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Ion channels in T cells: from molecular pharmacology to therapy

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Summary

Ion channels of a variety of cell types, such as cardiac and smooth muscle cells and neurons, serve as targets for many drugs used in therapy. T cells also express an assortment of ion channels that are in the focus of intensive research, as they may provide efficient ways to specifically manipulate T cell function and, consequently, immune responses. T cell activation relies on the operation of voltage-gated and Ca^{2+}-activated potassium channels and Ca^{2+} release-activated Ca^{2+} channels. Many peptide toxin and small molecule blockers of these channels are known, but inhibitors of even higher affinity and selectivity would be needed for safe and effective clinical use. The recent discovery that the expression pattern of potassium channels in T cells is subset specific emphasizes the potential that these proteins have in immunomodulation. Compounds that could suppress T cells involved in autoimmunity without affecting T cells in normal immune responses would be of enormous value. In this paper the basic properties of these channels and compounds known to influence their operation are reviewed.

Key words: **T cell • activation • potassium channels • CRAC channels • channel blockers**

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INTRODUCTION

T cells are known to express a wide variety of ion channels, including voltage-gated and Ca^{2+}-activated K^+ channels, Ca^{2+} release-activated Ca^{2+} (CRAC) channels, osmotically activated small-conductance and possibly two other types of larger-conductance Cl^- channels, and even the expression of a non-voltage-gated L-type Ca^{2+} channel and voltage- and ligand-gated Na^+ channels was also suggested[59, 71]. The role of these channels in cellular functions has been extensively studied mostly relying on the patch-clamp technique, and the involvement of ion channel activity in T cell activation, proliferation, volume regulation, and apoptosis has been recognized[44, 53]. Some of the earliest observations following the discovery of voltage-gated K^+ channels in T lymphocytes implicated their participation in T cell proliferation[7, 11]. Since T cells are key players in cellular immunity, the pharmacological modulation of their activity promises to open up new possibilities in controlling certain immune responses, especially autoimmune disorders.

Ion channels in a variety of cell types have long been popular targets for therapeutic compounds, such as local anesthetics, sedatives, and antiarrhythmic and vasodilator agents. Due to the diverse repertoire of T cell ion channels and their crucial roles in cellular processes, these channels were also destined to be in the focus of pharmacological research in the field of immunology. The increasing body of knowledge gathered about the structure, biophysical properties, and cell biological function of T lymphocyte ion channels stimulated the search for compounds that selectively bind to these channels with high affinity and consequently affect T cell function to achieve a desired clinical effect. In turn, the compounds discovered this way proved invaluable research tools in mapping the structure and exploring the biophysical and cell biological characteristics of the channels, in addition to their potential use in therapy.

In this review we give an overview of pharmacological agents of diverse chemical structure that alter the function of different T cell ion channels and discuss how these may be employed in medicine.

ION CHANNELS INVOLVED IN T CELL ACTIVATION

The signal transduction pathway leading to the activation of T cells begins with the binding of the antigenic peptide-loaded major histocompatibility complex molecule of an antigen-presenting cell to the T cell receptor-CD3 complex in the plasma membrane of T cells. This first signaling step results in the activation of kinases and phospholipase C (PLC)γ. Following activation, PLCγ cleaves phosphatidylinositol 4,5-biphosphate (PiP_2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), and at this point two signaling pathways of lymphocyte activation diverge. DAG activates protein kinase C (PKC), which phosphorylates several intracellular substrates and triggers transcription via the assembly of the Fos/Jun (AP-1) transcription factor. IP_3 releases Ca^{2+} from the endoplasmic reticulum (ER) by binding to its receptor, which raises the free Ca^{2+} concentration ($[Ca^{2+}]_i$) from the resting level of 100 nM to a peak of about 500 nM. This initial rise in $[Ca^{2+}]_i$ is a necessary but not sufficient criterion of the completion of the activation process. Full execution of the signal transduction cascade requires sustained elevation of $[Ca^{2+}]_i$, which is accomplished by Ca^{2+} influx from the extracellular space through CRAC channels. The opening of these channels is initiated by the emptying of the intracellular Ca^{2+} stores. The Ca^{2+} signal activates the phosphatase calcineurin, which dephosphorylates the transcription factor NF-AT (nuclear factor of activated T cells). NF-AT then accumulates in the nucleus and acts synergistically with AP-1 from the PKC pathway by forming complexes to regulate gene expression[37].

The sustained Ca^{2+} signal requires long-lasting Ca^{2+} influx through the CRAC channels. This, however, can only be achieved by the participation of the voltage-gated Kv1.3 and the Ca^{2+}-activated IKCa1 potassium channels, since Ca^{2+} influx depolarizes the cells and reduces the driving force for further Ca^{2+} entry. Kv1.3 channels are activated by membrane depolarization and IKCa1 channels by the rise of $[Ca^{2+}]_i$, both of which are accomplished by Ca^{2+} influx. The activation of the two K^+ channels restores the sufficiently negative membrane potential required for sustained Ca^{2+} entry. Thus, these two potassium channels are as crucial to the activation process as the CRAC channel itself. This simple view of the participation of Kv1.3 channels in lymphocyte activation has been challenged recently by showing that Kv1.3 channels are in the physical vicinity of the T cell receptor-CD3 complex[46] and that Kv1.3 channels are recruited into the immunological synapse, the signaling complex formed between cytotoxic and target cells[51]. The physical vicinity of the channels and the protein kinases in the signaling complex may allow a reciprocal regulation of these proteins and thereby modulate the efficacy of the antigen recognition process[52].

THE KV1.3 CHANNEL

Kv1.3 is a voltage-gated channel of the *Shaker* family with high selectivity for K^+. It is assembled from 4 identical subunits[38], each containing 6 membrane-

-spanning α-helices. Part of the linker section between the 5th and 6th transmembrane segments from each subunit forms the majority of the ion conduction pore through the membrane. A short, narrow region of the pore near the extracellular mouth of the channel is called the selectivity filter, which lends the channel its high selectivity. Here dehydrated potassium ions pass through in single file at a very fast rate. Inward of the selectivity filter is a water-filled cavity, and near the intracellular end of the pore is the gate that opens and closes the channel[13]. This gate in Kv1.3 is controlled by transmembrane voltage in such a way that the channels begin to open at membrane potentials of –60 to –50 mV, and their opening probability steeply increases with increasing depolarizations. During long-lasting depolarized periods, Kv1.3 channels enter a non-conducting inactivated state, which results from a cooperative conformational change of the 4 subunits near the extracellular mouth of the pore[48, 50]. Gating transitions of *Shaker* channels are affected by changes in the composition of the intra- and extra-cellular solutions[35, 68, 69, 74, 78] and that of the cell membrane as well[23, 39, 47, 73].

