POSTER SESSION

D

INTRACELLULAR Ca^{2+} STORES AND Ca^{2+} SIGNALLING

INVESTIGATING THE MECHANISM OF AMYLOID β-PEPTIDE-INDUCED Ca^{2+} RELEASE

ALLAN L.E.[1], BULTYNCK G.[2], AMIJEE H.[3], BOOTMAN M.D.[1], RODERICK H.L.[1,4]

1 Lab. of Mol. Signalling, The Babraham Institute, Cambridge, CB22 3AT, UK.
2 Lab. of Mol. and Cell. Signalling, K.U. Leuven, B-3000 Leuven, Belgium.
3 Senexis Limited, Babraham Research Campus, Cambridge CB22 3AT, UK
4 Dept. Pharmacology, Univ. Cambridge, Cambridge,CB2 1PD, UK.

Alzheimer's disease (AD) is a fatal neurodegenerative disorder leading to severe memory loss and a decline in cognitive abilities. It has been hypothesised that the amyloid beta-peptide (Aβ) is central to the pathogenesis of AD but its exact mode of action is yet to be fully elucidated. Increasing evidence implicates a disruption of intracellular calcium (Ca^{2+}) homeostasis in AD. Here we investigate the immediate effects of oligomeric Aβ on cellular Ca^{2+} levels.

Aβ oligomers were prepared according to published work and their toxicity validated in MTT assays prior to imaging experiments. To investigate the effects of Aβ on intracellular Ca^{2+} homeostasis, SH-SY5Y cells loaded with fura-2 were imaged following extracellular application of Aβ for 25 minutes. Carbachol (CCH) was then applied to ascertain whether Ca^{2+} stores had remained intact. Aβ oligomers elicited Ca^{2+} transients in a concentration-dependent manner. An inverse relationship between the responses to Aβ and to CCH was also observed, suggesting that Aβ induced depletion of intracellular Ca^{2+} stores. This conclusion was supported by the lack of Ca^{2+} signals in cells in which ER Ca^{2+} stores had been depleted by thapsigargin. Moreover, Aβ-induced Ca^{2+} signals persisted when extracellular Ca^{2+} was removed. Inhibition of IP_3Rs with caffeine significantly reduced the peak amplitude ($p = 0.002$) and the area under the curve ($p < 0.0001$) of Aβ-induced Ca^{2+} transients but did not abolish them. This indicates that Aβ-induced Ca^{2+} transients do not only occur via IP_3Rs. Western blot analysis and imaging experiments showed that SH-SY5Y cells did not express RyRs, indicating the involvement of another mechanism. Adenoviral infection of SH-SY5Y cells with IP_3 5-phosphatase and FlexStation experiments using permeabilised DT40 and TKO DT40 cells now aim to corroborate the involvement of IP_3Rs in Aβ-induced Ca^{2+} release. FlexStation experiments will also help to establish whether Ca^{2+} leak from the ER is affected by Aβ.

Keywords: Alzheimer's disease, SH-SY5Y cells, amyloid beta-peptide, IP_3 receptor

EFFECT OF DIFFERENT SPHINGOLIPIDS AND AGELASINE B ON INTRACELLULAR CALCIUM MOBILIZATION IN BREAST CANCER CELLS (MCF-7)

BENAIM G.[1,2], PIMENTEL A.[1], NIEVES J.[1], ARVELO F.[1,2], SOJO F.[2], ROJAS H.[3], COMPAGNONE R.[2] & SUAREZ A.[4]

[1] Centro de Biociencias y Medicina Molecular, Instituto de Estudios Avanzados (IDEA),
[2] Instit. de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela
[3] Centro de Biofisica, IVIC, and
[4] Facultad de Farmacia, Universidad Central de Venezuela,
Caracas, Venezuela.

Sphingolipids regulate different cellular functions. We have demonstrated, using Fura-2 fluorescence, that ceramide, ceramide 1-P (Cer-1-P), sphingosine and sphingosine-1-P (S-1-P) are all involved in Ca^{2+} mobilization in many cell lines. However, their effect varies depending on the cell type. In the present work we show that these sphingolipids are able to increase the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in breast cancer cells (MCF-7). Both, ceramide and Cer-1-P, increased $[Ca^{2+}]_i$ from intracellular compartments, which in turn activate a capacitative Ca^{2+} entry at the plasma membrane, as corroborated by the use of the CRAC inhibitor BTP-2. Confocal microscopy revealed that both ceramide and Cer-1-P released Ca^{2+} from the endoplasmic reticulum. However, Cer-1-P was more potent than ceramide. Addition of ceramine, a non-hydrolysable analogue of ceramide, reproduced its effect, thus ruling out that it was due to conversion of ceramide to sphingosine. On the other hand, the effect of ceramide was additive to that of sphingosine, which also induced an increase in the $[Ca^{2+}]_i$. However, the effect of sphingosine was observed even after the endoplasmic reticulum was depleted of Ca^{2+}, by addition of thapsigargin. This result indicated that this sphingolipid by itself provoked an opening of a plasma membrane Ca^{2+} channel.

We also show in this work the effect of agelasine B in breast cancer cells. This compound was purified from a sea sponge (Agelas clathrodes), showing differential cytotoxic properties in MCF-7 (IC_{50} 2.22 µg/ml.) with respect to fibroblasts (IC_{50} 23.43 µg/ml), Agelasine B was able to induce an increase in the $[Ca^{2+}]_I$ in MCF-7. This was due to the liberation of Ca^{2+} from the endoplasmic reticulum, as could be seen by the use of confocal microscopy. We also demonstrated that agelasine B is able to inhibit the activity of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), which could explain its deleterious effect on these cell line.

Keywords: sphingolipids, Ca^{2+} signalling, agelasine B

ALTERATION OF Ca^{2+} DEPENDENCE OF SYNAPTOSOMAL PLASMA MEMBRANE Ca^{2+} ATPASE IN HUMAN BRAIN AFFECTED BY ALZHEIMER'S DISEASE

BERROCAL M., MARCOS D. & MATA A.M.

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Avda. de Elvas s/n, E-06071 Badajoz, Spain.

Considerable evidence supports a cause-effect relationship between Ca^{2+} dysregulation, amyloid β-peptide (Aβ) toxicity and neuronal degeneration in Alzheimer's disease (AD).

In this study, effects of Ca^{2+}-dependence on the activities of the plasma membrane Ca^{2+}-ATPase (PMCA) and intracellular Ca^{2+} pumps from the endoplasmic reticulum (SERCA) and the secretory pathway (SPCA) were assayed in non-affected (control) and AD human brains. Our results indicate a Ca^{2+} sensitivity decrease of the total Ca^{2+}-ATPase activity in AD compared with control brains. However, only the PMCA activity was affected, while SERCA and SPCA pumps showed similar Ca^{2+} affinity in both tissues. This could be a consequence of a change in PMCA conformation, which may result from its binding to the toxic Aβ which is present in AD brains, because PMCA activities in control human membranes and pig synaptosomes were less sensitive to Ca^{2+} changes only in the presence of Aβ.

These data point out a specific alteration in the functioning of PMCA with respect to Ca^{2+} dependence in human brains affected with AD, which may be intimately linked to the toxic effect of Aβ and may result or be a consequence of dysregulation in neuronal Ca^{2+} homeostasis.

Supported by Ministerio de Educación y Ciencia (Grant Ref. BFU2005-00663) and Fundación Marcelino Botín, Spain.

Keywords: Alzheimer's disease, PMCA, brain, amyloid β-peptide

EFFECTS OF HTLV-1 p13 PROTEIN ON Ca^{2+} HOMEOSTASIS

BIASIOTTO R., AGUIARI P., D'AGOSTINO D.M., SILIC-BENUSSI M., CANNIZZARO E., RIZZUTO R., PINTON P. & CIMINALE V.

Department of Oncology and Surgical Sciences, Oncology Section, University of Padova, Italy.
Department of Experimental and Diagnostic Medicine, Section of General pathology, University of Ferrara, Italy.

Human T-cell Leukemia/Lymphoma virus type-1 (HTLV-1) is the ethiological agent of adult T-cell leukemia/lymphoma, an aggressive neoplasm of mature $CD4^+$ T cells. Previous studies showed that p13, an 87-amino acid protein of HTLV-1, is targeted to the inner mitochondrial membrane, alters mitochondrial morphology, affects cell turnover in vitro and exerts antitumor effects in vivo. Assay employing synthetic p13 and isolated mitochondria showed that p13 triggers an inward K^+ current which induces mitochondrial depolarization, accompanied by increased respiratory chain activity.

The present study was aimed at understanding the effects of p13 on Ca^{2+} homeostasis. We studied the effects of p13 on mitochondrial, cytosolic, and endoplasmic reticulum (ER) Ca^{2+} content using aequorin probes targeted to these compartments. Results demonstrated that, following histamine stimulation, p13-expressing HeLa cells exhibit decreased mitochondrial Ca^{2+} content and increased duration of the cytosolic Ca^{2+} transient compared to control cells.

In contrast, no major effect of p13 was seen on ER filling. These results suggest that p13 might exert its main effect on mitochondrial Ca^{2+} uptake. Interestingly, this effect was not observed with p13 mutants that are incapable of inducing K^+ influx in mitochondria. To gain insight on the possible mechanism of this effect, we studied the effect of p13 on mitochondrial membrane potential in living cells using p13-GFP fusion proteins and labelling with tetramethylrodamine, a mitochondrial membrane potential-specific fluorescent probe. Results showed that p13 induces mitochondrial depolarization, but the extent of this effect varies among different cells, suggesting that p13 expression is necessary but not sufficient to induce mitochondrial depolarization. Current studies are aimed at studying the effects of p13 on Ca^{2+} signaling and the interplay between p13 and p12, an HTLV-1 ER-localizing protein promoting ER Ca^{2+} release.

Keywords: HTLV-1, mitochondria

GLUCOSE AND PHARMACOLOGICAL MODULATORS OF ATP-SENSITIVE K^+ CHANNELS CONTROL $[Ca^{2+}]_c$ BY DIFFERENT MECHANISMS IN ISOLATED MOUSE PANCREATIC α-CELLS

CHENG-XUE R.[1], QUOIX N.[1], MATTART L.[1], ZEINOUN Z.[1], GUIOT Y.[2], BEAUVOIS M.C.[1], HENQUIN J.C.[1] & GILON P.[1]

Units of [1] Endocrinology and Metabolism, and [2] Pathology, University of Louvain, Faculty of Medicine, B-1200, Brussels, Belgium.

Changes in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) are implicated in the control of glucagon release by pancreatic α-cells, but the mechanisms by which glucose and pharmacological modulators of K_{ATP} channels modify α-cell $[Ca^{2+}]_c$ remain controversial.

To address this issue, we used our mouse model named GYY expressing EYFP under the control of the glucagon promoter to identify α-cells by their specific fluorescence. Single β-cells were studied for comparison. Isolated islets or single cells were cultured for 1-3 days in RPMI medium containing 7 mM glucose. Four parameters were monitored: glucagon secretion from islets, $[Ca^{2+}]_c$ (fura-PE3), the K_{ATP} current (perforated mode) and cell metabolism (NAD(P)H fluorescence) from single α-cells.

Increasing the glucose concentration from 0.5 to 15 mM strongly inhibited glucagon secretion from islets of GYY mice. When isolated islet cells were perifused with 0.5 mM glucose, $[Ca^{2+}]_c$ oscillated in some α-cells and was low and stable in the others. Increasing glucose to 15 mM decreased oscillating $[Ca^{2+}]_c$ by ~30%. α-cell I_{KATP} was inhibited by tolbutamide and activated by diazoxide or the mitochondrial poison azide, as in β-cells. Tolbutamide increased α-cell $[Ca^{2+}]_c$ whereas diazoxide and azide lowered it. Surprisingly, increasing glucose from 0.5 to 15 mM did not change I_{KATP} and NAD(P)H fluorescence in α-cells although it reversibly affected both parameters in β-cells. GABA and zinc did not decrease α-cell $[Ca^{2+}]_c$ whereas insulin very modestly lowered it.

In conclusion, K_{ATP} channels and cell metabolism control α-cell $[Ca^{2+}]_c$ in a similar way as in β-cells. However, contrary to β-cells, glucose does not affect α-cell I_{KATP} and only modestly decreases α-cell $[Ca^{2+}]_c$. Glucose-induced inhibition of glucagon secretion in situ probably results from a combination of a K_{ATP} channel-independent direct effect of glucose on α-cells and an indirect effect of factors released by non-α-cells, possibly involving insulin, but not GABA or zinc.

