Ion Channels and Calcium Signaling in Mast Cells[a]

MARKUS HOTH, CRISTINA FASOLATO, AND REINHOLD PENNER

Department of Membrane Biophysics
Max-Planck-Institute for Biophysical Chemistry
Am Fassberg
D-3400 Göttingen, Germany

INTRODUCTION

In response to an external stimulus mast cell granules fuse with the plasma membrane and release substances like histamine, serotonin, and heparin, which can lead to different hypersensitivity reactions such as asthma and allergies.[1] In basophils and mast cells, secretion can be induced by oligomerization of specific IgE receptor by the corresponding antigen. Other physiological stimuli include substance P and tachykinins; mast cells are often clustered around neuropeptide-secreting nerve endings. Secretion can also be induced by non-physiological agents such as compound 48/80 or mastoparan, which are thought to bypass the receptor level and directly activate membrane G proteins.[2] The degranulation mediated by IgE has been reported not to require opening of ion channels,[3] but nevertheless, there is evidence that antigenic stimuli require extracellular calcium[1,4] and ion channels may be involved in stimulus-secretion coupling in mast cells.[5-8]

Calcium plays an important role in the stimulus-secretion coupling of neurons, exocrine and endocrine cells, and also in cells of the immune system. Changes in the intracellular calcium concentration, $[Ca^{2+}]_i$, in response to receptor stimulation usually show a biphasic behavior: an initial Ca^{2+} spike followed by a sustained plateau phase. The former is mainly caused by release of Ca^{2+} from internal stores in response to inositol 1,4,5-trisphosphate production ($InsP_3$), the latter is mainly due to Ca^{2+} entry across the plasma membrane. In mast cells, $[Ca^{2+}]_i$ is believed to have at least a modulatory effect on the signal transduction cascade that leads to secretion. It was shown that the transient increase in $[Ca^{2+}]_i$ is neither sufficient[6,9] nor necessary[6,10,11] to trigger exocytosis. However, a sustained increase in the basal calcium concentration enhances the rate of secretion when combined with an additional stimulus.[12-15] Recent studies

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Address correspondence to: Dr. Reinhold Penner, Department of Membrane Biophysics, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg, D-3400 Göttingen, Germany.

have revealed the mechanisms underlying calcium influx in mast cells. Similar mechanisms designed to maintain elevated plateaus of $[Ca^{2+}]_i$ might also be expressed in other cell types.

