POSTER SESSION

A

PLASMA MEMBRANE CHANNELS AND Ca^{2+} ENTRY

PROBING SUBSARCOLEMMAL Ca^{2+} THROUGH CALCIUM-SENSITIVE ION TRANSPORT IN CARDIAC MYOCYTES

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In cardiac cells, Ca^{2+}-induced Ca^{2+}-release from the sarcoplasmic reticulum (SR) is a local process that occurs in the subsarcolemmal space at the T-tubules. Direct examination of local Ca^{2+} changes during triggered release is hampered due to the small volume of the restricted space. In this study we estimated the subsarcolemmal Ca^{2+} level through its effect on the activities of the L-type Ca^{2+} channel (I_{CaL}) and Na^+/Ca^{2+} exchanger (NCX), as these transporters are expressed at the T-tubules and modulated by Ca^{2+}.

METHODS: In pig ventricular myocytes, membrane currents were recorded using whole-cell voltage-clamp with Fluo-3 as indicator for global Ca^{2+}. SR Ca^{2+} release was triggered through activation of I_{CaL} during a depolarizing step from -70 to -35 mV. At this potential, I_{CaL} showed Ca^{2+} release dependent inactivation and recovery. The step at -35 mV was interrupted at different time intervals by a 10 ms step to -70 mV followed by a step to 0 mV to measure the time course of NCX current and availability of I_{CaL} respectively. NCX tail currents at -70 mV were converted to subsarcolemmal Ca^{2+} levels using the steady-state dependence of NCX on global cytosolic Ca^{2+} during caffeine application.

Subsarcolemmal Ca^{2+} sensed by NCX reached its peak value immediately after the trigger pulse and decreased linearly with time. In contrast, global Ca^{2+} increased more slowly and to a lesser extent. Maximal inactivation and recovery of I_{CaL} had a faster time course than the accompanying changes in global Ca^{2+}, but was delayed by 10-20 ms compared to maximal NCX activation and peak subsarcolemmal Ca^{2+}. This delay was observed in 11 of 16 cells. During inhibition of NCX, the degree of I_{CaL} inactivation and recovery was not significantly different.

CONCLUSION: Changes in local Ca^{2+} sensed by NCX and I_{CaL} during triggered release considerably differ from changes in global Ca^{2+}. The discrepancy in the time course of Ca^{2+}-dependent modulation between these currents is unexpected and might be related to different kinetics.

Keywords: cardiomyocytes, Na/Ca^{2+} exchanger, subsarcolemmal Ca^{2+}

PKC-ßII REGULATION COUNTERACTS ATP-MEDIATED EFFECTS ON TRPV6 CALCIUM CURRENTS AND POINTS TO A FUNCTIONAL DIFFERENCE BETWEEN TRPV6 POLYMORPHIC ALLELES

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Transient receptor potential vanilloid 6 (TRPV6) belongs to the cation conducting family of TRP ion channels and exhibits a high selectivity profile for calcium as well as an unusually accelerated protein evolution for one of its polymorphic alleles in humans. Whether this unusual polymorphism correlates with functional differences and whether and how calcium influx depends on the metabolic status is unclear.

Here we show that TRPV6 activity is regulated by intracellular ATP and protein phosphorylation in an opposing manner: While ATP prevents both inactivation and run-down of TRPV6-mediated currents, stimulation of protein kinase C by the phorbol ester PMA but not by its inactive analog 4α-PDD prevents this ATP-dependent current stabilization. By using a combination of different inhibitors and by expression of different isotypes of PKC, we show that this regulation requires a specific splice variant of PKC, namely PKC-ßII. One of the polymorphic sites within TRPV6 removes a potential phosphorylation site from the ancestral allele and these channels become less sensitive towards regulation by PKC. We have mapped one functionally relevant site for regulation by ATP to the finger loop between the intracellular ankyrin repeat domains 3 and 4. Of the functionally relevant phosphorylation sites, two also lie within this region with additional important sites in the N- as well as C-terminus. Our data ties into a model where ATP stabilizes the channel possibly by tethering the C-and N-termini of the subunits closer together. Phosphorylation is likely to disrupt the interaction with ATP and yields the channels susceptible to run-down.

Keywords: TRPV6, Protein Kinase C, ATP

EXPRESSION AND LOCALIZATION OF CLASSICAL TRANSIENT RECEPTOR POTENTIAL CHANNELS (TRPCS) IN MAMMARY GLAND CELLS

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Ca^{2+} is taken up by mammalian gland cells for secretion into milk or for intracellular Ca^{2+} signaling. However, the mechanism of Ca^{2+} influx is still unknown. Classical transient receptor potential channels (TRPCs), G protein-coupled receptors in the TRP family, have been proposed as the means for basolateral Ca^{2+} influx into the mammary epithelial cells.

This study showed an abundant expression of TRPC1 and TRPC 6 mRNA in the human breast cancer cells MCF-7, whereas TRPC3 was predominant in HC-11, the normal mouse mammary epithelial cells. Twenty-four hour treatment of 1 μg/ml prolactin, the lactating hormone, had no effect on their expression. Although all TRPC mRNAs were observed in rat mammary tissue, they showed differential patterns of expression. TRPC2, 3 and 4 were suppressed from 7 days before parturition through lactation. In contrast, expression of TRPC1, 5, 6 and 7 was increased on or after the day of parturition, then decreased on day 14 of lactation. Immunofluorescent study showed TRPC1 and 3 to be localized in the cytosol, while TRPC3-6 were diffusely distributed in MCF-7. In rat mammary tissue, TRPCs were expressed homogeneously in the cytosol on parturition day with more confined localization at the apical membrane on day 14 of lactation. By using Ca^{2+}-bound fluorescent dye Fluo-3, 2-amino-ethoxydiphenylborate (2-APB), TRPC inhibitor, was found to suppress an ATP-evoked increase in intracellular Ca^{2+}.

This differential expression of TRPCs in human and mouse mammary epithelial cells and rat mammary tissue at various physiological states, imply specific functions for TRPCs in the mammary gland.

Keywords: TRPC channels, mammary gland

CFTR PROTEIN DOWN REGULATES THE DAG-DEPENDENT Ca^{2+} INFLUX MEDIATED BY TRPC6 IN TRACHEAL EPITHELIAL CELLS

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In the disease cystic fibrosis (CF), the most common genetic mutation results in a deletion of phenylalanine at position 508 in the Cystic Fibrosis Transmembrane conductance Regulator protein (F508del-CFTR). The misfolded F508del-CFTR protein is trapped in the endoplasmic reticulum (ER). In homozygous F508del-CFTR cells, the histamine-induced Ca^{2+} mobilization is abnormally increased. The influx induced by calcium store depletion, named capacitative Ca^{2+} entry (CCE), represents the major Ca^{2+}-influx mechanism in cells. During CCE, Ca^{2+} influx can be mediated by one or several of the 7 isoforms of transient receptor potential canonical channels (TRPC) and each isoform have a different regulation.

In the present work, we studied the CCE in human CF tracheal gland serous CF-KM4 cells (F508del-CFTR homozygous cell) compared to non-CF human tracheal MM39 cells. Our results show: (i) TRPC1 and C6 are expressed in both cell lines. (ii) In CF-KM4 cells, we observed an increased Ca^{2+} influx (Ca^{2+} measurement and patch-clamp technique). (iii) The store-operated Ca^{2+}-channel activity is similar in CF and non-CF cells. (iv) In contrast, the DAG-dependent Ca^{2+} currents are abnormally increased in CF-KM4 cells. (v) After correction of abnormal F508del-CFTR trafficking by the pharmacological corrector miglustat like in CF-KM4 reverted cells (stably transfected with wild type CFTR), the Ca^{2+} influx is normalized to a similar level as in non-CF cells.

Our results add to the understanding of the role of Ca^{2+} signalling in CF cells compared to corrected CF cells. In CF cells the DAG-dependent Ca^{2+} influx (TRPC6) is dramatically increased compared to non-CF cells. These observations show that presence at the plasma membrane of CFTRwt or F508del-CFTR down regulates the TRPC6-mediated Ca^{2+} influx. These data reveal a new regulation of ionic channel by the CFTR protein.

Supported by Vaincre La Mucoviscidose and CNRS.

Keywords: CFTR, TRPC6, store-operated Ca^{2+} entry

LOCAL AND GLOBAL CALCIUM SIGNALS ASSOCIATED WITH THE OPENING OF NEURONAL α7 NICOTINIC ACETYLCHOLINE RECEPTORS

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Neuronal nicotinic acetylcholine receptors (nAChRs) are Ca^{2+}-permeable ligand-gated channels widely expressed in the central and peripheral nervous system whose most Ca^{2+} selective isoform is the homopentameric α7-nAChR implicated in schizophrenia. The activity of α7-nAChRs is usually recorded electrophysiologically, which limits the amount of information obtained.

