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REVIEW

Ion channels regulating mast cell biology

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Summary

Mast cells play a central role in the pathophysiology of asthma and related allergic conditions. Mast cell activation leads to the degranulation of preformed mediators such as histamine and the secretion of newly synthesised proinflammatory mediators such as leukotrienes and cytokines. Excess release of these mediators contributes to allergic disease states. An influx of extracellular Ca^{2+} is essential for mast cell mediator release. From the Ca^{2+} channels that mediate this influx, to the K^+, Cl^- and transient receptor potential channels that set the cell membrane potential and regulate Ca^{2+} influx, ion channels play a critical role in mast cell biology. In this review we provide an overview of our current knowledge of ion channel expression and function in mast cells with an emphasis on how channels interact to regulate Ca^{2+} signalling.

Introduction

Mast cells play a central role in the pathophysiology of allergic diseases such as asthma, rhinitis, urticaria and anaphylaxis [1]. In response to allergens and many non-immunological stimuli mast cells release a battery of both preformed and newly synthesised mediators including granule-derived histamine and proteases, newly generated products of arachidonic acid metabolism such as prostaglandins and leukotrienes, and cytokines. Excess release of these mediators as a result of aberrant mast cell activation contributes to allergic disease states.

IgE dependent activation of mast cells is characterised by an influx of extracellular Ca^{2+} that is essential for the release of these mediators. In addition to the Ca^{2+} channels responsible for this influx, mast cells express K^+, Cl^- and transient receptor potential (TRP) channels that, by regulating cell membrane potential, also play an important role in cell activation. Because of their role in mast cell mediator release, migration and survival, ion channels have emerged as attractive therapeutic targets for the treatment of allergic diseases. In this review we provide an overview of our current knowledge of ion channel expression and function in mast cells with an emphasis on how channels interact to regulate Ca^{2+} signalling.

Ca^{2+} channels

The major pathway through which Ca^{2+} influx occurs in mast cells is through calcium release activated Ca^{2+} (CRAC) channels, also known as store operated channels (SOC). These channels are activated by the inositol 1,4,5-triphosphate (IP_3) mediated depletion of the endoplasmic reticulum (ER) Ca^{2+} stores that occurs following cell surface receptor-dependent activation of phospholipase C (Fig. 1). CRAC channels were first described in rodent mast cells by Hoth and Penner [2] using electrophysiological methods. They and others subsequently defined the properties of these channels [3, 4]. Channels are highly selective for Ca^{2+}, resulting in currents (I_{CRAC}) that are strongly inwardly rectifying. In addition the single channel conductance is very low resulting in currents that under physiological conditions are only very small.

It was not until 2005 that the molecular components of CRAC channels began to be identified. STIM1 was the first to be identified using RNA_i screens of Drosophila S2 cells [5]. It acts to sense the ER Ca^{2+} concentration and transmit this information to the CRAC channel pore (Fig. 1). ORAI1 (also known as CRACM1) was then identified through RNA_i screens as a regulator of Ca^{2+} signalling [6]. At the same time it was identified as the gene responsible for immunodeficiency in patients with a defect in CRAC channel function [7]. ORAI1 was subsequently identified as the Ca^{2+}-selective pore forming protein in the plasma membrane [8–10]. The gene for ORAI1 encodes a membrane protein with four transmembrane domains, with channels functional as tetramers. Mammalian cells express two further homologues, ORAI2 and ORAI3, which show a high

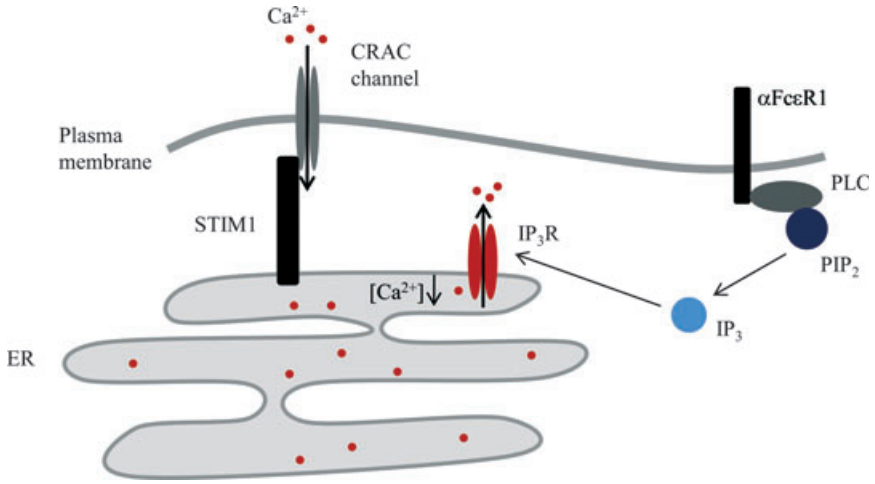

Fig. 1. Ca^{2+} entry into mast cells through store operated (CRAC) channels. Activation of cell surface receptors e.g. the high affinity IgE receptor (αFcεR1) leads to the generation of inositol 1,4,5-triphosphate (IP_3) as a result of activation of phospholipase C (PLC). IP_3 activates IP_3 receptors (IP_3R) on the endoplasmic reticulum (ER) membrane leading to the depletion of the ER Ca^{2+} stores. STIM1 senses the reduced ER Ca^{2+} concentration and interacts with and activates CRAC channels located in the plasma membrane resulting in the influx of extracellular Ca^{2+} into the cell.

degree of sequence homology with ORAI1 but have distinct functional properties [11, 12]. Heterodimerisation between CRAC channel subunits has been reported in heterologous expression systems [11, 13], but it is not yet known whether this also occurs in mast cells.