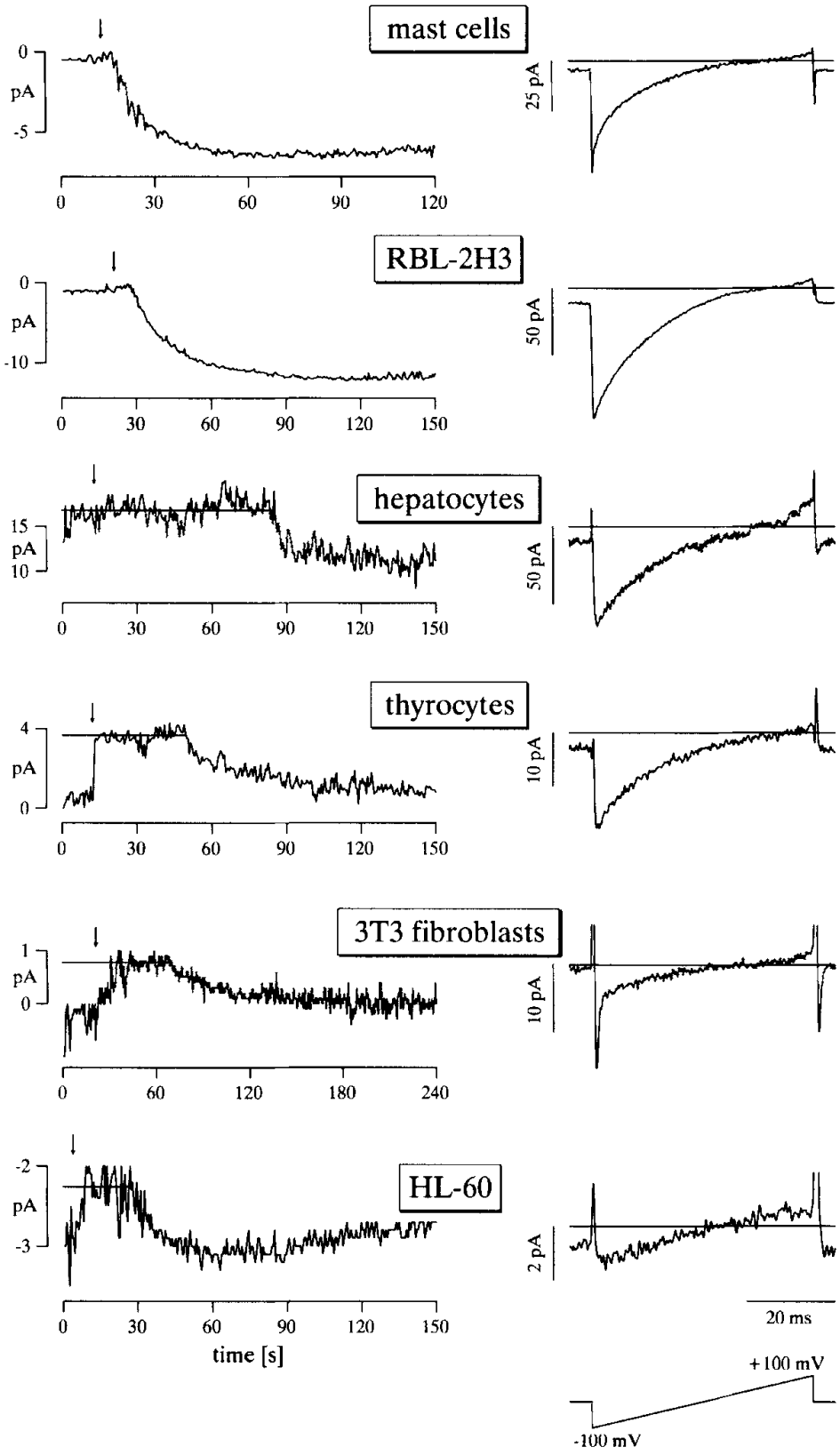
In mast cells and many other non-excitable cells, there appear to exist two main pathways for Ca^{2+} influx. One mechanism, known as "capacitative" Ca^{2+} entry,[16] is linked to the filling state of Ca^{2+} stores and, upon depletion of cellular Ca^{2+} pools, results in activation of a highly Ca^{2+}-selective current (I_{CRAC} = Calcium Release-Activated Calcium current).[17,18] Another mechanism is provided by nonspecific cation channels, which may be classified as receptor- or second messenger-activated channels.[19] Both Ca^{2+} influx mechanisms appear to be voltage-independent and would provide larger Ca^{2+} entry at hyperpolarizing membrane potentials. Hyperpolarization of the cells could be mediated by potassium or chloride channels, both of which are also found in mast cells.[5,20–22] Furthermore, these channels might also be involved in the degranulation process in a more direct way, as indicated by biochemical studies.[7,8]

CALCIUM RELEASE-ACTIVATED CALCIUM CURRENT

In rat peritoneal mast cells, depletion of internal Ca^{2+} stores activates a Ca^{2+} current.[17,18] This calcium release-activated calcium current (I_{CRAC}) can be activated by at least three different experimental procedures that all result in store depletion ($InsP_3$, ionomycin, Ca^{2+} chelators). Since these procedures share no apparent common mechanism, except for depleting Ca^{2+} stores, it seems unlikely that they involve a direct gating of the current by inositol phosphates or the released Ca^{2+}. This contrasts with previous findings in Jurkat cells[23] and recent work in lobster olfactory receptor neurons[24] where $InsP_3$ appears to activate Ca^{2+} permeable channels directly.

I_{CRAC} is a very selective Ca^{2+} influx pathway with a permeability ratio P_{Ca2+}/P_{M+} ($P_{calcium}/P_{monovalents}$) similar to that of voltage-operated Ca^{2+} channels.[18] Since I_{CRAC} is activated by depletion of Ca^{2+} stores and since it is highly selective for Ca^{2+} over monovalents, it is very likely that I_{CRAC} is the long-sought after Ca^{2+} current that is responsible for "capacitative" Ca^{2+} entry, which had been postulated to exist in a variety of nonexcitable cells. In fact, I_{CRAC} appears to be widely distributed, being found in almost all non-excitable cells that we have tested so far (FIG. 1), including rat peritoneal mast cells, RBL-2H3 (rat basophilic leukemia cells, a mucosal mast cell line), hepatocytes, dissociated thyrocytes, Swiss 3T3 fibroblasts, and HL-60 cells (a human leukemia cell line). Recent evidence suggests that a current with almost identical properties as I_{CRAC} is also present in MDCK cells (an epithelial cell line from kidney),[25] in Jurkat cells[26] (A. Zweifach and R. S. Lewis, personal communication), and in A431 cells (A. Lückhoff and D. Clapham, personal communication). Interestingly, the latter two happen to be cells in which a direct gating of cation channels by $InsP_3$ has also been reported. FIGURE 1 depicts I_{CRAC} in different cell types at 0 mV (left panel) and over the whole voltage range (right panel). In these examples, store depletion was achieved by internal perfusion of $InsP_3$.

