

A Patch-Clamp Study of Histamine-secreting Cells

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ABSTRACT The ionic conductances in rat basophilic leukemia cells (RBL-2H3) and rat peritoneal mast cells were investigated using the patch-clamp technique. These two cell types were found to have different electrophysiological properties in the resting state. The only significant conductance of RBL-2H3 cells was a K^+ -selective inward rectifier. The single channel conductance at room temperature increased from 2–3 pS at 2.8 mM external K^+ to 26 pS at 130 mM K^+ . This conductance, which appeared to determine the resting potential, could be blocked by Na^+ and Ba^{2+} in a voltage-dependent manner. Rat peritoneal mast cells had a whole-cell conductance of only 10–30 pS, and the resting potential was close to zero. Sometimes discrete openings of channels were observed in the whole-cell configuration. When the Ca^{2+} concentration on the cytoplasmic side of the membrane was elevated, two types of channels with poor ion specificity appeared. A cation channel, observed at a Ca^{2+} concentration of $\sim 1 \mu M$, had a unit conductance of 30 pS. The other channel, activated at several hundred micromolar Ca^{2+} , was anion selective and had a unit conductance of ~ 380 pS in normal Ringer solution and a bell-shaped voltage dependence. Antigenic stimulation did not cause significant changes in the ionic conductances in either cell type, which suggests that these cells use a mechanism different from ionic currents in stimulus-secretion coupling.

INTRODUCTION

Basophils and mast cells initiate allergic and inflammatory reactions by secreting histamine and other mediators. The plasma membrane of these cells contains receptors for the F_c portion of immunoglobulin E (IgE). If a multivalent antigen or anti-IgE is added to cells primed with the appropriate IgE, the receptors are cross-linked, which leads to the fusion of the secretory granules with the plasma membrane, and thus granular material is released (Ishizaka and Ishizaka, 1984). Since mast cells and basophils are stimulated through the same antigenic mechanism, it has been widely assumed that these two cell types are closely related.

The electrophysiology of secretory cells has been studied in squid giant synapse (Katz and Miledi, 1967; Llinas, 1984), chromaffin cells (Fenwick et al., 1982a,

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b), and GH₃ cells (Hagiwara and Ohmori, 1982; Fernandez et al., 1984*a*). These cells have a negative resting potential typically determined by K⁺-selective ion channels. During an action potential, the cell is depolarized, which causes the transient opening of voltage-dependent, Ca²⁺-selective channels. The entry of Ca²⁺ through these channels eventually leads to secretion. It has been shown that the binding of acetylcholine in chromaffin cells opens ion channels, which leads to depolarization of the cell membrane (Fenwick et al., 1982*a*).

Similar mechanisms have been discussed for histamine secretion from mast cells and basophils (Kanner and Metzger, 1983; Sagi-Eisenberg and Pecht, 1984). It has been reported that stimulation through the IgE system requires extracellular Ca²⁺ (Foreman and Mongar, 1975; Beaven et al., 1984*a, b*), and an increase of the intracellular Ca²⁺ concentration has been observed after stimulation (White et al., 1984; Beaven et al., 1984*b*; Moore et al., 1984). Models involving the entry of extracellular Ca²⁺ have been proposed that assume the opening of Ca²⁺ channels by cross-linking of the F_c receptors (Cockroft and Gomperts, 1979; Crews et al., 1981; Ishizaka and Ishizaka, 1984; Mazurek et al., 1984).

We have investigated the electrophysiology of both cell types using patch-clamp techniques and have found that rat peritoneal mast cells and rat basophilic leukemia cells have quite different properties. RBL-2H3 cells have a negative resting potential caused by K⁺-selective, inwardly rectifying ion channels. In contrast, resting rat peritoneal mast cells have neither a defined resting potential nor significant ion conductances in the plasma membrane. The conductance and the resting potential in both cell types are not significantly affected by the binding of IgE or cross-linking of the F_c receptors. Therefore, it appears that these cells use a mechanism different from ionic channels in stimulus-secretion coupling.

MATERIALS AND METHODS

RBL-2H3 Cell Culture

The RBL-2H3 cell line was obtained from T. Jovin (Max-Planck-Institut, Göttingen, Federal Republic of Germany [FRG]). The cells were kept frozen in small aliquots at -80°C. The aliquots were transferred to culture flasks (250 ml) and the cells were grown in Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum (Gibco Laboratories, Grand Island, NY) and inoculated with 2% streptomycin-penicillin solution (Gibco Laboratories). The cell cultures were maintained at 37°C in a humidified atmosphere of 10% CO₂ and 90% air and grown until confluency. Cells were transferred to a new flask every 7–10 d. The ability of the RBL-2H3 cell line to secrete was tested by loading the cells with radioactive serotonin and measuring the amount released into the extracellular medium after antigenic stimulation (Kanner and Metzger, 1983). In two separate experiments, a release of up to 57% of the total available serotonin was obtained. For patch-clamp experiments, cells were plated on the glass bottom of the measuring chamber and then incubated with the same medium. Only confluent cells were used in these experiments. All experiments were performed at room temperature.

Mast Cell Preparation

Large rats (400–500 g) were killed and 20–50 ml of external saline (see below) was injected into their peritoneal cavities. After some gentle massage of the rat abdominal region, the peritoneal cavity was surgically exposed and ~10–20 ml of fluid was aspirated.

This fluid contained macrophages, neutrophils, and a significant number of mast cells. Aliquots of ~1 ml were directly transferred to coverslips in petri dishes. Cells were incubated at 37°C for at least 30 min before use. Under these conditions, most cells kept in the incubator at 37°C were responsive for up to 30 h.

Recording Configurations

The experiments were done with cell-free patches and in the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). The patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, FRG) was used in the current-clamp and voltage-clamp modes without series resistance compensation.

In most experiments, the command input of the EPC-7 was controlled by a computer (PDP 11/23 or Apple IIe) via a digital-analog converter. The current was sampled simultaneously at an appropriate rate after filtering at the Nyquist frequency. When a voltage pulse was given, a total of 256 or 1,024 points were recorded and stored on a disk. In some experiments, current and voltage were continuously recorded using an FM tape recorder (Store 4, Racal Recorders, Inc., Corina, CA).

The reference electrode was an Ag/AgCl pellet connected to the bath solution through a 1 M KCl or 150 mM KCl/agar bridge.

Solutions

The internal solution for RBL-2H3 cells was: 100 mM K-glutamate, 20 mM Na-glutamate, 7 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, 10 mM HEPES, adjusted to pH 7.2–7.3 with KOH. The final K concentration was ~135 mM. This solution was stored at –18°C. Before the experiments, Na₂ATP was added from a 500-mM stock solution in 1 M Tris-Cl (pH 7.2) to the thawed solution to give a final concentration of 5 mM ATP.

The internal solution for mast cells was: 150 mM K-glutamate, 7 mM MgCl₂, 10 mM HEPES, adjusted to pH 7.2–7.3 with NaOH. This solution was stored frozen until use. After thawing, EGTA and Na₂ATP were added to a final concentration of 100 μM EGTA and 100 μM Na₂ATP.

The external solution was: 142 mM NaCl, 2.8 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM HEPES, adjusted to pH 7.2–7.3 with NaOH and ~30 mM glucose to adjust the osmolarity. The K concentration was increased in some cases by replacing certain amounts of NaCl with KCl. In some experiments, Na was replaced with *N*-methyl-glucamine. The bath solution could be changed during the recordings by bath perfusion.

