

REVIEW

Human mast cell ion channels

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Mast cells in asthma

Mast cells play a significant role in the pathophysiology of asthma through their immunomediator-secretory activities as a response to their activation by both allergen and other secretory stimuli [1]. The autacoid mediators histamine, PGD_2 and LTC_4 induce bronchoconstriction, mucus secretion and mucosal oedema, and thus contribute to the symptoms of asthma. Mast cells also secrete a number of proteases and proinflammatory cytokines including IL-4, IL-5 and IL-13, which regulate both IgE synthesis and the development of eosinophilic inflammation [2]. Importantly, mast cells are present in an 'activated' secretory state within the asthmatic bronchial mucosa, with evidence of ongoing mediator release and cytokine synthesis [3–5]. Furthermore, mast cells present in the bronchoalveolar lavage (BAL) fluid of asthmatic subjects exhibit increased spontaneous and IgE-dependent mediator release [4, 6, 7]. Interestingly, mast cells are also activated by aeroallergens through an IgE-independent mechanism [8], suggesting they may not only contribute to the chronic airway inflammatory response and symptomatology, but potentially have a central role at the initiation of the allergic immune response, i.e. providing signals inducing B-cell IgE synthesis and Th2 lymphocyte differentiation [9, 10].

Potential mechanisms of mast cell hypersecretion in asthma

Although allergen exposure is strongly linked to the development of asthma, once established, allergen avoidance usually has a minor effect on the state of established disease, which appears to become 'self-perpetuating'. This is typified in cases of occupational asthma, where asthma and mast cell activation will persist if the patient is not removed from the offending agent early in the course of the disease. Mast cell activation in chronic asthma may therefore not only reflect continuing allergen exposure, but may have additional explanations such as (i) activation by other inflammatory cell types, cytokines and proteases, or (ii) intrinsic abnormalities in the signal transduction pathway for mediator release. Irrespective of the point from where pathological secretion is sustained, we hypothesize that all mechanisms will change the activity of the final 'effector' ion channels involved in normal stimulus-secretion coupling (Fig. 1). It is therefore important to identify these critical molecular effectors of human mast cell secretion, and to study them in asthmatic vs. normal populations.

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Ion channels and mast cell secretion

IgE-dependent activation of both human and rodent mast cells is characterized by an influx of extracellular Ca^{2+} , which is essential for subsequent release of both pre-formed (granule-derived) mediators and newly generated autacoids and cytokines [11]. However, flow of ions such as K^+ and Cl^- is likely to play an important role in mast cell secretory responses through their effect on membrane potential and thus Ca^{2+} influx. This is because the store-operated Ca^{2+} channels (SOCC) that carry Ca^{2+} into cells conduct larger currents at negative membrane potentials [12]. This principal is highlighted in T cells where specific inhibition of the voltage-dependent K^+ channel Kv1.3 by the scorpion toxin margatoxin inhibits T cell proliferation, IL-2 secretion and delayed-type hypersensitivity responses [13].

Several ion currents have been identified in rodent mast cells, but the function of most of these remains unclear. At rest in rodent 'mucosal' mast cells an inwardly rectifying K^+ channel (K_{IR}) is open [14], which sets a resting membrane potential close to the K^+ equilibrium potential at approximately -90 to -60 mV (Fig. 2). Several activation-dependent currents have been identified in response to various secretagogues, including a non-selective cation current carrying Ca^{2+} and Na^+ [15], specific Ca^{2+} influx through store-operated calcium channels (SOCC) [12], and an outwardly rectifying Cl^- conductance [16]. Interestingly, adenosine activates an outwardly rectifying K^+ channel (K_{OR}) in a GTP-dependent and pertussis toxin-sensitive manner, which polarizes the cell membrane potential and could therefore explain how adenosine potentiates IgE-dependent degranulation [17, 18]. Despite these observations, there are important differences between rodent and human mast cells with respect to mediator content, and secretory and pharmacological responsiveness, so from a clinical perspective it is essential that studies be performed on human cells.