Keywords: glucose, pancreatic α-cells, glucagon

MOLECULAR CHARACTERISATION OF A NOVEL SEA URCHIN ADP-RIBOSYL CYCLASE

CHURAMANI D., RAMAKRISHNAN L., BOULWARE M.J., GEACH T.J., MARTIN A.C.R., VACQUIER V.D., MARCHANT J.S., DALE L. & PATEL S.

University College London, London, UK.
University of Minnesota, Minneapolis, Minnesota, USA.
Scripps Institution of Oceanography, San Diego, California, USA.

ADP-ribosyl cyclases are multifunctional enzymes capable of synthesizing several molecules involved in calcium homeostasis (Lee et al., 2000; Schuber and Lund, 2004). We have recently reported the molecular cloning of an extended family of ADP-ribosyl cyclases from the sea urchin and provided evidence that one of these enzymes (SpARC1) is a luminal endoplasmic reticulum protein (Churamani et al., 2007).

Here we characterize SpARC2. Recombinant SpARC2 was N-glycosylated and catalysed both the base-exchange and cyclization reactions using NADP and NGD, respectively. Comparison of the ratio of these activities with that of SpARC1 suggests that SpARC2 may be a preferential base-exchanger. SpARC2 heterologously expressed in Xenopus oocytes localized almost exclusively to the cell surface and underwent internalisation upon maturation. SpARC2 was also localized to the cell surface in HEK cells, whereas a truncated construct lacking its C terminus was secreted. SpARC2 was released upon bacterial phospholipase C treatment of HEK cells indicating that it is likely a glycosylphosphatidylinositol-anchored plasma membrane protein. Transcripts for SpARC2 are detectable in sea urchin eggs, and following phospholipase C treatment a major fraction of base-exchange activity was released from membranes. Our data reveals a plasma membrane location of a novel sea urchin ADP-ribosyl cyclase and suggests that base-exchange is its preferred activity.

Churamani et al. (2007) PloS ONE 2, e797.
Lee (2000) Chem. Immunol. 75, 39-59.
Schuber and Lund (2004) Curr. Mol. Med. 4, 249-261.

Keywords: ADP-ribosyl cyclase, sea urchin, base-exchange

EXPRESSION OF SARCO/ENDOPLASMIC RETICULUM Ca^{2+} ATPASE (SERCA) 3 PROTEINS IN TWO MAJOR CONFORMATIONAL STATES IN NATIVE HUMAN MEMBRANES

CORVAZIER E., BREDOUX R., KOVACS T. & ENOUF J.

CRCIL, Hôpital Lariboisière, F-75475 Paris Cedex 10, France and Hungarian Academy of Sciences, Hungary.

The SERCA family includes 3 genes (SERCA1-3), each of which giving rise to various isoforms. To date, detailed structural data is only available for the SERCA1a isoform.

Here, kinetic analysis of controlled trypsinolysis of either human platelet membranes (mainly expressing the SERCA3a isoform) or recombinant SERCA3a expressed in HEK-293 cells followed by Western Blotting using antibodies raised against different regions of the SERCA3a protein revealed two distinct proteolytic profiles. The earlier one uses many tryptic sites while the later one uses an unique tryptic site. Using site-directed mutagenesis, Arg334, Arg396, Arg638 and Arg198 were directly assigned to the early and late tryptic profiles, respectively. Arg671, Lys712/Lys713 and Lys728 were also found to modulate the early tryptic fragmentation. By testing SERCA inhibitors thapsigargin and TBHQ favoring E2 conformation of the enzyme, differential inhibition of the two tryptic profiles was observed. Studies of the trypsinolysis of recombinant SERCA3b-3f isoforms revealed: i) two tryptic profiles similar to SERCA3a, with variations of the length of the C-terminal fragments; ii) Arg1002 as an additional tryptic site restricted to SERCA3b-3e isoforms.

Taken together, the two SERCA3 fragmentation profiles sign the still undescribed co-expression of SERCA3 proteins in two conformational states in various cell membranes presenting similarities with the E2P- and E1-like conformations of SERCA1a. These findings may help to understand the apparent low Ca^{2+} affinity of SERCA3s documented in functional studies performed both in membrane preparations or living cells.

Keywords: SERCA, catalytic cycle

EXPRESSION OF CD38 IN PANCREATIC AR42J CELLS IS ESSENTIAL FOR NAADP SIGNALING

COSKER F. & CANCELA J.M.

Centre National de la Recherche Scientifique, Institut de Neurobiologie Alfred Fessard-FRC2118, Laboratoire de Neurobiologie Cellulaire et Moléculaire-UPR9040, F-91198 Gif sur Yvette, France.

In rat pancreatic acinar cells, the gut peptide cholecystokinin (CCK) induces digestive enzyme secretion through Ca^{2+} release from internal stores. It has been shown that CCK-evoked Ca^{2+} signals require the NAADP receptor activation. The second messenger NAADP triggers the Ca^{2+} signal by mobilizing Ca^{2+} from an acidic lysosome-related store. At least in vitro the surface antigen CD38 is capable of NAADP synthesis but its role in intact pancreatic acinar cells is not understood.

Here we show that in the pancreatic exocrine cell line AR42J, CCK-evoked Ca^{2+} signal does not require the NAADP pathway but mainly the IP_3 pathway. Surprisingly, these cells do not express endogenously CD38. Therefore, we took advantage of the lack of CD38 expression to use it as an expression cell system to investigate the putative role of CD38 in Ca^{2+} signaling. Firstly, transient expression of CD38 shows a distribution to the plasma membrane and to the acidic vesicles among them the lysosomes. We found that CD38 expression restores ryanodine sensitivity to the CCK-evoked Ca^{2+} signal. Then we looked at the NAADP pathway using the protocol of desensitization of its own receptor by high concentrations of NAADP which is a well documented approach in mammalian cells. Our experiments show that incubation of rat pancreatic acinar cells with 100 µM NAADP decreases the amplitude of CCK-induced Ca^{2+} response. We have also shown that the CD38 expression restores Gly-Phe-β-naphtylamide (GPN) sensitivity, suggesting that lysosomes are now recruited likely by NAADP.

All together, we conclude that the expression of CD38 in AR42J cells is clearly required for recruitment of the NAADP pathway by the neuropeptide CCK.

Keywords: CD38, NAADP, pancreatic acinar cells, Ca^{2+} signalling

SLP-2 IS A NEW MODULATOR OF MITOCHONDRIAL CALCIUM HOMEOSTASIS

DA CRUZ S.[1], DE MARCHI U.[2], PARONE P.A.[1], MARTINOU J.-C.[1], FRIEDEN M.[2] & DEMAUREX N.[2]

[1] Department of Cell Biology, University of Geneva, 30 Quai E. Ansermet, CH-1211 Geneva 4, Switzerland.
[2] Department of Cell Physiology and Metabolism, University of Geneva, 1 Rue Michel-Servet, CH-1211 Geneva 4, Switzerland.

Mitochondria are eukaryotic organelles that play a major role in cellular calcium homeostasis. The transport of calcium ions in and out of the mitochondrial matrix is regulated by a variety of mitochondrial inner membrane ion channels. Although the properties of these channels have been studied for decades, their molecular identity remains unknown.

Recently, we identified SLP-2 (stomatin-like protein 2) as a component of the mitochondrial inner membrane proteome. SLP-2, also called paraslipin, is a member of the stomatin gene family, of which the founding member, stomatin, is a highly conserved plasma membrane protein that regulates the activity of ion channels. Since no function has been ascribed to SLP-2, we investigated whether SLP-2 could modulate the activity of mitochondrial ion channels.

We show that SLP-2 is involved in the regulation of mitochondrial calcium homeostasis. Following a rise in intracellular calcium, the capacity of mitochondria from SLP-2 over-expressing cells to store calcium was increased and the maximum Ca^{2+} efflux rate decreased compared to mitochondria from control cells. In contrast, the storage capacity of mitochondria from SLP-2 depleted cells was decreased and the maximum Ca^{2+} efflux rate increased compared to control cells. Treatment of SLP-2 depleted cells with CGP 37157, an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger partly restored this altered capacity.

We suggest that SLP-2 may modulate mitochondrial calcium efflux by negatively regulating the mitochondrial Na^+/Ca^{2+} exchanger.

Keywords: SLP-2, mitochondria, Na^+/Ca^{2+} exchanger

ACTIVATION OF ATP RELEASE BY CX43 HEMICHANNELS INVOLVES CALMODULIN-ARACHIDONIC ACID AND ROS/NO

DE VUYST E., DE BOCK M., WANG N., DECROCK E. & LEYBAERT L.

Dept. Basic Medical Sciences - Physiology Group,
Faculty of Medicine and Health Sciences, Ghent University, Ghent, B-9000, Belgium.

Connexins (Cx) are transmembrane proteins that form hexameric structures known as hemichannels. The docking of apposing hemichannels on neighboring cells results in the formation of functional gap junction (GJ) channels, but recent evidence indicates that hemichannels not interacting with their counterparts may also exist and are regulated by various influences. Connexin hemichannels are activated by various stimuli including depolarization and oxidative stress. Recently, we showed that elevating intracellular calcium induces the opening of Cx32 hemichannels.

The aim of the present work was to further investigate the signaling events leading from an increase of cytoplasmic calcium ($[Ca^{2+}]_i$) to hemichannel-mediated ATP release. Involvement of Cx43 hemichannels was concluded on the basis of gene silencing and the exclusion of other release mechanisms. Elevating $[Ca^{2+}]_i$ with the ionophore A23187 triggered ATP release that peaked around 500 nM $[Ca^{2+}]_i$ and was absent at low or high (~1000 nM) concentrations. A23187-triggered ATP responses were blocked by antagonists of calmodulin (CaM), CaM-dependent kinase II, arachidonic acid (AA) metabolism and ROS/NO signaling. CaM activation with CALP1 triggered ATP release that was again blocked by interfering with AA metabolism or ROS/NO signaling. CALP1-triggered ATP responses had a S-shaped concentration dependence while the responses triggered by A23187 were bell-shaped. AA applied exogenously also triggered ATP release that was blocked by ROS/NO inhibition. The results indicate Ca^{2+}/CaM-mediated activation of hemichannels via AA-ROS/NO signaling and CaM-independent signaling pathways for hemichannel inactivation at high $[Ca^{2+}]_i$.

Keywords: connexins, hemichannels, ATP, arachidonic acid, NO, calmodulin

WHAT CAN WE LEARN FROM THE IRREGULARITY OF Ca^{2+} OSCILLATIONS?

DUPONT G.[1], ABOU-LOVERGNE A.[2] & COMBETTES L.[2]

[1] Unité de Chronobiologie Théorique, Université Libre de Bruxelles, Faculté des Sciences, Brussels, Belgium
[2] Institut National de la Santé et de la Recherche Médicale, Unité UMR-S757, Univ. Paris-Sud, Orsay, France

Signal-induced Ca^{2+} oscillations have been observed in many cell types and play a primary role in cell physiology. Although it is the regular character of these oscillations that first draws the attention, a closer look at time series of Ca^{2+} oscillations reveals that the fluctuations on the period during individual train spikes are far from being negligible.

We first measure the average standard deviation on the period in freshly isolated hepatocytes stimulated by noradrenaline. We next investigate in which conditions Gillespie simulations taking into account the known numbers of $InsP_3$ receptors and free Ca^{2+} ions in hepatocytes allow to reproduce the experimentally measured standard deviations on the period. This leads us to make predictions about the spatial distribution of $InsP_3$ receptors in this cell type. We next address the question of how other factors such as the ratios between the concentrations of the different isoforms of the $InsP_3$ receptors or the possible accompanying $InsP_3$ oscillations may affect the robustness of signal-induced Ca^{2+} oscillations.

We conclude that the arrangement of Ca^{2+} channels in hepatocytes can be viewed as a compromise between regularity in the period and sensitivity to low levels of signal.

Keywords: hepatocytes, Ca^{2+} oscillations, stochasticity, IP_3 receptor, computational modelling

CALCIUM "TRANSPORTOME" OF THE PLANKTONIC CRUSTACEAN DAPHNIA

ELLIOTT A.C., HANNA J. & PENNINGTON N.

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, UK.

The increasing sequencing of whole genomes from model organisms, including invertebrates, allows examination of protein families implicated in Ca^{2+} signalling across many years of evolution.