Liquid Junction Potential

All potentials were corrected for the liquid junction potential that develops at the tip of the pipette if it is immersed into the bath solution (Fenwick et al., 1982a). The liquid junction potential between the normal internal and external solutions was ~10 mV, mainly because of the different mobilities of the Cl and glutamate anions.

Sensitization and Stimulation of the Cells

Cells were incubated for at least 1 h at 37°C in Ringer solution containing 1 μg/ml mouse IgE before stimulation experiments. The passively sensitized cells were stimulated by the addition of dinitrophenyl bovine serum albumin with 43 mol dinitrophenyl per mole bovine serum albumin (DNP₄₃-BSA, kindly supplied by H. Metzger, National Institutes of Health, Bethesda, MD). This procedure led to visible degranulation of rat peritoneal mast cells, as judged from light microscopy, and to the release of radiolabeled serotonin from RBL-2H3 cells. In the patch-clamp experiments, 10 μl of a DNP₄₃-BSA solution (100 μg/ml in phosphate-buffered saline) was dropped into the recording chamber in the

vicinity of the observed cells. The antigen was added in the presence of 2 mM CaCl₂ and 100 μM phosphatidylserine (Sigma Chemical Co., St. Louis, MO).

RESULTS

Rat Basophilic Leukemia Cells

Whole-cell current clamp. The resting potential of the cells was determined in the whole-cell configuration, using the current-clamp mode of the patch-clamp amplifier (zero-current potential). We recorded the resting potential with external solutions containing different K⁺ concentrations (the sum of Na⁺ and K⁺ was kept constant). Fig. 1A shows that the resting potential followed the external K⁺ concentration. For a 10-fold change in [K⁺]_{out}, the resting potential changed by

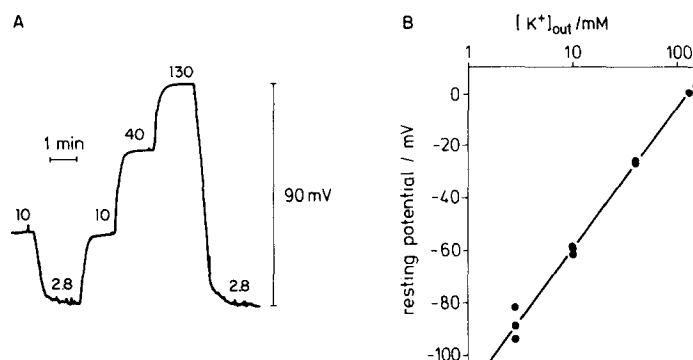


FIGURE 1. Influence of the external K⁺ concentration on the resting potential of RBL-2H3 cells. (A) Recording of the resting potential in a perfusion experiment. The numbers refer to the extracellular K⁺ concentrations in millimolar. (B) Dependence of the resting potential on the external K⁺ concentration. At [K⁺]_{out} = 130 mM, the measured resting potential was always 0 ± 5 mV. This potential was taken to be exactly zero in each experiment by shifting the data points along the y-axis.

54 ± 2 mV in this experiment. In four experiments, slopes between 52 and 58 mV/decade were obtained (Fig. 1B). RBL-2H3 cells thus behave like an ideal K⁺ electrode. At the “normal” K⁺ gradient (130 mM inside, 2.8 mM outside), the typical resting potential was about -90 mV.

Whole-cell voltage clamp. Fig. 2, A–C, shows whole-cell currents evoked by pulses from a holding potential of -10 mV to different potentials between -150 and +80 mV. Strong inward rectification is obvious. At low external K⁺ concentrations (high Na⁺ concentration), inactivation of the membrane conductance occurred (Fig. 2, A and B). Elevation of the external K⁺ concentration by replacing Na⁺ increased the currents and decreased the amount of inactivation (Fig. 2, B and C). A rising phase in the millisecond time scale was observed at small current amplitudes with all concentrations.

Whole-cell current-voltage relationships at different K⁺ concentrations are shown in Fig. 2D. The currents measured at the end of 200-ms-long pulses were

used for this figure (steady state current). For $[K^+]_{out} = 2.8$ mM, the current 7 ms after the onset of the pulse is also shown. For convenience, we plotted the current at this fixed time instead of determining the actual peak current since both values are equal within $\sim 10\%$. Under these conditions (2.8 mM K^+ , 145 mM Na^+), the isochronal current I_7 was linearly dependent on voltage below E_K ; however, the steady state current displayed voltage-dependent inactivation. At all K^+ concentrations, a considerable conductance was observed at the resting potential. The conductance increased with hyperpolarization until inactivation

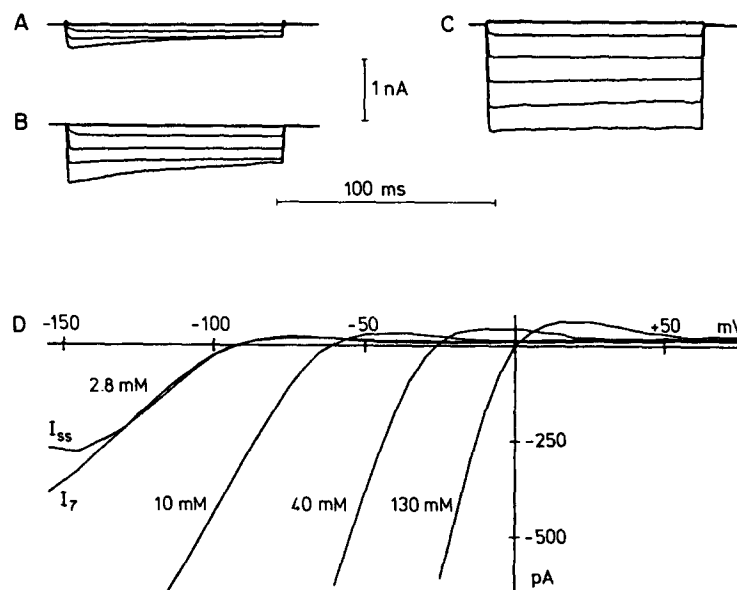


FIGURE 2. (A–C) Whole-cell currents at different K^+/Na^+ concentrations. The sum $[K^+]_{out} + [Na^+]_{out}$ was kept constant (148 mM). Pulses were given from a holding potential of -10 mV to different potentials between -150 and $+60$ mV in 10-mV increments. For clarity, only every third pulse is shown. $[K^+]_{out} = 10$ (A), 30 (B), and 100 (C) mM. (D) Current-voltage relationship at different Na^+/K^+ concentrations, measured in a cell with exceptionally high conductance owing to the anomalous rectifier. The steady state current (I_{ss}) was determined as the current at the end of 200-ms-long pulses. At $[K^+]_{out} = 2.8$ mM, isochronal currents 7 ms after the onset of the pulse (I_7) were also measured. The data points were taken every 5 mV and are connected by straight lines.

occurred. With depolarization, the conductance rapidly decreased so that the current-voltage relationship shows only a small "bump" in the outward direction. The same behavior has been observed for the inward rectifier in other cell types (Hagiwara et al., 1976; Sakmann and Trube, 1984a). The slope conductance evaluated from the peak currents at potentials at least 25 mV below E_K was ~ 5 nS at 2.8 mM external K^+ in the experiment of Fig. 2D. This limiting slope conductance was approximately proportional to the square root of the external K^+ concentration, in agreement with the results obtained for the anomalous

rectifier in other cells (Hagiwara and Takahashi, 1974; Sakmann and Trube, 1984a). The conductance at the resting potential of the cell was about half of this limiting value. The current observed at +80 mV corresponds to a nonspecific leakage of ~100 pS in the experiment shown in Fig. 2.