Resting T cells express 2-300 Kv1.3 channels that are the main determinants of the membrane potential in these cells[11]. Membrane potential control by Kv1.3 channels is accomplished by a negative feedback mechanism, since depolarization opens the gate, resulting in K^+ efflux and consequent hyperpolarization, which then closes the gate. The pivotal role of Kv1.3 channels in the membrane potential control and mitogenesis of T lymphocytes was demonstrated by the use of channel-blocking compounds of various chemical structure[7, 11, 12, 19, 76]. These agents blocked Kv1.3 channels, depolarized the cells, and inhibited thymidine incorporation, interleukin (IL)-2 production, and proliferation. These classical blockers, such as tetraethylammonium and 4-aminopyridine, were non-specific for Kv1.3 or IKCa1 channels and had low affinities for Kv1.3, so to clarify the role of each of the channels in T cell activation, more selective blockers with higher affinity were needed. This need was fulfilled by the discovery that the venoms of a variety of animals, especially scorpions, blocked these channels and often contained several high-affinity peptide toxin components that could be purified from the venom[56, 57, 58]. Charybdotoxin (ChTx), a 37-amino acid peptide isolated from the venom of the scorpion *Leiurus quiquestratus*[42], was the first toxin shown to block voltage-gated and IKCa1 channels of lymphocytes with affinities several orders of magnitude higher than those of the classical blockers[21, 66]. ChTx blocked Kv1.3 currents and suppressed IL-2 production and lymphocyte proliferation at nanomolar concentrations[17, 62].

Since ChTx blocked both Kv1.3 and IKCa1 channels, it did not provide a way to differentiate between the involvement of the two channels in the activation process. Soon two peptide toxins selective for Kv1.3 over IKCa1, noxiustoxin and margatoxin, were discovered and found to depolarize resting T cells and prevent their activation similarly to ChTx. This observation proved that in resting T cells the membrane potential is set by Kv1.3 channels and the contribution of IKCa1 channels is negligible[33]. Margatoxin was also used to first demonstrate that the inhibition of Kv1.3 suppresses immune responses *in vivo*[30].

In addition to these toxins, several other high-affinity toxin blockers of K^+ channels were identified, including kaliotoxin[9], agitoxins 1, 2 and 3[18], maurotoxin[65], Heterometrus spinnifer toxin HsTX1[32], and Pi toxins 1, 2, and 3[56, 58]. These toxins block Kv1.3 by a simple pore-blocking mechanism: one toxin molecule binds to one channel and occludes the ion conduction pore much like a cork in a bottle. The very high affinity of these toxins for the channel arises from the large complementary contact surfaces between the toxin and the channel. Analysis of data obtained by complementary mutagenesis of Kv1.3 and some toxins has revealed the existence of a shallow vestibule at the external entrance to the pore which is approximately 28–32 Å wide and 4–8 Å deep. The contact surface between the channel and the docking toxin is not restricted to the amino acids forming the base of this vestibule; amino acids located at the side walls of the vestibule also contribute to the high-energy binding[20]. For even higher affinity, most toxins also employ a positively charged lysine residue that protrudes into the pore and is "immersed" into the transmembrane electric field. Generally an aromatic residue nearby is also present, forming a diad with the lysine, which seems to be a hallmark of high affinity K^+ channel-blocking toxins. However, there are exceptions to these rules. A recently discovered toxin that blocks Kv1.3 with nanomolar affinity lacks both the lysine and the aromatic residue[3]. Hanatoxin is a toxin isolated from spider venom that does not use the usual pore-blocking mechanism. As many as four hanatoxin molecules can bind to voltage-gated K^+ channels simultaneously outside the pore region and modify their voltage-dependent gating[72].

Although the binding affinity of most of these toxins is very high for Kv1.3 (dissociation constants in the pM–nM range), their selectivity for Kv1.3 over K^+ channels, especially other members of the Kv1 family, is not sufficient for safe therapeutic application without the risk of serious side effects. In order to produce selective blockers for a particular channel one must take advantage of the minute structural dif-

ferences between closely related channel types. For this, the interaction surface between the toxin and the channel must be known in fine detail, and then mutations must be introduced into the toxin sequence to tailor it to the unique properties of the targeted channel. The selectivity of ShK, a toxin extracted from a sea anemone, which blocks several Kv1 family members with low, picomolar affinity, was increased for Kv1.3 using this strategy. The critical lysine in ShK was replaced by the positively charged, non-natural amino acid diaminopropionic acid (ShK--Dap)[22] based on topological information of the channel pore and the toxin to create a polypeptide that was slightly less effective at blocking Kv1.3 than the wild-type toxin, but which was much more selective[26]. A more recent study, however, found ShK-Dap[22] much less effective at inhibiting T cell activation than ShK[41]. The usefulness of ShK-Dap[22] as a potential immunosuppressant was also questioned because ShK-Dap[22] was shown to block heteromultimeric Kv1.1-Kv1.2 channels with very high affinity, raising the possibility of undesired side effects.