We have been exploring the existence and structure of putative homologues of Ca^{2+} channels and transporters in the recently-completed genome of the freshwater microcrustacean Daphnia. Daphnia, commonly termed waterfleas, are grazing freshwater zooplankton. In evolutionary terms, they are intermediate between decapod crustaceans and insects (arthropoda, hexapods). Daphnia are a critical part of freshwater ecosystems, consuming algae and constituting a common prey species for fish, and are widely used in ecotoxicological assessments and biotoxicity assays.

Searches of the Daphnia pulex genome using gene ontologies or sequence homology show an extensive repertoire of Ca^{2+} channels and transporters. The genome encodes at least half a dozen voltage-gated Na^+ or Ca^{2+} channel (Na_V or Ca_V) α subunits (identity to insect sequences 45-75%). Multiple sequences encoding members of the NCX (K^+-independent) and NCKX (K^+-dependent) Na^+-Ca^{2+} exchanger families are present. There appears to be only a single Daphnia gene each for Orai/CRACM, $InsP_3R$, RyR, PMCA and SERCA. The SERCA sequence shows approximately equal homology (80-85% identity) to either insect or decapod (e.g. lobster) SERCA, with a sequence identity to human SERCA1 of 70%.

The Daphnia genome also contains a group of putative TRP channels, with at least one sequence homologous to each known TRP family except TRPP. Homology to insect channels is lower for TRPs (40-65%) than for Ca_Vs. As in insects (notably Drosophila), there are multiple putative TRP channels with >4 N-terminal ankyrin repeats (4/5 putative TRPA homologues and one TRPN).

The sequence data used for this analysis were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov

Keywords: invertebrates, TRP channels, SERCA, Orai1, IP_3 receptor, ryanodine receptor, Na^+/Ca^{2+} exchanger, Daphnia

SMOOTH MUSCLE CELL FUNCTION IN MOUSE AORTA SEGMENTS LACKING THE SERCA2a ISOFORM

FRANSEN P.[1], VANGHELUWE P.[2], VAN ASSCHE T.[1], VAN HOVE C.[1], RAEYMAKERS L.[2], WUYTACK F.[2] & BULT H.[1]

[1] Division of Pharmacology, University of Antwerp, Antwerp, Belgium.
[2] Laboratory of Ca^{2+}-transport ATPases, Katholieke Universiteit Leuven, Leuven, Belgium.

Preventing the alternative splicing of the mouse Atp2a2 transcript encoding the sarco-endoplasmic reticulum calcium (Ca^{2+}) ATPase-2 (SERCA2) to the SERCA2a isoform by bi-allelic modification results in SERCA2b/b mice expressing the house-keeping SERCA2b isoform only. The animals show a clear cardiac structural and functional phenotype.

The consequences of the SERCA2a to SERCA2b swap in vascular smooth muscle cells (SMC) was, however, not yet addressed. Real-time PCR on RNA isolated from SMCs of thoracic aorta revealed that only 5% of the SERCA2 gene was transcribed to SERCA2a in control SERCA2WT mice, but none in SERCA2b/b mice. Endothelium-denuded SERCA2b/b segments displayed enhanced cytosolic Ca^{2+} (Fura-2 fluorimetry) and force responses to depolarisation with 50 mM K^+, and higher force for similar Ca^{2+} mobilisation if stimulated with the alfa-adrenoceptor agonist phenylephrine. Supplementary addition of cyclopiazonic acid (10 µM, SERCA inhibitor) caused a large additional increase of cytosolic Ca^{2+}, which was not different between the strains, but which was accompanied by a significantly larger force increase in SERCA2b/b compared with SERCA2WT segments. SERCA2b/b segments displayed enhanced endothelium-dependent (ATP) or -independent (nitrite, diethylamine NONOate) relaxation, suggesting that SERCA2b/b SMCs were significantly more sensitive to endogenous or exogenous NO than SERCA2WT SMCs. Furthermore, the higher relaxing capacity of NO in SERCA2b/b mice was paralleled by a larger Ca^{2+} decrease during relaxation. Since addition of the cGMP analogue 8-Br-cGMP caused similar relaxation in both strains, the above results suggest a higher NO-sensitivity of SERCA2b compared with SERCA2a.

In summary, results of the present study indicate that despite the presence of only 5% of the total SERCA2 transcript as 2a in mouse thoracic aorta SMCs, this isoform plays an important role in contractile behaviour and in determining the relaxing capacity of NO.

Keywords: vascular myocytes, NO, SERCA

THE RELAXING CAPACITY OF NITRIC OXIDE IN MOUSE AORTA SMOOTH MUSCLE CELLS: ROLE OF INTRACELLULAR CALCIUM

VAN HOVE C.E., HERMAN A.G., BULT H. & FRANSEN P.

Division of Pharmacology, University of Antwerp, Antwerp, Belgium.

The present study investigated whether mechanisms of calcium (Ca^{2+}) mobilisation in smooth muscle cells (SMCs) influence the vasodilator activity of nitric oxide (NO). Isometric contractions evoked by depolarisation (high potassium, K^+) and α-adrenoceptor stimulation (phenylephrine, PE), and relaxations by acetylcholine (ACh), diethylamine NONOate (DEANO) and glyceryl trinitrate (GTN) were measured in aorta segments of C57Bl6 mice. Segments under PE stimulation were more sensitive to endothelium-derived (ACh) or exogenous NO (DEANO, GTN) than under K^+ depolarisation. The difference in sensitivity was independent of the source of NO or the resting potential of endothelial cells or SMCs. The NO-elicited relaxation was paralleled by a decrease of SMC intracellular Ca^{2+} in PE-, but not in high K^+-stimulated segments. The decline of Ca^{2+} was blocked by inhibition of the sarcoplasmic reticulum (SR) Ca^{2+} uptake with cyclopiazonic acid (10 µM), but was not influenced by soluble guanylate cyclase (sGC) inhibition (ODQ, 50 µM). Stimulation (BAY K8644, 30 nM) of L-type Ca^{2+} influx in SMCs diminished, while inhibition (nifedipine, 1-100 nM) augmented the relaxing capacity of NO.

It is concluded that in mouse aorta SMCs relaxation by NO occurred via at least two pathways. Relaxation of contractions initiated by L-type Ca^{2+} influx upon SMC depolarisation by high K^+ is not accompanied by a decrease of SMC Ca^{2+} and occurs presumably via stimulation of sGC and increase of cGMP. Relaxation of contractions initiated by mobilisation of Ca^{2+} from inside the SMC as with PE, is associated with NO-stimulated uptake of Ca^{2+} to the SR and concomitant stimulation of sGC. The direct stimulation by NO of the Ca^{2+} uptake to the SR may be responsible for the larger relaxing capacity of NO under PE stimulation compared with high K^+ contraction. Furthermore, these findings indicate that inhibition of L-type Ca^{2+} channels by Ca^{2+}-entry blockers increases SMC sensitivity to the vasodilator NO.

Keywords: vascular myocytes, NO

NAADP-MEDIATED CALCIUM MOBILIZATION IN CD4 EFFECTOR T CELLS AS NOVEL TARGET FOR IMMUNOMODULATION

GUSE A.H.[1], FLÜGEL A.[2], HOHENEGGER M.[3] & POTTER B.V.L.[4]

[1] University Medical Center Hamburg-Eppendorf, Germany.
[2] Max-Planck-Institute for Neurobiology, Germany.
[3] University of Vienna, Austria.
[4] University of Bath, UK.

NAADP is the most potent Ca^{2+}-mobilizing compound known so far. Recent work in T cells indicates biphasic kinetics of endogenous NAADP upon TCR/CD3 stimulation (Gasser et al., 2006), also after gene silencing of CD38 (submitted). NAADP released Ca^{2+} from the endoplasmic reticulum of T cells (Steen et al., 2007), most likely via activation of ryanodine receptors (RyR; Langhorst et al., 2004; Dammermann and Guse, 2005). In addition to Ca^{2+} release, NAADP appears to be involved in regulation of Ca^{2+} entry (Langhorst et al., 2004).

We developed a small molecule inhibitor, which specifically blocked Ca^{2+} mobilization by inferring with NAADP-induced channel opening of the type 1 ryanodine receptor (RyR1). The NAADP antagonist effectively inhibited T cell receptor-driven pro-inflammatory cytokine production and proliferation in antigen-experienced $CD4^+$ effector T cells. Naïve and memory T cells, which express lower levels of RyR1 were significantly less susceptible. Experimental autoimmune encephalomyelitis, the classical T cell-mediated model for multiple sclerosis, was significantly ameliorated. Molecular analyses and live two-photon imaging revealed that NAADP antagonists induced a state of transient non-responsiveness of effector T cells and prevented efficient reactivation of the autoaggressive T cells within their target organ resulting in reduced inflammation and paralytic disease (submitted).

Gasser et al. (2006) J. Biol. Chem. 281, 16906-16913.
Steen et al. (2007) J. Biol. Chem. 282, 18864-18871.
Langhorst et al. (2004) Cell. Signal. 16, 1283-1289.
Dammermann and Guse (2005) J. Biol. Chem. 280, 21394-21399.

Keywords: NAADP, ryanodine receptor, lymphocytes, immunomodulation, $CD4^+$

Ca^{2+}-HANDLING PHENOTYPE OF CARDIOMYOCTYES WITH IMPAIRED MITOCHONDRIAL FUNCTION IS REVERSED BY N-ACETYLCYSTEINE (NAC)

HÄNNINEN S.L., RONKAINEN J.J., KORHONEN T. & TAVI P.

Institute of Biomedicine, Department of Physiology and Biocenter Oulu, University of Oulu, P.O. Box 5000, SF-90014-University of Oulu, Finland.

Cardiac cells are heavily energy-dependent, and impaired mitochondrial function has been shown to manifest as hypertrophic cardiomyopathy, arrhythmias, and sudden cardiac death. Chronic respiratory chain impairment in humans and animal models demonstrates the ability of these cells to survive through compensatory changes at the cellular level.

Here, we show that the phenotype of cardiac myocytes is altered in response to chronic mitochondrial stress induced by FCCP. Ca^{2+} transients of treated cells were significantly altered, but there was no change in the amplitude or frequency of action potentials. The observed Ca^{2+}-handling phenotype was associated with the specific downregulation of genes involved in Ca^{2+} handling and contraction, and there was a significant decrease in ATP levels. Downregulation of the marker gene cardiac calsequestrin (CASQ2) was dose-dependent and attenuated by the reactive oxygen species (ROS) scavenger N-acetylcysteine (NAC), which decreased FCCP-induced ROS. Importantly, NAC also rescued the Ca^{2+}-handling phenotype of the cells, with Ca^{2+} signals of cells exposed to FCCP and NAC similar to controls, indicating the role of ROS in the cardiomyocyte response.

We have shown a clear adaptive response of neonatal cardiomyocytes to chronic mitochondrial inhibition, in which the genetic program is changed to downregulate the most energy-consuming processes, namely Ca^{2+} signaling and contraction, to prolong survival.

Keywords: cardiomyocyte, FCCP, N-acetylcysteine, mitochondria, calsequestrin, ROS, Ca^{2+} signaling

GLUCOCORTICOIDS NEGATIVELY REGULATE IP_3R-ASSOCIATED KINASES FYN AND LCK TO MODULATE TCR-INDUCED CALCIUM SIGNALS

HARR M.W. & DISTELHORST C.W.

Case Western Reserve University and University Hospitals of Cleveland, Department of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-1712, USA.

Glucocorticoids are potent immunosuppressive agents that suppress early signaling events required for T cell activation, thereby inhibiting IP_3-mediated calcium signaling and IL-2 transcription. In previous studies, we have shown that the strength of T cell activation determines the pattern of calcium signaling induced by anti-CD3 antibody in WEHI7.2 T cells. For example, a high concentration of anti-CD3 (i.e., strong activation) generates a transient broad calcium elevation that peaks 1-2 minutes after stimulation, whereas a low concentration of anti-CD3 (i.e., weak activation) induces calcium oscillations or spikes that persist up to 60 minutes after stimulation.

Here we show that short-term pre-treatment of WEHI7.2 cells with dexamethasone (10^{-8} M) converts transient broad calcium elevations into oscillations and spikes following strong T cell activation. cDNA microarray analysis revealed that Src family kinases Fyn and Lck, which together are required for TCR signaling, were downregulated by dexamethasone. In stimulated cells, short-term pre-treatment with 10^{-8} M dexamethasone was sufficient to reduce total Fyn levels and Lck phosphorylation at Y394, the site that positively regulates its kinase activity. To determine if changes in calcium signaling patterns were dependent on Fyn and Lck, we decreased their expression using a siRNA-mediated knock-down approach. Knock-down of either Fyn or Lck was sufficient to convert transient broad calcium elevations, induced by strong T cell activation, to calcium oscillations and spikes. Additionally, both Fyn and Lck were in complex with IP_3 receptors and loss of Fyn and Lck expression or activity resulted in downregulation of IP_3R1.