It has been shown that the inactivation of the anomalous rectifier at strong hyperpolarization is due to blockage by Na ions (Ohmori, 1978). Fig. 3A shows that the inactivation was nearly abolished when Na⁺ was replaced with *N*-methylglucamine, whereas the peak inward current remained essentially unchanged. The whole-cell conductance is efficiently blocked by Ba ions (Ohmori, 1980; Sakmann and Trube, 1984b). Fig. 3B shows the results of an experiment where the solution contained 100 mM K⁺, no Na⁺, and different Ba²⁺ concentrations. In the presence of Ba²⁺, the peak inward current was also nearly unaffected, whereas the blockage was much faster and more complete than in the case with Na⁺ (Fig. 3A). The blockage during a pulse to -150 mV at 100 μM Ba²⁺ was

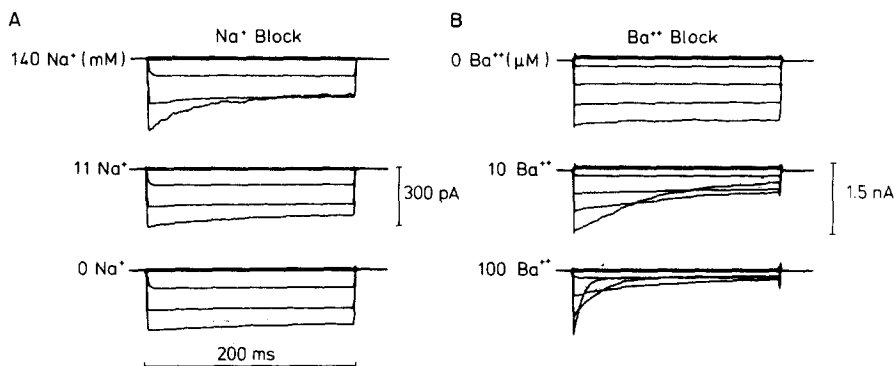


FIGURE 3. Voltage-dependent blocking of the anomalous rectifier by Na⁺ and Ba²⁺. The holding potential was +20 mV at 100 μM Ba²⁺ and -10 mV in all other cases. Pulses were given to potentials between -150 and +60 mV. (A) [K⁺]_{out} = 10 mM; [Na⁺]_{out} as indicated. Na was replaced by *N*-methylglucamine. (B) [K⁺]_{out} = 100 mM, no Na⁺; Ba²⁺ concentrations as indicated.

~10 times faster than at 10 μM Ba²⁺, which would be in agreement with a single binding site. To attain the full peak amplitude of the current at 100 μM Ba²⁺, it was necessary to hold the cell at +20 mV, which indicates that at -10 mV the channels were already partly blocked. In the presence of 1 mM Ba²⁺, the current-voltage relationship (steady state current) was linear, corresponding to a nonspecific leakage of ~70 pS (data not shown). When Ba²⁺ was added, the cell's resting potential usually collapsed to values close to zero and showed large fluctuations. The effect of Ba²⁺ was reversible.

Outside-out patches. Single channel events corresponding to the anomalous rectifier were observed in outside-out patches. A patch usually contained two to four channels. Fig. 4 shows recordings from a patch containing at least three channels with a symmetric K⁺ concentration of 130 mM. Pulses of 2 s duration were given from a holding potential of -10 mV. At -150 mV, the channels were mostly in the open state, interrupted by short closures. As in the case of

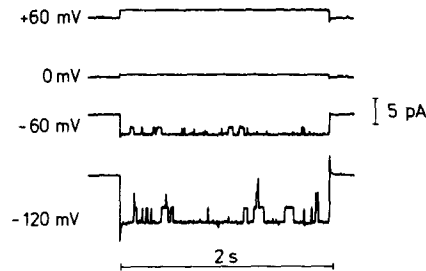


FIGURE 4. Single channel events of the anomalous rectifier recorded from an outside-out patch obtained from an RBL-2H3 cell membrane. $[K^+]_{out} = 130$ mM. Pulses were given from a holding potential of -10 mV to the indicated potentials.

the macroscopic currents (Figs. 1–3), single channel events were observed in the inward direction only. The blockage by Ba ions was also observed at the single channel level. 1 mM Ba^{2+} reversibly blocked all the inward rectifying channels of the type seen in Fig. 4.

This channel was thus identified as the channel responsible for the inward rectifying conductance observed in the whole-cell configuration. Single channel amplitudes were measured at different potentials and at different K^+ concentrations. The results are shown in Fig. 5A. The extrapolated reversal of the single channel current was always close to E_K . For a 10-fold shift in $[K^+]_{out}$, the extrapolated single channel reversal changed by ~ 46 mV, which is very similar to the corresponding value obtained for the anomalous rectifier of guinea pig heart cells (Sakmann and Trube, 1984a). We determined a slope conductance of 26 pS at 130 mM symmetric K^+ . As shown in Fig. 5B, the dependence of the single channel conductance (γ) on the external K^+ concentration can be described by the relation

$$\gamma = A \cdot [K^+]_{out}^{0.56},$$

with $A = 1.7$ pS/M $^{0.56}$. This relationship, and the absolute conductance values,

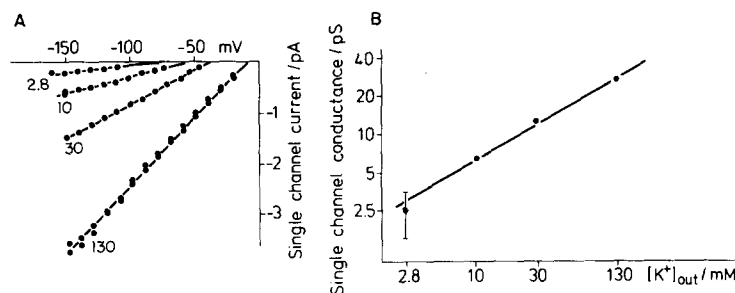


FIGURE 5. (A) Single channel current-voltage relationships from outside-out patches at different extracellular K^+ concentrations. The numbers refer to $[K^+]_{out}$ in millimolar. External NaCl was partially replaced by KCl to increase the K^+ concentration. (B) Dependence of the single channel conductance on the external K^+ concentration in a double-logarithmic plot. The straight line has a slope of 0.56 .

are in excellent agreement with those reported for the anomalous rectifier in guinea pig heart cells (Sakmann and Trube, 1984a).

Effect of stimulation. The effect of stimulation through the IgE system was investigated in whole-cell experiments. The current-to-voltage relationships of RBL-2H3 cells sensitized with IGE were identical to those of nonsensitized cells. In one experiment, the whole-cell configuration was formed with a nonsensitized cell and IgE was added (final concentration, $\sim 1 \mu\text{g/ml}$). The current-voltage relationship did not change during 40 min after the addition of IgE.

Experiments were carried out in which DNP₄₃-BSA was added to passively sensitized cells. The external K⁺ concentration was 2.8 or 5 mM (near normal). In most cases, we did not observe a change of the resting potential after stimulation. In some experiments, however, the resting potential decreased by 10–20 mV, but this was usually observed with cells that also showed fluctuations of the resting potential before stimulation. From the current-voltage relationship of Fig. 2D, it is obvious that at low external K⁺ concentrations, a small nonspecific leakage leads to a marked depolarization of the cell.