Besides peptide toxins, many small-molecule blockers of Kv1.3 have been discovered[10]. Due to the smaller contact area and less specific interactions with the channel, small-molecule blockers tend to have lower affinity and selectivity for Kv1.3 than peptide toxins. Many such blockers of diverse chemical structure have been identified, with varying blocking efficiencies and selectivities. Among the first small-molecule blockers shown to inhibit K1.3 channels at submicromolar concentrations with the concomitant suppression of IL-2 production were the iminodihydroquinolines WIN 17317-3 and CP-319,818[45, 83]. However, both compounds lacked the required selectivity, as they also blocked voltage-gated Na channels and Kv1.4, a cardiac and a neuronal Kv channel, respectively[45, 83]. Classical immunosuppressants were also found to block lymphocyte K^+ channels[49, 80], but these agents affect a plethora of other ion channels, transporters, and other biomolecules as well. Alkoxypsoralens that lack the phototoxicity characteristic of psoralens and block Kv1.3 with high affinity were synthesized, but need further development due to their equally high affinity for Kv1.2 channels[86]. Other compounds shown to inhibit Kv1.3 channels in the micromolar-submicromolar range, but not possessing the desired selectivity over other Kv1.x family members, include UK-78,282 (a piperidine compound), correolide (a nortriterpene of plant origin), certain furo- and pyranoquinoline derivatives, and khellinone (acetyl-dimethoxy-hydroxybenzofuran) derivatives[2, 5, 25, 29]. A disubstituted cyclohexyl derivative, named PAC, blocked Kv1 family members with 300 nM affinity and a Hill coefficient of 2. In an effort to increase its selectivity, substitutions at the C-1 ketone of PAC were made to produce cis and trans isomer pairs. The trans isomers were found to distinguish between members of the Kv1 family based on their rates of C-type inactivation[67]. A very promising molecule was identified in a series of synthesized 5-phenylalkoxypsoralens, termed Psora-4, which blocked Kv1.3 with an EC_{50} of 3 nM and a Hill coefficient of 2 and displayed great selectivity over other Kv1 family members and many other channel types tested, except the cardiac channel Kv1.5[79]. Although its modest, 2.5-fold, selectivity for Kv1.3 over Kv1.5 raises concerns of potential side effects, Psora-4 displayed no signs of acute toxicity when it was administered to rats.

The blocking mechanism and the site of action of these compounds are much more varied than those of peptide toxins. Some bind to the extracellular vestibule of the channel, others bind extracellularly, but outside the pore region[77], and yet others bind to the internal water-filled cavity below the selectivity filter[24]. The binding stoichiometry of the blockers also varies: one[25, 45], two[2, 67, 79] or even four[77] blocking molecules may bind to a single channel protein.

Thus, even though the affinity and selectivity of the small-molecule blockers needs to be improved, many templates exist as starting points for developing compounds of advantageous properties. The different mechanisms and sites of action may be exploited for various clinical applications.

THE IKCA1 CHANNEL

Along with Kv1.3, the other potassium channel responsible for cation efflux during the sustained phase of the Ca^{2+} signal that maintains the driving force for Ca^{2+} influx is the intermediate-conductance Ca^{2+}-activated K^+ channel, hIKCa1. It is encoded by the hKCa4 gene and is also referred to as $K_{Ca}3.1$[34]. The basic structure of this channel is very much like that of Kv1.3, with the major difference of the missing voltage-sensor region. Instead, a molecule of the Ca^{2+}-binding protein calmodulin binds to each of the 4 channel subunits and they cooperatively act as a Ca^{2+}-sensor to control the gating of the channel[14, 87]. The channel is activated by the rise in $[Ca^{2+}]_i$ with a Hill coefficient of 4, implying very sensitive dependence on $[Ca^{2+}]_i$. The midpoint of the activation curve is at about 300–400 nM $[Ca^{2+}]_i$[21, 81].

Due to the similar pore regions, many peptide blockers of Kv1.3 also block IKCa1 channels, often with similar potencies. Nevertheless, there are peptide toxins preferentially binding to one of these channels.

A potent blocker of IKCa1 that is also highly selective for IKCa1 over Kv1.3 is maurotoxin, a short polypeptide purified from scorpion venom[6, 28]. Significantly more IKCa1-selective blockers have been identified among small-molecule compounds. The antimycotic clotrimazole exhibits nanomolar potency for IKCa1 channels and was used in therapy, but it also inhibited cytochrome P450 enzymes, producing side effects. To overcome this disadvantageous property, derivatives of clotrimazole were synthesized that did not inhibit cytochrome P450, yet blocked IKCa1 channels with high affinity and selectivity. One of these compounds was TRAM-34, a pyrazole-substituted triarylmethane, which blocked cloned and native IKCa1 channels of human T cells with a K_d of 20–25 nM and displayed a 200- to 1500--fold selectivity over other ion channels[85]. The other compound also developed from clotrimazole was ICA17043, which blocked the Gardos channel of red blood cells with nanomolar potency and inhibited the dehydration of cells in mice with sickle cell disease[70]. Another group of compounds, a triazole, a cyclohexadiene, and rac-11 (a 4-phenyl-4H-pyran derivative), was shown to potently block IKCa1 and may represent a new pharmacological approach to attenuate acute brain damage caused by traumatic brain injury[40, 75].

The role of IKCa1 channels in setting the membrane potential in resting T cells and in other immune cells is negligible due to their low number (5–20) and a $[Ca^{2+}]_i$ level that is below their activation threshold[21, 22]. Even during Ca^{2+} signaling when the elevated $[Ca^{2+}]_i$ activates the few channels present, their effect on the membrane potential is low. However, following activation, naïve ($CCR7^+CD45RA^+$ phenotype, based on the classification of T cells by the expression of the chemokine receptor CCR7 and the phosphatase CD45RA) and central memory ($CCR7^+CD45RA^-$) cells upregulate IKCa1 channels to as many as 500 per cell[4, 84]. These cells then become reliant on IKCa1 channels in membrane potential control and, accordingly, blockers of IKCa1 are required to suppress further activation of these cells. In contrast, activated effector memory (T_{EM}) cells ($CCR7^-CD45RA^-$) upregulate Kv1.3 channels to ~1500 per cell with little increase in IKCa1 expression. As a result, the proliferation of activated effector memory cells is efficiently inhibited by Kv1.3 blockers.

T cells involved in autoimmune reactions such as multiple sclerosis or type-1 diabetes were found to be mostly of the T_{EM} phenotype expressing high numbers of Kv1.3 channels[36, 82]. The differential expression of K^+ channels by different T cell phenotypes makes it possible to manipulate only a subset of T cells using specific channel blockers.