In non-excitable cells, Ca^{2+} influx following store depletion is often studied using Mn^{2+} as a Ca^{2+} tracer, taking advantage of its ability to permeate through Ca^{2+} channels and to quench Fura-2 fluorescence.[27,28] We have recently found that I_{CRAC} conducts a small but measurable Mn^{2+} current.[29] In the presence of intracellular BAPTA [1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid], a Mn^{2+} current


mast cells
0
pA
-5
0 30 60 90 120
25 pA
RBL-2H3
0
pA
-10
0 30 60 90 120 150
50 pA
hepatocytes
15
pA
10
0 30 60 90 120 150
50 pA
thyrocytes
4
pA
0
0 30 60 90 120 150
10 pA
3T3 fibroblasts
1
pA
0
0 60 120 180 240
10 pA
HL-60
-2
pA
-3
0 30 60 90 120 150
time [s]
2 pA
20 ms
+100 mV
-100 mV

through I_{CRAC} was recorded in isotonic $MnCl_2$ (100 mM). Its amplitude is 10% of that measured in a solution containing 10 mM Ca^{2+}. However, there was no significant quench of Fura-2 fluorescence due to the presence of intracellular BAPTA. A strong quench of Fura-2 fluorescence could be measured after store depletion when omitting intracellular BAPTA, so that all the incoming Mn^{2+} is captured by the fluorescent dye. These findings further strengthen the hypothesis that I_{CRAC} is a ubiquitous mechanism whereby cells accomplish Ca^{2+} influx to refill depleted calcium stores. In mast cells, I_{CRAC} is responsible for the largest part of the sustained Ca^{2+} plateau following receptor stimulation.[30]

NON-SELECTIVE CATION CHANNELS

In response to receptor stimulation (e.g., with compound 48/80) at least two different types of non-selective cation channels can be activated in rat peritoneal mast cells. The first one is a cation channel of 50 pS unitary conductance that is responsible for small whole cell currents (5–50 pA at a holding potential of −40 mV). Channel activation is likely mediated by a G protein as GTPγS mimicks and GDPβS inhibits receptor-mediated activation of these channels. Channel activity is subject to negative feedback inhibition through protein kinase C and high $[Ca^{2+}]_i$.[31,32] Although activation of 50 pS channels is often associated with Ca^{2+} mobilization from intracellular stores, the 50 pS channel is not directly activated by either Ca^{2+} or $InsP_3$.

FIGURE 2 depicts membrane currents measured in the whole cell configuration of the patch-clamp technique and changes in $[Ca^{2+}]_i$ measured with the fluorescent dye Fura-2 during application of 48/80. These experiments were carried out in the presence of intracellularly applied heparin to prevent $InsP_3$-mediated Ca^{2+} influx through I_{CRAC}. The figure demonstrates the relationship between the size of the 50 pS currents and the resulting changes in $[Ca^{2+}]_i$ at different external calcium concentrations for individual cells and the mean relationship. Although the change in $[Ca^{2+}]_i$ elicited by activation