The question of whether ligand-activated Ca channels are being opened by antigenic stimulation was investigated in voltage-clamp experiments. We used a holding potential of +5 mV, because peak currents occur at about this voltage in other preparations and possible changes in the seal resistance would only produce small current changes. If cross-linking of the F_c receptors would lead to the opening of voltage-independent Ca channels, as recently proposed (Mazurek et al., 1984), pronounced inward currents should be observed because of the large Ca gradient (2 mM outside, 10 nM inside). Under these conditions, only a small outward current was observed in the unstimulated state. The fluctuation level of the whole-cell current was ~ 1 pA at 20 Hz, and no inward current was observed within 30 min after the addition of DNP₄₃-BSA. Taken together, our experiments suggest that the membrane conductance of RBL-2H3 cells is not changed by cross-linking the IgE receptors.

We successfully tested a similar procedure of antigenic stimulation with a 5HT release assay (see Materials and Methods). In the patch-clamp experiments, however, we had no clear indication of whether the dialyzed cell responded to the DNP₄₃ stimulus, because the degranulation of RBL-2H3 cells was not accompanied by a clearly observable morphological change. Under the microscope, only minor changes of the cell membrane appearance were observed after stimulation. The fusion of the secretory granules should lead to an increase of the cell capacitance (Neher and Marty, 1982; Fernandez et al., 1984b). However, no significant change of the capacitance was observed after stimulation in the whole-cell configuration.

Rat Peritoneal Mast Cells

In contrast to RBL-2H3 cells, rat peritoneal mast cells respond to antigenic stimulation with a pronounced and rapid morphological transformation caused by the fusion of a large number of secretory granules with the plasma membrane. This phenomenon is easily observable under the microscope with Nomarski optics and indicates successful stimulation (Lindau and Fernandez, 1986).

Nonstimulated Cells. We were not able to record a stable negative resting potential in the whole-cell configuration. The zero-current potential usually showed slow fluctuations between 0 and -30 mV. This behavior is explained by the current-voltage relationship shown in Fig. 6A. The slope conductance of the whole cell between -100 and $+20$ mV was only 20 pS and seemed to be rather nonspecific. In the whole-cell configuration, the opening and closing of single channels was sometimes observed, and the opening of a selective channel would cause a slow change of the resting potential toward the new equilibrium potential. Because of the small number of these channels, however, their properties could not be investigated in detail.

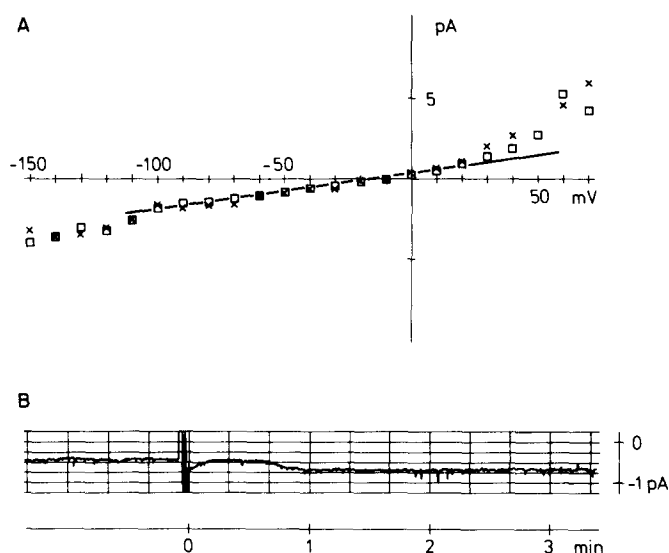


FIGURE 6. (A) Current-voltage relationship of an IgE-sensitized mast cell before (\times) and after (\square) stimulation. 200-ms-long pulses were given from -10 mV to the different potentials. The current was constant during the pulse and the currents plotted here are the average of the data points sampled during the pulse interval. (B) Effect of stimulation on the whole-cell current at -40 mV holding potential. DNP₄₅-BSA was added at time zero.

When the Ca concentration in the pipette was increased to ~ 1 μ M, sometimes the conductance increased in a stepwise manner within ~ 1 min. This conductance was due to the activation of ion channels. When the pipette was withdrawn from the cell, these channels could be investigated in an outside-out patch. The kinetics of opening and closing were slow (Fig. 7A) and appeared to be independent of the membrane potential. Fig. 7B shows that the current-voltage relationship was linear and the reversal was close to 0 mV with our standard solutions, which contained K-glutamate as the main ions on the intracellular side and NaCl as the main ions on the extracellular side of the membrane. The unit conductance was ~ 30 pS. From these data, the channel can be classified as a Ca-activated, nonselective cation channel, as observed in a number of other cell types

(Colquhoun et al., 1981; Yellen, 1982; Maruyama and Petersen, 1982; Maruyama et al., 1985).

In experiments where the pipette was filled with a modified internal solution containing 2 mM CaCl_2 and no EGTA, the whole-cell conductance increased dramatically. The withdrawal of the pipette resulted in the formation of outside-out patches containing up to four channels of large unitary conductance. Under

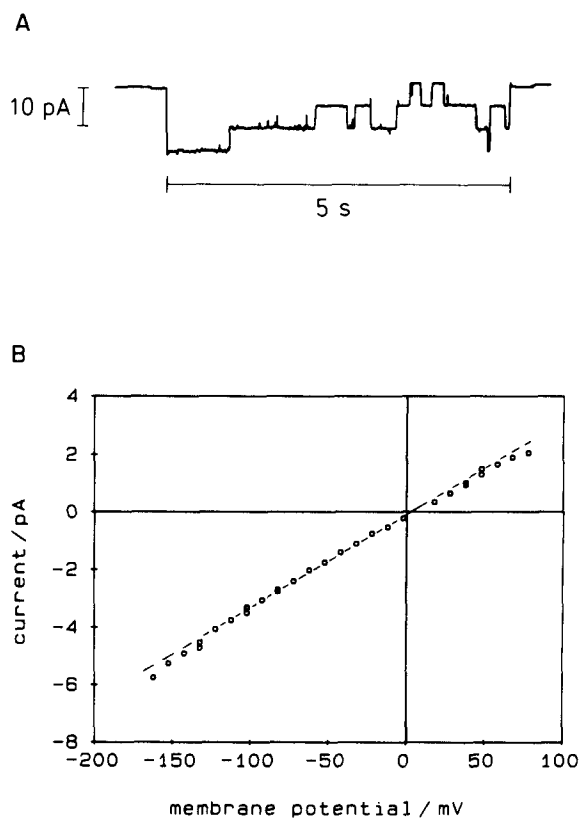


FIGURE 7. (A) Single channel recording of Ca-activated cation channels from an outside-out patch obtained from a rat peritoneal mast cell. A 5-s-long pulse to -160 mV was given from a holding potential of -10 mV. Standard external solution; the internal solution contained 0.85 mM CaCl_2 and 1 mM EGTA. (B) Single channel current-voltage relationship obtained from the same patch. The voltage was stepped from -10 mV to the different potentials for 5 s.

these conditions, where glutamate was the main anion on the intracellular side of the membrane, the large single channel events were observed only in the outward direction, which suggests that the channel is anion selective. The slope conductance between $+20$ and $+100$ mV was ~ 350 pS, with an extrapolated reversal of 0 mV. A similar behavior was observed for an anion-selective channel with large unitary conductance in cultured rat Schwann cells (Gray et al., 1984). The channel was also activated when the cell was dialyzed with external saline

and in inside-out patches when the cytoplasmic face of the membrane was exposed to the external solution. After activation, it appeared to be independent of the Ca concentration, because changing the bath solution to an external saline with $\sim 10^{-8}$ M free $[\text{Ca}^{2+}]$ did not affect the channel significantly over a period of 15 min.