Human mast cell ion channels

We have recently described for the first time ion channels present in the human mast cell line HMC-1, human peripheral blood-derived mast cells (HPBDMC), and human lung mast cells (HLMC) [19, 20]. Interestingly, in contrast to rat 'mucosal' mast cells, the majority of HLMC and HPBDMC are electrically silent at rest with resting membrane potential oscillating around 0 mV. We have never seen a K_{IR} current in any human mast cell (lung, skin, nasal, HPBDMC, HMC-1), although HLMC and HMC-1 do express mRNA for Kir2.1 and 2.2 (unpublished data).

Following IgE-dependent activation, 90% of HPBDMC cells respond acutely with the development of a Ca^{2+} -activated K^+

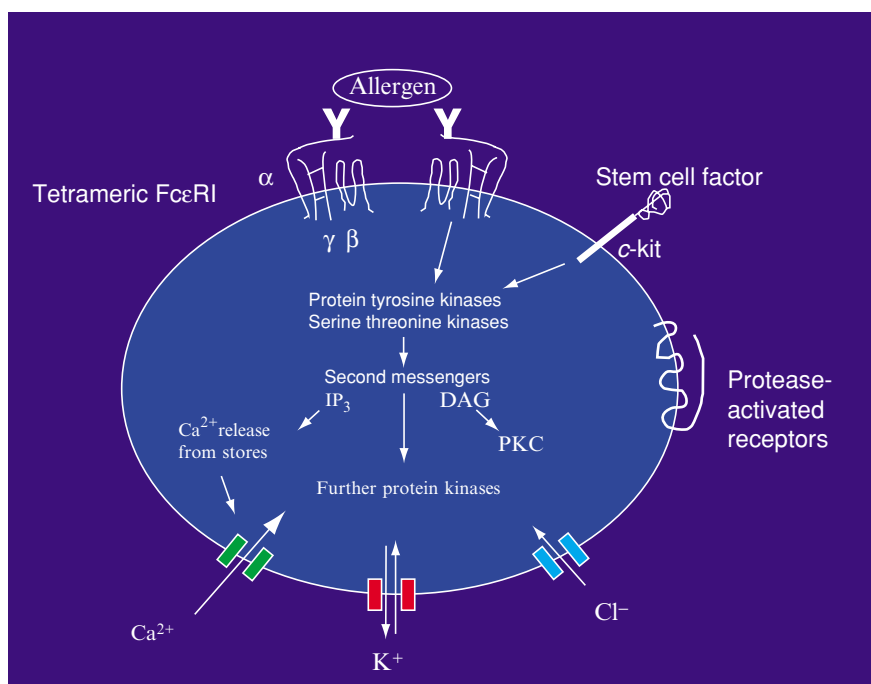


Fig. 1. Simplified outline of potential mechanisms sustaining mast cell secretion in asthma. Irrespective of the point from where pathological secretion is sustained, we hypothesize that all mechanisms will change the activity of the final 'effector' ion channels involved in normal stimulus-secretion coupling.