Both human and rodent mast cells express all three CRAC channels at least at the mRNA level [14, 15] (Table 1). The importance of ORAI1 to antigen evoked Ca^{2+} influx into mouse mast cells is revealed by studies of an ORAI1 knockout mouse [15]. Ca^{2+} influx was reported to be reduced by 70% in foetal liver-derived mast cells from the ORAI1 knockout. The remaining Ca^{2+} influx was blocked by CRAC channel inhibitors suggesting that ORAI2 and/or ORAI3 also contribute to Ca^{2+} influx in these cells. Degranulation, LTC_4 release, and TNFα production were all greatly reduced in the ORAI1 knockout indicating that ORAI1 is essential to mouse mast cell function [15]. STIM1 is also necessary for mouse mast cell function. Foetal liver-derived mast cells from a STIM1 knockout mouse showed impaired Ca^{2+} influx, and reduced degranulation, TNF, IL-6 and IL-13 production [16].

Studies of the actions of CRAC channel blockers on rodent mast cells further underline the importance of CRAC channels to Ca^{2+} influx in these cells. Treatment of cells of the rat basophilic leukaemia cell line RBL-1 with La^{3+} at concentrations reported to completely block CRAC channels reduces Ca^{2+} influx into these cells and also mast cell activation [17]. Similarly, treatment of mouse bone marrow-derived mast cells with *N*-(4-(3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (BTP-2) inhibited Ca^{2+} influx, degranulation and cytokine secretion [18]. Synta-66, a compound structurally related to BTP-2, suppressed antigen evoked Ca^{2+} influx in RBL-1 cells [19].

CRAC channels are also critical for Ca^{2+} influx into and the function of human mast cells. Human lung mast cells express CRAC-like currents following dialysis with IP_3 or activation through FcεRI [14] (Fig. 2). Block of CRAC channels in purified human lung mast cells by Synta-66 and GSK7975A (compound 36 from patent WO 2010/1222089) reduced Ca^{2+} influx, histamine release (degranulation), LTC_4 release and cytokine secretion [14] (Fig. 2). Consistent with this, there was a marked reduction of allergen-dependent bronchial smooth muscle contraction in grass allergen-sensitised human bronchial rings [14], suggesting that a similar inhibition of the mast cell-dependent early asthmatic reaction to allergen challenge will occur *in vivo*. Interestingly though, a substantial residual secretion of these mediators was observed using relatively high concentrations of the blockers. This appears to open up the possibility that other Ca^{2+} permeable channels and/or receptors may play at least some role in Ca^{2+} influx in human lung mast cells (see below). I_{CRAC}-like currents have also been observed in mast cells isolated from nasal polyps of patients with nasal polyposis [20].

The relative contributions of the three CRAC channels to Ca^{2+} influx in human mast cells are not currently known. Our recent finding of ORAI2 and ORAI3 mRNA and at least ORAI2 protein expression in human lung mast cells in addition to that of ORAI1, suggests that ORAI2 and ORAI3 may play at least some role in Ca^{2+} influx in these cells [14].

Because of the critical requirement for Ca^{2+} influx for mediator release from mast cells, CRAC channels are of great interest as novel targets for the development of

Table 1. Plasma membrane ion channels in mast cells

Channel	Selectivity	Mast cell origin*
Calcium channels		
ORAI1, -2 and -3	Ca^{2+}	Human: lung [14], nasal polyps [20], HMC-1 cell line [14] Mouse: foetal liver [15], bone marrow derived [18] Rat: RBL-1cell line [17]
Ca_v 1.2	Ca^{2+}	Mouse: bone marrow derived [24] Rat: RBL-2H3 cell line [24]
Ca_v 3.3	Ca^{2+}	Human#: lung [30]
P2X1–4, -6 and -7	Cations including Ca^{2+}	Human: cord blood [30, 31], lung [30, 32], skin [30], LAD2 cell line [32] Mouse: bone marrow derived [33] Rat: peritoneum [34]
TRP channels		
TRPC1	Cations including Ca^{2+}	Human#: skin [30] Mouse: bone marrow derived [41] Rat: RBL-2H3 cell line [44]
TRPC2, -3 and -7	Cations including Ca^{2+}	Rat: RBL-2H3 cell line [44]
TRPC5	Cations including Ca^{2+}	Mouse: bone marrow derived [41] Rat: RBL-2H3 cell line [44]
TRPC6	Cations including Ca^{2+}	Mouse: bone marrow derived [41]
TRPV1and -4	Cations including Ca^{2+}	Human#: HMC-1 cell line [40] Rat: RBL-2H3 cell line [42]
TRPV2	Cations including Ca^{2+}	Human#: cord blood [30], lung [30], skin [30], HMC-1 cell line [40] Mouse: bone marrow derived [42] Rat: RBL-2H3 cell line [42]
TRPV6	Ca^{2+}	Rat: RBL-2H3 cell line [42]
TRPM2	Cations including Ca^{2+}	Human#: cord blood [30], lung [30]
TRPM4	Monovalent cations	Mouse: bone marrow derived [43]
TRPM7	Cations including Mg^{2+}, Ca^{2+}	Human: lung [39], HMC-1 [39] and LAD2 [39] cell lines Rat: RBL-2H3cell line [42]
TRPM8	Cations including Ca^{2+}	Rat: RBL-2H3 cell line [45]
Potassium channels		
$K_{Ca}3.1$	K^+	Human: lung [27, 29], blood derived [27, 29], bone marrow derived [49] Mouse: bone marrow derived [50] Rat: RBL-2H3 cell line [56]
Kir2.1-2.4, 3.1, 3.2, 3.46.1, 6.2	K^+	Human#: lung [30, 51], skin [30] Rat: bone marrow derived [52], RBL-2H3 cell line [67, 68]
TWIK-2, TASK-2	K^+	†Mouse [53, 54]
Chloride channels		
ClC2	Cl^-	Rat: RCMC cell line [77]
CFTR	Cl^-	Human: LAD2 cell line [79] Rat: peritoneum [78], RCMC cell line [78]
††Anoctamins?	Cl^-	ND
††Bestrophins?	Cl^-	ND

*Primary mast cells unless otherwise stated.
†Source unspecified.
††Possible Ca^{2+} and/or volume regulated Cl^- conductances.
#mRNA only demonstrated.
ND not determined.

anti-secretory drugs. The pharmacology of CRAC channels is however still developing and there are currently no specific blockers of CRAC channels in clinical trials. Many of the compounds used to study CRAC channel function have subsequently been found to have additional targets of activity. BTP-2 for example, as well as blocking CRAC channels blocks TRPC3 and TRPC5 and is an activator of TRPM4 channels [21, 22]. Nevertheless, Synta-66 and GSK-7975A appear more specific and are examples of recent progress in this respect [14, 19].