Fig. 8A illustrates the effect of voltage on this channel. Pulses of 2 s duration

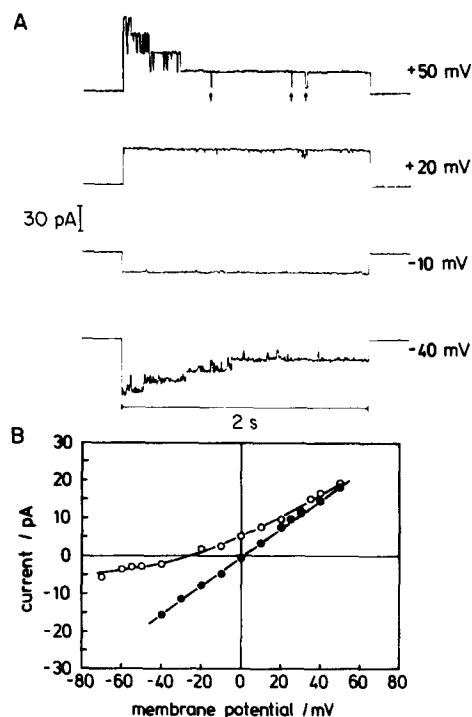


FIGURE 8. (A) Single channel recordings of large anion channels from an outside-out patch obtained from a rat peritoneal mast cell. Standard external solution was used on both sides of the membrane. 2-s voltage pulses were given from 0 mV to the indicated potentials. At +50 mV, four channels are open at the onset of the pulse. All channels are closed at the arrows. (B) Single channel current-voltage relationships under symmetrical and nonsymmetrical conditions. Solid symbols: standard external solution on both sides of the membrane; data points are averages from five patches. Open symbols: standard external solution on the extracellular side and fivefold-diluted external solution on the cytoplasmic side. Data points are averages from three inside-out patches.

were given from 0 mV to the indicated potentials. When the pulse potential was close to 0 mV (+20 and -10 mV in Fig. 8A), the channels stayed open most of the time. When the voltage was stepped to -40 or +50 mV, the channels closed. The upper trace in Fig. 8A shows that only a very small leakage current was left when all channels were closed (arrows). A similar bell-shaped voltage dependence has recently been reported for anion channels of large unitary conductance in

other cell types (Blatz and Magleby, 1983; Schwarze and Kolb, 1984; Gray et al., 1984). At negative potentials, the transitions between the open and closed state are less regular and the channel appears to adopt substates of reduced conductance (-40 mV trace in Fig. 8A). This phenomenon has not been investigated. The existence of conductance sublevels has also been described for this channel type in the other preparations.

The single channel conductance and the selectivity have been investigated using inside-out and outside-out patches. Fig. 8B shows the current-voltage relationship under symmetrical and nonsymmetrical conditions. When external saline was used on both sides of the membrane, the current-voltage relationship was linear and the reversal was close to 0 mV. The data points in the case of symmetric solutions are averages from five patches. We obtained a slope conductance of 378 ± 4 pS and a zero-current potential of $+1.2 \pm -0.3$ mV. When the solution on the extracellular side was diluted fivefold, the reversal shifted in the negative direction by ~ 25 mV. Outward rectification was observed under these conditions, as expected from constant field theory (Cooper et al., 1985). For a perfectly anion-selective channel, a shift of 41 mV is calculated if activity coefficients are neglected. The smaller shift indicates that Cl^- is only slightly more permeable than Na^+ . From our data, we obtained a permeability ratio $P_{\text{Cl}}/P_{\text{Na}} = 5 \pm 2$ ($n = 4$).

Effect of stimulation. Mast cells sensitized with IgE showed the same conductance properties as nonsensitized cells. The data shown in Fig. 6A are derived from a sensitized cell. The effect of stimulation was studied in voltage-clamp experiments. At a holding potential of -40 mV, stimulation of the cell did not induce a significant inward current, as shown in Fig. 6B. If there was any stimulation-induced inward current, it was ≤ 0.25 pA (Fig. 6B). As shown in Fig. 6A, the current-voltage relations of the cell remained unchanged. Similar results were obtained in six experiments.

The addition of DNP_{43} -BSA to sensitized mast cells led to degranulation of most cells as observed under the microscope. The cell dialyzed with the patch pipette, however, did not degranulate (Lindau and Fernandez, 1986). Considering that all the cells around the dialyzed cell were degranulating, it is most likely that stimulation with DNP_{43} -BSA effectively cross-linked the F_c receptors on all the cells. In the whole-cell configuration, however, an essential intracellular component mediating stimulus-secretion coupling was probably washed out, preventing degranulation (Fernandez et al., 1984b; Lindau and Fernandez, 1986).

DISCUSSION

The Anomalous Rectifier of RBL-2H3 Cells

We have shown that the only significant conductance of RBL-2H3 cells is attributable to a K^+ -selective, inwardly rectifying channel. Recently, the existence of the anomalous rectifier in these cells has independently been reported in abstract form (Ikeda and Weight, 1984). The conductance depends on the electrochemical K^+ potential rather than on the membrane voltage. Under conditions where this conductance was blocked or closed, or if K^+ was removed

on the outside, only a nonspecific leakage of ~ 100 pS was observed in the whole cell.

Similar anomalous rectifiers have been studied previously, mainly in eggs of starfish and tunicates (Hagiwara and Takahashi, 1974; Hagiwara et al., 1978; Ohmori, 1978, 1980; Fukushima, 1982), frog skeletal muscle fibers (Standen and Stanfield, 1978, 1979; Leech and Stanfield, 1981), and guinea pig heart cells (Sakmann and Trube, 1984*a, b*). The properties of the channel in RBL-2H3 cells are very similar to those reported for the inwardly rectifying K^+ current in the other cell types.

The channel was blocked by Na^+ in a voltage-dependent manner, as observed in tunicate eggs (Ohmori, 1978, 1980; Fukushima, 1982) and frog skeletal muscle (Standen and Stanfield, 1979). It was efficiently blocked by Ba^{2+} in micromolar concentrations. The blockage with Ba^{2+} was also voltage dependent. This has been described for eggs of starfish (Hagiwara et al., 1978) and tunicate (Ohmori, 1980), frog skeletal muscle (Standen and Stanfield, 1978), guinea pig atrial cardiac myoballs (Bechem et al., 1983), and guinea pig heart cells (Sakmann and Trube, 1984*a*).

The macroscopic conductance was roughly proportional to the square root of the external K^+ concentration. This empirical square root dependence has also been found for the anomalous rectifier of egg cells (Hagiwara and Takahashi, 1974) and of guinea pig heart cells (Sakmann and Trube, 1984*a*). The square root dependence was also observed for the single channel conductance in the RBL-2H3 cells, as in eggs (Ohmori, 1978; Fukushima, 1982) and guinea pig heart cells (Sakmann and Trube, 1984*a*).

The absolute single channel conductance at 100 mM external K^+ and room temperature can be estimated to be 7–15 pS in eggs (Ohmori, 1978; Fukushima, 1982) and frog skeletal muscle (Schwartz et al., 1981). These values are somewhat lower than the conductance of ~ 22 pS that we obtained under these conditions for RBL-2H3 cells. The single channel conductance values found for the inwardly rectifying K^+ channel in guinea pig heart cells (Sakmann and Trube, 1984*a*) are identical to our results at all K^+ concentrations, which indicates that the same channel may be present in both cell types.