FIGURE 1. Activation of I_{CRAC} in different non-excitable cells. The left panel depicts the temporal pattern of activation of an inward current recorded at 0 mV holding potential during perfusion with the standard pipette solution supplemented with $InsP_3$ (Amersham, 10 μM) and the Ca^{2+} chelator EGTA (10 mM). Establishment of the whole cell mode of the patch clamp technique is indicated by the arrow. Immediately after breaking the patch, voltage ramps from −100 mV to +100 mV (duration 50 msec) were applied. The right panel shows these voltage ramps after activation of the inward current corrected by voltage ramps before activation of the inward current. For details of the pulse protocol see Hoth and Penner.[17] **Methods and solutions.** For details see von zur Mühlet *et al.*[11] and Hoth and Penner.[17] Patch-clamp experiments were done in the tight-seal whole-cell configuration[48] at 23–27°C in standard Ringer's solution containing (in mM): NaCl 140, KCl 2.8, $CaCl_2$ 10, $MgCl_2$ 2, glucose 11, HEPES-NaOH 10, pH 7.2. Sylgard-coated patch pipettes had resistances between 2–5 MΩ after filling with standard internal solution which contained (in mM): K-glutamate 145, NaCl 8, $MgCl_2$ 1, Mg-ATP 0.5, HEPES-KOH 10, pH 7.2. Fura-2 pentapotassium salt (Molecular Probes) was regularly added to the internal solution (100 μM). Extracellular solution changes (in case of the application of compound 48/80) were made by local application from a wide-tipped micropipette. The $[Ca^{2+}]_i$ was monitored (using the fluorescent dye Fura-2) with a photomultiplier-based system.[49]

of 50 pS channels increased with increasing external calcium, the amount of current declined with increasing calcium (FIG. 2, C). This behavior was also observed in a single cell when alternately perfused with Ca^{2+}-free and Ca^{2+}-containing medium. This effect of decreasing current amplitude with increased $[Ca^{2+}]_o$ arises from a decrease in the overall channel open probability accompanied by a minor reduction in the slope conductance.[30] A further attenuation of cation currents is due to the inhibitory effect of elevated $[Ca^{2+}]_i$.[32] Thus, cation channels are tightly regulated by Ca^{2+} ions, exerting efficient negative feedback control on Ca^{2+} influx through 50 pS cation channels.

In about 50% of the cells prolonged applications (tens of seconds) of compound 48/80 evoke a very large inward current, even at resting or buffered $[Ca^{2+}]_i$, probably through non-selective cation channels.[29] The current activates abruptly, in bursts, and only rarely returns to the prestimulus level. The nature and physiological role of this current is still unknown; a cation current this large is expected to cause cell death. Indeed a large conductance, characterized by weak cation selectivity, has been observed in the presence of extracellular ATP (ATP^{4-}). Low concentrations of ATP stimulate Ca^{2+} entry and exocytosis while high doses induce large pores responsible for cell lysis