In addition to CRAC, mast cells express a number of other ion channels/receptors that may allow the entry of extracellular Ca^{2+}. At least in rodent mast cells, these include L-type voltage gated Ca^{2+} channels. Voltage gated Ca^{2+} channels (Ca_v) comprise as a minimum an α_1 subunit that contains the channel pore and voltage sensor. In the Ca_v1 and Ca_v2 sub-families additional subunits ($\alpha_2\delta$, β, γ) regulate trafficking to the membrane and channel gating [23]. In rodent mast cells L-type channels, most likely to be $Ca_v1.2$, may be involved in Ca^{2+} influx independent of endoplasmic reticulum Ca^{2+} store emptying following mast cell activation [24]. Interestingly though $Ca_v1.2$ channels have been shown to interact with and be regulated by STIM1 in a manner that is reciprocal to the regulation of CRAC channels by STIM1 [25, 26]. Ca_v-like currents have not to date been observed in human mast cells [27–29] although human lung mast cells express mRNA for $Ca_v3.3$ (T-type) and the $\alpha_2\delta_2$ subunit [30].

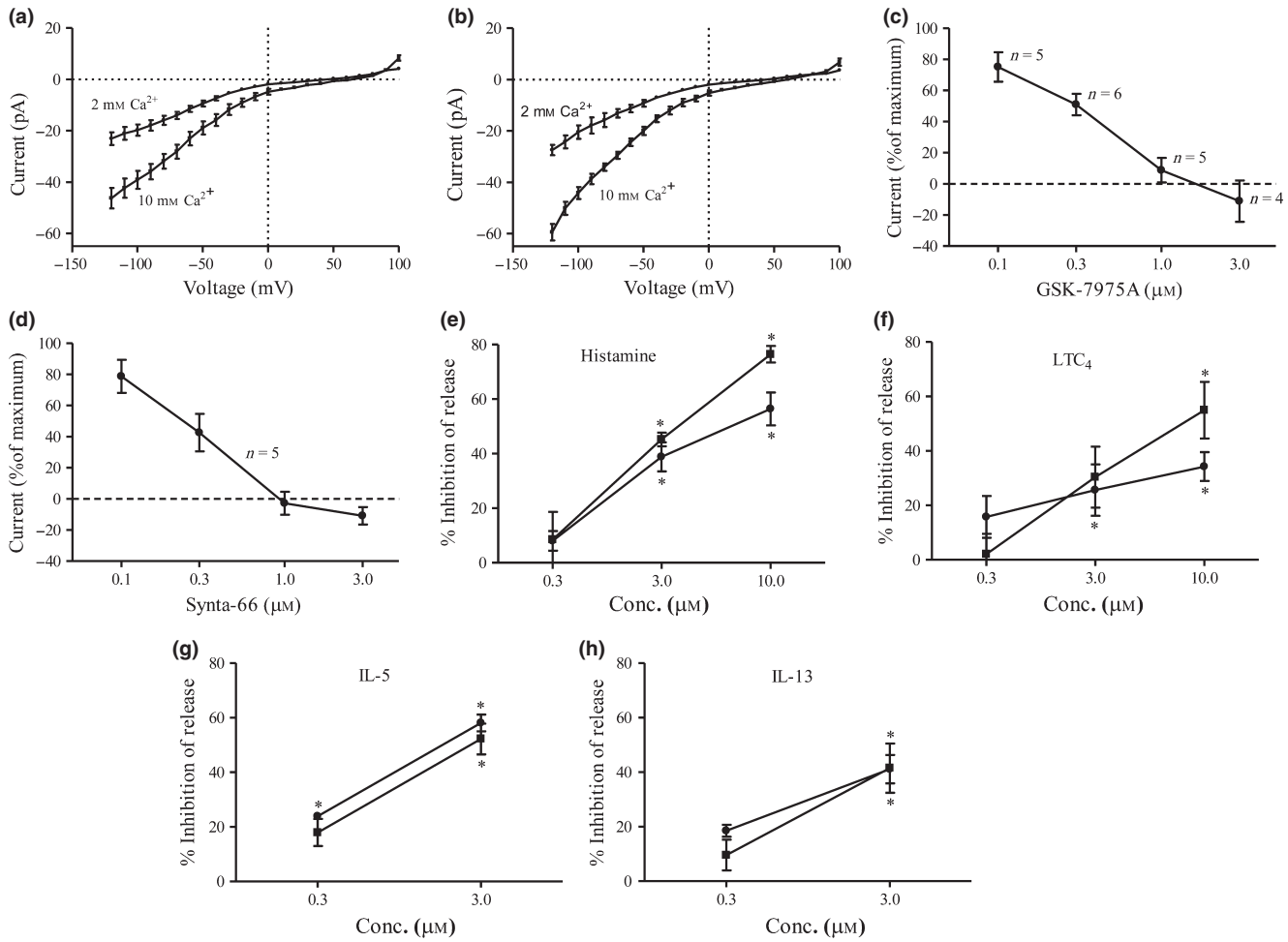

Fig. 2. Human lung mast cells express CRAC-like currents, block of which results in reduced mast cell mediator release. (A and B) Subtracted whole cell patch clamp current voltage (I–V) curves from (A) HLMCs 4 min after dialysis with IP_3 (mean ± SEM, $n = 6$) and (B) from HLMCs within 4 min of FcεR1-dependent activation (mean ± SEM, $n = 7$) in 2mM and then 10mM external Ca^{2+}. (C and D) Inhibition of IP_3-dependent whole cell current by the CRAC channel blockers GSK-7975A (C) and (D) Synta-66 ($n = 4$–6). A negative value implies a contribution from CRAC channels to the baseline whole cell current. (E–H) GSK-7975A (■) and Synta-66 (●) inhibit release of HLMC histamine (E), LTC_4 (F), and cytokines IL-5 (G) and IL-13 (H) dose dependently following FcεR1-dependent activation (mean ± SEM; $n = 5$ for histamine and LTC_4; $n = 3$ for IL-5 and IL-13). *$P < 0.05$ using repeated measures ANOVA with Bonferroni post hoc test on raw data. Adapted from J. Allergy Clin Immunol, 2012; 129, Ashmole I, Duffy SM, Leyland ML, Morrison, Begg M and Bradding P, CRACM/Orai ion channel expression and function in human lung mast cells, 1628–1635. Copyright (2012), with permission from Elsevier

IP_3 receptors also function as Ca^{2+} channels, though in an intracellular location [31]. They are the major conduit for Ca^{2+} released from the ER stores following mast cell activation. Mast cells express all three IP_3 receptor subtypes based on a study of RBL-2H3 cells [32]. The IP_3R_2 subtype is reported to be the predominant form, comprising 70% of the total [33]. Ca^{2+} is an important regulator of IP_3 receptor activity, with high concentrations inhibiting and low concentrations enhancing receptor activity respectively [34]. The importance of IP_3 receptors to mast cell function is supported by the application of xestospongin C, an IP_3 receptor inhibitor, to RBL-2H3 cells [35]. Both Ca^{2+} influx and degranulation were inhibited [35].

P2X receptors