THE CRAC CHANNEL

Following the initial Ca^{2+} release induced by IP_3 binding to its receptor in the membrane of the endoplasmic reticulum, Ca^{2+} enters the cell through CRAC channels. Ca^{2+} current in Jurkat cells activated by thapsigargin was indistinguishable from that activated by the cross-linking of the T cell receptor, suggesting that these channels are activated by the depletion of the internal Ca^{2+} stores rather than directly by IP_3[91]. Similar results were obtained in human T cells, which exhibited low amplitude Ca^{2+}--selective currents in response to intracellular store depletion by intracellular perfusion of IP_3 or thapsigargin or extracellular perfusion of ionomycin[54].

The CRAC channel has high selectivity for Ca^{2+}, but also conducts some other divalent ions, e.g. Ba^{2+}, and it is not blocked by internal Mg^{2+} as a similar low--conductance cation channel, Mg^{2+}-inhibited cation (MIC). The MIC channel, which has somewhat similar properties but much greater conductance, used to be often mistaken for CRAC[27]. The single-channel conductance of CRAC is very low: 21–24 fS for Ca^{2+}, and ~200 fS for Na^+ in divalent free solutions, which makes its electrophysiological characterization difficult[31, 61]. The CRAC channel has not been cloned yet, so its molecular identity is still unknown. At present the most likely candidates are members of the transient receptor potential cation channels, which are expressed in a variety of tissues and cell types and perform a wide range of functions[43], but none of these candidates has been clearly confirmed as the real CRAC channel.

The linking mechanism between store depletion and channel opening is also elusive, although several different models have been proposed. Diffusion of a "calcium influx factor" to transmit the signal[64], insertion of functional CRAC channels into the membrane from vesicles following store depletion[15], and direct physical coupling between the IP_3 receptor and the CRAC channel have all been suggested as possible mechanisms. A very recent model hypothesizes that Ca^{2+} released from internal stores is taken up by nearby mitochondria, which in turn release adenosine diphosphate ribose (ADPR) or nicotinamide adenine dinucleotide (NAD^+). Then ADPR (or NAD^+) diffuses to the membrane and opens store-operated Ca^{2+} channels[1]. It is possible that several different mechanisms, such as conformational coupling or signaling by a diffusible factor, exist for initiating capacitative Ca^{2+} entry, and the actual

mechanism used may vary by cell type or may even occur by a combination of different mechanisms[63].

The pharmacology of CRAC channels lags behind that of T cell potassium channels. The organic blocker SKF-96365 was found to inhibit the Ca^{2+} current in a dose-dependent fashion in Jurkat T cells at micromolar concentrations and inhibit [^{3}H]-thymidine incorporation and IL-2 synthesis in peripheral blood lymphocytes[8]. More recently, the reversible block of CRAC channels by SKF-96365 was verified[31, 61]. However, neither the potency nor the selectivity of SKF-96365 is adequate to consider it a potential immunosuppressive agent, since it was shown to block other cation channels as well[88]. Capsaicin and its analogs also blocked Ca^{2+} entry through ion channels in Jurkat cells, but the experimental conditions did not rule out the involvement of MIC channels, so the compound's effect on CRAC channels remains doubtful[16]. Furthermore, capsaicin also blocked Kv1.3 channels in the same study at similar potency, questioning its usefulness as a CRAC inhibitor. 2-Aminoethoxydiphenyl borate appears to have opposite, concentration-dependent effects on CRAC channels and also affects other molecules involved in Ca^{2+} signaling[55, 60].

Recently, the bistrifluoromethyl pyrazole derivative BTP2 was shown to inhibit CRAC channels in T cells with an IC_{50} of about 10 nM. It also suppressed Ca^{2+} signals and the proliferation of T lymphocytes. It demonstrated high specificity for CRAC channels by being ineffective on Ca^{2+} pumps, mitochondrial Ca^{2+} signaling, ER Ca^{2+} release, K^+ and even MIC channels[90]. Thus, BTP2 is the first compound to block CRAC channels with high affinity and selectivity. The compound was found to act from the outside; however, its on-rate seems to be very slow, requiring hours of pre-incubation for full effect and the mechanism of action is also vague, as reversibility of the block has not been demonstrated. Its selectivity should also be further tested on other ion channels with vital roles, such as cardiac voltage-gated K^+ channels. Diethylstilbestrol (DES) also reversibly blocked CRAC currents in rat basophilic leukemia cells with an IC_{50} ≈0.5 μM and was ineffective on MIC channels[89]. On the other hand, DES is also known to block a variety of other channels and pumps.

Thus, a truly potent and selective blocker of CRAC channels is yet to be discovered. The large pool of known peptide toxins may serve as the source for further research, or the inhibitors of CRAC identified thus far could serve as templates for structure-based design of blockers with higher efficacy. This, however, would require structural information, which relies on the identification of the channel molecule. Finding highly selective blockers of CRAC does not appear to be an easy task due to the enormous variety and very wide tissue distribution of low-conductance cation channels sharing similar biophysical properties with CRAC.

CONCLUDING REMARKS

The key roles that ion channels play in T cell activation make them promising targets of future drugs used in the therapy of autoimmune diseases. Great advances have been achieved in the field of K^+ channel inhibitors with the discovery of new peptides and, especially, new small molecules synthesized based on structural information of the channel and the blocking molecule. Many inhibitors of Kv1.3 and IKCa1 channels are now available, but further improvement of their affinity and selectivity is required if they are to be used in therapy. Nevertheless these compounds can serve as starting points for compounds with superior properties. The discovery that channel expression in T cells is subset specific especially underlines the importance of highly selective blockers which could target cell subsets that are responsible for the pathogenesis of autoimmune diseases.

We will probably have to wait a few more years for the advent of CRAC channel blockers of similar potential due to the limited information about the channel itself. Once the structure and the tissue distribution of CRAC channels are known, the design of a new class of immunosuppressants may become possible.