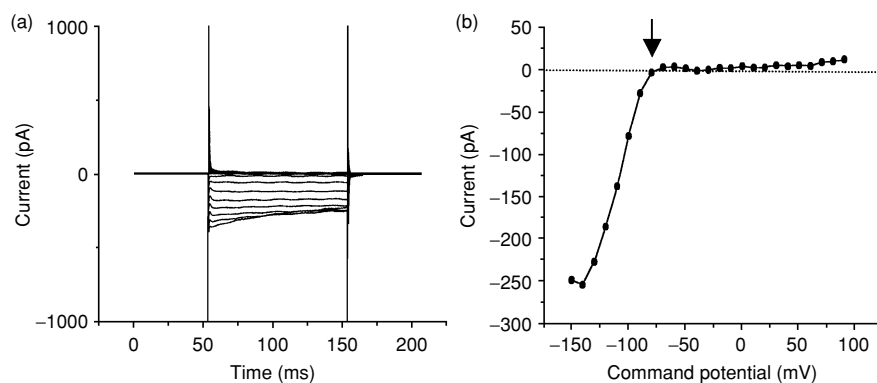


Fig. 2. Typical whole cell electrophysiological recording of electrical current in a resting rat basophilic leukaemia (RBL) cell. (a) raw current, (b) current-voltage relationship. There is a whole cell current demonstrating marked inward rectification, with reversal potential of about -75 mV (arrow), indicating it is carried by K^+ . The channel passing this current is Kir2.1, a member of the inwardly rectifying family of K^+ channels. Currents were evoked by applying voltage commands from -150 to $+100$ mV in 10 mV steps from a holding potential of -70 mV. The standard pipette solution contained 140 mM KCl; the standard extracellular solution contained 5 mM K^+ , 140 mM Na^+ , 2 mM Ca^{2+} and 151 mM Cl^- .

current (K_{CA}) exhibiting weak inward rectification, which polarizes the cell membrane potential to about -45 mV, and a smaller calcium-independent outwardly rectifying Cl^- conductance (Fig. 3). HLMC show more heterogeneity in their response to anti-IgE, with K_{CA} and calcium-independent Cl^- currents developing in about 50% of cells, with the Cl^- component more dominant. The electrophysiological properties of the K_{CA} current (inward rectification, immediate appearance on voltage steps, lack of inactivation during voltage pulses, block by charybdotoxin) indicate that it is carried by the intermediate conductance K_{CA} IKCA1 (also known as hSK4/hSKCA4/hIK1) or a closely related unidentified family member [21–24]. The activation-dependent Cl^- current has electrophysiological properties compatible with the presence of the voltage-dependent Cl^- -channel CIC-3 [25]. A Ca^{2+} -activated Cl^- channel is also unmasked in both HPBDMC and HLMC following activation with Ca^{2+} ionophore.

The HMC-1 cell line, which is often used as a model for human mast cells, shows a very different channel profile. At rest these cells express a relatively large Cl^- current demonstrating extreme outward rectification with electrophysiological

properties similar to the voltage-dependent Cl^- channels CIC-4 and CIC-5 [19,26,27] (Fig. 4). These cells express mRNA for CIC-5 but not CIC-4, suggesting this channel may well carry this Cl^- current. This is interesting because a CIC-4/5 current has never previously been recorded in the plasma membrane of a native cell. However, similar currents have been recorded in endothelial cells and gliomas [28,29], where they have been implicated in the dysregulation of cell proliferation. This same current is present at rest in about 10% of HLMC, and since it has recently been demonstrated that a subpopulation of HLMC do indeed proliferate [30], it is interesting to speculate that cells expressing this channel are the cells with proliferative potential.

The role of the K_{CA} and Cl^- channels following IgE-dependent activation

K_{CA} opening results in a negative shift in membrane potential, which is likely to increase Ca^{2+} influx both by increasing the electrical driving force for Ca^{2+} entry, but perhaps more