Here, we used fluorescence imaging to record Ca^{2+} transients associated with activation of the α7-nAChR in neuroblastoma cells stably expressing human α7-nAChRs. Application of nicotine (50 μM) consistently evoked transient (30 sec), stereotyped Ca^{2+} responses that were inhibited by the selective α7-nAChRs antagonists methyllycaconitine (MLA) and α-bungarotoxin, and greatly increased and prolonged by the allosteric modulator PNU-120596 (1 μM). Unexpectedly, brief (1-5 sec) miniature Ca^{2+} transients were observed in filipodia of cells expressing α7-nAChR. PNU-120596 increased the frequency and slowed the decay kinetics of these miniature Ca^{2+} events, which were insensitive to ryanodine, were preserved by hyperpolarisation with valinomycin, and were prevented by MLA, α-bungarotoxin, or Ca^{2+} removal. Global Ca^{2+} responses were also recorded in ganglion cells of embryo chicken retina during co-application of PNU-120596 and nicotine, together with whole-cell currents and brief current bursts. These data demonstrate that Ca^{2+} signals generated by the α7-nAChRs can be recorded optically both in cell lines and in intact tissues. The possibility to image miniature Ca^{2+} signals enables to map the location of functional α7-nAChR channel clusters within cells and to analyze their single channel properties optically. Deciphering the rich pattern of intracellular Ca^{2+} signals generated by the activity of the α7-nAChRs will reveal the physiological role of these receptor-channels.

Keywords: nicotine, nicotinic acetylcholine receptors, neurons

REGULATION OF NOX2 BY STORE-OPERATED Ca^{2+} ENTRY AND AN ALTERNATIVE PATHWAY: ROLE OF PKC/PI3K IN RAC2-DEPENDENT NOX2 ACTIVITY

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The requirement of Ca^{2+} entry for NADPH oxidase (NOX2) regulation of neutrophils is clearly established. However, its role in the signalling pathway leading to NOX2 activation is still elusive. 1-oleoyl-2-acetyl-sn-glycerol (OAG) causes an increase in NOX2 activity and has been shown to modulate directly Ca^{2+} channels unrelated to the well-known store-operated Ca^{2+} entry (SOCE) mechanism.

In our study, we have investigated the potential role of OAG in Ca^{2+} influx-mediated NOX2 activity in neutrophil-like differentiated HL-60 cells to further characterize second signals involved in the regulation of NOX2. OAG inhibited fMLF- and thapsigargin-induced Ca^{2+} entry, a phenomenon which was not restored by PKC or PI3K blockade. Further, addition of OAG resulted in a rapid decrease of maximal intracellular Ca^{2+} concentration. Both results suggest that OAG has an inhibitory effect, independent of PI3K and PKC, on the regulation of SOCE. In contrast to SOCE inhibition, OAG-induced NOX2 activation was mediated by PKC and PI3K.

Our data establish that both kinases exert their effects through the regulation of Rac2 activity. Activation of NOX2 by fMLF was potentiated by OAG. In the same line, OAG also led to a substantial activity of NOX2 triggered by thapsigargin.

Our results reveal finally that PKC and PI3K-independent signal acts in synergy with Ca^{2+} influx to trigger NOX2 activation. This signal might have opposite actions in activating NOX2 and inhibiting SOCE.

Keywords: store-operated Ca^{2+} entry, NADPH oxidase, neutrophils

THE CALCIUM CHANNEL $Ca_v\beta4$ SUBUNIT ACTS AS AN INDEPENDENT TRANSCRIPTION FACTOR

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β subunits ($Ca_v\beta$) are auxiliary subunits of voltage-dependent calcium channels that are involved in trafficking functions and in normalizing channel properties. Their implication in new and channel-independent signaling pathways is an emerging concept.

Here, we report that, upon neuronal differentiation, $Ca_v\beta4$ acts as a transcription factor by forming a channel-independent molecular complex with phosphatase 2A (PP2A) through a conformational-sensitive binding onto B56δ, a nuclear regulatory subunit of PP2A. Binding of $Ca_v\beta4$ to B56δ requires an intact intra-molecular interaction between the SH3 and GK domains of $Ca_v\beta4$. Formation of this complex greatly favors nuclear targeting of $Ca_v\beta4$ which is then found associated to the nucleosomes. The $Ca_v\beta4$ / B56δ / PP2A / nucleosome complex allows histone dephosphorylation to occur, a crucial step involved in changes in transcriptional activity. Interestingly, $Ca_v\beta4$ also binds onto the thyroid receptor α (TRα), a nuclear receptor, and decreases its T3-dependent and -independent transcriptional input as assessed by a DR4-luciferase reporter system. These findings have also pathological relevance since a human truncated mutant, $Ca_v\beta1$-481 which lacks the C-terminal 38 amino acids of $Ca_v\beta4$, and that is responsible for juvenile myoclonic epilepsy, has lost the ability to interact with B56δ and forms no complex with PP2A. As a consequence, this mutant is defective for two properties, nuclear localization and $Ca_v\beta4$-mediated histone dephosphorylation. These changes translate into important modifications in the transcriptional program of $Ca_v\beta4$.

Keywords: Ca^{2+} channel, β subunit, PP2A, transcription factor

TARGETING DIHYDROPYRIDINE RECEPTORS EXPRESSED BY Th2 CELLS PREVENTS EXPERIMENTAL ASTHMA

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Allergic asthma is a chronic inflammatory disease of the lungs which prevalence and severity are both increasing. $CD4^+$ T cell subsets include Th1 cells that produce interferon-γ (IFNγ) and Th2 cells that produce interleukin 4, 5 and 13. Th2 cells are considered as orchestrating the inflammatory reaction characteristic of asthma. Signalling pathways and especially calcium regulation differ between Th1 and Th2 cells which could offer an opportunity for the development of new therapies targeting calcium signalling. Dihydropyridine-sensitive calcium channels are specifically expressed by Th2 cells while absent in Th1 cells. These channels are involved in TCR-dependent calcium response and in Th2-cytokine production (IL-4, IL-5, IL-10 and IL-13). In vitro and in vivo studies showed that the inhibition of these channels inhibits Th2 cell functions with a beneficial effect in experimental asthma.

Altogether these data indicate that dihydropyridine-sensitive calcium channels are crucial in Th2 cell signalling. Targeting these channels is a rationale for the development of new therapies in the treatment of allergic asthma.

Keywords: $CD4^+$, Th2 cells, asthma, L-type Ca^{2+} channels

FUNCTIONAL ANALYSIS OF THE CACNA1A MUTATION Y1245C ASSOCIATED TO CHILDHOOD PERIODIC SYNDROMES AND HEMIPLEGIC MIGRAINE

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Mutations in the gene encoding the P/Q Ca^{2+} channel α_{1A} subunit (CACNA1A) linked to familial hemiplegic migraine locate to the pore and voltage sensor regions and normally involve gain of channel function and increased neurotransmitter release. This anomaly favors cortical spreading depression (CSD) initiation and propagation that in turn will lead to neurogenic inflammation and pain via the activation of the trigeminovascular system. CACNA1A Y1245C is the first missense mutation described in a subject affected with childhood periodic syndromes, who displayed a changing, age-specific phenotype that finally evolved into hemiplegic migraine (HM). Y1245C is also the first amino acid change described in the S1 segment of any domain of CACNA1A in any patient with HM.

Due to its interesting location and clinical relevance we investigated the functional consequences of this mutation on P/Q Ca^{2+} channels expressed in HEK 293 cells. Mutation Y1245C induced a 10 mV left shift in the current-voltage activation curve, which was accompanied by accelerated activation kinetics and slowed deactivation kinetics within a wide range of voltage depolarizations. α_{1A}(Y1245C) channels also left-shifted the voltage dependence of steady-state inactivation with a significant increase in steepness, suggesting a direct effect on the P/Q Ca^{2+} channel voltage sensor. The formation of a new disulfide bridge between cysteines may contribute to the effect of the Y1245C mutation on channel activation, as it is partially reverted by the sulphydryl-reducing agent dithiothreitol. Finally we found that mutation Y1245C reduced P/Q Ca^{2+} channel inhibition by G protein β-γ subunits at voltages that may be attained during CSD.

Our data suggest that mutation Y1245C induces a structural change in the α_{1A} voltage sensor producing an overall gain of channel function that may explain the observed clinical phenotypes.

Supported by Fundació la Marató de TV3, Ministerio de Educación y Ciencia, Fondo de Investigación Sanitaria and Generalitat de Catalunya.

Keywords: P/Q-type Ca^{2+} channels, CACNA1A mutation, hemiplegic migraine, channelopathy

8-Br-ADPR: A NOVEL ANTAGONIST OF TRPM2

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ADP-ribose (ADPR) can be produced by the ecto-enzyme CD38 from its substrate nicotinamide adenine dinucleotide (NAD+). It has been shown to control cation entry through the plasma membrane cation channel TRPM2 (Perraud et al., 2001; Gasser et al., 2006). Activation of Ca^{2+} and Na^{+} influx through TRPM2 channels might play an important role in cell regulation, e.g. apoptosis induction in T cells (Gasser et al., 2006) or neutrophil chemotaxis and migration (Partida-Sanchez et al., 2007).