Together, these results suggest that Fyn and Lck regulate IP_3 receptor expression, and hence, govern the pattern of calcium signaling induced by strong T cell activation. Thus, glucocorticoids may function to suppress immune responses, in part, by diminishing the strength of the TCR signal.

Keywords: IP_3 receptor, lymphocytes, dexamethasone, T cell activation, Src kinases

NAADP SIGNALLING IN SKELETAL MUSCLES

HOHENEGGER M.[1], WANKE-JELINEK L.[1], PUSCH O.[2] & WEIGL L.[3]

[1] Institute of Pharmacology, [2] Center for Anatomy and Cell Biology, [3] Dept. of Anesthesia, Intensive Care Medicine and Pain Control, Medical Univ., Vienna, Austria.

Nicotinic acid adenine dinucleotidephosphate, NAADP, is a potent Ca^{2+}-mobilizing second messenger. Although it is generally accepted that NAADP provokes an intracellular Ca^{2+} release, the target receptor and the primary source of Ca^{2+} depletion is still controversial (Yamasaki et al., 2005; Fliegert et al., 2007). In skeletal muscle NAADP is capable to directly activate the purified ryanodine receptor of the sarcoplasmic reticulum (SR) (Hohenegger et al., 2002). In fractions enriched with SR an ADP-ribosyl cyclase is detectable which supports the base exchange reaction to form NAADP (Bacher et al., 2004). A novel aspect of NAADP signalling has been introduced by extracellular application of NAADP to astrocytes and neurons, which resulted in intracellular Ca^{2+} release (Heidemann et al., 2005). As skeletal muscle belongs to the excitable cell types, we have investigated this novel signalling pathway and compared the susceptibility of primary human skeletal muscle cells (hSKM) to extracellular application of NAADP.

In undifferentiated hSKM cells extracellular NAADP mobilized intracellular Ca^{2+} in a concentration-dependent manner. Chelation of extracellular Ca^{2+} was not sufficient to prevent NAADP-induced Ca^{2+} release. However, spontaneous Ca^{2+} release activity in the absence of NAADP was virtually abolished. Immunohistochemical detection revealed the presence of connexin 43 hemichannels at the plasma membrane of undifferentiated hSKM cells. In order to control for a translocation of NAADP, specific inhibition of these channels suppressed the NAADP-induced Ca^{2+} release by approximately 70%. In a similar manner under conditions where the ryanodine receptor is blocked, no NAADP-induced Ca^{2+} release was observable.

In differentiated hSKM cells spontaneous and NAADP-induced Ca^{2+} release is reduced by about 40% compared to undifferentiated hSKM cells. This is accompanied by reduced expression of connexin 43. Thus, these data suggest a differentiation-dependent difference in NAADP signalling in hSKM cells.

Bacher et al. (2004) Biochem. J. 381, 147-154.
Fliegert et al. (2007) Biochem. Soc. Trans. 35,109-114.
Heidemann et al. (2005) J. Biol. Chem. 280, 35630-35640
Hohenegger et al. (2002) Biochem. J. 367, 423-431.
Yamasaki et al. (2005) FEBS J. 272, 4598-606.

Keywords: NAADP, skeletal muscle, ryanodine receptor, connexins

IP_3-DEPENDENT POST-TETANIC CALCIUM TRANSIENTS INDUCED BY ELECTROSTIMULATION IN ADULT SKELETAL MUSCLE FIBERS

CASAS M., BUVINIC S., FIGUEROA R., GARCÍA I., MOLGO J. & JAIMOVICH E.

Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile.
NBCM, CNRS, Gif sur Yvette, France.

Tetanic electrical stimulation induces two separate calcium signals in rat skeletal myotubes, a fast one dependent on RyRs and related to contraction, and a slow signal, dependent on dihidropyridine receptors (DHPR) and IP_3Rs, related to transcriptional events.

We searched for slow calcium signals in adult muscle fibers; we used isolated adult Flexor digitorum brevis fibers from 4-6 weeks-old mice, loaded with Fluo-3. When stimulated with 6 s trains of 0.3 ms pulses at 45 Hz, we observed a fast calcium tetanus (associated with contraction) and a second, slower signal, similar to those described in cultured myotubes. The slow signal (more than the fast one) was inhibited by 25 μM nifedipine, suggesting a role for DHPR in its onset, by the IP_3R inhibitor Xestospongin-C (5 μM) and was partly dependent on extra cellular calcium. The post-tetanic calcium transients depend on both tetanus frequency and duration; extracellular ATP also appears to have a role in the genesis of the slow (and partly the fast) calcium signal, because its depletion by incubating the fibers with 2 U/ml apyrase had also an inhibitory effect. In mouse hemi-diaphragm preparations, we demonstrated that apyrase reduces time-dependently both twitch- or tetanus-evoked increase in tension. As in myotubes, a role for purinergic receptors in the onset of IP_3-dependent calcium signals appears to be likely. Moreover, using immunofluorescence, we have found that all three IP_3R isoforms are present in adult muscle at different levels and that IP_3R-1 is differentially expressed in different types of muscle fibers, being higher in a subset of fast-type fibers. These results support the idea that different calcium kinetics for the slow signals mediated by IP_3R may exist in different types of muscle fibers and participate in the activation of specific transcriptional programs of slow and fast phenotype.

Supported by FONDAP 15010006, Bicentenario-PSD24, FONDECYT 1080120 and CNRS-CONICYT.

Keywords: skeletal muscle, IP_3 receptor, gene expression

8-BROMO-CYCLIC INOSINE DIPHOSPHORIBOSE: A CALCIUM-MOBILIZING ANALOGUE OF THE SECOND MESSENGER CYCLIC ADP-RIBOSE IN T-LYMPHOCTES

KIRCHBERGER T.[1], MOREAU C.[2], WAGNER G.K.[2,3], FLIEGERT R.[1], POTTER B.V.L.[2] & GUSE A.H.[1]

[1] University Medical Center Hamburg-Eppendorf, Institute of Biochemistry and Molecular Biology I, D-20246 Hamburg, Germany.
[2] University of Bath, Dept. of Pharmacy and Pharmacology, Bath, BA2 7AY, UK.
[3] University of East Anglia, Norwich, NR4 7TJ, UK.

Cyclic ADP-ribose (cADPR) is a calcium mobilizing second messenger active in many cell types and tissues (Guse et al., 2004). Recently 8-bromo-cyclic inosine diphosphoribose (8-Br-N1-cIDPR), a cADPR agonist, was introduced (Kirchberger et al., 2006; Wagner et al., 2003).

Here we describe the calcium mobilization by this cADPR analogue in Fura2-loaded Jurkat T-cells. Microinjection or addition of 8-Br-N1-cIDPR resulted in a calcium signal consisting of a rapid and high calcium peak followed by a lower, but sustained plateau phase. A calcium free/calcium reintroduction protocol was used to separate calcium release and calcium entry. Under calcium-free conditions, a robust calcium release upon 8-Br-N1-cIDPR addition was observed. Readdition of extracellular calcium resulted in a huge calcium entry overshoot followed by a sustained plateau phase. Co-microinjections of 8-Br-N1-cIDPR with ryanodine receptor (RyR) inhibitor ruthenium red reduced the initial calcium peak and completely blocked the sustained calcium plateau phase indicating that the initial peak partially and the sustained phase fully requires calcium release via RyR. By the use of inhibitors of calcium entry, Gd^{3+} or SKF96365, upon addition of 8-Br-N1-cIDPR, a robust calcium release was observed but under calcium-free conditions calcium entry was inhibited. In presence of 8-Br-cADPR, a cADPR antagonist, the calcium release was only slightly, but the influx strongly inhibited. Kolisek et al. (2005) showed that cADPR together with ADPR activates unspecific cation channel TRPM2, indicating that the inhibition of calcium entry by 8-Br-cADPR resulted from an inhibition of the TRPM2 channel. Presumably, the 8-Br-N1-cIDPR-mediated entry was mainly evoked by activation of TRPM2 channels and less by CRAC-channels.

Guse (2004) Curr. Mol. Med. 4, 239-248.
Kirchberger et al. (2006) Br. J. Pharmacol. 149, 337-344.
Kolisek et al. (2005) Mol. Cell. 18, 61-69.
Wagner et al. (2003) Chem. Commun. 7, 1944-1945.

Keywords: cADPR, Ca^{2+} signalling, lymphocytes

Ca^{2+}-RELEASE CHANNELS IN PARAMECIUM TETRAURELIA

LADENBURGER E.M., SEHRING I.M., KORN I. & PLATTNER H.

Department of Biology, University of Konstanz, D-78457 Konstanz, Germany.

There are various well characterized Ca^{2+}-regulated processes in the ciliated protozoan, Paramecium tetraurelia (Plattner & Klauke, 2001), but the channels mediating such signals are largely unknown. A database search of the Paramecium genome (Aury et al., 2006) reveals the presence of 34 genes coding for proteins related to Ca^{2+}-release channels of the ryanodine- or IP_3-receptor type (RyR, IP3R). Phylogenetic analyses show that the 34 possible Ca^{2+}-release channels (CRCs) can be subdivided in six groups (CRC-I to CRC-VI); each showing characteristic features regarding their relationship to IP_3Rs and RyRs.

Two families of CRCs, CRC-II-1 -also designated as IP_3RN (Ladenburger et al., 2006)- and CRC-IV-1 were analysed on a molecular level. While CRC-II-1 possesses all features of IP_3Rs, CRC-IV-1 types differ, despite their highly conserved channel domains, in terms of the ligand-binding domain. Immunofluorescence analyses show that both types of CRCs are localized to different cellular compartments. CRC-IV-1 could be localized to membranes of the endoplasmic reticulum and of cortical Ca^{2+} stores, while antibodies against CRC-II-1 unexpectedly stain membranes of the osmoregulatory system.

Functional analyses by gene silencing experiments reveal that knockdown of either one of these CRCs results in a reduced exocytotic capacity of dense core vesicles, so called trichocysts. Whereas silencing of CRC-II-1 leads to decreasing amounts of trichocyst matrix proteins, suggesting that the biogenesis of trichocysts is affected due to altered Ca^{2+} homeostasis, CRC-IV-1 silenced cells are incompetent for exocytosis, suggesting an involvement of these channels in signal transduction. Ca^{2+}-imaging studies confirm this findings, as knockdown of CRC-IV-1 results in an impaired release of Ca^{2+} from cortical stores of silenced cells.

The identification of aberrant protozoan IP_3R- and RyR-related CRCs in Paramecium is particularly relevant considering the unclear situation in their close pathogenic relatives, Plasmodium and Toxoplasma (Nagamune & Sibley, 2008).

Aury et al. (2006) Nature 444, 171-178.
Ladenburger et al. (2006) J. Cell Sci. 119, 3705-3717.
Nagamune and Sibley (2008) Mol. Biol. Evol. 23, 1613-1627.
Plattner and Klauke (2001) Int. Rev. Cytol. 201, 115-118.

Keywords: Paramecium, IP_3 receptor, ryanodine receptor

EXPRESSION OF THE SECRETORY PATHWAY Ca^{2+} ATPASE 1 (SPCA1) IN VASCULAR SMOOTH MUSCLE CELLS (A7r5) CULTURED UNDER NORMAL & DIABETIC GLUCOSE CONDITIONS

LAI P.F. & MICHELANGELI F.

School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

The secretory pathway Ca^{2+} ATPase (SPCA) is involved in calcium homeostasis, a process often associated with diabetes mellitus-related vascular pathology, which has not previously been investigated for their involvement in the disease to a great extent.

Here we show SPCA1 mRNA expression levels differ significantly between rat aortic smooth muscle cells (A7r5) cultured in normal (5.55 mM) and high (25.0 mM) glucose-supplemented media. Sarco/endo-plasmic reticulum Ca^{2+} ATPase (SERCA) mRNA expression was also shown to differ to a significant extent between these two conditions. However, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme, and β-actin, a contractility marker, did not significantly differ in its level of mRNA expression. Protein expression levels also showed that SPCA but not SERCA had decreased on their expression, but were not to the same relative extent as their mRNA levels.