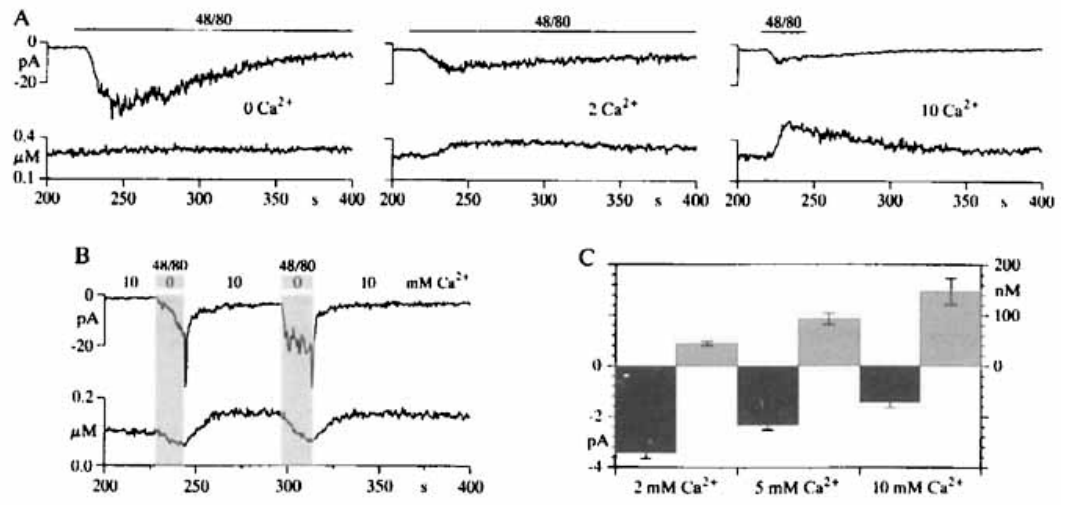


FIGURE 2. Relation among current through 50 pS cation channels and $[Ca^{2+}]_i$. (**A**) Examples of membrane current (upper traces) activated by application of compound 48/80 (5 μg/ml), and the simultaneously determined $[Ca^{2+}]_i$ (lower traces) from three different rat peritoneal mast cells at the indicated concentrations of external calcium. The duration of secretagogue application is indicated by the line above the traces. Heparin (low molecular weight, Sigma, 500 μg/ml) was present in the pipette solution in all three cells to suppress I_{CRAC} (heparin prevents Ca^{2+} release from internal stores through $InsP_3$ which is transiently produced after stimulation with compound 48/80). The holding potential in each case was −40 mV. The inward current elicited by compound 48/80 is due to activation of non-specific cation channels (50 pS channels). (**B**) Examples of membrane current (upper trace) and $[Ca^{2+}]_i$ for a rat peritoneal mast cell bathed in 10 mM external Ca^{2+}. Compound 48/80 was applied in a 0-Ca^{2+} Ringer's solution at the time indicated by the shaded regions. The pipette solution contained 500 μg/ml heparin, and the holding potential was −40 mV. (**C**) Summary of the average increase in membrane current (black bars) and $[Ca^{2+}]_i$ (shaded bars) elicited by compound 48/80 at 2, 5, and 10 mM external Ca^{2+} concentration. The vertical lines indicate ±SEM (n = 16–29). (**A**: From Fasolato *et al.*[30] Reprinted with permission.)

and death.[33] Lindau and Fernandez[22] have described another nonselective cation current in rat peritoneal mast cells with a conductance of about 30 pS which, in contrast to the 50 pS channel, the large cation current, and the ATP-gated channel, is activated by an increase in $[Ca^{2+}]_i$. Janiszewski *et al.* reported that substance P activates large transient currents in RBL-2H3 cells,[34] reaching hundreds of pA, with some cation selectivity. The response was strictly dependent on extracellular Ca^{2+} and could be mimicked by Ca^{2+} ionophores. It is possible that this may reflect positive feedback of Ca^{2+} influx through one influx pathway, which then maintains the activation of cation channels by $[Ca^{2+}]_i$. Evidence for more than one cation channel has also been found in rat peritoneal mast cells by Kuno and Kimura using noise analysis.[35]

Theoretically, all non-selective cation channels could play a role in Ca^{2+} influx provided the current amplitudes are large enough. We have determined the Ca^{2+} selectivity of the 50 pS channels in peritoneal mast cells and found a permeability ratio of P_{Ca2+}/P_{M+} of around 2 in physiological solutions with 2 mM Ca^{2+} (taking activity coefficients into account). This means that 3–4% of the current through 50 pS channels is carried by Ca^{2+} ions. Since inward currents following 48/80 stimulation average less than 4 pA at −40 mV (FIG. 2,C), 50 pS cation channels can at best account for one third of the Ca^{2+} influx necessary to sustain the Ca^{2+} plateaus typically observed in rat peritoneal mast cells.[30]

During the last year, evidence has accumulated that inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) may be involved in Ca^{2+} influx.[36,37] Although $InsP_4$ is unable to activate Ca^{2+} influx by itself, it may enhance Ca^{2+} entry in conjunction with other factors. Thus, in endothelial cells, $InsP_4$ increases the open probability of Ca^{2+}-activated cation channels,[38] whereas in *Xenopus* oocytes[39] and lacrimal gland cells[40] the additional presence of $InsP_3$ is required to produce the synergistic enhancement of Ca^{2+} influx by $InsP_4$. In a variety of other cell types no actions of $InsP_4$ could be detected. In rat peritoneal mast cells and RBL-2H3 cells, we found neither an effect of $InsP_4$ on I_{CRAC} nor interference with non-selective cation channels.[17,31]

OTHER CHANNELS

In addition to the Ca^{2+}-permeable channels described in the previous two sections, chloride (Cl^-) channels and voltage-activated potassium (K^+) channels are found in mast cells.[3,20–22] These may be activated and modulated following receptor stimulation or secondary to changes in membrane potential. One of the major functions of the conductances may rest on their ability to set the membrane potential to hyperpolarized levels to support Ca^{2+} influx through voltage-independent cation and calcium currents.