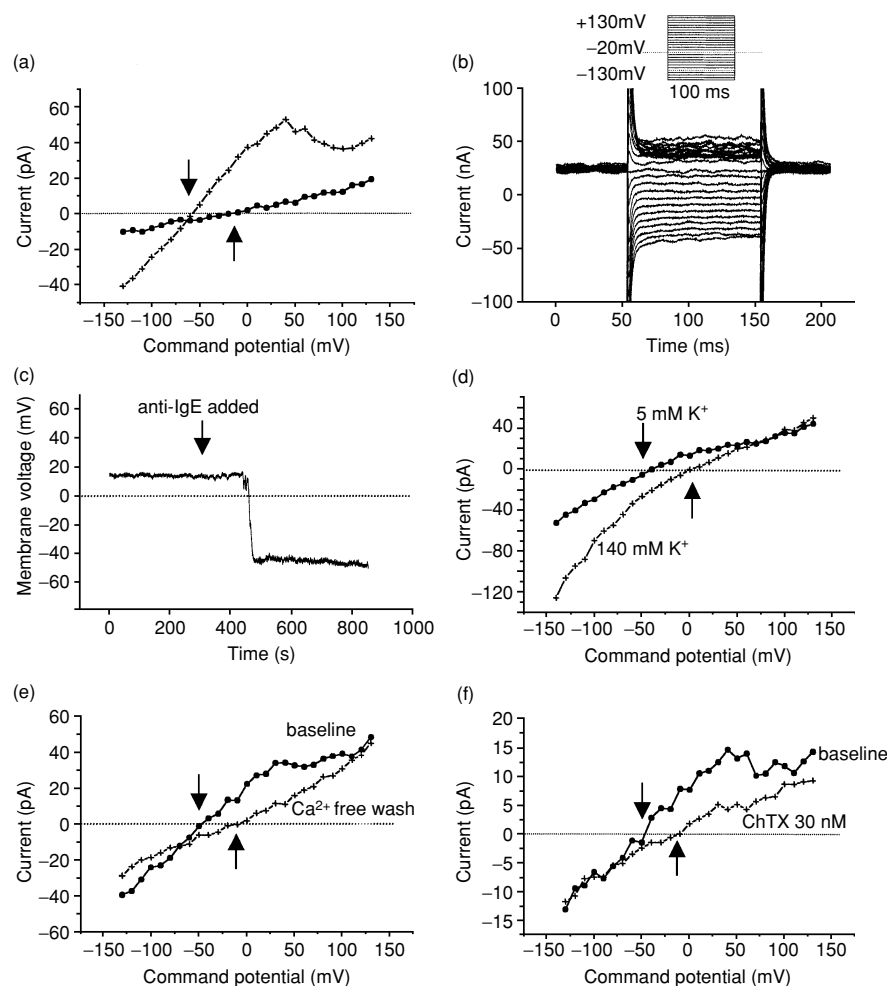


Fig. 3. Whole cell electrophysiological recording of membrane potential and electrical currents in HPBDMC at rest and following IgE-dependent activation. (a) Current-voltage curves from a cell at rest and 2 min post-IgE-dependent activation. The cell exhibits a small linear current at rest with reversal potential about -5 mV (arrow). Following addition of anti-IgE to the bath a current develops that demonstrates weak inward rectification. Note the negative shift in reversal potential to -60 mV (arrow), indicating that the current is likely to be carried predominantly by K^+ . (b) Raw current from the same cell as in (a) post-anti-IgE, demonstrating appearance of current immediately following voltage steps and no decay during a 100 msec pulse. The voltage protocol is shown inset. Currents were evoked by applying voltage commands from -130 to $+130$ mV in 10 mV steps from a holding potential of -20 mV. (c) Continuous recording of membrane potential in current-clamp mode, demonstrating a stable resting membrane potential around $+15$ mV, with an acute negative shift to -45 mV within 1 min of adding anti-IgE to the bath solution. (d) A further cell post-anti-IgE recorded in 5 mM and 140 mM external K^+ . Inward current increases, outward current decreases and reversal potential shifts from -50 mV to 0 mV (arrows) on switching from 5 to 140 mM external K^+ , confirming the presence of a K^+ current. Note also the inward rectification in 140 mM K^+ . (e) Another cell post-anti-IgE demonstrating the typical whole cell current. Following removal of extracellular Ca^{2+} there is a marked decrease in the whole cell current with a shift in reversal potential from -50 to -5 mV (arrows) indicating that the K^+ component is carried predominantly by a Ca^{2+} -activated K^+ channel. A smaller Ca^{2+} -insensitive current remains. This is carried in part by Cl^- and possibly in part by a mixed cation current. (f) A further cell post-IgE-dependent activation before and after addition of ChTX 30 nM. The current is decreased and reversal potential shifts from -45 to -5 mV, indicating partial blockade of the K^+ current. In all experiments the standard pipette solution contained 140 mM KCl; the standard extracellular solution contained 5 mM K^+ , 140 mM Na^+ , 2 mM Ca^{2+} and 151 mM Cl^- .