The Resting Potential of RBL-2H3 Cells

We have shown that the resting potential of RBL-2H3 cells followed the K^+ equilibrium potential caused by the high conductance of the anomalous rectifier at E_K . However, at low K^+ concentrations (<10 mM), the resting potential became very unstable in some cells. From Fig. 2*D*, it can be estimated that at $[K^+]_{out} = 2.8$ mM, a nonspecific increase of the seal or membrane conductance by 100–200 pS would depolarize the cell completely, and in many cells the whole-cell conductance of the anomalous rectifier is even smaller than that shown in Fig. 2*D*. If a certain nonspecific leakage conductance is added to the anomalous rectifier, a current-voltage relationship can be obtained that shows zero current at two different potentials. In fact, we observed spontaneous switching between two states of the resting potential in some cells. In this case, one state was close to E_K and the other was close to zero. In an intact cell, however, the anomalous

rectifier will determine the resting potential to be equal to the K^+ equilibrium potential.

The resting potential and its change as a consequence of stimulation have previously been studied using the lipophilic ion tetraphenylphosphonium (Kanner and Metzger, 1983; Sagi-Eisenberg and Pecht, 1984). Kanner and Metzger arrived at a resting potential of -90 mV, which changed after stimulation to -70 mV, whereas Sagi-Eisenberg and Pecht calculated a resting potential of about -60 mV and ascribed the observed changes to secondary effects across the mitochondrial membrane. In most experiments, we observed no change of the resting potential after stimulation, but in a few cells, depolarization by 10–20 mV was observed. In one of these cells, the conductance of the anomalous rectifier decreased slightly after stimulation, whereas in another cell the anomalous rectifier was not changed, but some additional nonspecific leakage was observed. From the current-voltage relationship shown in Fig. 2D, it can be estimated that at $[K^+]_{out} = 2.8$ mM, a whole-cell inward current of 10–20 pA would depolarize a cell completely. In contrast to these results, stimulation of chromaffin cells with acetylcholine leads to the generation of action potentials and to Ca currents of several hundred picoamperes (Fenwick et al., 1982a, b).

Conductance and Resting Potential of Mast Cells

Rat peritoneal mast cells appeared to be strikingly different from RBL-2H3 cells. In mast cells, no inwardly rectifying currents were observed, even in experiments where we used the same internal solution as for RBL-2H3 cells. Mast cells had only a very small whole-cell conductance, which was usually as low as 10–30 pS.

The resting potential normally fluctuated between 0 and about -30 mV. Large fluctuations of the resting potential have previously been observed in chromaffin cells, which have an input resistance of 5–10 G Ω , and such fluctuations have been predicted to be a general phenomenon displayed by cells with a small resting conductance (Fenwick et al., 1982a). The whole-cell conductance that we observed in mast cells corresponds to an input resistance of 30–100 G Ω , which is comparable to typical values reported for the resistance of the pipette-membrane seal (Hamill et al., 1981). If the observed whole-cell conductance of mast cells was due mainly to the imperfect seal, no ion channels of significant conductance were open in the cell membrane. In such a cell, the zero-current potential is determined by other electrogenic ion translocating systems, such as the Ca/Na exchanger or the Na/K pump. Such ion movements could contribute inward and outward currents, generating a rather small net current. This current could be short-circuited by the imperfect seal, leading to a zero-current potential close to 0 mV. The unit conductance of many channel types is of the same order of magnitude as the measured whole-cell conductance. The opening of one single channel would cause a change of the zero-current potential to a new value, determined by the selectivity of the channel and by the ratio of the channel conductance to the leakage conductance. The potential should change with the membrane time constant, which is in the range of hundreds of milliseconds in mast cells. If the channel opening is shorter than this time constant, the new equilibrium value will not be reached, and after a small hyperpolarization the

cell should return to 0 mV. We have observed this behavior in whole-cell current-clamp experiments. Accordingly, single channel openings were sometimes observed in whole-cell voltage-clamp recordings.

We cannot exclude a resting potential caused by electrogenic ion pumps, which might have been short-circuited by the leakage conductance of the imperfect seal. However, in this case, very small currents must be involved. An outward current of 1 pA would lead to a measured resting potential of -50 mV in a $50\text{-G}\Omega$ cell. If the true resting potential of mast cells has been shunted by the leak in our experiments, then the current had to be significantly smaller than 1 pA.

The electrogenic Na/K pump in isolated heart cells has been demonstrated to generate a current of $\sim 1\ \mu\text{A}/\text{cm}^2$ at 0 mV under conditions of elevated intracellular Na^+ and ATP concentrations and a low internal K^+ concentration (Gadsby et al., 1985). The membrane area of these cells has been estimated from capacitance measurements. The capacitance of a rat peritoneal mast cell is typically 5–10 pF (Lindau and Fernandez, 1986). If the same current density is assumed, a current of 5–10 pA would thus be expected at 0 mV in mast cells under the same conditions. The Na/K pump current in heart cells was not detectable at 1 mM internal Na^+ (Gadsby et al., 1985). Under our standard conditions (100 μM ATP, ~ 3 mM Na^+), the pump current would be expected to be rather small and could have been below the limit of 1 pA. Further experiments are required to determine the existence and density of electrogenic ion pumps in the mast cell membrane and their possible involvement in generating a stable negative resting potential.

In a microelectrode study of rat mesentery mast cells, resting potentials between -6 and -24 mV, with an average of -14.3 mV, have been reported (Tasaka et al., 1970). From this report, it is not clear whether the resting potential was stable in these cells or whether spontaneous fluctuations were observed. Since the leakage conductance introduced by the microelectrode impalement is usually larger than the leakage conductance of the patch pipette seal, the resting potential recorded with a microelectrode would be expected to be strongly attenuated. This could be taken as evidence that certain ion channels or electrogenic pumps had been washed out or inactivated by our internal solution. However, in the slow whole-cell configuration (Lindau and Fernandez, 1986), where the patch is not disrupted but instead is permeabilized (patch resistance, 200–2,000 $\text{M}\Omega$), we also recorded a fluctuating resting potential. In this configuration, the cells are not fully dialyzed and remain responsive to external stimulation, which indicates that the cytoplasmic composition remains intact. Therefore, it is very unlikely that the unstable resting potential is an artifact caused by our artificial internal solution. The membrane conductance of mast cells was not changed by cross-linking the IgE receptors under conditions where degranulation occurred in the surrounding cells.

We observed two channel types in mast cells that are normally inactive. One type was activated in some cells if the cytoplasmic Ca concentration was elevated to $\sim 1\ \mu\text{M}$. This channel was cation selective and voltage independent and had a unitary conductance of ~ 30 pS. Very similar Ca-dependent cation channels have been observed previously in cultured cardiac cells (Colquhoun et al., 1981),

neuroblastoma (Yellen, 1982), pancreatic acinar cells (Maruyama and Petersen, 1982), and rat thyroid follicular cells (Maruyama et al., 1985). The pattern of opening and closing observed in these other cell types shows striking similarities to the channel we found in mast cells. We determined the single channel conductance to be 30 pS, which is similar to the 22–35 pS reported for this channel in the other cell types. It has been shown that the cytoplasmic Ca concentration sometimes increases transiently to the micromolar range after antigenic stimulation of mast cells (Neher and Almers, 1986). Using the novel slow-whole-cell mode of the patch-clamp technique, we have observed a transient conductance increase after stimulation in some cells (Lindau and Fernandez, 1986). The Ca-dependent channel reported here is a likely candidate to link the conductance transients to the Ca transients.