Here we describe 8-Br-ADPR, a novel compound that specifically inhibits ADPR-activated cation entry. To analyze the specificity of the potential TRPM2 antagonist 8-Br-ADPR, patch-clamp experiments and calcium imaging were performed. TRPM2 currents were recorded from Jurkat T-lymphocytes in the whole-cell mode. ADPR and 8-Br-substituted compounds were added to the intracellular solution in the patch pipette. The TRPM2 current activated by ADPR in Jurkat T-lymphocytes was blocked by the simultaneous intracellular application of 8-Br-ADPR in a 3-fold excess. This effect was shown to be specific as TRPM2 currents were not significantly inhibited by 8-Br-cADPR or 8-Br-NAD. In calcium imaging experiments, co-injection of 8-Br-ADPR did not influence calcium signals evoked by the calcium mobilizing compounds IP_3, cADPR or NAADP. These results indicate that 8-Br-ADPR specifically antagonizes the activation of TRPM2 by ADPR.

Gasser et al. (2006) J. Biol. Chem. 281, 2489–2496.
Partida-Sanchez et al. (2007) J. Immunol. 179, 7827–7839.
Perraud et al. (2001) Nature 411, 595–599.

Keywords: Ca^{2+} entry, TRPM2, ADPR

UNDERTAKER, A DROSOPHILA JUNCTOPHILIN, LINKS DRAPER AND DRCED-6 TO CALCIUM HOMEOSTASIS DURING PHAGOCYTOSIS

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Phagocytosis is important during development and innate immunity. We report that Undertaker/Retinophilin (UTA), a junctophilin-related protein is required for phagocytosis. Junctophilins couple Ca^{2+} channels at the plasma membrane to those of the endoplasmic reticulum, the ryanodine receptors. UTA acts with the ryanodine receptor, Rya-r44F, and behaves as a junctophilin. dSTIM, the ER Ca^{2+} sensor, and dOrai, the Ca^{2+}-release activated Ca^{2+} channel, which promote store-operated Ca^{2+} entry, are also required for phagocytosis in the same pathway as UTA and Rya-r44F.

In C. elegans, the ced-1, 6, 7, and ced-2, 5, 10 and 12 genes control phagocytosis of apoptotic cells. The CED-2/5/10/12 pathway regulates actin, but the role of the CED-1/6/7 pathway is unclear. We placed UTA into the DRPR (CED-1 homologue)/drCed-6 pathway, and found that DRPR and drCed-6 are required for store-operated Ca^{2+} entry. Thus, our results implicate a junctophilin in phagocytosis, and link DRPR and drCed-6 to Ca^{2+} homeostasis during phagocytosis.

Keywords: phagocytosis, junctophilins, store-operated Ca^{2+} entry

EFFECTS OF IRRITANT BITTER COMPOUNDS ON TRP CHANNELS OF THE CHEMOSENSORY SYSTEM

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Mammalian taste perception relies on the segregated detection of five distinct qualities sweet, bitter, salty, sour and umami. However, ordinary gustatory experiences result from complex interactions between these taste modalities. We have previously shown that TRPM5, a Ca^{2+}-activated cation channel having a crucial role in taste transduction, is inhibited by the bitter tastants quinine and quinidine. Therefore, this channel constitutes a locus for the known inhibition of sweet taste perception by these chemicals. To test the generality of this mechanism, we examined whether TRPM5 is affected by other common bitter compounds. We found that nicotine inhibits TRPM5 currents with an EC_{50} of 1-3 mM. In contrast, TRPM5 was not affected by saccharine, naringin, 6-n-propylthiouracil (PROP) nor by the xanthines caffeine, theophylline and theobromine. Nicotine also inhibits TRPM4, a close relative of TRPM5, and critical regulator of the immune response of mast cells.

Notably, taste perception can be also modified by irritant chemicals activating trigeminal chemosensory nerves. However, the underlying molecular determinants are not entirely known. Here we tested the effects of the known trigeminal irritant caffeine, and the related compounds theophylline and theobromine on TRPV1 and TRPA1, two broadly tuned chemosensory ion channels present in trigeminal neurons. There was no effect on TRPV1, but all three compounds reversibly activated TRPA1. This activation was not secondary to Ca^{2+} release from intracellular stores and caused crossed desensitization with the canonical TRPA1 activator allyl isothiocyanate (mustard oil). Altogether, our results demonstrate the key role of TRP channels as molecular loci for interactions between distinct sensory pathways.

Keywords: taste, TRP channels, chemosensation

CAN WE DISCRIMINATE BETWEEN THE IONIC CURRENTS ACTIVATED BY AGONIST OR STORE DEPLETION IN ENDOTHELIAL CELLS?

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Ca^{2+} entry in endothelial cells (EC) is a key signaling event required for the majority of cellular functions. Among the mechanisms proposed that lead to Ca^{2+} entry, two major pathways are proposed: a store-operated Ca^{2+} entry (SOCE), directly linked to the Ca^{2+} filling state of the endoplasmic reticulum (ER), and a receptor-activated Ca^{2+} entry (RACE), that requires the presence of an agonist to be stimulated. The contribution of those two pathways during physiological stimulation as well as their electrophysiological signatures are not known in EC.

The aim of this study is the electrophysiological characterization and comparison of the currents activated by an agonist (histamine) versus store depletion. The patch clamp method in whole-cell configuration (perforated patch) was used on endothelial cells derived from human umbilical vein (Ea.hy926).

In presence of 10 mM extracellular Ca^{2+}, the whole-cell current activated by agonist shows a strong outward rectification with a reversal potential around -25 mV. This current is blocked by 10 µM KB-R7943, an inhibitor of the Na^{+}/Ca^{2+} exchanger (NCX), which leads us postulating the involvement of NCX working in the reverse mode as being part of the Ca^{2+} entry process activated by histamine. On the contrary, in presence of 10 mM extracellular Ba^{2+} (with 2 mM Ca^{2+}), the histamine-activated current is inwardly rectifying with a reversal potential around 25 mV. On the other hand, passive depletion of the ER by thapsigargin (TG) activates an inwardly rectifying current with a positive reversal potential (around 25 mV) both in presence of 10 mM Ca^{2+} as well as 10 mM Ba^{2+}, the current being larger in Ba^{2+} medium.

In conclusion, histamine and TG activated different currents when recorded in 10 mM Ca^{2+}, while in presence of 10 mM Ba^{2+} both currents are difficult to differentiate. The reason for this discrepancy remains to be investigated, but points to the existence of different currents supporting RACE and SOCE in endothelial cells.

Keywords: receptor-activated Ca^{2+} entry, endothelium, store-operated Ca^{2+} entry

STIM1 AND STIM2 ARE PRESENT IN MOUSE BRAIN NEURONS AND SHOW PUNCTA-LIKE COLOCALIZATION WITH ORAI1 UPON CALCIUM DEPLETION OF THE ER

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Store Operated Calcium Entry (SOCE) is well known in non-excitable cells. It is based on the interaction of ER calcium sensors STIM1 or STIM2 with the plasma membrane calcium channel protein ORAI1. Although SOCE is ubiquitous in non-excitable cells, it is also crucial for the nervous system. Its alterations cause deregulation of calcium homeostasis in the cell and may lead to pathology like Alzheimer's and Huntington's disease.

We analyzed and compared the distribution of STIM1 and STIM2 in mouse brain and in cultured cortical and hippocampal neurons using various techniques. We show that the protein and mRNA levels of STIM1 and STIM2 vary in different brain regions. For STIM1 the highest level is present in the cerebellum, and for STIM2 in the hippocampus. Immunohistochemistry of brain sections shows a distinct distribution of both proteins mostly in the hippocampus, cerebellum and amygdala. We also demonstrate that STIM1 and STIM2 are present in cultured neurons and their expression is accumulated mainly in the cell bodies. However, strong dendritic immunostaining is observed for STIM1, but not for STIM2.

Our data revealed that depletion of the calcium store in cultured cortical neurons induces a change in the localization of YFP-STIM1, YFP-STIM2 and ORAI1 from disperse, in untreated, to puncta-like in thapsigargin-treated cells. We propose that, in neurons, just as in non-excitable cells, the ORAI1 and STIM proteins are involved in store operated calcium entry.

* Both authors contributed equally to this work.

Keywords: store-operated Ca^{2+} entry, STIM1, STIM2, ORAI1, neurons

TRPA1 ACTS AS A COLD SENSOR IN VITRO AND IN VIVO

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TRPA1 functions as an excitatory ionotropic receptor in sensory neurons. It was originally described as a cold-activated channel, but its cold sensitivity has been disputed in later studies and the contribution of TRPA1 to (noxious) cold sensing is currently a matter of strong debate.

Here, we provide several lines of evidence to establish that TRPA1 acts as a cold sensor in vitro and in vivo. First, we reconfirm that heterologously expressed TRPA1 is activated by cold in a Ca^{2+}-independent manner, and show that the influence of temperature on TRPA1 gating can be described using a two-state model previously used for other temperature-sensitive TRP channels. Second, we identify a specific subset of cold- and mustard oil-sensitive sensory neurons that is fully absent in neuronal preparations from TRPA1-deficient mice. Finally, we report a characteristic TRPA1-dependent nociceptive response to cold in mice.

We conclude that TRPA1 acts as a sensor for noxious cold.