Overall, the work to be discussed suggests a novel possibility for the involvement of SPCA in the effects of diabetes mellitus in vascular smooth muscle cells due to the sensitivity of its expression to glucose. SERCA's contribution to the disease, as suggested by results from the current study, will be discussed.

Keywords: SPCA, SERCA, diabetes mellitus, vascular myocytes, glucose

HIPPOCAMPAL SPCA Ca^{2+} ATPASE: EFFECT OF ISCHEMIC PRECONDITIONING ON GENE EXPRESSION AND OXIDATIVE DAMAGE IN RATS

LEHOTSKY J., PAVLÍKOVÁ M, KAPLAN P., SIVONOVA M. & TATARKOVÁ Z.

Comenius Univ., Jess. Fac. Med., Department of Med. Biochem., Martin, Slovakia.

Ischemic preconditioning (IPC) represents a phenomenon of adaptation of CNS to sub-lethal ischemia, which results in increased tolerance to the lethal ischemia. Ischemia/reperfusion (IRI) initiates a cellular catastrophic cascade in which many subcellular organelles play important role. The Golgi apparatus, as a part of secretory pathways (SP) and Ca^{2+} store, regulates secretion of neurotransmitters and secretory proteins for growth/reorganization of neuronal circuit by secretory Ca^{2+}ATPases (SPCA1). In addition, SP are involved in stress sensing and transduction of apoptotic signals.

In this study we have determined the effect of ICP on IRI-associated alterations of mRNA and protein levels of SPCA1 in the rat hippocampus. Global ischemia was induced by 4-vessel occlusion for 15 min. Rats were preconditioned by 5 min of sub-lethal ischemia and 2 days later, lethal ischemia was induced. RT-PCR and Western blot analysis clearly detected expression of SPCA1 gene in injured area after IRI. In addition, injured tissue responded by the increase of mRNA expression in the reperfusion period. IPC did not change significantly the expression profile, however the tissue response was elevated. Similar pattern was observed on the translational level by Western blot. Protein level of SPCA1 was highest in the reperfusion time and IPC initiated elevation of tissue response. In addition, both the in vitro oxidative stress and IRI induced lipo- and protein oxidation as well as depression of SPCA activity which were partially reversed by IPC. Our results showed that IPC affects SPCA gene expression and post-translational changes induced by ischemia.

This suggests a potential role of secretory processes involved in neuronal damage and/or remodelling of neuronal circuits as response to a pre-ischemic challenge.

Supported by VEGA 3380/06 and MVTS COST B30.

Keywords: ischemia, neurons, SPCA

CONNEXIN HEMICHANNEL INVOLVEMENT IN Ca^{2+} OSCILLATIONS/WAVES IN BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS

DE BOCK M.[1], DE VUYST E.[1], WANG N.[1], DECROCK E.[1], CULOT M.[2], CECCHELLI R.[2] & LEYBAERT L.[1]

[1] Dept. Basic Medical Sciences - Physiology Group, Faculty of Medicine and Health Sciences, Ghent University, B-9000 Ghent, Belgium.
[2] Laboratoire de Physiopathologie de la Barrière Hémato-Encéphalique, Université d'Artois, F-62307 Lens, France.

The blood-brain barrier (BBB) is an endothelial cell (EC) barrier that protects the brain from the blood. The intracellular calcium concentration ($[Ca^{2+}]_i$) in BBB EC is an important regulator of BBB permeability but little is on the effect of Ca^{2+} oscillations or waves on BBB function.

Here, we investigated various conditions that trigger Ca^{2+} oscillations and waves and determined the involvement of connexin (Cx) hemichannels (HC), which are half gap junction channels that may be involved in the release of paracrine messengers like ATP. Exposure of immortalized rat brain EC (RBE4) or primary bovine BBB EC (BCEC) to solutions with lowered $[Ca^{2+}]_e$, a known trigger for HC opening, caused Ca^{2+} waves and oscillations which were significantly reduced by the Cx mimetic peptide Gap27. The same solutions also elicited uptake of propidium iodide and the release of ATP suggesting involvement of HC. Exposure of RBE4 and BCEC to bradykinin (BK) induced Ca^{2+} oscillations but not waves. The number of oscillatory cells was reduced in the presence of carbenoxolone (Cbx), Gap26, suramin and apyrase, indicating a role for extracellular ATP, presumably released via HC. Exogeneous application of ATP also induced Ca^{2+} oscillations (not waves), but remarkably, here the number of oscillating cells was not reduced by Cbx or Gap26 suggesting a different oscillation mechanism.

Overall our data suggest that ATP release via HC is involved in the induction of Ca^{2+} oscillations brought about by BK. HC responses display a bell-shaped dependence on $[Ca^{2+}]_i$ and we propose a scenario whereby a small $[Ca^{2+}]_i$ change triggered by BK induces HC-related ATP release that acts in an autocrine manner to amplify the $[Ca^{2+}]_i$ change thereby shutting-off the HC responses again – repetition of this cycle then generates the oscillations. Further work is under way to characterize the interplay between other Ca^{2+} oscillation mechanisms, e.g. those governed by the bell-shaped response of $InsP_3$ receptor channels.

Keywords: connexins, hemichannels, Ca^{2+} oscillations, Ca^{2+} waves, endothelium, blood-brain barrier

DOES Ca^{2+} SIGNALLING PLAY A ROLE IN SHIGELLA INVASION?

ZHANG J.[1,2], LIU B.[1], PIERRE F.[1,2], ERNEUX C.[3], COMBETTES L.[1,2] & TRAN VAN NHIEU G.[2]

[1] UMR-S757, Université Paris-Sud, Orsay, France.
[2] Unité de Pathogénie Microbienne Moléculaire, INSERM U786, Institut Pasteur, Paris, France.
[3] IRIBHM, Université Libre de Bruxelles, Campus Erasme, Brussels, Belgium.

Shigella, the causative agent of bacillary dysentery, induces Ca^{2+} responses during epithelial cell invasion that are dependent on a functional bacterial type III secretory system and sensitive to the PLC inhibitor U73122. These Ca^{2+} responses are involved in the opening of connexin (Cx) hemichannels, allowing an ATP-dependent paracrine signaling that favors Shigella invasion and dissemination in epithelial cells (Tran Van Nhieu et al., 2003). Extracellular ATP, through the stimulation of purinergic receptors, is known to induce Ca^{2+} signaling and to also regulate cytoskeletal dynamics.

We set up to determine whether Ca^{2+} increase is required for cytoskeletal rearrangements during Shigella entry. The $InsP_3$ 5'-Pase, which overexpression was shown to drastically reduce the levels of $InsP_3$ and to inhibit Ca^{2+} increase induced by $InsP_3$-dependent agonists (Dupont et al., 2003), was stably transfected in HeLa (HeLa/IP5P), or HCx26 cells (HCx26/IP5P). As expected, Ca^{2+} responses induced by $InsP_3$-dependent agonists were significantly inhibited in these cells line, while no obvious modification of the cell cytoskeleton could be detected, consistent with a specific inhibition of $InsP_3$-dependent signaling. Shigella invasion was two-fold more efficient in HCx26/IP5P compared to parental HeLa cells. Invasion, however, was significantly reduced and to similar levels in HeLa/IP5P or HCx26/IP5P, indicating that $InsP_3$ signaling is involved in Shigella internalization as well as in bacterial-induced hemichannel signaling. To further address the role of Ca^{2+} in Shigella invasion, cells were perfused with Ca^{2+}-free medium in the presence of thapsigargin. These conditions prevented the Ca^{2+} increase normally observed by agonist treatment or challenge with invasive Shigella. In spite of the absence of detectable Ca^{2+} responses, bacterial invasion was not significantly affected suggesting a role for $InsP_3$ but independent of global Ca^{2+} increase in Shigella entry. The role of local Ca^{2+} responses is under study.

Dupont et al. (2003) FEBS Lett. 534, 101-105.
Tran Van Nhieu et al. (2003) Nat. Cell Biol. 5, 720-726.

Keywords: connexins, hemichannels, paracrine communication, Shigella, IP_3, bacteria

OVEREXPRESSION OF TRPC3 REDUCES THE CONTENT OF INTRACELLULAR CALCIUM STORES IN HEK-293 CELLS

LÖF C., BLOM T. & TÖRNQUIST K.

Department of Biology, Abo Akademi University, Biocity, Artillerigatan 6, 20520 Turku, Finland.

The mammalian canonical transient receptor channels (TRPCs) are considered to be candidates for store-operated calcium channels (SOCCs). Many studies have addressed how TRPC3 channels are affected by depletion of intracellular calcium stores. Conflicting results have been shown for TRPC3 regarding its function, and this has been linked to its level of expression in various systems. In the present study, we have investigated how overexpression of TRPC3 interferes with the regulation of intracellular calcium stores.

We demonstrate that overexpression of TRPC3 reduces the mobilization of calcium in response to stimulation of the cells with thapsigargin (TG) or the G-protein coupled receptor agonist sphingosine-1-phosphate (S1P). Our results indicate that this is the result of the expression of TRPC3 channels in the endoplasmic reticulum (ER), thus depleting ER calcium stores. OAG evoked calcium entry in cells overexpressing TRPC3, indicating that functional TRPC3 channels were also expressed in the plasma membrane.

Taken together, our results show that overexpression of the putative SOCC, TRPC3, actually reduces the calcium content of intracellular stores, but does not enhance agonist-evoked or store-dependent calcium entry. Our results may, in part, explain the conflicting results obtained in previous studies on the actions of TRPC3 channels.

Keywords: Ca^{2+} stores, TRPC3, Ca^{2+} entry

FUNCTIONAL MODULATION OF CALCIUM PUMPS BY AMYLOID β-PEPTIDE IN PRIMARY CULTURES OF HIPPOCAMPAL NEURONS

MARCOS D., BERROCAL M. & MATA A.M.

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Avda. de Elvas s/n, E-06071 Badajoz, Spain.

The regulation of Ca^{2+} is essential for neuronal development and survival. The amyloid β-peptide (Aβ) seems to be closely related to disruption of cellular Ca^{2+} homeostasis exhibited by affected neurons in Alzheimer's disease although the mechanism involved in this disorder is not known.

To investigate the involvement of Ca^{2+}-dependent proteins in the effects of Aβ in cellular responses, we have studied the activities of calcium pumps, a protein family essentially implicated in high-affinity Ca^{2+} regulation, after the extracellular application of Aβ and other compounds in primary neuronal cultures from mouse hippocampus.

We have observed that only the activity of the plasma membrane (PMCA) calcium ATPase was reduced in cells grown in the presence of Aβ, while the activities of the intracellular calcium ATPases from sarco(endo)plasmic reticulum (SERCA) and the secretory pathway (SPCA) were not affected. The addition of calmodulin and cholesterol to cell cultures blocked the inhibitory effect of Aβ. Cell viability assays and morphologic observations showed a protective effect of these substances against the cytotoxic action of Aβ.

These results suggest a PMCA-Aβ interaction, being the inhibition of PMCA activity by the peptide closely associated to dysregulation of Ca^{2+} homeostasis as a mechanism of cellular damage.

Supported by Ministerio de Educación y Ciencia (Grant Ref. BFU2005-00663) and Fundación Marcelino Botín, Spain.

Keywords: PMCA, amyloid β-peptide, neurons, Alzheimer's disease

INVERSE DISTURBANCE OF CALCIUM HOMEOSTASIS IN SPHINGOSINE KINASE-1- AND SPHINGOSINE-1-PHOSPHATE LYASE-DEFICIENT CELLS

MEYER ZU HERINGDORF D.[1,2], HEGEN B.[1], TER BRAAK M.[1], HARDEL V.[1], HLA T.[3], JAKOBS K.H.[1] & VAN VELDHOVEN P.P.[4]

[1]Universitätsklinikum Essen and [2] Universitätsklinikum Frankfurt am Main, Germany.
[3] University of Connecticut, Farmington, CT, USA.
[4] LIPIT, K.U. Leuven, Belgium.

Sphingosine-1-phosphate (S1P) regulates cell growth and survival, migration and adhesion in many cell types by activation of specific G protein-coupled receptors. It is formed by sphingosine kinases (SPHKs), degraded back to sphingosine by phosphatases, and irreversibly cleaved by S1P lyase.

Since SPHK-mediated S1P formation has been implicated in agonist-induced Ca^{2+} mobilization, we have studied Ca^{2+} signalling in mouse embryonic fibroblasts (MEFs) deficient in SPHK1 (Sphk1$^{-/-}$) or S1P lyase (Sgpl1$^{-/-}$).