REFERENCES

1. Ayub K. and Hallett M. B. (2004): The mitochondrial ADPR link between Ca^{2+} store release and Ca^{2+} influx channel opening in immune cells. FASEB J., 18, 1335–1338.
2. Baell J. B., Gable R. W., Harvey A. J., Toovey N., Herzog T., Hansel W. and Wulff H. (2004): Khellinone derivatives as blockers of the voltage-gated potassium channel Kv1.3: synthesis and immunosuppressive activity. J. Med. Chem., 47, 2326–2336.
3. Batista C. V., Gomez-Lagunas F., Rodríguez de la Vega R. C., Hajdu P., Panyi G., Gaspar R. and Possani L. D. (2002): Two novel toxins from the Amazonian scorpion Tityus cambridgei that block Kv1.3 and Shaker B K^+-channels with distinctly different affinities. Biochim. Biophys. Acta, 1601, 123–131.
4. Beeton C., Wulff H., Barbaria J., Clot-Faybesse O., Pennington M., Bernard D., Cahalan M. D., Chandy K. G. and Beraud E. (2001): Selective blockade of T lymphocyte K^+ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. Proc. Natl. Acad. Sci. USA., 98, 13942–13947.
5. Butenschon I., Moller K. and Hansel W. (2001): Angular

methoxy-substituted furo- and pyranoquinolinones as blockers of the voltage-gated potassium channel Kv1.3. J. Med. Chem., 44, 1249–1256.

6. Castle N. A., London D. O., Creech C., Fajloun Z., Stocker J. W. and Sabatier J. M. (2003): Maurotoxin: a potent inhibitor of intermediate conductance Ca^{2+}-activated potassium channels. Mol. Pharmacol., 63, 409–418.

7. Chandy K. G., Decoursey T. E., Cahalan M. D., McLaughlin C. and Gupta S. (1984): Voltage-gated potassium channels are required for human T lymphocyte activation. J. Exp. Med., 160, 369–385.

8. Chung S. C., McDonald T. V. and Gardner P. (1994): Inhibition by SK&F 96365 of Ca^{2+} current, IL-2 production and activation in T lymphocytes. Br. J. Pharmacol., 113, 861–868.

9. Crest M., Jacquet G., Gola M., Zerrouk H., Benslimane A., Rochat H., Mansuelle P. and Martin-Eauclaire M. F. (1992): Kaliotoxin, a novel peptidyl inhibitor of neuronal BK-type Ca^{2+}--activated K^+ channels characterized from Androctonus mauretanicus mauretanicus venom. J. Biol. Chem., 267, 1640–1647.

10. Damjanovich S., Gaspar R. J. and Panyi G. (2004): An alternative to conventional immunosuppression: small-molecule inhibitors of Kv1.3 channels. Mol. Intervent., 4, 251–254.

11. DeCoursey T. E., Chandy K. G., Gupta S. and Cahalan M. D. (1984): Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? Nature, 307, 465–468.

12. Deutsch C., Krause D. and Lee S. C. (1986): Voltage-gated potassium conductance in human T lymphocytes stimulated with phorbol ester. J. Physiol., 372, 405–423.

13. Doyle D. A., Morais Cabral J., Pfuetzner R. A., Kuo A., Gulbis J. M., Cohen S. L., Chait B. T. and MacKinnon R. (1998): The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science, 280, 69–77.

14. Fanger C. M., Ghanshani S., Logsdon N. J., Rauer H., Kalman K., Zhou J., Beckingham K., Chandy K. G., Cahalan M. D. and Aiyar J. (1999): Calmodulin mediates calcium-dependent activation of the intermediate conductance KCa channel, IKCa1. J. Biol. Chem., 274, 5746–5754.

15. Fasolato C., Hoth M. and Penner R. (1993): A GTP-dependent step in the activation mechanism of capacitative calcium influx. J. Biol. Chem., 268, 20737–20740.

16. Fischer B. S., Qin D., Kim K. and McDonald T. V. (2001): Capsaicin inhibits Jurkat T-cell activation by blocking calcium entry current I(CRAC). J. Pharmacol. Exp. Ther., 299, 238–246.

17. Freedman B. D., Price M. and Deutsch C. (1992): Evidence for voltage modulation of IL2 production by human blood lymphocytes. J. Immunol., 149, 3784–3794.

18. Garcia M. L., Garcia-Calvo M., Hidalgo P., Lee A. and MacKinnon R. (1994): Purification and characterization of three inhibitors of voltage-dependent K^+ channels from Leiurus quinquestriatus var. hebraeus venom. Biochemistry, 33, 6834–6839.

19. Gaspar R. Jr., Varga Z., Bene L., Marcheselli F., Pieri C. and Damjanovich S. (1996): Effect of acetylcholine on the electrophysiology and proliferative response of human lymphocytes. Biochem. Biophys. Res. Commun., 226, 303–308.

20. Goldstein S. A., Pheasant D. J. and Miller C. (1994): The charybdotoxin receptor of a Shaker K^+ channel: peptide and channel residues mediating molecular recognition. Neuron, 12, 1377–1388.

21. Grissmer S., Nguyen A. N. and Cahalan M. D. (1993): Calcium-activated potassium channels in resting and activated human T lymphocytes. Expression levels, calcium dependence, ion selectivity, and pharmacology. J. Gen. Physiol., 102, 601–630.

22. Hajas G., Zsiros E., Laszlo T., Hajdu P., Somodi S., Rethi B., Gogolak P., Ludanyi K., Panyi G. and Rajnavolgyi E. (2004): New phenotypic, functional and electrophysiological characteristics of KG-1 cells. Immunol. Lett., 92, 97–106.

23. Hajdu P., Varga Z., Pieri C., Panyi G. and Gaspar R. Jr. (2003): Cholesterol modifies the gating of Kv1.3 in human T lymphocytes. Pflugers Arch., 445, 674–682.

24. Hanner M., Green B., Gao Y. D., Schmalhofer W. A., Matyskiela M., Durand D. J., Felix J. P., Linde A. R., Bordallo C., Kaczorowski G. J., Kohler M. and Garcia M. L. (2001): Binding of correolide to the K(v)1.3 potassium channel: characterization of the binding domain by site-directed mutagenesis. Biochemistry, 40, 11687–11697.

25. Hanson D. C., Nguyen A., Mather R. J., Rauer H., Koch K., Burgess L. E., Rizzi J. P., Donovan C. B., Bruns M. J., Canniff P. C., Cunningham A. C., Verdries K. A., Mena E., Kath J. C., Gutman G. A., Cahalan M. D., Grissmer S. and Chandy K. G. (1999): UK-78,282, a novel piperidine compound that potently blocks the Kv1.3 voltage-gated potassium channel and inhibits human T cell activation. Br. J. Pharmacol., 126, 1707–1716.