Keywords: TRPA1, cold sensor, sensory neurons

T-TYPE CALCIUM SIGNALLING IN NOCICEPTION

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Ca^{2+} signaling plays a basic role in the functioning in neuronal networks that transmit sensory messages including the nociceptive ones to higher brain structures. This process is beginning at the level of dorsal root ganglia (DRG) neurons which are of different size and connected to corresponding types of primary afferents. Nociceptors are found only among medium and small sized neurons, and both types (contrary to large ones) express low-voltage activated (T-type) Ca^{2+} channels. Therefore these channels may play a definite role in nociceptive signaling.

A specific expression of these channels has been demonstrated in our experiments: in medium-sized neurons connected to Aγδ afferent fibers (capacitance 60±4.8 pF) the T-type Ca^{2+} channels corresponded to $Ca_v3.2$ (α_{1H}) type characterized by slow kinetics of activation and inactivation, while in small-sized ones connected to C-type afferent fibers (capacitance 26±1.6 pF) corresponded to $Ca_v3.3$ (α_{1I}) type characterized by fast kinetics. Obviously both types of DRG neurons participate in the transmission of pain messages of different type, and the activation of indicated types of T-type Ca^{2+} channels definitely determines the generation of pain sensations. An important finding came from analysis of the effects of external acidosis on the described T-type Ca^{2+} channels. It has been found that neurons of the middle size such acidification significantly and reversibly decreased LVA Ca^{2+}-current densities and shifted the potential of maximal activation to more positive voltages with significant reduction of its voltage-dependence. In small cells acidosis decreased LVA Ca^{2+} currents.

These results confirm the conclusion that these two types of Ca^{2+} channels with different sensitivity to external pH changes can be differently involved in the origin of neuropathic changes. This conclusion is supported by our findings of different pharmacological sensitivity of both types of primary nociceptive neurons.

Keywords: T-type Ca^{2+} channels, nociception, Ca^{2+} signalling

PRESENILIN 1 RELATED DISTURBANCES OF CALCIUM SIGNALING IN B CELLS ISOLATED FROM POLISH PATIENTS SUFFERING FROM ALZHEIMER'S DISEASE

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Dysregulation of calcium homeostasis is considered to be one of possible accelerating factors or even a cause of pathological changes observed in Alzheimer's disease (AD). Mutations in the gene encoding presenilin 1 (PS1) result in development of familial Alzheimer's disease (FAD) and were demonstrated to alter cellular calcium signaling. Cells bearing PS1 FAD mutations have been reported to display increased ER calcium content and attenuated capacitative calcium entry (CCE).

We investigated effects of 5 pathogenic FAD mutations (P117R, M139V, H163R, S170F, I213F) and non-pathogenic polymorphism (E318G) in PS1 gene on calcium homeostasis of B cells isolated from Polish patients. Ratiometric analysis revealed attenuation of CCE in cells with FAD mutations in comparison to lymphocytes from healthy individuals while thapsigargin-induced release of calcium ions from ER remained unchanged.

To elucidate the mechanism of observed disturbances in B cells calcium signaling, we assessed intracellular localization and expression levels of PS1 and STIM1/2 proteins, the latter being main signaling molecules involved in CCE activation. Our preliminary experiments revealed that in case of analysed PS1 FAD mutations cellular level of STIM2 protein was decreased while the amount of STIM1 and PS1 remained unchanged.

Decreased level of STIM2 protein may provide plausible explanation for attenuated CCE in cells bearing PS1 FAD mutations.

Keywords: presenilins, store-operated Ca^{2+} entry, Alzheimer's disease, STIM2

IDENTIFICATION OF A CRITICAL AMINO ACID FOR STIM1-MEDIATED GATING OF CRACM1

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Stromal interaction molecule (STIM1) in the endoplasmic reticulum (ER) and CRACM1 (Orai1) in the plasma membrane are essential molecules for controlling the store-operated CRAC current (I_{CRAC}). Combined over-expression of both proteins reconstitutes amplified CRAC currents and all three mammalian CRAC channel homologs (CRACM1, CRACM2 and CRACM3) represent functional store-operated channels. STIM1 proteins function as Ca^{2+} sensors in the ER and store depletion causes STIM1 to redistribute from a diffuse localization throughout the ER into puncta near the plasma membrane. The proximity of STIM1 puncta to the plasma membrane (10–25 nm) is close enough to permit local interactions with proteins in the plasma membrane, so that physical interactions may occur, directly or indirectly, between STIM1 and CRACM molecules in their respective membranes. However, little is known about the critical domains that participate in the interaction between these proteins. We have investigated this question using site-directed mutagenesis, chimeric constructs, protein truncations and tested for both store-operated and store-independent activation of CRACM1 and CRACM3. We identify a single amino acid residue in the N-terminus of CRACM1 that is critical for store-operated activation of CRACM1 but retains store-independent gating by 2-aminoethoxydiphenyl borate (2-APB) when probed in CRACM1/CRACM3 chimeras. In addition, we find that C-terminal deletions below amino acid Q285 in CRACM1 and K279 in CRACM3 result in complete loss of both store-operated and non store-operated gating modes.

Keywords: STIM1, Orai1, store-operated Ca^{2+} entry

THE STIM1-ORAI1 INTERACTION - A POTENTIAL THERAPUTIC TARGET IN THE TREATMENT OF PSORIASIS?

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Interactions between cytoplasmic calcium (Ca^{2+}_i), mitochondrial calcium (Ca^{2+}_{mt}) and store-operated calcium entry (SOCE) play a central role in regulating physiological and pathological responses. Psoriasis is a complex disease characterised by immune activation and epidermal hyperproliferation. Cultured keratinocyte from psoriatic patients display fundamental defects in Ca^{2+} signalling (Karvonen et al., 2000). Dithranol is an established topical treatment which induces rapid clearance of psoriatic plaques. As we have previously shown that dithranol localises to mitochondria, dissipates mitochondrial membrane potential ($\Delta\Psi m$) and induces apoptosis (McGill et al., 2005), we examined the effect of dithranol on Ca^{2+}_i, Ca^{2+}_{mt} and SOCE.

Pre-treatment with 5 μM dithranol significantly inhibited both UTP-induced Ca^{2+} release from the endoplasmic reticulum (ER) and also UTP-induced Ca^{2+} entry in HaCaT keratinocytes ($P<0.001$). In contrast, dithranol did not reduce the pool of Ca^{2+} mobilised from the ER by thapsigargin (Tg) in Ca^{2+}-free medium. However, in the presence of extracellular Ca^{2+}, dithranol reduced Tg-induced Ca^{2+} entry (SOCE). As $\Delta\Psi m$ regulates Ca^{2+} entry into mitochondria, we assessed its effects on $[Ca2+]_{mt}$ using Rhod 2. As expected dithranol induced ~10% reduction in resting $[Ca^{2+}]_{mt}$ over 20 min and inhibited Tg-induced increase in $[Ca^{2+}]_{mt}$. However, danthrone, a dithranol analogue that does not affect $\Delta\Psi m$, also impaired SOCE. As STIM1 and ORAI1 have been implicated in SOCE, we examined the effect of dithranol on their interaction. Confocal microscopy showed impairment of Tg-induced translocation of STIM1-cherryFP to the plasma membrane and association of STIM1-cherryFP and ORAI1-GFP by dithranol ($P<0.001$, Manders co-localisation co-efficient).

In conclusion, our data indicate that dithranol inhibits both Ca^{2+} release and SOCE. Dissipation of $\Delta\Psi m$ by dithranol may account in part for this but also suggest important cross-talk between mitochondria and ER Ca^{2+}.

Karvonen et al. (2000) J. Invest. Dermatol. 114, 693-700.
McGill et al. (2005) FASEB J. 19, 1012-1014.

Keywords: psoriasis, dithranol, mitochondria, store-operated Ca^{2+} entry

CONNEXIN HEMICHANNELS-MEDIATED Ca^{2+} ENTRY RESULTS IN NO PRODUCTION IN IN SITU INJURED ENDOTHELIAL CELLS

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Injury-induced Ca^{2+} signal in intact rat aorta consists of an initial peak, mainly mediated by Ca^{2+} release from inositol-1,4,5-trisphosphate-sensitive receptors, which is followed by a prolonged Ca^{2+} entry (Berra-Romani et al., 2008). The latter is abolished by putative gap-junction inhibitors, a result which led to the hypothesis that connexin (Cx) hemichannels mediate Ca^{2+} inflow into cells nearby wound edge.

In the present study, we investigated the effect of specific Cx mimetic peptides on injury-induced Ca^{2+} influx and assessed whether injury-induced Ca^{2+} entry may result in the synthesis of nitric oxide (NO), whose role is central to Ca^{2+}-dependent endothelial repair. These goals were accomplished by loading rat aortic endothelial cells (ECs) with the Ca^{2+}-sensitive fluorochrome, Fura-2/AM, and the NO-sensitive fluorescent dye, diaminofluorescein-2 (DAF-2)/FM diacetate. Pre-incubation (30 min) with 37,43Gap 27 (300 μM), which selectively inhibit Cx37 and Cx43, significantly reduced injury-induced Ca^{2+} entry nearby the lesion site. Similar results were obtained following acute application of both drugs. In a subsequent set of experiments, injury was found to cause a L-NAME-sensitive increase in DAF-2 fluorescence in ECs surrounding the lesion site. Injury-induced NO synthesis was not affected by previous depletion of intracellular Ca^{2+} stores with thapsigargin (2 μM), but disappeared in absence of extracellular Ca^{2+}. Pre-incubation with BTP-2 (20 μM), a selective blocker of capacitative calcium entry, did not alter injury-induced NO production. In contrast, injury-induced increase in NO levels was significantly reduced by a number of putative gap-junction inhibitors and by 37,43Gap 27 and 40Gap 27 (300 μM).