The other channel type is slightly selective for Cl^- over small cations and has a large unitary conductance of ~ 380 pS. Channels of this type have recently been observed in cultured rat muscle (Blatz and Magleby, 1983), cultured rat Schwann cells (Gray et al., 1984), macrophages and myotube membranes (Schwarze and Kolb, 1984), and frog rod plasma membrane (Kolesnikov et al., 1984). The single channel conductance of 380 pS is very similar to the values of 340–450 pS that were reported for this channel in other cell types when similar solutions were used (Blatz and Magleby, 1983; Schwarze and Kolb, 1984; Gray et al., 1984). The anion channel in the mast cell plasma membrane has a permeability ratio of $P_{\text{Cl}}/P_{\text{Na}} = 5$, which is in agreement with the results reported for the Cl channel in rat Schwann cells (Gray et al., 1984), macrophages, and myotubes (Schwarze and Kolb, 1984). The channel was usually open at potentials close to 0 mV and closed if the membrane was either hyperpolarized or depolarized. A similar bell-shaped voltage dependence was found for the large anion channels in the other cell types. We observed that the channel appeared when the cytoplasmic side of the membrane was exposed to a Ca concentration of ~ 1 mM, but after activation it was not affected by reducing the Ca concentration to 10^{-8} M. This result should be compared with the finding that the large anion channels in other cell types were not directly Ca dependent (Blatz and Magleby, 1983; Schwarze and Kolb, 1984; Gray et al., 1984). However, in the cell-attached configuration, the opening probability increased significantly after the addition of a Ca ionophore (Schwarze and Kolb, 1984).

The observed Ca-dependent cation-selective channel and the Ca-induced anion channel could be involved in the osmotic regulation of the cell, as recently suggested for Ehrlich ascites tumor cells (Hoffmann et al., 1984).

The Problem of the Missing Ca Channels

The entry of Ca into the cell after stimulation has generally been believed to be the crucial event leading to secretion in mast cells and RBL-2H3 cells (Ishizaka and Ishizaka, 1984). From experiments on 2H3 cells using ^{45}Ca and the Ca indicator quin-2, the Ca influx has been reported to require the presence of ATP (Beaven et al., 1984*b*). Recently, however, a cromoglycate binding protein has been isolated from RBL cell membranes and has been incorporated into lipid bilayers. This protein was found to form a Ca-selective channel if at least two proteins were linked together by a multivalent antibody or through the IgE

receptor system (Mazurek et al., 1984). No additional soluble or membrane-bound component was required. This Ca channel was found to be voltage independent and to have a single channel conductance of 2 pS. A 2H3 cell contains ~100,000 of these membrane proteins. The opening of only 200 of these channels should depolarize the cell completely under our experimental conditions. In the voltage-clamp experiments at +5 mV, no inward current was observed after cross-linking the F_c receptors, despite the fact that the Ca concentration was 2 mM outside and 10 nM inside. In these experiments, the opening of only 30 of these voltage-independent Ca channels should generate an inward current of 10 pA, which is one order of magnitude above our detection limit. The cell dialyzed with the patch pipette is probably not secreting in response to antigenic stimulation caused by the washout of soluble components. However, it is unlikely that such soluble components are required for cross-linking the F_c receptors, as this process is caused by binding steps on the extracellular side of the plasma membrane. In contrast to previous expectations, our data do not support the presence of ligand-activated ion channels in the native RBL-2H3 membrane.

Our recent data on intact, degranulating mast cells have shown that they can degranulate in response to antigenic stimulation without a detectable increase in the membrane conductance (Lindau and Fernandez, 1986). Any undetected conductance increase had to be <150 pS (Lindau and Fernandez, 1986). The data on fully dialyzed cells presented here confirm and extend these results. The stimulation-induced inward current was ≤ 0.25 pA under conditions where all the surrounding cells did degranulate, which further supports the arguments against a direct role of plasma membrane Ca channels. This conclusion is also supported by experiments showing that antigenic stimulation within 1 min after removing extracellular Ca^{2+} led to normal degranulation (Lindau and Fernandez, 1986) and to large intracellular Ca^{2+} transients (White et al., 1984; Neher and Almers, 1986). We cannot exclude, however, that under certain conditions, stimulation leads to a small Ca influx by a Ca-translocating mechanism different from an ionic channel, for many reports suggest that the elevation of intracellular Ca^{2+} involves the biochemical machinery of the cell (Cockcroft and Gomperts, 1979; Morita and Siraganian, 1981; Beaven et al., 1984*a, b*; Berridge and Irvine, 1984; Sagi-Eisenberg et al., 1985).

The universality of Ca as the main second messenger has been recently questioned in a number of preparations, as in the case of photoreceptors (Kaupp and Koch, 1986). In mast cells, the elevation of intracellular Ca has been shown to be neither sufficient (Fernandez et al., 1984*b*) nor necessary for secretion to occur (Neher and Almers, 1986). In view of these considerations, ligand-activated Ca channels have lost their original appeal as an elegant explanation for the mechanism of antigenic stimulation. Moreover, growing amounts of data appear to disprove the Ca hypothesis of stimulus-secretion coupling in histamine-secreting cells.

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REFERENCES

- Beaven, M. A., J. P. Moore, G. A. Smith, T. R. Hesketh, and J. C. Metcalfe. 1984a. The calcium signal and phosphatidylinositol breakdown in 2H3 cells. *Journal of Biological Chemistry*. 259:7137-7142.
- Beaven, M. A., J. Rogers, J. P. Moore, T. R. Hesketh, G. A. Smith, and J. C. Metcalfe. 1984b. The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. *Journal of Biological Chemistry*. 259:7129-7136.
- Bechem, M., H. G. Glitsch, and L. Pott. 1983. Properties of an inward rectifying K channel in the membrane of guinea-pig atrial cardioballs. *Pflügers Archiv*. 399:186-193.
- Berridge, M. J., and R. F. Irvine. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. 312:315-321.
- Blatz, A.L., and K. L. Magleby. 1983. Single voltage-dependent chloride-selective channels of large conductance in cultured rat muscle. *Biophysical Journal*. 43:237-241.
- Cockroft, S., and B. D. Gomperts. 1979. Evidence for a role of phosphatidylinositol turnover in stimulus-secretion coupling. *Biochemical Journal*. 178:681-687.
- Colquhoun, D., E. Neher, H. Reuter, and C. F. Stevens. 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature*. 294:752-754.
- Cooper, K., E. Jakobsson, and P. Wolynes. 1985. The theory of ion transport through membrane channels. *Progress in Biophysics and Molecular Biology*. 46:51-96.
- Crews, F. T., Y. Morita, A. McGivney, F. Hirata, R. P. Siraganian, and J. Axelrod. 1981. IgE-mediated histamine release in rat basophilic leukemia cells: receptor activation, phospholipid methylation, Ca²⁺ flux, and release of arachidonic acid. *Archives of Biochemistry and Biophysics*. 212:561-571.
- Fenwick, E. M., A. Marty, and E. Neher. 1982a. A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *Journal of Physiology*. 331:577-597.
- Fenwick, E. M., A. Marty, and E. Neher. 1982b. Sodium and calcium channels in bovine chromaffin cells. *Journal of Physiology*. 331:599-635.
- Fernandez, J. M., A. P. Fox, and S. Krasne. 1984a. Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH₃) cells. *Journal of Physiology*. 356:565-585.
- Fernandez, J. M., E. Neher, and B. D. Gomperts. 1984b. Capacitance measurements reveal stepwise fusion events in degranulating mast cells. *Nature*. 312:453-455.
- Foreman, J. C., and J. L. Mongar. 1975. Calcium and the control of histamine secretion from mast cells. In *Calcium Transport in Contraction and Secretion*. E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, editors. Elsevier/North-Holland, Amsterdam. 175-184.
- Fukushima, Y. 1982. Blocking kinetics of the anomalous potassium rectifier of tunicate egg studied by single channel recording. *Journal of Physiology*. 331:311-331.
- Gadsby, D. C., J. Kimura, and A. Noma. 1985. Voltage dependence of Na/K pump current in isolated heart cells. *Nature*. 315:63-65.
- Gray, P. T. A., S. Bevan, and J. M. Ritchie. 1984. High conductance anion-selective channels