importantly by increasing the open probability of the store-operated Ca^{2+} channels (SOCC). These latter channels that carry Ca^{2+} into the cell demonstrate inward rectification at negative membrane potentials [12], and thus carry larger Ca^{2+} currents at more negative potentials. This would therefore be predicted to increase mediator release and influence Ca^{2+} -dependent gene transcription. Indirect evidence supporting a pro-secretory role for K_{CA} channel opening in mast cells is provided by the observation that HPBDMC, in which the K_{CA} current predominates, always release $> 40\%$ of total cellular histamine, while HLHC in which the K_{CA} current is less marked, show marked heterogeneity, releasing 0 to 30% of total cellular histamine. Although ChTX produces significant block of the whole cell current under the conditions used for patch clamping, the

effect of this peptide on histamine and LTC_4 release was very variable in HLHC and minimal in HPBDMC [20]. However, ChTX is susceptible to degradation in cell culture by mast cell-derived reactive oxygen species and proteases, and might bind to serum proteins present in culture media, so further study using more stable channel blockers such as clotrimazole or specific channel 'knockout' is required to delineate further the role of this channel in secretory responses.

Interpreting the role of Cl^- currents during cell activation is difficult because intracellular Cl^- concentration, which varies widely between cells, is not known for functionally mature human mast cells. Physiological extracellular Cl^- concentration is about 100 mM, so if intracellular Cl^- concentration is in the region of 30 mM as has been estimated for RPMC [31],

Cl^- currents will contribute to membrane polarization as reversal potential for Cl^- at these concentrations is about -40 mV, and this will theoretically promote Ca^{2+} influx. In support of this, putative blockers of rodent mast cell Cl^- channels such as 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and 4,4'-diisothiocyanato-2,2'-disulphonic acid (DIDS) attenuate histamine secretion but only in the μM range [31, 32]. However, NPPB also inhibits Ca^{2+} influx through SOCC [32], and although DIDS attenuates secretion and blocks Cl^- channels, it does not inhibit antigen-induced $^{36}\text{Cl}^-$ uptake in RPMC, which occurs rapidly, while the appearance of Cl^- channel activity is delayed [31]. Furthermore, although sodium cromoglycate is a potent blocker of the IgE-dependent Cl^- conductance in RBL cells [16], it is only a weak antagonist of secretion from HLMC. Thus the role of the delayed Cl^- current remains unclear.

Conversely, if intracellular Cl^- concentration in HLMC is similar to extracellular Cl^- concentration, then Cl^- channel opening will depolarize the cell and antagonize Ca^{2+} influx. This may indeed be the case, as in HMC-1 we have estimated that the intracellular Cl^- concentration is about 66 mM from the extrapolated reversal potential of the single channel conductance of the resting Cl^- current [19]. With this scenario, one could hypothesize that since the appearance of the Cl^- current is delayed following IgE-dependent activation, it actually

represents a negative feedback pathway to provide a brake to the secretory response. An alternative role for these Cl^- channels may be to regulate cell volume following activation, perhaps in concert with the K_{CA} as described in other cell types [25, 33]. Firm molecular identification of the channels present and selective inactivation, for example with antisense oligodeoxynucleotides, will answer these questions.

Hypothetical mast cell electrical 'excitation' cycle

Because mast cells recover and regranulate following IgE-dependent activation, we envisage a hypothetical cycle of electrical activity analogous to that in excitable tissue such as ventricular myocardium, where a co-ordinated cell activation response requires an orderly sequence of ion channel gating and regulation of membrane potential. However, rather than occurring over milliseconds, in mast cells this electrical cycle would evolve over hours or even days (Fig. 5), although rapid millisecond side-cycles may operate to afford specific rapidly alternating channel activity where tight control of ion flow is required, e.g. between Ca^{2+} -activated K^+ or Cl^- channels with store-operated Ca^{2+} channels (Fig. 5, red arrows). It follows from this, that if mast cell ion channels can be maintained in, or