In conclusion, these findings demonstrate that Cx hemichannels-mediated Ca^{2+} entry results in NO synthesis in injured endothelium in situ. NO, in turn, might promote tissue repair by inducing endothelial migration and proliferation.

Berra-Romani et al. (2008) Cell Calcium, doi:10.1016/j.ceca.2007.12.007

Keywords: connexin, hemichannels, Ca^{2+} entry, NO

COUPLING OF THE PUTATIVE COILED–COIL DOMAIN OF ORAI PROTEINS WITH STIM1 MEDIATES ORAI CHANNEL ACTIVATION

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STIM1 and ORAI1 (also termed CRACM1) are essential components of the classical calcium-release-activated calcium (CRAC) current, however, the mechanism of the transmission of information of STIM1 to the CRAC/ORAI1 channel is as yet unknown.

Here we demonstrate by Förster Resonance Energy Transfer (FRET) microscopy a dynamic coupling of STIM1 and ORAI1 that culminates in the activation of Ca^{2+} entry. FRET imaging of living cells provided insight in the time-dependence of crucial events of this signalling pathway comprising Ca^{2+} store depletion, STIM1 multimerization and STIM1-ORAI1 interaction. Accelerated store depletion allowed resolving a significant time lag between STIM1-STIM1 and STIM1-ORAI1 interactions. Store refilling reversed both STIM1 multimerization and STIM1-ORAI1 interaction. The cytosolic STIM1 C-terminus itself was able, in vitro as well as in vivo, to associate with ORAI1 and to stimulate channel function, yet without ORAI1-STIM1 cluster formation. This dynamic interaction occurred via the C-terminus of ORAI1 that includes a putative coiled-coil domain structure. An ORAI1 C-terminus deletion mutant as well as a mutant (L273S) with impeded coiled-coil domain formation lacked both interaction as well as functional communication with STIM1, and failed to generate Ca^{2+} inward currents. Further data of ORAI2 and ORAI3 will be presented to support the concept of coiled–coil motif mediating communication between STIM1 and ORAI.

Supported by PhD-Program W1201 from the FWF, Austrian Academy of Science, FWF P18169.

Keywords: STIM1, Orai1, store-operated Ca^{2+} entry, FRET

MODULATION OF PMCA ACTIVITY BY LOCAL CALCIUM MICRODOMAIN NEAR CRAC CHANNEL IS CONTROLLED BY SUB-PLASMA MEMBRANE MITOCHONDRIA AFTER FORMATION OF THE IMMUNOLOGICAL SYNAPSE

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Formation of the immunological synapse (IS) is essential for T cell activation. The IS activates several signaling cascades including a rise in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through activation of Ca^{2+} release-activated Ca^{2+} (CRAC)/ORAI channels. Both mitochondria and plasma membrane Ca^{2+} ATPase (PMCA) are major targets of Ca^{2+} influx through CRAC/ORAI channels. The local microdomain of Ca^{2+} near CRAC/ORAI channels have been reported to induce channel inactivation if either mitochondrial Ca^{2+} uptake or mitochondrial translocation to the IS are disrupted. Sub-plasma membrane mitochondria control the steady state between CRAC/ORAI inactivation and the local Ca^{2+} microdomain, which has been shown to modulate the activity of PMCA locally as well.

By modulating the rate of Ca^{2+} influx, we changed the amplitude and extension of the Ca^{2+} microdomain and analyzed PMCA activity. In the absence of IS formation the local up-regulation of PMCA activity increased with increased Ca^{2+} influx through CRAC/ORAI channels. Upon IS formation, however, PMCA activity did not increase with increased Ca^{2+} influx. We hypothesized that the large amount of mitochondria close to the IS (< 200 nm from the plasma membrane) reduced the amplitude and extension of the Ca^{2+} microdomain by taking up much of the incoming Ca^{2+}. Indeed, the correlation between local modulation of PMCA activity and increased Ca^{2+} influx could be recovered by disrupting mitochondrial translocation to the IS, mitochondrial Ca^{2+} uptake or IS formation.

Our results suggest that mitochondria not only maintain a long lasting Ca^{2+} influx through CRAC/ORAI channels, but in addition decrease the Ca^{2+} extrusion through PMCA by reducing the amplitude and expansion of the Ca^{2+} microdomain near CRAC/ORAI channels following IS formation. Both mechanisms increase $[Ca^{2+}]_i$ and the subsequent Ca^{2+}-dependent activation and proliferation of T cells.

Keywords: mitochondria, PMCA, T cell activation, Orai1, Ca^{2+} microdomain, store-operated Ca^{2+} entry, lymphocytes

SLOW PHASE CYTOSOLIC CALCIUM CHANGES IN CULTURED CEREBELLAR ASTROCYTES DO NOT CORRELATE WITH VOLUME CHANGES OR GLUTAMATE UPTAKE

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Astrocytes modulate neuronal excitability and administrate brain electrolyte homeostasis. Under conditions of elevated neuronal cytosolic Ca^{2+} ($[Ca^{2+}]_i$) astrocyte $[Ca^{2+}]_i$ rises in parallel, a mechanism associated with feedback regulation of neurotransmission. Astrocytes are also essential for efficient extracellular glutamate clearance and water transport to the blood vessels.

We studied the effects of capacitative calcium entry and slow $[Ca^{2+}]_i$-changes in cultured cerebellar astrocytes and the effects of $[Ca^{2+}]_i$ on glutamate uptake and astrocyte volume changes. ATP-mediated $[Ca^{2+}]_i$ responses have little effect on glutamate uptake whereas endothelin-1 (ET-1) stimulation decreases uptake. Inhibition of the plateau phase of $[Ca^{2+}]_i$ elevation (capacitative Ca^{2+} entry) of ET-1 responses does not modulate glutamate uptake. Similarly, inhibitors or modulators of Ca^{2+} entry do not interfere with regulatory volume decrease (RVD) after hypotonic stress.

We conclude that the slow phase (e.g. activation of store-operated channels) of $[Ca^{2+}]_i$ homeostasis is not of central importance for volume homeostasis and glutamate transport in cultured cerebellar astrocytes. Due to Na^+/Ca^{2+} exchange, however, a sustained rise in $[Ca^{2+}]_i$ may give a rise in cytosolic Na^+ and indirectly lower the glutamate uptake capacity.

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Keywords: astrocytes, glutamate, store-operated Ca^{2+} entry

FAST Ca^{2+}-DEPENDENT INACTIVATION OF ORAI1/STIM1-MEDIATED CURRENT

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Orai1, a plasma membrane protein, and stromal interaction molecule 1 (STIM1), an EF hand containing, Ca^{2+}-binding polypeptide which senses the decrease in Ca^{2+} in the ER, are the recently cloned molecular components of the store-operated Ca^{2+} entry mechanism. Ectopically expressed Orai1 polypeptides that interact with STIM1 form store-operated Ca^{2+} channels with properties similar to that of the Ca^{2+}-release activated Ca^{2+} (CRAC) channels, which are present in hematopoietic cell lines. One of the properties that has been used to identify and distinguish I_{CRAC} from other types of Ca^{2+} currents in non-excitable cells is fast Ca^{2+}-dependent inactivation. However, the molecular mechanisms underlying fast inactivation of I_{CRAC} are poorly understood. Recent studies using heterologous expression of Orai1 and STIM1 show that mutations affecting selectivity of the channel also reduce or even abolish fast Ca^{2+}-dependent inactivation of the current (Yamashita et al., 2007).

In this work we show that fast Ca^{2+}-dependent inactivation of Orai1/STIM1-mediated current depends on relative expression levels of STIM1 and Orai1. Currents recorded from cells transfected with a mixture of cDNA encoding STIM1 and Orai1, show strong fast Ca^{2+}-dependent inactivation when STIM1 is in excess. In contrast, when Orai1 is in excess, Orai1/STIM1-mediated currents show strong activation at negative potentials. Moreover, activating and inactivating Orai1/STIM1-mediated currents show different selectivity for Ba^{2+} and Sr^{2+}, different levels of inhibition by external Ca^{2+}, and different levels of potentiation by 2-APB.

Overall, the results suggest that depending on the expression levels, Orai1 and STIM1 can form complexes of different stoichiometry.

Yamashita et al. (2007) J. Gen. Physiol. 130, 525-540.

Keywords: Orai1, STIM1, Ca^{2+}-dependent inactivation, store-oparated Ca^{2+} entry

α1-SYNTROPHIN AND ITS N-TERMINAL ARE CRUCIAL FOR REGULATING STORE-OPERATED CATION ENTRIES AND ASSOCIATE WITH TRPC1/TRPC4 CHANNELS IN MUSCLE

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A tight control of intracellular Ca^{2+} is primordial for the survival and normal function of skeletal muscle. The lack of dystrophin in Duchenne muscular dystrophy results in calcium mishandling and subsequent fiber necrosis. These are thought to involve uncontrolled store-operated Ca^{2+} channels (SOCs) currents supported by TRPCs.