- in rat cultured Schwann cells. *Proceedings of the Royal Society of London B Biological Sciences*. 221:395-409.
- Hagiwara, S., S. Miyazaki, W. Moody, and J. Patlak. 1978. Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *Journal of Physiology*. 279:167-185.
- Hagiwara, S., S. Miyazaki, and N. P. Rosenthal. 1976. Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *Journal of General Physiology*. 67:621-638.
- Hagiwara, S., and H. Ohmori. 1982. Studies of calcium channels in rat clonal pituitary cells with patch electrode voltage clamp. *Journal of Physiology*. 331:231-252.
- Hagiwara, S., and K. Takahashi. 1974. The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *Journal of Membrane Biology*. 18:61-80.
- Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv*. 391:85-100.
- Hoffmann, E. K., L. O. Simonsen, and I. H. Lambert. 1984. Volume-induced increase of K⁺ and Cl⁻ permeabilities in Ehrlich ascites tumor cells. Role of internal Ca²⁺. *Journal of Membrane Biology*. 78:211-222.
- Ikeda, S. R., and F. F. Weight. 1984. Inward rectifying potassium currents recorded from rat basophilic leukemia cells by whole-cell patch-clamp. *Society for Neuroscience Abstracts*. 10:870.
- Ishizaka, T., and K. Ishizaka. 1984. Activation of mast cells for mediator release through IgE receptors. *Progress in Allergy*. 34:188-235.
- Kanner, B. I., and H. Metzger. 1983. Crosslinking of the receptors for immunoglobulin E depolarizes the plasma membrane of rat basophilic leukemia cells. *Proceedings of the National Academy of Sciences*. 80:5744-5748.
- Katz, B., and R. Miledi. 1967. A study of synaptic transmission in the absence of nerve impulses. *Journal of Physiology*. 192:407-436.
- Kaupp, U. B., and K.-W. Koch. 1986. Mechanism of photoreception in vertebrate vision. *Trends in Biochemical Sciences*. 11:43-47.
- Kolesnikov, S. S., A. L. Lyubarsky, and E. E. Fesenko. 1984. Single anion channels of frog rod plasma membrane. *Vision Research*. 24:1295-1300.
- Leech, C. A., and P. R. Stanfield. 1981. Inward rectification in frog skeletal muscle fibres and its dependence on membrane potential and external potassium. *Journal of Physiology*. 319:295-309.
- Lindau, M., and J. M. Fernandez. 1986. IgE-mediated degranulation of mast cells does not require the opening of ion channels. *Nature*. 319:150-153.
- Llinas, R. R. 1984. The squid giant synapse. *Current Topics in Membranes and Transport*. 22:519-546.
- Maruyama, Y., D. Moore, and O. H. Petersen. 1985. Calcium-activated cation channel in rat thyroid follicular cells. *Biochimica et Biophysica Acta*. 821:229-232.
- Maruyama, Y., and O. H. Petersen. 1982. Single-channel currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature*. 299:159-161.
- Mazurek, N., H. Schindler, T. H. Schuerholz, and I. Pecht. 1984. The cromolyn binding protein constitutes the Ca channel of basophils opening upon immunological stimulus. *Proceedings of the National Academy of Sciences*. 81:6841-6845.
- Moore, J. P., A. Johannsson, T. R. Hesketh, G. A. Smith, and J. C. Metcalfe. 1984. Calcium signals and phospholipid methylation in eukaryotic cells. *Biochemical Journal*. 221:675-684.

- Morita, Y., and R. P. Siraganian. 1981. Inhibition of IgE-mediated histamine release from rat basophilic leukemia cells and rat mast cells by inhibitors of transmethylation. *Journal of Immunology*. 127:1339–1344.
- Neher, E., and W. Almers. 1986. Fast calcium transients in rat peritoneal mast cells are not sufficient to trigger exocytosis. *EMBO (European Molecular Biology Organization) Journal*. 5:51–53.
- Neher, E., and A. Marty. 1982. Discrete changes of cell membrane capacitance observed under conditions of enhanced secretion in bovine adrenal chromaffin cells. *Proceedings of the National Academy of Sciences*. 79:6712–6716.
- Ohmori, H. 1978. Inactivation kinetics and steady-state current noise in the anomalous rectifier of tunicate egg cell membranes. *Journal of Physiology*. 281:77–99.
- Ohmori, H. 1980. Dual effects of K ions upon the inactivation of the anomalous rectifier of the tunicate egg cell membrane. *Journal of Membrane Biology*. 53:143–156.
- Sagi-Eisenberg, R., H. Lieman, and I. Pecht. 1985. Protein kinase C regulation of the receptor-coupled calcium signal in histamine-secreting rat basophilic leukemia cells. *Nature*. 313:59–60.
- Sagi-Eisenberg, R., and I. Pecht. 1984. Resolution of cellular compartments involved in membrane potential changes accompanying IgE-mediated degranulation of rat basophilic leukemia cells. *EMBO (European Molecular Biology Organization) Journal*. 3:497–500.
- Sakmann, B., and G. Trube. 1984a. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *Journal of Physiology*. 347:641–657.
- Sakmann, B., and G. Trube. 1984b. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *Journal of Physiology*. 347:659–683.
- Schwartz, W., B. Neumcke, and P. T. Palade. 1981. K-current fluctuations in inward-rectifying channels of frog skeletal muscle. *Journal of Membrane Biology*. 63:85–92.
- Schwarze, W., and H.-A. Kolb. 1984. Voltage-dependent kinetics of an anionic channel of large unit conductance in macrophages and myotube membranes. *Pflügers Archiv*. 402:281–291.
- Standen, N. B., and P. R. Stanfield. 1978. A potential- and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. *Journal of Physiology*. 280:169–191.
- Standen, N. B., and P. R. Stanfield. 1979. Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *Journal of Physiology*. 294:497–520.
- Tasaka, K., K. Sugiyama, S. Komoto, and H. Yamasaki. 1970. Dissociation of degranulation and depolarization of the rat mesentery mast cell exposed to compound 48/80 and ATP. *Proceedings of the Japan Academy*. 46:826–830.
- White, J. R., T. Ishizaka, K. Ishizaka, and R. I. Sha'afi. 1984. Direct demonstration of increased intracellular concentration of free calcium as measured by quin-2 in stimulated rat peritoneal mast cell. *Proceedings of the National Academy of Sciences*. 81:3978–3982.
- Yellen, G. 1982. Single Ca^{2+} -activated nonselective cation channels in neuroblastoma. *Nature*. 296:357–359.