P2X receptors are ligand-gated non-selective cation channels that are activated by extracellular ATP. Although they are primarily of interest in mast cell function due to their permeability to Ca^{2+}, they are also permeable to Na^+ and K^+. P2X receptors have been shown to be expressed in both human and rodent mast cells. In humans, cord blood-derived and lung mast cells express mRNA for P2X1, P2X4 and P2X7 [30, 36, 37]; while P2X1 and P2X4 were found to be expressed in skin mast cells [30] (Table 1). In addition there is electrophysiological evidence for the presence of functional P2X1, -4 and -7 receptors in human lung mast cells and human mast

cell lines [37] (Fig. 3). P2X1-4 and P2X6 and -7 are expressed in mouse bone marrow-derived mast cells [38]. There is also pharmacological evidence for the presence of P2X7 receptors in rat peritoneal mast cells [39].

Increased local ATP concentrations are likely to be present around mast cells in inflamed tissues due to its release through cell injury or death and platelet activation [40]. Furthermore, mast cells themselves store ATP within secretory granules, which is released upon activation [41]. There is therefore the potential for significant Ca^{2+} influx into mast cells through P2X receptors. Members of the P2X family differ in both the ATP concentration they require for activation and the degree to which they desensitise following agonist activation [37, 38]. This opens up the possibility that by expressing a number of different P2X receptors mast cells may be able to tailor their response to ATP in a concentration dependent manner [37].

Transient receptor potential channels

The TRP family of ion channels contains 28 mammalian members which are subdivided into six main subfamilies based on sequence homology: TRPC (canonical); TRPV (vanilloid); TRPM (melastin); TRPP (polycystin); TRPML (mucolipin); and TRPA (ankyrin) [42]. The channels function predominantly as non-selective cation channels, most of which are permeable to Ca^{2+}. The selectivity of TRP channels for Ca^{2+} however varies greatly both across and within subfamilies. Prior to the discovery of the molecular components of CRAC channels, TRP channels, particularly members of the TRPC and TRPV subfamilies, were thought to be candidates to underlie I_{CRAC}. There are however substantial differences between the characteristics of I_{CRAC} and the functional properties of TRP channels and they are now considered to be separate entities. It remains possible however that TRP channels contribute an additional pathway for Ca^{2+} influx into mast cells. Such a pathway may be store operated though the evidence for this is at best mixed [43].

Both human and rodent mast cells appear to express an array of TRP channels (Table 1). Differentiated human mast cells express mRNA at least for TRPC1 (skin), TRPV2 (skin, cord blood and lung), and TRPM2 (cord blood and lung), and also functional TRPM7 channels (lung) [30, 44]. In addition the human mast cell line HMC-1 expresses TRPV1, -2 and -4 and TRPM7 [44, 45]. TRPM7 expression is also reported in the human mast cell line LAD2 [44]. In rodents TRPC1, -5 and -6, TRPV2, and TRPM4 are expressed in mouse bone marrow-derived mast cells [46–48]. In addition TRPC1, -2, -3, -5 and -7, TRPV1, -2, -4 and -6, TRPM7 and TRPM8 are expressed in RBL-2H3 cells [47, 49, 50].