Our recent work demonstrated that mini-dystrophin and α1-syntrophin restored normal capacitative calcium entries (CCEs) in dystrophic myotubes and that sarcolemmal TRPC1 channels are associated with dystrophin and also α1-syntrophin and its PDZ domain. This study shows by siRNA silencing that mini-dystrophin could not regulate CCEs in absence of α1-syntrophin. This suggests that α1-syntrophin is essential for regulating CCEs in muscle cells. Moreover, the PDZ-containing domain of α1-syntrophin is necessary for restoring normal CCEs in dystrophic cells. TRPC1 and TRPC4 channels are expressed at the plasma membrane and form a molecular complex in muscle. Both channels constitute SOCs when co-expressed in myotubes and increase CCEs. A molecular association was also found between TRPC1/TRPC4 channels and the α1-syntrophin/dystrophin complex.

We argue that TRPC1/TRPC4 channels form sarcolemmal SOC anchored to the dystrophin cytoskeleton, and that α1-syntrophin is necessary for maintaining normal regulation of TRPC-dependent CCE in skeletal muscle, and a low subsarcolemmal calcium microdomain.

Keywords: α1-syntrophin, TRPC1, TRPC4, store-operated Ca^{2+} entry, muscular dystrophy

SDMA, A UREMIC RETENTION SOLUTE, ENHANCES Ca^{2+} ENTRY IN MONOCYTES VIA STIMULATION OF STORE-OPERATED Ca^{2+} CHANNELS

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Deterioration of kidney function leads to development of the uremic syndrome, a complex clinical picture caused by retention of a series of solutes. A common feature in uremic cells/tissues is an increased intracellular calcium level ($[Ca^{2+}]_i$) and chronic kidney disease has been referred to as a state of calcium toxicity.

In this study the retention solute symmetric dimethylarginine (SDMA) was evaluated for its effect on $[Ca^{2+}]_i$ handling in monocytes. In a flow cytometric assay heparinized whole blood was loaded with the Ca^{2+} indicator Fluo-3 followed by the addition of a PE-labelled CD45 antibody to identify the leukocytes. Samples were incubated with saline (control) or SDMA at uremic concentration and $[Ca^{2+}]_i$ was measured at baseline and after exposure to fMLP, a chemotactic peptide stimulating the cells via a G-protein coupled receptor. Solutions without extracellular Ca^{2+} (Ca^{2+}_{ex}) contained in addition 5 mM EGTA. Thapsigargin and removal/addition of Ca^{2+}_{ex} was used to investigate the contribution of store-operated Ca^{2+} channels (SOCs). Pathophysiological consequences of SDMA were tested by analysing fMLP-triggered ROS production. We found that SDMA increased the amplitude of the peak $[Ca^{2+}]_i$ change triggered by fMLP. This potentiating effect was dependent on the presence of Ca^{2+}_{ex}, suggesting that SDMA enhances Ca^{2+} entry. Depletion of the intracellular stores with thapsigargin in the absence of Ca^{2+}_{ex}, followed by re-addition of Ca^{2+}_{ex} triggered Ca^{2+} entry that was significantly larger after SDMA treatment. SDMA potentiation of Ca^{2+} entry in these experiments was prevented with the SOC inhibitor SKF96365. At the pathophysiological level, SDMA potentiated fMLP-triggered ROS production and this effect was also dependent on the presence of Ca^{2+}_{ex}.

In conclusion, our experiments indicate that SDMA enhances Ca^{2+} entry via SOCs, an effect that lies at the origin of an increased ROS production and possibly contributing to enhanced oxidative stress in uremic patients.

Keywords: store-operated Ca^{2+} entry, uremic syndrome, SDMA

2-AMINOETHOXYDIPHENYL BORATE ALTERS SELECTIVITY OF Orai3 CHANNELS BY INCREASING THEIR PORE SIZE

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Stim1 in the endoplasmic reticulum and the three Orai (also termed CRACM) channels in the plasma-membrane are main components of native Ca^{2+} release activated Ca^{2+} channels. A pharmacological hallmark of these channels is their distinct sensitivity to 2-aminoethoxydiphenyl borate (2-APB).

Here we report using whole-cell patch clamp technique that Orai3 currents can be robustly stimulated by 75 µM 2-APB independently of Stim1, while 2-APB at similar concentrations inhibited store-operated Orai1 currents. 2-APB did not only promote currents through Orai3 channels but also dramatically altered ion selectivity of Orai3 channels, enabling permeation of monovalent cations both in the inward as well as outward direction, which is in sharp contrast to the high Ca^{2+} selectivity of store-operated Orai3 currents. An Orai3-R66W mutant, which, in analogy to the severe combined immune deficiency mutant Orai1-R91W, lacked store-operated activation was found to be resistant to 2-APB stimulation. The change in selectivity by 2-APB was associated with an increase in Orai3 minimum pore size from about 3.8 Å to more than 5.34 Å.

In line with a potential interaction of 2-APB with the Orai3 pore, among three pore mutants tested particularly the Orai3 E165Q mutant resembled in its permeation properties those of 2-APB-stimulated Orai3 and additionally exhibited a reduced response to 2-APB. A chimera where the 2nd loop of Orai1 was substituted into Orai3 protein was also robustly stimulated by 2-APB. Further deletion mutants of Orai3 proteins will be discussed. In aggregate, stimulation of Orai3 currents by 2-APB occurred along with an alteration of the permeation pathway which represents a unique mechanism for regulating ion channel selectivity by chemical compounds.

Supported by the Austrian Science Foundation (FWF) projects P18169 and INTAS project 05/1000008-8223 to C.R.

Keywords: Orai3, 2-APB, Ca^{2+} selectivity

FUNCTIONAL CHARACTERIZATION OF THE CACNA1A A454T MUTATION: RELEVANCE TO MIGRAINE CLINICAL PHENOTYPE

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Familial hemiplegic migraine-causing mutations in the gene encoding the P/Q Ca^{2+} channel α1A subunit (CACNA1A) locate to the pore and voltage sensor regions and normally involve gain of channel function that favor cortical spreading depression initiation and propagation.

We now report on a mutation identified in the first intracellular linker (ILI-II) of CACNA1A (α1A(A454T)) that is associated to the absence of sensorimotor symptoms in a migraine with aura pedigree. α1A(A454T) channels showed weakened regulation of voltage-dependent steady-state inactivation by CaVβ subunits. α1A(A454T) also presented accelerated inhibition by G protein β-γ dimers following facilitatory depolarization. Furthermore, the A454T mutation impairs interaction of syntaxin 1A and SNAP-25 with α1A, thus abolishing P/Q channel modulation by SNARE proteins and decreasing secretion efficiency.

Our results reveal the importance of ILI-II structural integrity in the interaction between P/Q Ca^{2+} channels and plasma membrane SNAREs and that genetic variation in CACNA1A may be not only a cause but also a modifier of migraine phenotype.

Supported by Fundació la Marató de TV3, Ministerio de Educación y Ciencia, Fondo de Investigación Sanitaria and Generalitat de Catalunya.

Keywords: P/Q-type Ca^{2+} channels, CACNA1A mutation, SNARE, hemiplegic migraine, channelopathy

STIM1 OLIGOMERIZATION IS A RAPID AND REVERSIBLE EVENT REGULATED BY PHYSIOLOGICAL CHANGES IN ER CALCIUM CONCENTRATION

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Stromal interacting molecule 1 (STIM1) is an endoplasmic reticulum (ER) Ca^{2+} sensor that redistributes to ER-plasma membrane junctions to activate store-operated Ca^{2+} entry (SOCE) channels upon ER Ca^{2+} depletion. STIM1 oligomerization is the critical event that triggers SOCE (Luik et al., 2008), but how $[Ca^{2+}]_{ER}$ dynamically control STIM1 oligomerization is unclear.

Here, we quantify the $[Ca^{2+}]_{ER}$ dependence of STIM1 oligomerization during physiological stimulation of HeLa cells, using the D1ER Ca^{2+} indicator to measure $[Ca^{2+}]_{ER}$ and FRET between CFP-STIM1 and YFP-STIM1 to monitor STIM1 oligomerization. Thapsigargin increased the STIM1 FRET signal as soon as $[Ca^{2+}]_{ER}$ decreased below basal values. Ca^{2+} removal caused a smaller drop in $[Ca^{2+}]_{ER}$ mirrored by a proportional increase in FRET. In Ca^{2+}-free medium, histamine decreased $[Ca^{2+}]_{ER}$ to ~200 µM and evoked a maximal increase in FRET, which was reversed by restoring $[Ca^{2+}]_{ER}$. Increasing $[Ca^{2+}]_{ER}$ above basal values further decreased the FRET signal, indicating that STIM1 formed oligomers at resting $[Ca^{2+}]_{ER}$. This prompted us to check whether the expression of exogenous STIM1 impacted on our $[Ca^{2+}]_{ER}$ measurements. STIM1 expression did not alter the Ca^{2+} sensitivity of the D1ER probe or the $[Ca^{2+}]_{ER}$ responses evoked by thapsigargin or by histamine in Ca^{2+}-free medium. In the presence of external Ca^{2+} however, STIM1 expression blunted the decrease in $[Ca^{2+}]_{ER}$ evoked by histamine and STIM1 oligomerization was barely detectable.