In rat peritoneal mast cells, externally applied secretagogues activate a slowly developing Cl^- current.[21,31] This delayed outward-rectifying current can also be activated by internally applied adenosine-3′,5′-cyclic monophosphate (cAMP) or guanosine 5′-*O*-3-thiotriphosphate (GTPγS) as well as elevated $[Ca^{2+}]_i$. The effect of Ca^{2+} is slow and incomplete however, suggesting that the current is not due to Ca^{2+}-activated Cl^- channels, such as those observed in lacrimal gland cells or *Xenopus* oocytes. Moreover, with elevated cAMP levels, current activation also occurs in the presence of 2 mM EGTA. The current is reduced by the chloride-channel blocker 4,4′-diisothiocyano-2,2′-stilbenedisulfonate (DIDS). The single-channel conductance of this chloride chan-

nel was estimated to a lower limit of 1–2 pS using noise analysis.[21] Since activation of chloride currents would hyperpolarize the membrane potential, it could serve to provide a larger driving force for Ca^{2+} entry via the I_{CRAC} mechanism or through non-selective cation channels. Chloride channels could therefore play a supporting role in the degranulation of mast cells.

Chloride channels can also be found in rat basophilic leukemia (RBL) cells where they are activated by cross-linking of IgE receptors and have a slope conductance of 32 pS.[7] The open-probability increases with depolarizing potentials and the channels are blocked by the Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and by the antiallergic drug cromolyn, both in the μM range. NPPB not only inhibits the Cl^- channel but also the serotonin release of these cells with almost the same dose-response relationship. Whether this effect is due to a reduction in driving force for Ca^{2+} influx resulting from the block of Cl^- channels or to some other process controlled by Cl^- channels remains to be determined. It is clear however that depolarizing RBL cells results in impairment of mediator release.[4]

Two main types of K^+ channels have so far been identified in RBL-2H3 cells.[20,22,41] The major resting conductance of this cell line is an inwardly rectifying K^+ channel. This channel seems to be responsible for setting the membrane potential of these cells to negative values (ranging between −50 and −90 mV), which provides a large driving force for Ca^{2+} influx. In physiological K^+ concentrations, the single channel conductance is around 2–3 pS.[22] An increase in $[Ca^{2+}]_i$ leads to a decrease in the open probability of this inward rectifier without affecting the single channel conductance.[41] McCloskey and Cahalan[20] found that this K^+ channel is controlled by a pertussis-sensitive G-protein. Lewis *et al.*[42] showed that injection of messenger RNA derived from RBL-2H3 cells into *Xenopus* oocytes resulted in the expression of an inwardly rectifying potassium channel, but cloning of inwardly rectifying potassium channels has so far succeeded only in plant cells.[43] RBL cells also possess another type of K^+ channel (outward rectifier type), which is modulated by non-hydrolyzable GTP analogs.[20] Different GTP-binding proteins seem to regulate the two different potassium channel types such that during activation of these G-proteins, the inward rectifier closes and the outward rectifier opens.

In rat peritoneal mast cells K^+ channel activity was reported by Matthews *et al.*[21] Less than 5% of the cell preparations showed large conductance, Ca^{2+} and voltage-dependent channels. The reversal potential of the current was more negative than that of the delayed Cl^- current and was affected by changes in external K^+. In a few rare cases, one may detect outward currents in mast cells with the kinetic behavior of an outward rectifying slow-inactivating K^+ channel (FIG. 3). This current may not be seen when clamping the cell to the usual holding potential of 0 mV since it is inactivated at this potential. Changing the holding potential to negative values (−70 mV) lets the channels recover from inactivation and therefore they can be activated by depolarizing voltage pulses. Since this current is observed so rarely, it has not been characterized in great detail. Pharmacological evidence also supports the existence of K^+ channels in the membrane of rat peritoneal mast cells, since K^+ channel blockers induce histamine release.[8]