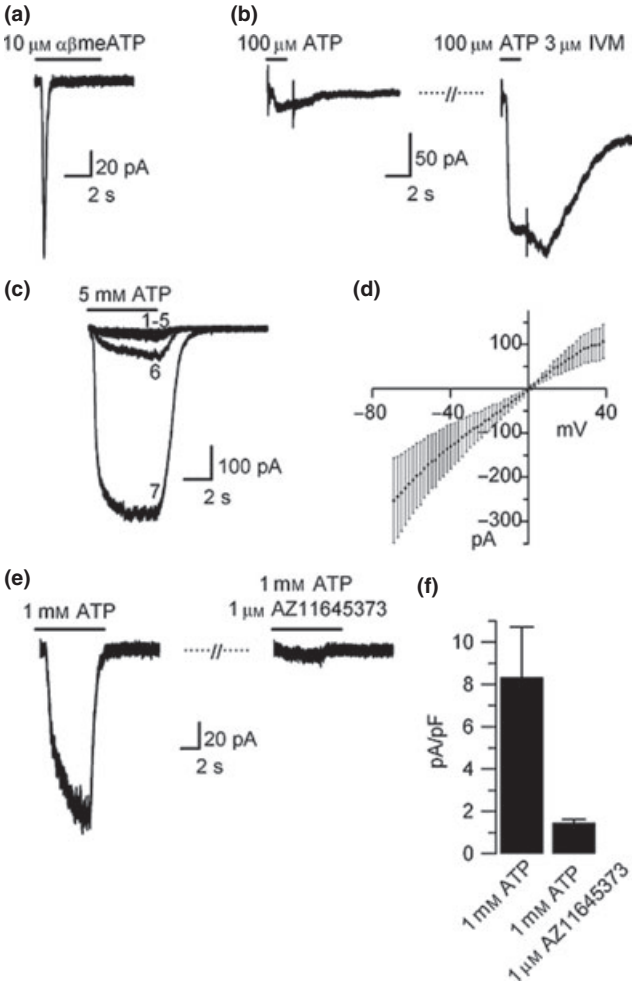

Fig. 3. P2X currents in human lung mast cells. (A) Rapidly desensitizing P2X1-like current seen in response to αβmethylene ATP (αβmeATP). (B) Example of a P2X4-like current elicited by ATP (100 μM) showing a characteristic increase in amplitude and duration following treatment with the P2X4 potentiator ivermectin (IVM) [3 μM superfused for 3 min before application of ATP (100 μM) + ivermectin (3 μM)]. (C) Facilitating P2X7-like currents elicited by repeated application of 5 mM ATP (1 min intervals, labelled 1–6). Maximum facilitation of the response (labelled 7) was achieved following a 1 min continuous application of 5 mM ATP. (D) Current–voltage (I–V) relationship of ATP (5 mM) induced current, means ± SEM (n = 5). (E) Antagonism of ATP (1 mM) induced currents by the P2X7-selective antagonist AZ 11645373 (1 min application of antagonist prior to ATP + antagonist application). (F) Summary of antagonism of ATP (1 mM) induced current by AZ11645373, expressed as mean currents ± SEM (n = 6). Recordings made in low divalent external solution to maximize currents through P2X7 receptors. Nucleotides applied for durations indicated by bars drawn over the traces. Currents were recorded at −60 mV unless otherwise stated. Reproduced with permission from Wiley, from Wareham K, Vial C, Wykes RC, Bradding P and Seward EP, 'Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells', Br J Pharmacol, 2009; 157:1215–1224.

A number of studies support a role for TRP channels in Ca^{2+} influx in mast cells, though these studies have largely been conducted in mast cell lines. Partial knockdown of TRPC1 in mouse bone marrow-derived mast cells impaired both Ca^{2+} influx and degranulation [46]. Similarly, knockdown of TRPC5 in RBL-2H3 cells reduced Ca^{2+} influx and degranulation [49]. Treatment of RBL-2H3 cells with noxious heat to activate TRPV2 channels is reported to promote both Ca^{2+} influx and degranulation from these cells [47]. Treatment of RBL-2H3 cells with measures designed to activate TRPM8 channels (cold, menthol) increased Ca^{2+} influx and degranulation, while partial knockdown of TRPM8 reduced them [50].

The function of TRPM7 channels in mast cells may depend on its permeability to Mg^{2+}, a permeability that is rare amongst ion channels. Functional TRPM7 channels are essential for human lung mast cell and HMC-1 cell survival and may be important for Mg^{2+} homeostasis in these cells [44] (Fig. 4). A similar role for TRPM7 channels has been suggested in DT-40 chicken B cells [51].

Activation of TRP channels as nonselective cation channels results in depolarisation of the cell membrane potential due to influx of cations. In mast cells this would be expected to reduce the driving force for Ca^{2+} influx through CRAC channels and therefore the amount of Ca^{2+} entry. This appears to be the function of TRPM4 channels in mouse mast cells [48]. Bone marrow-derived mast cells from a TRPM4 knockout mouse showed both increased Ca^{2+} entry and increased release of mediators (histamine, leukotrienes and tumor necrosis factor) compared to their wild type counterparts [48]. In addition, mast cell migration is impaired in bone marrow-derived mast cells from the TRPM4 knockout [52]. TRPM4 channels though not themselves permeable to Ca^{2+} are activated by increased cytosolic free Ca^{2+} concentrations allowing the possibility of a regulatory negative feedback loop between TRPM4 and CRAC channels [53]. A role in the determination of cell membrane potential may contribute to the function of other TRP channels expressed in mast cells. It is perhaps noteworthy in this regard that several of the TRP channels expressed in mast cells are activated by changes in cytosolic Ca^{2+} concentration [42].