We conclude that: 1) STIM1 oligomerization is a reversible event that occurs immediately when $[Ca^{2+}]_{ER}$ drops below resting levels and that is maximal when $[Ca^{2+}]_{ER}$ reach ~200 µM, 2) STIM1 can form labile oligomers even in cells with replete stores, suggesting that ER Ca^{2+} overload might prevents the basal activity of SOCE channels, and 3) enforced STIM1 expression blunts $[Ca^{2+}]_{ER}$ depletion during physiological stimulations, probably because the additional STIM1 molecules facilitate the refilling of ER Ca^{2+} stores.

Luik et al. (2008) Nature, in press (published online 2 July 2008).

Keywords: STIM1, store-operated Ca^{2+} entry, FRET, Ca^{2+} stores

MITOCHONDRIAL Ca^{2+} UPTAKE DURING STORE-OPERATED Ca^{2+} INFLUX

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The inwardly rectifying ruthenium red-sensitive Ca^{2+} uniporter, carrying Ca^{2+} into the mitochondrial matrix, has low affinity for Ca^{2+}. Mitochondrial Ca^{2+} uptake during cytosolic Ca^{2+} signalling has been attributed to high-Ca^{2+} microdomains (HCMD) formed between mitochondria and the mouth of Ca^{2+} channels either in the apposing endoplasmic reticulum (ER) or the plasma membrane. However, our studies in adrenal glomerulosa and ovarian luteal cells have indicated that store-operated Ca^{2+} influx (SOC) induces mitochondrial Ca^{2+} signals without the formation of HCMD (Rohács et al., 1997; Szabadkai et al., 2001; Szanda et al., 2006). Here we examined the role of HCMD in the induction of mitochondrial Ca^{2+} signals during Ca^{2+} influx via SOC in COS-7 cells.

According to current views, STIM1 molecules located in the ER membrane serve as sensors of intraluminal [Ca^{2+}]. ER Ca^{2+} depletion is followed by clustering of STIM1 molecules and translocation to subplasmalemmal ER regions (puncta formation). This also leads to clustering of the Orai1 Ca^{2+} channels in the corresponding plasma membrane regions leading to activation of Ca^{2+} influx (Putney, 2007).

In the present study COS-7 cells were transfected with Orai1, STIM1-mRFP and the mitochondrially targeted Ca^{2+}-sensitive fluorescent protein, mt-inverse-Pericam. Mitochondrial [Ca^{2+}] ([Ca^{2+}]$_m$) (Pericam) and STIM-1 movement and puncta formation (mRFP) within the 100 nm subplasmalemmal space were monitored with Total Internal Reflection Fluorescence (TIRF) microscopy. ATP + thapsigargin-induced Ca^{2+} depletion in a Ca^{2+}-free medium evoked rapid puncta formation in subplasmalemmal ER structures. Readdition of Ca^{2+} rapidly increased [Ca^{2+}]$_m$. Pericam-labelled mitochondria were located between but not co-localized with the mRFP-labelled ER puncta. SOC-mediated changes in [Ca^{2+}]$_m$ were inversely correlated with mRFP fluorescence indicating that mitochondria are not present in the HCMD formed between STIM1 and Orai1 molecules but are exposed to Ca^{2+} diffusing laterally from the sites of Ca^{2+} entry.

Putney (2007) J. Cell Sci. 120, 1959-1965.
Rohács et al. (1997) Biochem. J. 328, 525-528.
Szabadkai et al. (2001) Pflügers Arch. 441, 678-685.
Szanda et al. (2006) Cell Calcium 40, 527-537.

Keywords: mitochondria, STIM1, TIRF, Ca^{2+} microdomain, store-operated Ca^{2+} entry

NICOTINE ACTIVATES THE CHEMOSENSORY CHANNEL TRPA1

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Besides being used as a strong addictive, nicotine is employed in replacement therapies for smoking cessation. However, the mucosal and skin irritation produced by this compound is a common cause of treatment interruption. So far, nicotine-induced irritation is thought to be exclusively mediated by nicotinic acetylcholine receptors (nAChRs) expressed in chemosensory neurons.

Here we report that nicotine activates TRPA1, a cation channel that functions as a sensor of irritant chemicals in nociceptive nerve fibres. Patch-clamp experiments revealed that nicotine enhances TRPA1 currents in CHO cells with an effective concentration of 50 µM at -75 mV. This activation was also observed in intracellular Ca^{2+} imaging experiments in intact TRPA1-expressing CHO cells and in cell-free inside out patches. Closely resembling in vivo observations, nicotine-induced activation of TRPA1 causes self-desensitization, cross-desensitization with mustard oil and is inhibited by menthol, a compound popularly used to mask nicotine pungency. TRPA1 is also activated by the nicotine analogues nicotinic acid and nicotinamide, which constitute vitamin B3 and by anabasine, a compound that acts as insect repellent in plants. Notably, we found that mecamylamine, a nAChR antagonist commonly used to imply the role of these receptors in nicotine pungency, also inhibits TRPA1. In contrast, TRPA1 was not affected by the unspecific neuronal nAChR blocker hexamethonium. Nicotine stimulates a subset of mouse trigeminal neurons that largely overlaps with the TRPA1-expressing subpopulation. Neuronal responses to nicotine can be observed in the presence of hexamethonium and are strongly reduced in TRPA1 knockout mice. Our results indicate that TRPA1 contributes to nicotine-induced irritation and that its inhibition may help to alleviate the side effects of nicotine in smoke cessation therapies.

Keywords: TRPA1, nicotine, sensory neurons

SPHINGOSINE 1-PHOSPHATE AS AN AUTOCRINE ENHANCER OF CALCIUM ENTRY

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Calcium entry is one of the main regulators of intracellular signalling. In the present study we describe the importance of sphingosine, sphingosine kinase (SK) and sphingosine 1-phosphate (S1P) in regulating calcium entry through a protein kinase A (PKA) and phosphatase regulated entry pathway.

In cells incubated with the phosphatase inhibitor calyculin A (caly A) to evoke calcium entry, sphingosine inhibited calcium entry in a concentration-dependent manner. Furthermore, the SK inhibitor 2-(p-hydroxyanilino)-4-(p-chlorophenyl)thiazole (SKi) attenuated calcium entry. Addition of exogenous S1P restored the calcium entry in cells preincubated with caly A and SKi. Neither sphingosine nor SKi attenuated store-operated calcium entry. The cells express the S1P2 and S1P3 receptors, and blocking these with the S1P2-inhibitor JTE 013, but not with the S1P3-inhibitor VPC-23019, potently attenuated caly A-evoked calcium entry. The phospholipase C inhibitor U73122, but not the inactive analogue U73343, blocked calcium entry. In cells incubated with caly A and [^{3}H]-sphingosine, the production of [^{3}H]-S1P was increased by 24±6%, compared with vehicle-treated cells. Treating the cells with the ABCC1-inhibitor MK-571 attenuated caly A-evoked calcium entry, but the entry could be restored by addition of exogenous S1P. In cells overexpressing the wild type SK, caly A-evoked calcium entry was enhanced compared with mock-transfected cells. In cells transfected with the dominant negative G82D SK mutant, calcium entry was attenuated, but addition of exogenous S1P restored calcium entry.

Our results suggest that the PKA- and phosphatase dependent calcium entry pathway is blocked by sphingosine, but that SK and the production of S1P, probably through an autocrine mechanism, facilitates calcium entry by activation of the S1P2 receptor. This is a novel mechanism by which the sphingosine-S1P rheostat regulates cellular calcium homeostasis.

Keywords: sphingolipids, Ca^{2+} entry, thyroid

ASTROCYTES DETERMINE THE CALCIUM PERMEABILITY OF AMPA RECEPTORS OF MOTOR NEURONS BY REGULATING GluR2 EXPRESSION

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AMPA receptor-mediated excitotoxicity has been implicated in the pathogenesis of stroke, neurotrauma, epilepsy and many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disorder characterized by the selective death of motor neurons in the motor cortex, brainstem and spinal cord. In ALS animal models, AMPA receptor antagonists have a therapeutic effect and calcium influx through AMPA receptors is the major trigger for AMPA receptor-mediated motor neuronal death. The calcium permeability of this AMPA receptor is determined by the relative abundance of the GluR2 subunit. AMPA receptors containing the GluR2 subunit have a low calcium permeability, whereas GluR2-lacking AMPA receptors have a high calcium permeability. Cultured motor neurons are selectively vulnerable to AMPA receptor stimulation due to calcium influx through GluR2-lacking AMPA receptors.