26. Kalman K., Pennington M. W., Lanigan M. D., Nguyen A., Rauer H., Mahnir V., Paschetto K., Kem W. R., Grissmer S., Gutman G. A., Christian E. P., Cahalan M. D., Norton R. S. and Chandy K. G. (1998): ShK-Dap22, a potent Kv1.3-specific immunosuppressive polypeptide. J. Biol. Chem., 273, 32697–32707.

27. Kerschbaum H. H. and Cahalan M. D. (1999): Single-channel recording of a store-operated Ca^{2+} channel in Jurkat T lymphocytes. Science, 283, 836–839.

28. Kharrat R., Mabrouk K., Crest M., Darbon H., Oughideni R., Martin-Eauclaire M. F., Jacquet G., El Ayeb M., Van Rietschoten J., Rochat H. and Sabatier J. M. (1996): Chemical synthesis and characterization of maurotoxin, a short scorpion toxin with four disulfide bridges that acts on K^+ channels. Eur. J. Biochem., 242, 491–498.

29. Koo G. C., Blake J. T., Shah K., Staruch M. J., Dumont F., Wunderler D., Sanchez M., McManus O. B., Sirotina-Meisher A., Fischer P., Boltz R. C., Goetz M. A., Baker R., Bao J., Kayser F., Rupprecht K. M., Parsons W. H., Tong X. C., Ita I. E., Pivnichny J., Vincent S., Cunningham P., Hora D. Jr., Feeney W. and Kaczorowski G. (1999): Correolide and derivatives are novel immunosuppressants blocking the lymphocyte Kv1.3 potassium channels. Cell. Immunol., 197, 99–107.

30. Koo G. C., Blake J. T., Talento A., Nguyen M., Lin S., Sirotina A., Shah K., Mulvany K., Hora D., Cunningham P., Wunderler D. L., McManus O. B., Slaughter R., Bugianesi R., Felix J., Garcia M. L., Williamson J., Kaczorowski G. J., Sigal N. H., Springer M. S. and Feeny W. (1997): Blockade of the voltage-gated potassium channel Kv1.3 inhibits immune responses *in vivo*. J. Immunol., 158, 5120–5128.

31. Kozak J. A., Kerschbaum H. H. and Cahalan M. D. (2002): Distinct properties of CRAC and MIC channels in RBL cells. J. Gen. Physiol., 120, 221–235.

32. Lebrun B., Romi-Lebrun R., Martin-Eauclaire M. F., Yasuda A., Ishiguro M., Oyama Y., Pongs O. and Nakajima T. (1997): A four--disulphide-bridged toxin, with high affinity towards voltage-gated K^+ channels, isolated from Heterometrus spinnifer (Scorpionidae) venom. Biochem. J., 328 (Pt 1), 321–327.

33. Leonard R. J., Garcia M. L., Slaughter R. S. and Reuben J. P. (1992): Selective blockers of voltage-gated K^+ channels depolarize human T lymphocytes: mechanism of the antiproliferative effect of charybdotoxin. Proc. Nat. Acad. Sci. USA, 89, 10094–10098.

34. Logsdon N. J., Kang J., Togo J. A., Christian E. P. and Aiyar J. (1997): A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. J. Biol. Chem., 272, 32723–32726.

35. López-Barneo J., Hoshi T., Heinemann S. H. and Aldrich R. W. (1993): Effects of external cations and mutations in the pore region on C-type inactivation of *Shaker* potassium channels. Receptors and Channels, 1, 61–71.

36. Lovett-Racke A. E., Trotter J. L., Lauber J., Perrin P. J., June C. H. and Racke M. K. (1998): Decreased dependence of myelin basic protein-reactive T cells on CD28-mediated costimulation in multiple sclerosis patients. A marker of activated/memory T cells. J. Clin. Invest., 101, 725–730.

37. Macian F., Lopez-Rodriguez C. and Rao A. (2001): Partners in transcription: NFAT and AP-1. Oncogene, 20, 2476–2489.

38. MacKinnon R. (1991): Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature, 350, 232–235.

39. Martens J. R., Navarro-Polanco R., Coppock E. A., Nishiyama A., Parshley L., Grobaski T. D. and Tamkun M. M. (2000): Differential targeting of Shaker-like potassium channels to lipid rafts. J. Biol. Chem., 275, 7443–7446.

40. Mauler F., Hinz V., Horvath E., Schuhmacher J., Hofmann H. A., Wirtz S., Hahn M. G. and Urbahns K. (2004): Selective intermediate-/small-conductance calcium-activated potassium channel (KCNN4) blockers are potent and effective therapeutics in experimental brain oedema and traumatic brain injury caused by acute subdural haematoma. Eur. J. Neurosci., 20, 1761–1768.

41. Middleton R. E., Sanchez M., Linde A. R., Bugianesi R. M., Dai G., Felix J. P., Koprak S. L., Staruch M. J., Bruguera M., Cox R., Ghosh A., Hwang J., Jones S., Kohler M., Slaughter R. S., McManus O. B., Kaczorowski G. J. and Garcia M. L. (2003): Substitution of a single residue in Stichodactyla helianthus peptide, ShK-Dap22, reveals a novel pharmacological profile. Biochemistry, 42, 13698–13707.

42. Miller C., Moczydlowski E., Latorre R. and Phillips M. (1985): Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. Nature, 313, 316–318.

43. Minke B. and Cook B. (2002): TRP channel proteins and signal transduction. Physiol. Rev., 82, 429–472.

44. Nagy P., Panyi G., Jenei A., Bene L., Gáspár R., Matkó J. and Damjanovich S. (1995): Ion channel activities regulate transmembrane signaling in thymocyte apoptosis and T-cell activation. Immunol. Lett., 44, 91–95.

45. Nguyen A., Kath J. C., Hanson D. C., Biggers M. S., Cannif P. C., Donovan C. B., Mather R. J., Bruns M. J., Rauer H., Aiyar J., Lepple-Wienhues A., Gutman G. A., Grissmer S., Cahalan M. D. and Chandy K. G. (1996): Novel nonpeptide agents potently block the C-type inactivated conformation of Kv1.3 and suppress T cell activation. Mol. Pharmacol., 50, 1672–1679.