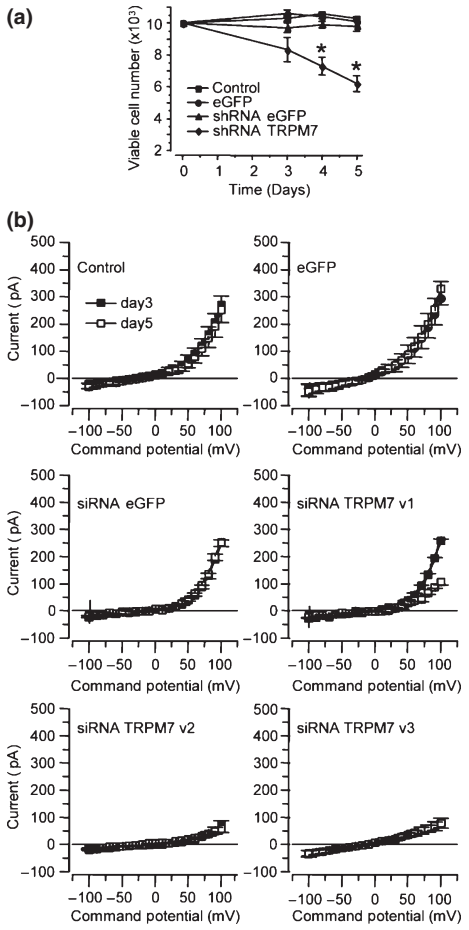

Fig. 4. TRPM7 currents are essential for HLMC survival. (A) Adenoviral transduction of HLMC with shRNA targetted against TRPM7 reduces cell survival, but shRNA against GFP and overexpressed eGFP have no effect. Mean of three experiments performed on HLMC from two donors is shown.$^{*}P < 0.05$ compared with control. (B) Adenoviral transfection of HLMC with shRNA against TRPM7 reduces the size of TRPM7 currents. Results for three different shRNAs (referred to as v1, v2, and v3) are shown. Mean data from two HLMC donors for TRPM7 v2 and v3 ($n = 6$ cells per condition), and from a separate HLMC donor for TRPM7 v1 ($n = 3$ cells per condition). Current amplitudes were unchanged under control conditions. Adapted with permission from Wykes RC, Lee M, Duffy SM, Yang W, Seward EP and Bradding P. J Immunol 2007; 17:4045–52. Copyright 2007 *The American Association of Immunologists, Inc.*

K^+ channels

The Na^+/K^+ ATPase in eukaryotic cells produces high concentrations of intracellular K^+ (~140 mM) compared to the extracellular fluid (~5 mM). Therefore, when K^+ selective channels are open, the outward movement of K^+ down its electrochemical gradient hyperpolarises the cell membrane potential towards the equilibrium potential for K^+ (E_{K^+}) which is approximately −80 mV. By regulating cell membrane potential, K^+ channels have a major influence on cell function through the regulation of Ca^{2+} signalling.

Mammalian K^+ channels fall into one of three structural classes: inward rectifier K^+ channels (Kir), subunits of which possess two transmembrane (TM) domains and a single pore-forming (P) loop; voltage-gated K^+ channels and their relatives which include Ca^{2+}-activated K^+ channels whose subunits possess six TM domains and a single P domain; and tandem pore K^+ channels subunits of which contain four TM and two P domains. Mast cells appear to express all three classes of K^+ channel

depending on the species, although human mast cells appear to only express functional intermediate conductance Ca^{2+} activated K^+ channels ($K_{Ca}3.1$) in the plasma membrane [27, 29, 30, 54–59] (Table 1).

$K_{Ca}3.1$

Though lacking a voltage sensor, $K_{Ca}3.1$channels are structurally related to the voltage-gated K^+ channels. They are activated by a rise in cytosolic free Ca^{2+} concentration. Ca^{2+} binds to calmodulin which is tightly bound near the C-terminus of the $K_{Ca}3.1$ subunit, thus serving as the Ca^{2+} sensor [60]. Under resting conditions with low basal cytosolic Ca^{2+}, channels are closed. In humans, there is strong electrophysiogical evidence for $K_{Ca}3.1$ expression in lung, blood- and bone marrow-derived mast cells [27, 29, 54] (Fig. 5). Human lung mast cells have also been shown to express $K_{Ca}3.1$ at the level of mRNA [29]. In rodents, $K_{Ca}3.1$ expression has been demonstrated only recently in mouse bone-marrow derived mast cells [55]. In addition there is pharmacological evidence for $K_{Ca}3.1$ expression in RBL-2H3 cells [61].

There appears to be a close functional relationship between $K_{Ca}3.1$ and CRAC channels in human mast cells. In these cells, the influx of extracellular Ca^{2+}, presumably through CRAC channels, activates $K_{Ca}3.1$channels by binding to the calmodulin located at its C-terminus [27, 29]. The resulting outward flow of K^+ acts in turn to hyperpolarise the cell membrane thus maintaining the driving force on the CRAC channels. In support of this relationship IgE-dependent Ca^{2+} influx and degranulation is significantly reduced in mouse bone-marrow derived mast cells derived from a $K_{Ca}3.1$ knockout mouse [55]. In addition the $K_{Ca}3.1$ knockout mouse has less severe systemic anaphylactic reactions [55]. In human lung mast cells block of $K_{Ca}3.1$channels by charybdotoxin reduces IgE-dependent degranulation while the $K_{Ca}3.1$ channel opener 1-ethyl-2-benzimidazolinone (1-EBIO) enhances IgE-dependent Ca^{2+} influx and degranulation [27, 29].

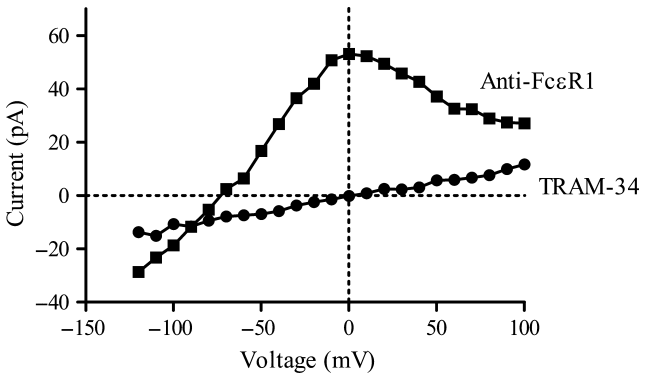

Fig. 5. Representative whole cell currents recorded from a single human lung mast cell. Currents were recorded with an external K^+ concentration of 5 mM, 2 min following FcεR1-dependent activation (■) and following the addition of 200 nM 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole (TRAM-34), a specific $K_{Ca}3.1$channel blocker (●). The I-V relationship obtained following mast cell activation is highly characteristic of that from $K_{Ca}3.1$channels.