In Sphk1$^{-/-}$ MEFs, agonist-induced $[Ca^{2+}]_i$ increases were variably altered while basal $[Ca^{2+}]_i$ was unaffected. Interestingly, $[Ca^{2+}]_i$ increases induced by the SERCA inhibitor, thapsigargin, were significantly reduced both in the presence and absence of extracellular Ca^{2+}, indicating reduced Ca^{2+} storage. Furthermore, Ca^{2+} mobilization by exogenous sphingosine was fully dependent on Sphk1, proving the previous hypothesis that it is mediated by S1P. In Sgpl1$^{-/-}$ MEFs, resting $[Ca^{2+}]_i$ was strongly elevated both in the presence and absence of extracellular Ca^{2+}, and agonist-induced $[Ca^{2+}]_i$ increases were augmented. Treatment with a SphK inhibitor reduced the elevated basal $[Ca^{2+}]_i$ levels, revealing the involvement of S1P accumulation. Importantly, $[Ca^{2+}]_i$ increases and Ca^{2+} mobilization induced by thapsigargin were augmented and accelerated, indicating enhanced Ca^{2+} storage in Sgpl1$^{-/-}$ cells.

Thus, cellular Ca^{2+} stores were inversely regulated by S1P lyase and SphK1, indicating that S1P plays a role not only in acute Ca^{2+} mobilization but also in long-term regulation of Ca^{2+} homeostasis.

Keywords: sphingosine-1-phosphate, Ca^{2+} release, sphingolipids

REAL TIME cAMP AND Ca^{2+} DYNAMICS IN THE OLFACTORY SYSTEM

MARITAN M.[1], MONACO G.[1], ZACCOLO M.[2] & LODOVICHI C.[1,3]

[1] Venetian Institute of Molecular Medicine, Padua, Italy.
[2] Dvn. Biochemistry and Molecular Biology, Glasgow, UK.
[3] Armenise Harvard CDA.

A unique aspect in the topographic organization of the olfactory system (OS) is the "dual" role of the odorant receptor (OR). The OR is involved in the transduction of chemical signals (odors), but it plays also an instructive role in the glomerular convergence of the olfactory sensory neuron (OSN) axons. The OR is, indeed, expressed not only on the cilia of the OSN, but also on their growth cone.

In the olfactory cilia, the OR transduction pathway is mediated by cAMP and Ca^{2+}. The intracellular events triggered upon the activation of the OR at the growth cone remain to be clarified.

To elucidate the functional properties and the signalling pathway coupled to the OR on the growth cone we have investigated the dynamics and the intracellular distribution of the second messenger cAMP and Ca^{2+} in living OSN in culture after transfection with genetically encoded, FRET-based sensor for cAMP and loading with Ca^{2+} indicators.

OSN were then challenged with pharmacological and physiological stimuli (odors). By analyzing the kinetics and spatial distribution of cAMP and Ca^{2+} we demonstrated that these second messengers are locally produced also at the axon termini growth cone level.

To assess whether the phenomena observed in vitro take place in vivo, we decided to perform Ca^{2+} imaging experiments on hemi-head preparations. In this preparation the mouse head is cut sagittally and the connection between the epithelium, the bulb and the brain is maintained along the medial axis. Olfactory sensory neurons were loaded in vivo with Calcium green-1. The dynamics of Ca^{2+} at the glomeruli level was then evaluated upon pharmacological and physiological stimuli (odors) focally applied at the olfactory bulb in the hemi head preparation.

A large increase of Ca^{2+} signal in the axon termini of the OSN was detected upon pharmacological and physiological stimuli (odors). These data indicate that the OR on the axon termini growth cone is a functional receptor coupled to cAMP and Ca^{2+}.

Keywords: olfactory system, FRET, Ca^{2+} signaling, cAMP, sensory neurons

UNEXPECTED PHOSPHORYL TRANSFER FROM PHOSPHORYLATED ASP351 TO FLUORESCEIN ATTACHED TO LYS515 IN SARCOPLASMIC RETICULUM Ca^{2+} ATPase

MONTIGNY C., CHAMPEIL P. & McINTOSH D.B.

CNRS, URA 2096 and CEA, iBiTec-S, Gif-sur-Yvette, France.
Institute of Infectious Diseases and Molecular Medicine, Division of Chemical Pathology, and National Health Laboratory Services, University of Cape Town, South Africa.

During the catalytic cycle of the Sarcoplasmic Reticulum (SR) Ca^{2+} ATPase, transient formation of an acyl phosphate occurs, at Asp351 in the rabbit skeletal muscle form (SERCA1a). Fluorescein isothiocyanate is a covalent inhibitor of this pump, known to specifically derivatise Lys515 in the nucleotide-binding site. It was previously found that an unusually stable, phosphorylated form of the derivatised ATPase, with low fluorescence, is obtained following Ca^{2+} loading of FITC-labeled SR vesicles with acetyl phosphate as energy source and then chelation with EGTA of Ca^{2+} on the cytosolic side (Pick, 1981; Champeil et al., 2001; Stokes et al., 2005).

Here we show that the phospho-linkage in this low fluorescent species is stable at alkaline pH, unlike the acyl-phosphate bond normally formed at Asp351. The low fluorescence and stable phosphoryl group track together in primary and secondary tryptic subfragments, separated by SDS-PAGE after denaturation, but normal fluorescence and absorbance are recovered upon treatment with alkaline phosphatase after extensive trypsinolysis. We conclude that the low fluorescent species is the result of the phosphoryl group being transferred from phosphorylated Asp351 to the bound fluorescein moiety during pump reversal, yielding fluorescein monophosphate tethered to Ca^{2+} ATPase (McIntosh et al., 2008). This unusual property might provide us with a new tool to investigate the catalytic cycle of Ca^{2+}-ATPase mutants.

Champeil et al. (2001) J. Biol. Chem. 276, 5795-5803.
McIntosh et al. (2008) Biochemistry, 47, 6386-6393.
Pick (1981) FEBS Lett., 123, 131-136.
Stokes et al. (2005) J. Biol. Chem. 280, 18063-18072.

Keywords: SERCA, catalytic cycle

ADAPTATION TO MICROGRAVITY BY THE DECREASE OF RYANODINE RECEPTOR SUBTYPE 1 EXPRESSION IN VASCULAR MYOCYTES

DABERTRAND F., MACREZ N. & MOREL J.L.

CNIC, CNRS Université de Bordeaux, Avenue des Facultés, F-33405 Talence, France.

In space, blood spreading is modified and induces the adaptation of the cardiovascular system. The main effect of microgravity on vascular function is the decrease of vascular tone leading to failure of orthostatic blood pressure after the spationaut return. Vascular contractility depends on Ca^{2+} signalling. Ryanodine receptor subtypes (RYR) are required in Ca^{2+} signalling implicated in vasoconstriction.

In the hindlimb-suspended rat, we have shown that a decrease of RYR expression was responsible for the lost of vascular tone. To know if the same result could be observed in cells cultured in microgravity, we used vascular cells cultured in specific hardware developped for space flight and boarded in taxi-flight to ISS. To measure the effect of gravity, a part of the samples is exposed to microgravity and the other is on a centrifuge reproducing gravity. Cells were fixed either at the beginning (flight effects) or the end of the flight (adaptation to microgravity) and expression of RYR subtypes (RYR1-3) were measured by RT-PCR and immunostaining. We show that RYR1 and RYR3 were expressed in cultured cells. The expressions of RYR1 and RYR3 subtypes were similar in cells on the centrifuge or maintained in laboratory. A long exposure to microgravity decreased only the expression of RYR1 whereas the flight did not modify the expression of RYR1.

In conclusion, we suggest that vascular myocytes can adapt their contractility to microgravity by a decrease of RYR1 that modifies the Ca^{2+} signalling. This work is the first evidence that microgravity during flight induces molecular adaptation of Ca^{2+} signalling in vascular cells.

Keywords: ryanodine receptor, microgravity, vascular myocytes

THE NON-KINASE ACTIVITY OF MYOSIN LIGHT CHAIN KINASE IN REGULATING SMOOTH MUSCLE CONTRACTION

NAKAMURA A.[1], XIE C.[1], MATSUMOTO A.[1], ZHANG Y.[1], GAO Y.[2], KOHAMA K.[1]

[1] Department of Molecular and Cellular Pharmacology, Faculty of Medicine, Gunma University, Graduate School of Medicine, 3-39-22 Showa-Machi, Maebashi, Gunma 371-8511, Japan.

[2] Department of Biochemistry, Dalian Medical University, Dalian, PR China.

A smooth muscle myosin light chain kinase (smMLCK), family of Ca^{2+}/calmodulin-dependent protein kinases, catalyzes the phosphorylation of regulatory light chain (MLC20) of myosin, and plays an important role in activating actomyosin-linked contractility in smooth muscle cell (Baraby, 1996). smMLCK also exhibits actin-binding and myosin-binding activities in addition to this kinase activity. These are called non-kinase activity. By the experiment of recombinant smMLCK fragments, the N-terminal actin-binding fragment inhibits myosin ATPase activity, and C-terminal myosin-binding fragment is activated (Ye et al., 1999).

In order to clarify those molecular mechanisms, we generated recombinant full-length smMLCK and succeeded in the high-level expression (5-10 mg/l culture medium) of full-length recombinant smMLCK by E. coli expression system using the cold shock promoter. Recombinant full-length smMLCK was in a soluble form and it phosphorylated MLC20. It also had non-kinase activity such as actin-binding and -bundling activities. We also generated the kinase-dead smMLCK by triple alanine mutation of the ATP-binding site of smMLCK (Gly731/Gly733/Gly736) (Nakamura et al., 2008).

The kinase-dead smMLCK abolished kinase activity in vitro. We examined the effect of the kinase-dead smMLCK on unphosphorylated or phosphorylated myosin. The kinase-dead smMLCK activated the Mg^{2+}-ATPase activity of unphosphorylated myosin. On the other hand, it inhibited it on phosphorylated myosin.

These results suggested that in addition to the kinase activity, smMLCK also has the non-kinase activities, which may play some regulatory role in smooth muscle contraction.

Baraby (1996) Biochem. Smooth Muscle Contraction, pp. 119-130.
Nakamura et al. (2008) Biochem. Biophys. Res. Commun. 369, 135-143.
Ye et al. (1999) Proc. Natl. Acad. Sci. USA 96, 6666-6671.

Keywords: smooth muscle, MLCK

Ca^{2+} SIGNALLING IN NITROGEN-FIXING BACTERIA NODULATING LEGUMES

NAVAZIO L.[1], MOSCATIELLO R.[1], ALBERGHINI S.[2], SQUARTINI A.[2] & MARIANI P.[1]

[1] Dipartimento di Biologia, Università di Padova, Via U. Bassi 58/B, I-35131 Padova, Italy.
[2] Dipartimento di Biotecnologie Agrarie, Università di Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy

The role of calcium as intracellular messenger, firmly established in eukaryotes, is much less defined in prokaryotes. Rhizobia are Gram-negative soil bacteria which can engage in a mutualistic association with leguminous plants, resulting in the formation of root nodules, where symbiotic rhizobia are able to convert atmospheric nitrogen into ammonia. During this interaction, the two partners enter into a molecular conversation that leads to the reciprocal recognition and the beginning of a successful symbiotic integration.

Ca^{2+} spiking has been demonstrated as an early key regulatory step in the transduction pathway which underlies plant responses to rhizobial informational molecules. Such involvement has so far been reported on the plant side but it is not yet known whether Ca^{2+} acts as transducer of plant signals in rhizobia. In order to verify that, two rhizobia (Mesorhizobium loti and Rhizobium leguminosarum bv. viciae) were transformed with a plasmid containing the aequorin cDNA, and challenged with symbiotic signals. Host plant root exudates, as well as specific flavonoids inducing bacterial nodulation (nod) genes, were found to trigger transient intracellular Ca^{2+} elevations. The Ca^{2+} responses activated by these plant-to-bacteria signal molecules were abolished by the Ca^{2+} channel blocker La^{3+}. Moreover, Ca^{2+} changes were not induced by non specific flavonoids unable to trigger nod gene expression.

The data are consistent with a critical role played by Ca^{2+} in the early signalling pathway activated in rhizobia and leading to a successful symbiosis.