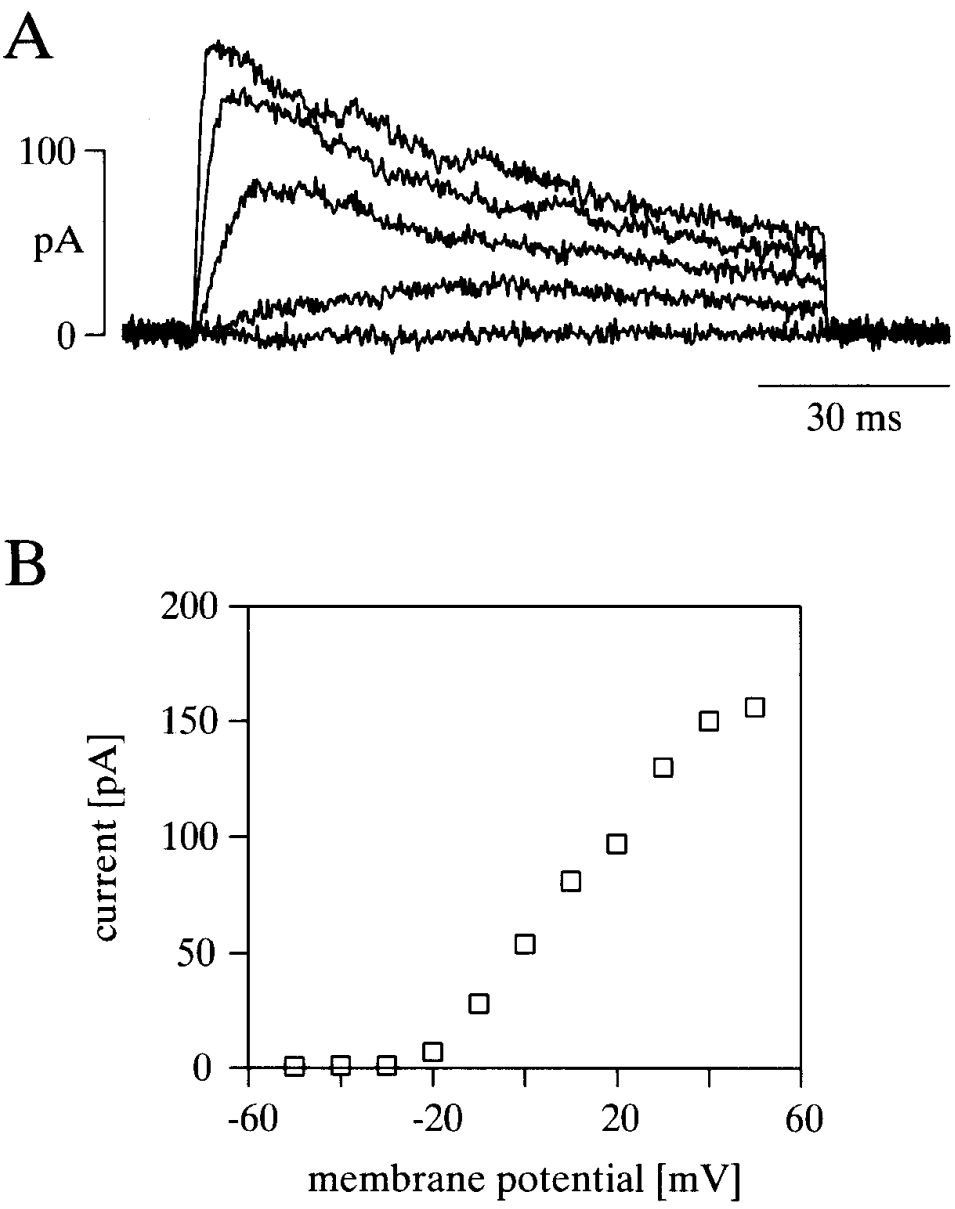


FIGURE 3. Outward currents in rat peritoneal mast cells. (**A**) Outward currents in response to depolarizing voltage pulses (ranging from −50 to + 50 mV, 20 mM increment) from a holding potential of −70 mV. (**B**) Current-voltage relationship plotting the peak-current derived from the experiment shown in **A** as a function of the membrane potential.

ION CHANNELS AND MAST CELL ACTIVATION

A simplified overview of the signal transduction pathways, ionic conductances, and second messengers involved in mast cell activation is shown in FIGURE 4. An agonist, such as substance P or compound 48/80, may activate more than one pathway, leading to phospholipase C (PLC) activation, Ca^{2+} release, and Ca^{2+} influx both through the selective pathway (I_{CRAC}) and the non-selective one (50 pS channels). Increased