In addition, $K_{Ca}3.1$ is important for human lung mast cell chemotaxis which is attenuated in the presence of $K_{Ca}3.1$ channel blockers [62]. This may be as a result of the impairment of the regulation of cell volume [55, 63]. The β_2-adrenoreceptor, the adenosine A_{2A} receptor and the EP_2 prostanoid receptor regulate $K_{Ca}3.1$ channels in human lung mast cells, with receptor activation resulting in channel closure [64–66]. This may explain at least in part the effect of activation of these receptors on mast cell secretion and chemotaxis [65–67].

The important role of $K_{Ca}3.1$ channels in mast cell function has led to its investigation as a target for asthma therapy [68]. $K_{Ca}3.1$ expression is not limited to mast cells but is widespread and it has been implicated in a number of other disease conditions in addition to asthma e.g. inflammatory bowel disease, atherosclerosis and sickle cell anaemia [69]. Use of $K_{Ca}3.1$ blockers in animal models of these disease states supports their therapeutic potential, see for example Toyama et al. [70]. One $K_{Ca}3.1$ blocker, ICA-17043 (also known as Senicapoc) has entered clinical trials, initially as a potential treatment for sickle cell anaemia. The drug was found to be safe and well tolerated, though the trials were eventually stopped due to lack of efficacy in reducing sickle cell crises [71]. ICA-17043 was subsequently found to inhibit the late airway response and the development of bronchial hyper-responsiveness following allergen challenge in a sheep model of asthma. This led to the use of ICA-17043 in two phase II clinical trials of patients with asthma. In both, the drug showed a favourable safety and tolerability profile. However in one of these trials studying the effect of ICA-17043 on exercise-induced asthma, no improvement in lung function was observed after four weeks of treatment [Icagen Inc, http://www.icagen.com]. In a second trial, there was no effect on the early reaction to allergen challenge which is mast cell-dependent, but there was a 30% reduction in the late asthmatic response.

Kir channels

Human mast cells express a number of different inward rectifier K^+ channels at the mRNA level. Expression of Kir2.1 and Kir2.4 has been observed in skin mast cells while Kir2.4 and Kir3.4 expression has been observed in lung mast cells [30]. In addition Kir2.1–2.4, 3.1, 3.2, 6.1 and 6.2 expression was detected in a study of human lung mast cells from a single donor [56]. In rodents, bone marrow-derived mast cells and RBL-2H3

cells express a robust inwardly rectifying K^+ current which has been ascribed to the expression of Kir2.1 in these cells [57, 72, 73]. However, we have never observed a Kir current in any human mast cell (cord skin, lung, adult peripheral blood-derived, bone marrow-derived) [27–29].

Tandem pore channels

Tandem pore K^+ channels give rise to so called background or leak currents which though small are important in stabilising the resting cell membrane potential [74]. There is evidence to suggest that TWIK-2 and TASK-2 are expressed in murine mast cells [58, 59], but Bradding et al. [30] did not detect any expression of a limited panel of tandem pore K^+ channels in human blood, skin or lung mast cells using gene arrays. There are currently no reports of tandem pore K^+ currents having been recorded in either rodent or human mast cells.

Chloride channels

It is only relatively recently that it has been fully appreciated that chloride (Cl^-) is not in electrochemical equilibrium but is actively transported in cells [75]. It is perhaps for this reason Cl^- channels have in general not been as extensively studied as cation channels [75]. This certainly appears to be the case for mast cells where there are relatively few studies of Cl^- channel expression and function and the molecular correlates underlying Cl^- conductances in mast cells remain largely to be identified. Cl^- channels may be broadly classified into five major families: voltage gated Cl^- channels (ClC); CFTR; anoctamins/TMEM16; bestrophins and neurotransmitter-gated GABA and glycine receptors. Of the nine mammalian members of the ClC family only four (ClC1, -2, -Ka and Kb) are actually channels. The remainder are functional as Cl^--H^+ exchangers, predominantly in intracellular membranes. At least two members of the anoctamin/TMEM16 family (anoctamin 1 and −2) are functional as plasma membrane Ca^{2+}-activated Cl^- channels [76, 77]. Anoctamin 7 is also reported to be a Ca^{2+}-activated Cl^- channel, but may function intracellularly [78]. Anoctamin 6 in contrast does not appear to be regulated by Ca^{2+} but has recently been shown to be a component of outwardly rectifying Cl^- channels (ORCC) [79]. The function of the remaining six members of the family is not clear, but they may function predominantly as intracellular channels [80]. The precise function of bestrophin channels is similarly unclear, but members of the family have also been suggested to function as Ca^{2+}-activated Cl^- channels or alternatively as volume-regulated Cl^- channels [75].

To date the only reported expression of a ClC channel (ClC2) in mast cells is from a rat mast cell line (RCMC) [81] (Table 1). Several of the Cl^--H^+ exchanger members of the ClC family were also found to be expressed in these cells. In contrast only ClC7, a Cl^--H^+ exchanger, was reported to be expressed in rat peritoneal mast cells [81]. Similarly only ClC Cl^--H^+ exchangers (ClC-3, -5, -7) have been identified in human mast cells [28, 30]. CFTR has been found to be expressed in rat peritoneal mast cells, and the RCMC and LAD2 cell lines [82, 83]. Expression data for the anoctamin/TMEM16 and bestrophin family is currently lacking in mast cells.