Keywords: rhizobium, bacteria, Ca^{2+} signalling

GLOBAL AND LOCAL CALCIUM SIGNALING UPON APC - T CELL INTERACTION

NEBEL M.[1], SCHMID F.[1], ZHANG B.[2], CORDIGLIERI C.[3], ODOARDI F.[3], POTTER B.V.L.[2], FLÜGEL A.[3] & GUSE A.H.[1]

[1] Institute of Biochemistry and Mol. Biology I, University Medical Centre Hamburg-Eppendorf, D-20426 Hamburg, Germany.
[2] University of Bath, Dept. of Pharmacy and Pharmacology, Bath, BA2 7AY, UK.
[3] Dept. of Neuroimmunology, Max-Planck-Institute for Neurobiology, D-82152 Martinsried, Germany.

Calcium signals play an important role in early T cell activation and are indispensable for proliferation, differentiation and apoptosis of T cells. Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent calcium releasing second messenger known so far (Lee et al., 1995; Gasser et al., 2006). Despite its importance for early T cell signaling, the enzyme to generate NAADP remains to be elucidated. Although its target receptor is controversially discussed, at least in T cells NAADP seems to act on the ryanodine receptor (RyR) at the endoplasmic reticulum (ER) (Steen et al., 2007; Dammermann et al., 2005; Langhorst et al., 2004).

To analyze the development of calcium signals inside the T cell under physiological conditions, we established an in vitro two-cell system including primary rat myelin basic protein (MBP)-specific $CD4^+$ T cells and the rat astrocyte cell line F 10. In contrast to standard T cell activation with anti-CD3-antibodies, this model relies on the antigen (Ag)-specific and direct interaction between the two cell types. Although astrocytes are non-professional Ag-presenting cells in vivo, presentation of specific Ag in context with major histocompatibility complex (MHC) II molecules can be induced on their surface in vitro resulting in remarkable interleukin-2 (IL-2) secretion and proliferation of Ag-specific T cells. After contact to Ag-MHCII complexes on the surface of astrocytes, T cells showed a strong, Ag-dependent increase in global cytosolic calcium concentration. In the absence of Ag, T cells exhibited only weak and delayed calcium signals. A recently developed NAADP-specific small molecule inhibitor is able to block calcium mobilization after Ag-recognition. Using high spatial and temporal resolution analysis on the single cell level, local subcellular calcium signals can be analyzed. The spatio-temporal pattern of NAADP-dependent calcium signals in relation to the immunological synapse is of special interest.

Dammermann et al. (2005) J. Biol. Chem. 280, 21394-21399.
Gasser et al. (2006) J. Biol. Chem. 281, 16906-16913.
Langhorst et al. (2004) Cell Signal. 16, 1283-1289.
Lee et al. (1995) J. Biol. Chem. 270, 2152-2157.
Steen et al. (2007) J. Biol. Chem. 282, 18864-18871.

Keywords: T cell activation, Ca^{2+} signalling, astrocytes, NAADP, lymphocytes, $CD4^+$

CANDIDATE CALCIUM CHANNELS FOR NAADP

BRAILOIU E., CHURAMANI D., DUN N.J. & PATEL S.

Temple University Medical School, Philadelphia, USA.
University College London, London, UK.

Recent studies have uncovered a new calcium signalling pathway regulated by the potent calcium mobilizing messenger NAADP. Unlike the "traditional" messenger molecules inositol trisphosphate and cyclic ADP-ribose, NAADP releases calcium from acidic stores of calcium. Moreover, NAADP has the unique ability to coordinate the activity of other intracellular calcium channels. However, despite the critical importance of NAADP in triggering agonist-evoked calcium signalling, the molecular identity of the target channel has yet to be unequivocally defined. This paucity is information is substantially hampering efforts to characterize the physiological roles of NAADP.

In this contribution, we present the preliminary molecular characterisation of two mammalian candidate calcium channels for NAADP. Our data are consistent with NAADP activating a unique calcium channel located on acidic stores.

Keywords: NAADP, Ca^{2+} release, NAADP receptor

RECRUITMENT OF NAADP-SENSITIVE CALCIUM STORES BY GLUTAMATE

PANDEY V., CHUANG C.C., LEWIS A.M., ALEY P.K., BRAILOIU E., DUN N.J., CHURCHILL G.C. & PATEL S.

University College London, London, UK.
University of Oxford, Oxford, UK.
Temple University Medical School, Philadelphia, USA.

NAADP is novel second messenger thought to mobilize acidic Ca^{2+} stores that are functionally coupled to the endoplasmic reticulum. Although NAADP-sensitive Ca^{2+} stores have recently been described in neurons, the physiological cues that recruit them are not known.

Here we show that in both hippocampal neurons and glia, extracellular application of glutamate in the absence of external Ca^{2+} evoked cytosolic Ca^{2+} signals that were inhibited by blockade of V-type ATPases or following osmotic bursting of lysosomes. The sensitivity of both cell types to glutamate correlated well with lysosomal Ca^{2+} content. However, interfering with acidic compartments was largely without effect on the Ca^{2+} content of the endoplasmic reticulum or Ca^{2+} signals in response to ATP. Glutamate but not ATP elevated cellular NAADP levels. Our data provide evidence for the agonist-specific recruitment of NAADP-sensitive Ca^{2+} stores by glutamate linking the actions of NAADP to a major neurotransmitter in the brain.

Keywords: NAADP, Ca^{2+} release, Ca^{2+} stores, glutamate, neurons

A NOVEL RYANODINE RECEPTOR ISOFORM EXPRESSED IN LIVER

PIEROBON N., GASPERS L. & THOMAS A.P.

University of Medicine and Dentistry of New Jersey (UMDNJ), Department of Pharmacology and Physiology, 185 South Orange Ave, Newark, NJ 07103, USA.

Hormone-evoked Ca^{2+} signaling in isolated hepatocytes appears as a series of periodic spikes in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$), with the frequency modulated by agonist concentration. The single cell temporal distribution is preserved in the intact liver where the spatial organization of the $[Ca^{2+}]_c$ response consists of organized Ca^{2+} waves that spread throughout the lobule. It is well known that the response is mediated by intracellular Ca^{2+} release through IP_3 receptors. However, there is conflicting evidence on whether hepatocytes express ryanodine receptor, a second intracellular Ca^{2+} channel.

In this study, we not only show that hepatocytes express ryanodine receptor, but also that it is a novel ryanodine receptor (RyR1b) that is fundamentally different from the known isoforms. We cloned the novel protein, a truncated form of the ryanodine receptor 1 missing about 50% of the total amino acids that make up the cytosolic domain, and showed the physiological relevance of the endogenous RyR1b in hepatic Ca^{2+} signaling. Ryanodine modulates the pattern of $[Ca^{2+}]_c$ oscillations by increasing oscillation frequency. Experiments in permeabilized hepatocytes showed that both ryanodine and cyclic ADP-ribose evoked a slow Ca^{2+} leak from intracellular stores and were able to increase the Ca^{2+} released response to a subthreshold dose of inositol 1,4,5-trisphosphate. The potential physiological relevance of RyR1b was tested in the intact organ where the protein may have a relevant role in the regeneration and propagation of the Ca^{2+} waves. Perfusion of ryanodine induced a dramatic increase of the frequency of the $[Ca^{2+}]_c$ oscillations induced by IP_3-linked hormone. The overexpression of the cloned RyR1b shows the correct targeting to the endoplasmic reticulum of a protein of about 220 kDa.

Keywords: ryanodine receptor, hepatocytes, Ca^{2+} signalling

THROMBIN-INDUCED INHIBITION OF INTERCELLULAR COMMUNICATION IN CORNEAL ENDOTHELIAL CELLS IS MEDIATED THROUGH THE C-TERMINUS OF Cx43

PONSAERTS R.[1], D'HONDT C.[1], SRINIVAS S.P.[2], VEREECKE J.[1], HIMPENS B.[1] & BULTYNCK G.[1]

[1] Laboratory of Molecular and Cellular Signaling, KULeuven, Leuven, Belgium.
[2] Indiana University, Bloomington, USA.

Activation of PAR-1 receptors by thrombin in bovine corneal endothelial cells (BCEC) inhibits intercellular Ca^{2+}-wave propagation (IC) by reducing connexin 43 (Cx43) hemichannel-mediated ATP-release. This inhibition was prevented by (-)-blebbistatin, a specific inhibitor of myosin II ATPase activity, indicating a role of actomyosin contractility (Ponsaerts et al., 2008).

To elucidate the mechanism by which actomyosin contractility regulates Cx43 hemichannels, we hypothesized that the C-terminal part of Cx43 may be involved in sensing actomyosin contractility through rearrangements of intramolecular Cx43 interactions and/or through changes in Cx43 interactions with cytoskeleton-linker proteins, e.g. ZO-1.

Therefore, we developed a set of cell-permeable TAT-peptides corresponding to the C-terminal region of Cx43 and investigated whether they affect thrombin-induced inhibition of IC elicited by mechanical stimulation of a single cell in a confluent monolayer. A peptide encompassing the last 10 a.a. of Cx43 (a.a. 373-382; SRPRPDDLEI) prevented the thrombin-induced inhibition of IC, whereas the peptide by itself had no significant effect on IC. The reverse sequence of the peptide did not affect the thrombin-induced inhibition of IC, suggesting a specific role for the C-terminal region of Cx43 in sensing enhanced actomyosin contractility. The last isoleucine in the C-terminal tail of Cx43 is crucial for Cx43 binding to ZO-1 as a PDZ2 ligand (Giepmans et al., 1998). A peptide lacking the last isoleucine also prevented the thrombin-induced inhibition of IC, suggesting that ZO-1 is not involved in linking actomyosin to Cx43 hemichannels. Moreover, using co-IP experiments, we found no specific interaction between ZO-1 and Cx43 in lysates of untreated and thrombin-treated BCEC.

In conclusion, our results indicate that the inhibitory effect of thrombin on hemichannel-mediated IC is mediated via a link between the myosin II ATPase activity of the cytoskeleton and the C-terminal tail of Cx43, without a major role for ZO-1.

Giepmans et al. (1998) Curr. Biol. 8, 931-934.
Ponsaerts et al. (2008) IOVS, doi:10.1167/iovs.07-1533.

Keywords: paracrine communication, thrombin, connexins, Ca^{2+} wave, hemichannel, myosin II ATPase, endothelium

MITOCHONDRIAL CALCIUM TRANSPORT AND MITOCHONDRIAL DYSFUNCTION AFTER GLOBAL BRAIN ISCHEMIA

RACAY P., TATARKOVÁ Z., CHOMOVÁ M., HATOK J., KAPLAN P. & DOBROTA D.

Institute of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Mala Hora 4, 03601 Martin, Slovak Republic.

Mitochondria are pivotal regulators of neuronal cell life and death through their role in energy production, involvement in apoptosis and control of neuronal Ca^{2+} homeostasis and signalling. Ischemia-induced mitochondrial Ca^{2+} overload and consequent dysfunction of mitochondria is considered to be an important event coupling cerebral blood flow arrest to neuronal cell death.

Here we report the effect of ischemia-reperfusion on mitochondrial Ca^{2+} uptake and activity of mitochondrial complexes I and IV in rat hippocampus. By performing 4-vessel occlusion model of global brain ischemia, we have observed that 15 minutes of global ischemia led to statistically significant decrease of capacity of mitochondria to accumulate Ca^{2+} to 80.8% of control whereas initial rate of mitochondrial Ca^{2+} uptake was not significantly changed. Reperfusion in duration of 1, 3 and 24 hours did not lead to significant changes in parameters of mitochondrial Ca^{2+} transport. However, ischemia induced significant progressive inhibition of complex I. The minimal activity of complex I was observed 24 hours after ischemia (63% of control). In addition, ischemia led to significant inhibition of complex IV activity to 80.6% of control observed 1 hour after ischemia.

To explain the discrepancy between impact of ischemia on rate of mitochondrial Ca^{2+} uptake and activities of complexes I and IV, we have performed titration experiments to study relationship between inhibition of particular complex and generation of mitochondrial transmembrane potential ($\Delta\Psi m$). Generation of threshold curves showed that complex I and IV activities must be decreased by approximately 40, and 60%, respectively, before significant decline in the $\Delta\Psi m$ was documented. Thus, mitochondrial Ca^{2+} uptake was not significantly affected by ischemia-reperfusion, apparently due to excess capacity of the complexes I and IV.

Keywords: mitochondria, ischemia, brain

LOCALISATION OF THE PUTATIVE NAADP-RECEPTOR

RIETDORF K.[1], RUAS M.[1], WATANABE K.[1], ZHU M.X.[2], PARRINGTON J.[1] & GALIONE A.[1]

[1] University of Oxford, Department of Pharmacology, Oxford, UK.
[2] Center of Molecular Neurobiology, The Ohio State University, Columbus, USA.