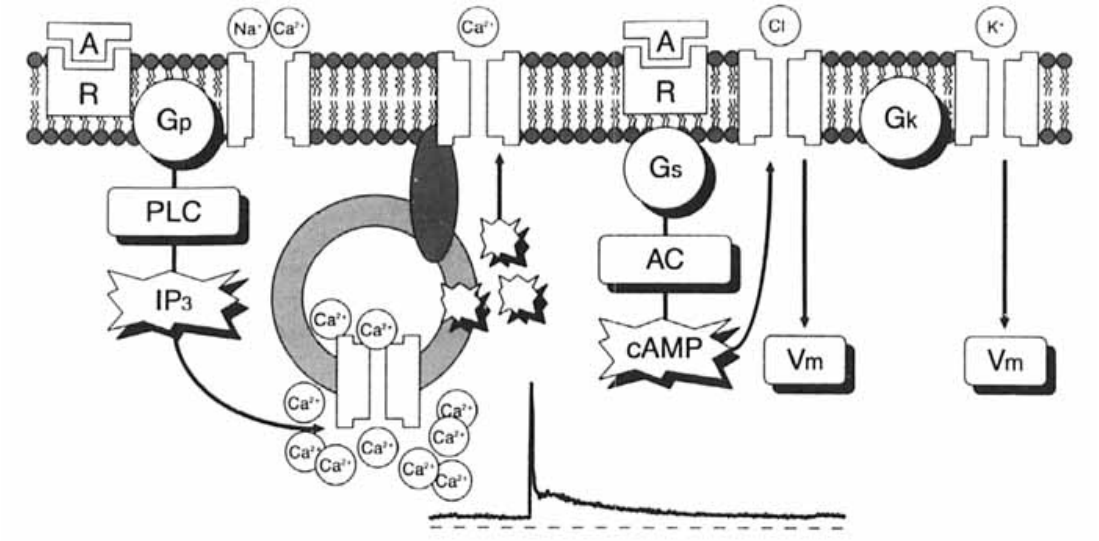


FIGURE 4. Scheme of the different types of channels found in mast cells. From left to right: Non-selective cation channels activated following agonist (A) -receptor (R) binding via a G-protein (Gp). At the same time, $InsP_3$ is produced through activation of phospholipase C (PLC). $InsP_3$ depletes internal Ca^{2+} stores, which leads to activation of I_{CRAC}. The signal transduction mechanisms that lead to activation of I_{CRAC} after store depletion are presently unknown. Cl^- channels can be activated by cAMP, which is increased following stimulation of a G-protein (Gs) that activates adenylate cyclase (AC). Voltage-activated K^+ channels may be regulated by G-proteins (Gk). Both Cl^- and K^+ channels can be responsible for negative membrane potentials (Vm). At the bottom of the figure a typical $[Ca^{2+}]_i$ trace in response to a stimulus like compound 48/80 is shown. The fast Ca^{2+} transient is followed by a sustained plateau phase due to Ca^{2+} influx across the plasma membrane.

levels of $[Ca^{2+}]_i$ and diacylglycerol support the secretory response through the protein kinase C pathway. The same agonist can also activate the delayed Cl^- conductance which, in non voltage-clamped cells, leads to membrane hyperpolarization with further increases of calcium and cation fluxes. It should be noted however that cAMP levels remain unchanged or decrease during 48/80 stimulation but increase with antigen as a stimulus. Since high levels of cAMP also reduce secretion by an unknown mechanism when applied through the patch pipette,[10] the size and the timing of activation of these transduction pathways may explain the ability of different agonists to induce or to suppress the secretory response.[44]

A further level of complexity arises when one considers that the granule content, once secreted, may further activate mast cells. The best known example is displayed by ATP. Mast cells secrete ATP together with histamine[45] and both ATP receptors, coupled to phospholipase C, and ATP-gated channels have been described in mast cells.[33] However, the role of ATP on secretion is still unclear since cellular responses to extracellular ATP, either released by immunocompetent cells or by nerve terminals, can range from $[Ca^{2+}]_i$ rises and membrane depolarization to cell death.[46]

Even within the same type of non-excitable cells, striking differences can be found in two tissue variants: the peritoneal mast cells of the connective tissue type and the basophilic mast cells of the mucosal type. Peritoneal mast cells, under resting conditions, have a very small whole-cell conductance with a resting membrane potential around

0 mV since their dominant conductance, the delayed Cl^- current, is silent. By contrast, the high negative resting potential of the RBL cells is governed by the inwardly rectifying K^+ current.[22] Both cell types are also endowed with the same highly selective Ca^{2+} current activated by store depletion and with a still undefined number of less selective cation pathways. These different channel equipments are likely involved in defining the secretory properties of the two cell types. While in peritoneal mast cells secretion occurs in a few seconds with dramatic morphological changes,[12] a slow release, lasting 20–30 minutes characterizes the secretory process in RBL-2H3 cells.[47]

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