A number of Cl^- conductances have been described in both human and rodent mast cells using electrophysiological techniques for which the underlying molecular correlate is currently unknown. These include Ca^{2+}-activated and volume regulated-Cl^- conductances and a strongly outwardly rectifying Cl^- conductance showing voltage dependence in both human lung mast cells and the HMC-1 cell [27, 28]. In addition, a moderately outwardly rectifying Cl^- conductance has been described in human lung mast cells, that develops only relatively slowly following mast cell activation, taking about 10 min to peak [27]. A similar current has been observed in rodent mast cells and RBL-2H3 cells [84–86]. CFTR and members of the anoctamin/TMEM16 and bestrophin families may account for at least some of these conductances.

Mast cell Cl^- channels potentially play an important role in the regulation of cell membrane potential. An influx of Cl^- would act to hyperpolarise the cell membrane, which would act to maintain the driving force for Ca^{2+} influx through inwardly-rectifying CRAC

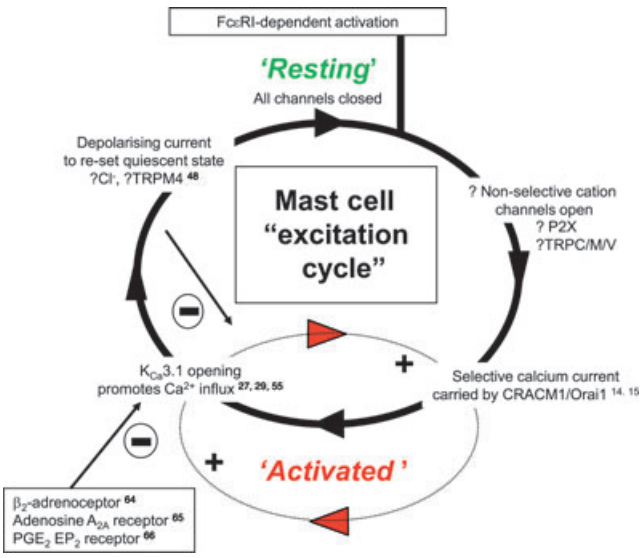

Fig. 6. Hypothetical cycle of ion channel activation in mast cells following FcεRI -dependent activation, and the inhibitory role of Gs-coupled stimuli. Figures in superscript refer to references in the text.

channels and therefore enhance cell mediator release. In support of this, degranulation from rodent mast cells and RBL-2H3 cells is reduced in the presence of the Cl^- channel blockers 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), 4,4′-diisothiocyanato-2,2′-disulphonic acid (DIDS) and the CFTR blocker diphenylamine-2-carboxylate [82, 85, 87, 88]. In addition, sodium cromoglycate reduces both the IgE-dependent Cl^- conductance and degranulation in RBL-2H3 cells [85]. Law et al. [86] have used a microelectrode array technique to measure Cl^- influx in anti-IgE activated rat peritoneal mast cells. Degranulation was found to be reduced in the presence of low Cl^-- or Cl^--free buffers [86]. It should be noted however that the direction of Cl^- currents in mast cells is a function of the intracellular Cl^- concentration and cell membrane potential. Friis et al. [87] have estimated the intracellular Cl^- concentration of rat peritoneal mast cells to be of the order of 29 mM, consistent with Cl^- influx during mast cell activation. Duffy et al. [28] however, have estimated the intracellular Cl^- concentration of HMC-1 cells to be significantly higher at 66 mM. Such a concentration would be consistent with Cl^- efflux during activation and as a result, membrane depolarisation.

The role of Cl^- channels at intracellular locations is not well understood in mast cells. In other cells intracellular Cl^- channels play a role in the exocytosis of secretory granules [89]. A similar role in mast cells remains plausible.

Conclusion and future perspectives

Recent years have seen a substantial increase in our understanding of the role of ion channels in mast cell function. The identification of CRAC channels as the main pathway for Ca^{2+} influx during FcεRI-dependent mast cell activation has been central to this increased understanding. This in turn has led to the identification of two feedback loops by which Ca^{2+} influx and therefore cell activation and mediator release may be regulated. The activation of $K_{Ca}3.1$ channels as a result of an increased cytosolic Ca^{2+} concentration acts to hyperpolarise the cell membrane, thereby maintaining the driving force for Ca^{2+} influx through CRAC channels. TRPM4 channels are also activated by the influx of Ca^{2+} in mice, but in contrast act to depolarise the cell membrane and so reduce the driving force for Ca^{2+} influx. In the future, further regulatory feedback loops between mast cell ion channels may become apparent. This is likely to require a greater understanding of the role of the remaining TRP channels expressed in mast cells together with the identification of the molecular correlates underlying the various Cl^- conductances that have been identified in mast cells. Because mast cells recover and regranulate following FcεRI-dependent activation, in 2002 we proposed the presence of a mast cell excitation cycle of channel activity taking the cell from rest, through activation and back to rest [90]. This is analogous to the action potential in neurons, but rather than occurring over milliseconds is likely to occur over several hours. Based on the experimental evidence accumulated in the ensuing decade, this cycle is now less speculative, and can be updated with clearly identified channel proteins (Fig. 6).

All of the channels identified in mast cells are potential targets for the treatment of allergic diseases. The recent use of the $K_{Ca}3.1$ blocker ICA-17 043 in clinical trials of patients with asthma though not showing clear efficacy, underlines the promise of channel blockers as therapeutic agents in this and related conditions. In the future the development of a specific pharmacology for CRAC channels together with an understanding of the individual roles of ORAI1, ORAI2 and ORAI3 in mast cells may allow the development of a novel potent inhibitor of mast cell secretion for which there is much clinical need.

Conflict of interests

Peter Bradding was a consultant for Icagen Inc and has received financial support from GlaxoSmithKline for attendance at scientific meetings and for speaker fees. Ian Ashmole does not have any conflicts of interests to declare.

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