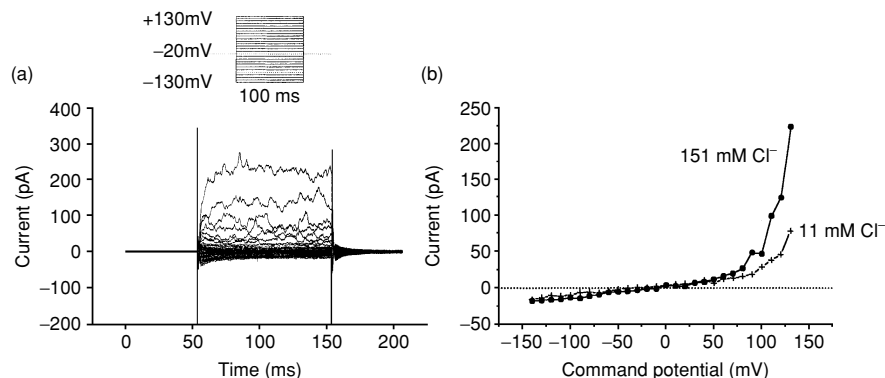


Fig. 4. Typical whole cell electrophysiological recording of electrical current in a resting HMC-1. (a) raw current, (b) current-voltage relationship. There is a whole cell current demonstrating marked outward rectification, with reversal potential of about 0 mV indicating it is likely to be carried by Cl^- . This is confirmed by lowering extracellular Cl^- concentration to 11 mM, which markedly reduces the outward current. Currents were evoked by applying voltage commands from -130 to $+130$ mV in 10 mV steps from a holding potential of -20 mV. The standard pipette solution contained 140 mM KCl; the standard extracellular solution contained 5 mM K^+ , 140 mM Na^+ , 2 mM Ca^{2+} and 151 mM Cl^- .

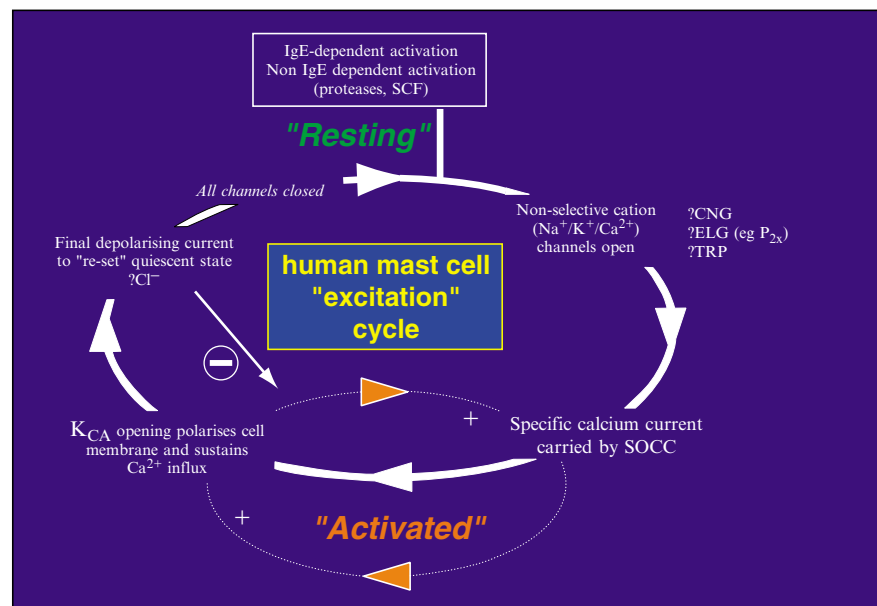


Fig. 5. Simplified hypothetical mast cell electrical 'excitation' cycle based on currents in human mast cells. In disease, the cells may be locked into the side-cycle indicated by the red arrows where membrane hyperpolarization secondary to opening of Ca^{2+} -activated K^+ channels increases Ca^{2+} influx, which in turn opens the K^+ channels, thus creating a positive feedback loop. The delayed Cl^- current might attenuate this process by producing membrane depolarization.

returned to, the resting state, then mast cell secretion associated with disease might be attenuated.

In summary, the ion channels present in human mast cells are clearly very different from those present in rodents, which is likely to have important consequences for various cellular responses. This profound difference between rodent and human mast cells may explain in part the significant phenotypic differences between species, and studies of ion channel regulation of mast cell function should therefore be limited to human cells. We believe these studies will identify new modulatory mechanisms for the release of mast cell mediators, which will aid the development of novel therapies for the treatment of asthma and other mast cell-mediated diseases by means of mast cell ion channel modulation.

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