (1983) J. Membrane Biol. 75, 97–104.
[17] Sagi-Eisenberg, R. and Pecht, I. (1984) EMBO J. 3, 497–500.
[18] Barajas-Lopez, C. and Huizinga, J.D. (1988) Can. J. Physiol. Pharmacol. 66, 328–331.