Here, we investigated the difference between two rat strains in the vulnerability of motor neurons to excessive AMPA receptor stimulation. Cultured motor neurons derived from Wistar rats were more susceptible to AMPA receptor stimulation than Holtzman motor neurons. AMPA receptors from Wistar motor neurons had a higher calcium permeability compared to AMPA receptors from motor neurons of Holtzman rats. Single cell RT-PCR experiments showed that Wistar motor neurons had a lower relative GluR2 mRNA expression level, indicating that GluR2 expression is regulated at the transcriptional level. Moreover, the astrocytic feeder layer determined the motor neuron behavior. Wistar motor neurons grown on a Holtzman feeder layer acquired the characteristics of Holtzman motor neurons, while Holtzman motor neurons cultured on a Wistar feeder layer behaved like Wistar motor neurons.

In conclusion, these results show that astrocytes play a crucial role in the regulation of the subunit composition of AMPA receptors, and thus in the vulnerability of neurons to excitotoxicity.

Keywords: amyotrophic lateral sclerosis, excitotoxicity, ionotropic glutamate receptor, motor neurons

DIFFERENTIAL INTERACTIONS OF Na^+ CHANNEL TOXINS WITH T-TYPE Ca^{2+} CHANNELS

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Voltage-gated Na^+ and Ca^{2+} channels are ubiquitously expressed in excitable cells across the animal kingdom and from an evolutionary standpoint have been proposed to have arisen from a common ancestor, primarily by gene duplication. Among the three subfamilies of Ca^{2+} channels encoded by the Ca_v genes (Ca_v1, Ca_v2, and Ca_v3), the Ca_v3 subfamily encoding low-threshold voltage-activated Ca^{2+} channels commonly referred to as T-type Ca^{2+} channels has been hypothesized to be the closest Ca^{2+} channel subfamily to the Na^+ channel genes. Tetrodotoxin (TTX) and saxitoxin (STX) are two structurally related heterocyclic guanidinium marine toxins that potently inhibit voltage-gated Na^+ channels by an interaction with several residues in the P-loop.

In view of the structural similarities within or near the pore region of the two classes of channels, we wondered whether these toxins also interact with T-type Ca^{2+} channels. We examined the effects of TTX and STX on calcium currents from Tsa-201 cells overexpressing $Ca_v3.1$, $Ca_v3.2$, or $Ca_v3.3$. While TTX produced no direct effect on I_{CaT} elicited by overexpression of $hCa_v3.1$ and $hCa_v3.2$, it significantly attenuated the block of this current by Ni^{2+}; in contrast, 30 µM TTX directly inhibited $hCa_v3.3$-induced I_{CaT} and the addition of 750 µM Ni^{2+} to the TTX-containing medium led to greater block of the current which was not significantly different from that produced by Ni^{2+} alone. On the other hand, 1 µM STX directly inhibited $Ca_v3.1$-, $Ca_v3.2$-and $Ca_v3.3$-mediated calcium currents but did not enhance the ability of Ni^{2+} to block these currents.

These findings provide important new implications for our understanding of structure-function relationships of T-type Ca^{2+} channels, and further extend the hypothesis of a parallel evolution of Na^+ and Ca^{2+} channels from an ancestor with common structural motifs.

Keywords: nickel, T-type Ca^{2+}channels, toxins, Na^+ channels

CHARACTERIZATION OF AN 'OLD' BUT POORLY UNDERSTOOD TRP MEMBER - TRPM1?

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TRPM1 is the first identified member of the mammalian TRPM (melastatin) subfamily and was originally named 'melastatin'. TRPM1 is proposed as a putative tumor suppressor protein. In melanoma cells TRPM1 expression is decreased, and it has been shown that there is an inverse correlation between melastatin expression and metastatic potential in some melanoma cell lines (Duncan et al., 1998). At present, surprisingly, no functional data have been reported for TRPM1.

We have investigated variants of the B16 murine melanoma line, B16-F1 and B16-F10. First, we validated the TRPM1 expression that is expressed at high levels in poorly metastatic variants of B16 melanoma and at much reduced levels in highly metastatic B16 variants. Patch clamp recordings showed a correlation between cDNA levels and the amplitude of an outward rectifying current. MicroRNAi developed against TRPM1 showed significant reduction of the outward rectifying current in B16-F1 cells. This outward rectifying current is unaffected by changes in extracellular [Ca^{2+}] and can be blocked by 0.1 mM La^{3+}. A similar outward rectifying current was observed in human epidermal melanocytes (HEM). Significant reduction in cDNA level and current was measured in HEM cells transfected with miRNA against TRPM1.

In conclusion, we observed a correlation between TRPM1 cDNA levels and the amplitude of an outward rectifying current in B16-F1 and HEM cells.

Duncan et al. (1998) Cancer Res. 58, 1515-1520.

Keywords: TRPM1, melanoma

ATP-INDEPENDENT TRANSLOCATION OF STIM1 AND FORMATION OF STIM1-ORAI1 COMPLEXES

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Stromal interacting molecule 1 (STIM1) is currently known to be a calcium sensor protein located in the endoplasmic reticulum (ER). Depletion of the ER calcium store triggers translocation of STIM1 into subplasmalemmal punctae where it activates calcium channels and initiates store operated calcium entry (SOCE). We show that inhibition of ATP production induced a slow calcium leak from the ER that was followed by formation of subplasmalemmal STIM1 puncta. Depletion of cytosolic ATP also initiated the loss of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) from the plasma membrane. Although STIM1 punctae formed by inhibition of ATP synthesis co-localised with clusters of ORAI1 channels these complexes did not efficiently facilitate calcium influx. Restoration of ER calcium levels in the absence of ATP permitted STIM1 re-translocation from subplasmalemmal punctae to the bulk of the ER. Therefore we suggest that dynamic re-arrangement of STIM1 and the formation of STIM1-ORAI1 complexes is an ATP-independent process that can occur under conditions of $PI(4,5)P_2$ depletion.

Keywords: Store-operated Ca^{2+} entry, STIM1, ATP, PIP_2

THE S218L FAMILIAL HEMIPLEGIC MIGRAINE MUTATION PROMOTES DEINHIBITION OF $Ca_v2.1$ CALCIUM CHANNELS DURING DIRECT G-PROTEIN REGULATION

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Familial hemiplegic migraine type 1 (FHM-1) is caused by mutations in CACNA1A; the gene encoding for the $Ca_v2.1$ subunit of voltage-gated calcium channels. Although various studies attempted to determine biophysical consequences of these mutations on channel activity, it remains unclear exactly how mutations can produce a FHM-1 phenotype. A lower activation threshold of mutated channels resulting in increased channel activity has been proposed. However, hyper-activity may also be caused by a reduction of the inhibitory pathway carried by G protein-coupled receptor activation.

The aim of this study is to determine functional consequences of the FHM-1 S218L mutation on direct G protein regulation of $Ca_v2.1$ channels. In HEK 293 cells, DAMGO activation of human μ-opioid receptors induced a 55% Ba^{2+} current inhibition through both wild-type and S218L mutant $Ca_v2.1$ channels. In contrast, this mutation considerably accelerates the kinetic of current deinhibition following channel activation by 1.7- to 2.3-fold depending on membrane potential values. Taken together, these data suggest that the S218L mutation does not affect G proteins association onto channel in the closed state, but promotes its dissociation from the activated channel thereby decreasing the inhibitory G-protein pathway. Similar results were obtained with the R192Q FHM-1 mutation, although of lesser amplitude, which seems in line with the less severe associated clinical phenotype in patients.

Functional consequences of FHM-1 mutations appear thus as the consequence of the alteration of both intrinsic biophysical properties and of the main inhibitory G protein pathway of $Ca_v2.1$ channels. The present study furthers molecular insight in the physiopathology of FHM-1.

Keywords: CACNA1A mutation, hemiplegic migraine, P/Q-type Ca^{2+} channels, G protein, channelopathy

FUNCTIONAL REGULATION OF CALCIUM HOMEOSTASIS IN ADULT MOUSE SKELETAL MUSCLE FIBRE BY SPECIFIC G-PROTEIN β-γ SUBUNITS

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G-protein β-γ dimer, an ubiquitous second messenger, allows functional coupling between external signals from G-protein coupled receptors and intracellular effectors such as voltage-gated calcium channels. This regulation is mainly recognized by a drastic current inhibition, associated in some cases with other biophysical modifications of the channel activity. Whereas inhibition of Ca_v2 channels is well documented, little is understood on the regulation of the Ca_v1 subfamily.

The aim of this study is to determine if $Ca_v1.1$ channels (dihydropyridine receptor, DHPR) could also undergo direct regulation by the G-protein β-γ dimer. To this aim, the different YFP-tagged G-protein β isoforms (β1 to β5) were overexpressed in vivo in adult mouse skeletal muscle in combination with the γ2 subunit. We show that the L-type calcium current density is selectively reduced in fibres overexpressing the G-protein β1-γ2 dimer. Moreover, this inhibition is associated with a reduction of voltage-activated calcium release, suggesting an alteration of the functional coupling between the DHPR and the ryanodine receptor (RyR). This effect is consistent with an expression pattern of the β1-γ2 dimer in the plasma membrane, as well as in the T-tubule membrane, as observed by confocal microscopy.

Taken together, these results demonstrate for the first time a specific regulation of the DHPR activity by the G-protein β-γ dimer and open new insights into possible implications of G-protein coupled receptors in the control of calcium homeostasis in skeletal muscle.

Keywords: G protein, skeletal muscle, L-type Ca^{2+} channel