46. Panyi G., Bagdany M., Bodnar A., Vamosi G., Szentesi G., Jenei A., Matyus L., Varga S., Waldmann T. A., Gaspar R. and Damjanovich S. (2003): Colocalization and nonrandom distribution of Kv1.3 potassium channels and CD3 molecules in the plasma membrane of human T lymphocytes. Proc. Nat. Acad. Sci. USA, 100, 2592–2597.

47. Panyi G., Berecki G., Gáspár R., Seres I., Fülöp T. and Damjanovich S. (1994): Peripheral blood lymphocytes display reduced K^+ channel activity in aged humans. Biochem. Biophys. Res. Commun., 199, 519–524.

48. Panyi G. and Deutsch C. (1996): Assembly and suppression of endogenous Kv1.3 channels in human T cells. J. Gen. Physiol., 107, 409–420.

49. Panyi G., Gáspár R., Krasznai Z., ter Horst J.J., Ameloot M., Aszalós A., Steels P. and Damjanovich S. (1996): Immunosuppressors inhibit voltage-gated potassium channels in human peripheral blood lymphocytes. Biochem. Biophys. Res. Commun., 221, 254–258.

50. Panyi G., Sheng Z. and Deutsch C. (1995): C-type inactivation of a voltage-gated K^+ channel occurs by a cooperative mechanism. Biophys. J., 69, 896–903.

51. Panyi G., Vamosi G., Bacso Z., Bagdany M., Bodnar A., Varga Z., Gaspar R., Matyus L. and Damjanovich S. (2004): Kv1.3 potassium channels are localized in the immunological synapse formed between cytotoxic and target cells. Proc. Natl. Acad. Sci. USA, 101, 1285–1290.

52. Panyi G., Vamosi G., Bodnar A., Gaspar R. J. and Damjanovich S. (2004): Looking through ion channels: recharged concepts in T cell signaling. Trends Immunol., 25, 565– 569.

53. Panyi G., Varga Z. and Gaspar R. (2004): Ion channels and lymphocyte activation. Immunol. Lett., 92, 55–66.

54. Partiseti M., Le Deist F., Hivroz C., Fischer A., Korn H. and Choquet D. (1994): The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. J. Biol. Chem., 269, 32327–32335.

55. Peppiatt C. M., Collins T. J., Mackenzie L., Conway S. J., Holmes A. B., Bootman M. D., Berridge M. J., Seo J. T. and Roderick H. L. (2003): 2-Aminoethoxydiphenyl borate (2-APB) antagonises inositol 1,4,5-trisphosphate-induced calcium release, inhibits calcium pumps and has a use-dependent and slowly reversible action on store-operated calcium entry channels. Cell Calcium, 34, 97–108.

56. Peter M. Jr., Hajdu P., Varga Z., Damjanovich S., Possani L. D., Panyi G. and Gaspar R. Jr. (2000): Blockage of human T lymphocyte Kv1.3 channels by Pi1, a novel class of scorpion toxin. Biochem. Biophys. Res. Commun., 278, 34–37.

57. Peter M. Jr., Varga Z., Panyi G., Bene L., Damjanovich S., Pieri C., Possani L. D. and Gaspar R. Jr. (1998): Pandinus imperator scorpion venom blocks voltage-gated K^+ channels in human lymphocytes. Biochem. Biophys. Res. Commun., 242, 621–625.

58. Peter M. J., Varga Z., Hajdu P., Gaspar R. J., Damjanovich S., Horjales E., Possani L. D. and Panyi G. (2001): Effects of toxins Pi2 and Pi3 on human T lymphocyte Kv1.3 channels: the role of Glu7 and Lys24. J. Membr. Biol., 179, 13–25.

59. Pieri C., Recchioni R., Moroni F., Balkay L., Marian T., Tron L. and Damjanovich S. (1989): Ligand and voltage gated sodium channels may regulate electrogenic pump activity in human, mouse and rat lymphocytes. Biochem. Biophys. Res. Commun., 160, 999–1002.

60. Prakriya M. and Lewis R. S. (2001): Potentiation and inhibition of Ca^{2+} release-activated Ca^{2+} channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP(3) receptors. J. Physiol., 536, 3–19.

61. Prakriya M. and Lewis R. S. (2002): Separation and characterization of currents through store-operated CRAC channels and Mg^{2+}--inhibited cation (MIC) channels. J. Gen. Physiol., 119, 487–507.

62. Price M., Lee S. C. and Deutsch C. (1989): Charybdotoxin inhibits proliferation and interleukin 2 production in human peripheral blood lymphocytes. Proc. Nat. Acad. Sci., USA, 86, 10171–10175.

63. Putney J. W. Jr., Broad L. M., Braun F. J., Lievremont J. P. and Bird G. S. (2001): Mechanisms of capacitative calcium entry. J. Cell Sci., 114, 2223–2229.

64. Randriamampita C. and Tsien R. Y. (1993): Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature, 364, 809–814.

65. Rochat H., Kharrat R., Sabatier J. M., Mansuelle P., Crest M., Martin-Eauclaire M. F., Sampieri F., Oughideni R., Mabrouk K., Jacquet G., Van Rietschoten J. and El Ayeb M. (1998): Maurotoxin, a four disulfide bridges scorpion toxin acting on K^+ channels. Toxicon, 36, 1609–1611.

66. Sands S. B., Lewis R. S. and Cahalan M. D. (1989): Charybdotoxin blocks voltage-gated K^+ channels in human and murine T lymphocytes. J. Gen. Physiol., 93, 1061–1074.

67. Schmalhofer W. A., Bao J., McManus O. B., Green B., Matyskiela M., Wunderler D., Bugianesi R. M., Felix J. P., Hanner M., Linde--Arias A. R., Ponte C. G., Velasco L., Koo G., Staruch M. J., Miao S., Parsons W. H., Rupprecht K., Slaughter R. S., Kaczorowski G. J. and Garcia M. L. (2002): Identification of a new class of inhibitors of the voltage-gated potassium channel, Kv1.3, with immunosuppressant properties. Biochemistry, 41, 7781–7794.

68. Somodi S., Varga Z., Hajdu P., Starkus J. G., Levy D. I., Gaspar R. and Panyi G. (2004): pH-dependent modulation of Kv1.3 inactivation: role of His399. Am. J. Physiol. Cell Physiol., 287, C1067–C1076.

69. Starkus J. G., Varga Z., Schonherr R. and Heinemann S. H. (2003): Mechanisms of the inhibition of Shaker potassium channels by protons. Pflugers Arch., 447, 44–54.

70. Stocker J. W., De Franceschi L., McNaughton-Smith G. A., Corrocher R., Beuzard Y. and Brugnara C. (2003): ICA-17043, a novel Gardos channel blocker, prevents sickled red blood cell dehydration *in vitro* and *in vivo* in SAD mice. Blood, 101, 2412–2418.

71. Stokes L., Gordon J. and Grafton G. (2004): Non-voltage-gated L--type Ca^{2+} channels in human T cells: pharmacology and molecular characterization of the major α pore-forming and auxiliary β-subunits. J. Biol. Chem., 279, 19566–19573.

72. Swartz K. J. and MacKinnon R. (1997): Mapping the receptor site for Hanatoxin, a gating modifier of voltage-dependent K^+ channels. Neuron, 18, 675–682.

73. Teisseyre A. (2000): Regulation of human T lymphocyte potassium channel function by intracellular second messengers – role of calcium, cyclic AMP and membrane lipid metabolites. Postepy Hig. Med. Dosw., 54, 381–390.

74. Teisseyre A. (2002): The patch-clamp technique in investigations on T lymphocyte potassium channel modulation. Postepy Hig. Med. Dosw., 56, 393–399.

75. Urbahns K., Horvath E., Stasch J. P. and Mauler F. (2003): 4--Phenyl-4H-pyrans as IK(Ca) channel blockers. Bioorg. Med. Chem. Lett., 13, 2637–2639.

76. Varga Z., Bene L., Pieri C., Damjanovich S. and Gaspar R. Jr. (1996): The effect of juglone on the membrane potential and whole-cell K^+ currents of human lymphocytes. Biochem. Biophys. Res. Commun., 218, 828–832.

77. Varga Z., Panyi G., Péter M. Jr., Pieri C., Csécsei G., Damjanovich S. and Gáspár R. Jr. (2001): Multiple binding sites for melatonin on Kv1.3. Biophys. J., 80, 1280–1297.

78. Varga Z., Rayner M. D. and Starkus J. G. (2002): Cations affect the rate of gating charge recovery in wild-type and W434F Shaker channels through a variety of mechanisms. J. Gen. Physiol., 119, 467–485.

79. Vennekamp J., Wulff H., Beeton C., Calabresi P. A., Grissmer S., Hansel W. and Chandy K. G. (2004): Kv1.3-blocking 5-phenylalkoxypsoralens: a new class of immunomodulators. Mol. Pharmacol., 65, 1364–1374.

80. Vereb G. Jr., Panyi G., Balázs M., Mátyus L., Matkó J. and Damjanovich S. (1990): Effect of cyclosporin A on the membrane potential and Ca^{2+} level of human lymphoid cell lines and mouse thymocytes. Biochim. Biophys. Acta, 1019, 159–165.

81. Verheugen J. A., Vijverberg H. P., Oortgiesen M. and Cahalan M. D. (1995): Voltage-gated and Ca^{2+})-activated K^+ channels in intact human T lymphocytes. Noninvasive measurements of membrane currents, membrane potential, and intracellular calcium. J. Gen. Physiol., 105, 765–794.

82. Viglietta V., Kent S. C., Orban T. and Hafler D. A. (2002): GAD65-reactive T cells are activated in patients with autoimmune type 1a diabetes. J. Clin. Invest., 109, 895–903.

83. Wanner S. G., Glossmann H., Knaus H. G., Baker R., Parsons W., Rupprecht K.M., Brochu R., Cohen C. J., Schmalhofer W., Smith M., Warren V., Garcia M. L. and Kaczorowski G. J. (1999): WIN 17317-3, a new high-affinity probe for voltage-gated sodium channels. Biochemistry, 38, 11137–11146.

84. Wulff H., Calabresi P. A., Allie R., Yun S., Pennington M., Beeton C. and Chandy K. G. (2003): The voltage-gated Kv1.3 K^+ channel in effector memory T cells as new target for MS. J. Clin. Invest., 111, 1703–1713.

85. Wulff H., Miller M. J., Hansel W., Grissmer S., Cahalan M. D. and Chandy K. G. (2000): Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^+ channel, IKCa1: a potential immunosuppressant. Proc. Natl. Acad. Sci. USA, 97, 8151–8156.

86. Wulff H., Rauer H., During T., Hanselmann C., Ruff K., Wrisch A., Grissmer S. and Hansel W. (1998): Alkoxypsoralens, novel nonpeptide blockers of Shaker-type K^+ channels: synthesis and photoreactivity. J. Med. Chem., 41, 4542–4549.

87. Xia X. M., Fakler B., Rivard A., Wayman G., Johnson-Pais T., Keen J.E., Ishii T., Hirschberg B., Bond C. T., Lutsenko S., Maylie J. and Adelman J. P. (1998): Mechanism of calcium gating in small-conductance calcium-activated potassium channels. Nature, 395, 503–507.

88. Yao X., Kwan H. Y. and Huang Y. (2002): A mechanosensitive cation channel in endothelial cells. J. Card. Surg., 17, 340–341.

89. Zakharov S.I., Smani T., Dobrydneva Y., Monje F., Fichandler C., Blackmore P. F. and Bolotina V. M. (2004): Diethylstilbestrol is a potent inhibitor of store-operated channels and capacitative Ca^{2+} influx. Mol. Pharmacol., 66, 702–707.

90. Zitt C., Strauss B., Schwarz E. C., Spaeth N., Rast G., Hatzelmann A. and Hoth M. (2004): Potent inhibition of Ca^{2+} release-activated Ca^{2+} channels and T-lymphocyte activation by the pyrazole derivative BTP2. J. Biol. Chem., 279, 12427–12437.

91. Zweifach A. and Lewis R. S. (1993): Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. Proc. Natl. Acad. Sci. USA, 90, 6295–6299.