Three second messengers that activate intracellular calcium release are known so far: IP_3, cADPR, and NAADP. Of these, NAADP is the most recently identified and the most potent second messenger. It was first discovered in sea urchin eggs, but its importance has since been shown in many mammalian tissues (Galione and Ruas, 2005). NAADP has been shown to act on acidic organelles (Churchill et al., 2002), but there is also evidence that it might act on the RyR on the ER (Galione and Petersen, 2005; Fliegert et al., 2007). So far, the identity of the NAADP-receptor is unknown.

We have recently identified a candidate for the NAADP-receptor. There are several isoforms of this protein in both, sea urchin and mammals. Here we show the localisation of the human and the sea urchin protein after heterologous expression in mammalian cells. One of the human isoforms co-localises with late endosomes / lysosomes, whereas the others colocalises with endosomes only. All the isoforms show no co-localisation with the endoplasmic reticulum, the golgi apparatus, and the mitochondria. For the sea urchin protein we also find co-localisation of one of the isoforms with late endosomes / lysosomes, whereas the other isoforms show a vesicular staining pattern, which only shows a little degree of overlap with late endosomes / lysosomes. All sea urchin isoforms do not show co-localisation with the golgi apparatus or the mitochondria. This localisation is in agreement with the previous findings that NAADP acts on an acidic store.

Churchill et al. (2002) Cell 111, 703-708.
Fliegert et al. (2007) Biochem. Soc. Trans. 35:109-114.
Galione and Petersen (2005) Mol. Interv. 5, 73-79.
Galione and Ruas (2005) Cell Calcium 38, 273-280.

Keywords: NAADP, NAADP receptor, lysosomes, endosomes

INTERACTION OF THE DIHYDROPYRIDINE RECEPTOR β SUBUNIT WITH RYR1 REGULATES EXCITATION-CONTRACTION COUPLING

BARBADO M., APPAIX F., BICHRAOUI H., DE WAARD M. & RONJAT M.

Grenoble Institut des Neurosciences, Inserm U836, team 3, Bât. Edmond J. Safra, Université Joseph Fourier, Site Santé de la Tronche, BP 170, F-38042 Grenoble, France.

Several studies have previously shown that, in skeletal muscle cell, the α1 subunit of the dihydropyridine receptor (DHPR) plays an important role in the bidirectional functional coupling of the DHPR with the ryanodine receptor (RyR).

We show here that the cytoplasmic β subunit of the DHPR also interacts directly with RyR1 and that this interaction controls the orthograde coupling of the DHPR with RyR1. The binding site for the DHPR β1a subunit was mapped to the M3201 - W3661 region of RyR1. Modification of the RyR1 interacting domain of the β subunit by mutagenesis induces the loss of the functional coupling between DHPR and RyR1.

We identified the domain of the β subunit involved in this interaction. The interaction of this domain with RyR1 depends on the conformational state of the β subunit. The conformation of β subunit switches between different states depending on intra molecular interactions between different domains of the β subunit. We will discuss the dynamic of these interactions during the excitation-contraction coupling process.

Keywords: L-type Ca^{2+} channel, β subunit, excitation-contraction coupling, ryanodine receptor

MOLECULAR AND FUNCTIONAL ANALYSIS OF THE INTERACTION BETWEEN POLYCYSTIN-2 AND THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR

SAMMELS E., DEVOGELAERE B., MISSIAEN L., PARYS J.B. & DE SMEDT H.

Laboratory of Molecular and Cellular Signaling, Department of Molecular Cell Biology, K.U.Leuven, Campus Gasthuisberg O/N1 Bus 802, B-3000 Leuven, Belgium.

Recent data indicate that polycystin-2 (TRPP2, an ion channel of the TRP-superfamily) can interact with the inositol 1,4,5-trisphosphate receptor (IP_3R). In most cell systems, polycystin-2 resides in the endoplasmic reticulum, but its function there still remains unclear.

In this study we aimed to specify the exact interaction sites for polycystin-2 as well as for the IP_3R and to investigate the functional relevance of their interaction. Via immunoprecipitation of polycystin-2 from extracts of the LLC-PK_1 renal cell line stably expressing full-length polycystin-2, we could detect interaction with full-length IP_3R3, which is the main IP_3R isoform expressed in these cells. Further analysis by GST-pull-down assays revealed that the C-terminal cytoplasmic tail of polycystin-2 (a.a. 679-966) strongly interacted with the N-terminal ligand-binding domain (LBD) of the IP_3R1 (a.a. 1-581). Moreover, the C-terminal tail of polycystin-2 inhibited the binding of IP_3 to this LBD with an IC_{50} ~350 nM. We demonstrated that the suppressor region of IP_3R1 (a.a. 1-225) was necessary for this inhibitory effect of polycystin-2 on IP_3 binding, which indicates the suppressor as the main site for interaction with polycystin-2. Next, when full-length polycystin-2 is re-introduced in $TRPP2^{-/-}$ renal epithelial cells, there is a clear stimulation of ATP-induced intracellular Ca^{2+} release. This might indicate that the polycystin-2 channel itself is activated by a local Ca^{2+} increase.

We therefore conclude that direct interaction between polycystin-2 and the IP_3R can be important for modulating intracellular Ca^{2+} signalling. Disturbance of the intracellular Ca^{2+} signalling could therefore be one mechanism for the development of autosomal dominant polycystic kidney disease caused by loss-of-function mutations in polycystin-2.

Keywords: polycystin-2, IP_3 receptor, TRPP2, polycystic kidney disease, Ca^{2+} release

NAADP: A SECOND MESSENGER INVOLVED IN MODULATION OF MOUSE CARDIAC MYOCYTE CALCIUM TRANSIENTS

SIEBRANDS C.C.[1], SCHLEGEL S.[1], ZHANG B.[3], GRENDEL J.[1], BRUHN S.[1], POHLMANN L.[2], CARRIER L.[2], ESCHENHAGEN T.[2], POTTER B.V.L.[3] & GUSE A.H.[1]

[1] Inst. of Biochemistry and Molecular Biology I, and
[2] Inst. of Experimental and Clinical Pharmacology and Toxicology,
University Medical Centre Hamburg-Eppendorf, Germany.
[3] Wolfson Laboratory of Medicinal Chemistry, Dept of Pharmacy and Pharmacology,
University of Bath, UK.

The free cytosolic calcium concentration plays a major role in cardiac excitation-contraction coupling. Here we provide evidence for a modulatory role of nicotinic acid adenine dinucleotide phosphate (NAADP) in ventricular cardiac myocyte calcium signaling. NAADP is an endogenous nucleotide that mobilizes calcium from intracellular stores at low nanomolar concentrations.

Primary ventricular cardiomyocytes from adult mice were used for calcium imaging and combined imaging and patch-clamp experiments.

Controlled infusion of NAADP into cardiac myocytes resulted in calcium release sensitive to the specific NAADP antagonist BZ194. When cardiac myocytes were plated on the basement membrane protein, laminin 111, and electrically stimulated, isoprenaline (200 nM) provoked spontaneous additional calcium transients occurring shortly after the first calcium spike. In contrast, such spontaneous calcium transients were not observed when the cells were plated on a plastic surface under otherwise identical conditions. The NAADP antagonist BZ194 reduced the additional calcium transients seen on laminin 111 in a concentration-dependent manner. Our data suggest that laminin-myocyte interaction sensitizes ryanodine receptors via intracellular NAADP. Further, if this sensitization is accompanied by increased sarcoplasmic reticulum calcium loading upon β-adrenergic stimulation, these two mechanisms in concert cause spontaneous additional calcium transients. This mechanism may be involved in ventricular arrhythmias during remodeling of cardiac tissue.

Keywords: cardiomyocytes, NAADP, laminin

ASSEMBLY AND DYNAMICS OF SARCOPLASMIC RETICULUM DOMAINS IN SKELETAL MUSCLE CELLS

CUSIMANO V., BENINI F., GIACOMELLO E., ROSSI D. & SORRENTINO V.

Molecular Medicine Section, Department of Neuroscience, University of Siena, Siena, Italy.

The sarcoplasmic reticulum (SR) of skeletal muscle cells is a complex network of tubules and cisternae that share a common lumen delimited by a single continuous membrane. The SR contains longitudinal and junctional domains characterized by distinctive patterns of protein localization, but how SR proteins reach or are retained at these sites is not known. Here we report that the organization of longitudinal SR proteins is a slow process characterized by temporally distinct patterns of protein localization. In contrast, junctional SR proteins rapidly and synchronously assembled into clusters. These clusters of junctional proteins merged later into mature triadic junctions, but only after completion of longitudinal SR proteins organization. FRAP experiments indicated that organization of the SR was accompanied by significant changes in the dynamic properties of longitudinal and junctional proteins. The reduced mobility that accompanied organization of longitudinal SR proteins, with the exception of SERCA pumps, may result from retention at specific sites through interactions with external structures, like the cytoskeleton or myofibrils. Establishment of junctional SR domains was accompanied by a more significant decrease in the mobility of junctional proteins, which agrees with the assembly of these proteins into a large multiprotein complex. Deletion analysis indicated that the intraluminal region of triadin mediated the stable association of this protein to the junctional SR domain. Thus, organization of proteins within SR domains appears to result from a process of membrane reorganization accompanied by the establishment of multiple protein-protein interactions with intrinsic and extrinsic cues.

Keywords: sarcoplasmic reticulum, protein dynamics, skeletal muscle

Mg^{2+} SIGNALING AND MITOCHONDRIAL Ca^{2+} UPTAKE

SZANDA G.[1], RAJKI A.[2], GALLEGO-SANDÍN S.[3], GARCIA-SANCHO J.[3] & SPÄT A.[1,2]

[1] Dept. of Physiol., Faculty of Medicine, Semmelweis University, and
[2] Lab. of Neurobiochem. and Mol. Physiol., Hungarian Academy of Sciences, Budapest, Hungary.
[3] Dept. of Physiol., Faculty of Medicine, IBGM,University of Valladolid, Valladolid, Spain.

Knowing that Mg^{2+} inhibits the Ca^{2+} uptake of isolated mitochondria we raised the questions whether changes of cytosolic [Mg^{2+}] in the physiological range would affect mitochondrial Ca^{2+} uptake.

HEK-293T cells, expressing mitochondrially targeted GFP-enhanced aequorin, were permeabilized and stepwise elevations of submicromolar [Ca^{2+}] and various [Mg^{2+}] in the cytosol-like media were applied to determine Ca^{2+} uptake and efflux rates. Mg^{2+} attenuated mitochondrial Ca^{2+} uptake without affecting Ca^{2+} efflux. The inhibition was most pronounced in the physiologically relevant 0.25-1.0 mM [Mg^{2+}] range.

To reveal changes in cytosolic [Mg^{2+}] ([Mg^{2+}]$_c$) during Ca^{2+} signaling, intact HEK-293T cells were loaded with Ca^{2+}-and Mg^{2+}-sensitive dyes and fluorescent signals were monitored by laser confocal microscopy. Resting [Mg^{2+}]$_c$ was around 0.3 mM. Extracellular ATP, acting on P2Y receptors, evoked cytosolic Ca^{2+} response that was accompanied with fast Mg^{2+} signal falling in the 0.25-0.75 mM range. ATP and store-operated Ca^{2+} influx induced comparable Ca^{2+} signal but the latter was associated only with negligible Mg^{2+} response implying that Mag-Fluo-4 predominantly reflects [Mg^{2+}]. The Mg^{2+} to Ca^{2+} ratio during ATP-induced signaling was unaltered when intracellular ATP was depleted suggesting that ATP consumption is not the main source of the initial Mg^{2+} response. Despite of comparable Ca^{2+} signals, UV-photolysis of caged Ca^{2+} was less effective in inducing Mg^{2+} signal than stimulation with ATP. This difference is most probably due to IP_3-induced Mg^{2+} release since Mg^{2+}, in addition to Ca^{2+}, was also mobilized by IP_3 in permeabilized cells.

Our data show that Mg^{2+} inhibits mitochondrial Ca^{2+} uptake in the concentration range in which [Mg^{2+}]$_c$ changes in intact, ATP-stimulated cells. Displacement of Mg^{2+} from cytosolic binding sites by the Ca^{2+} signal and IP_3 sensitive stores were identified as major sources of Mg^{2+}.

Keywords: mitochondria, Ca^{2+} uptake, magnesium, Ca^{2+} release

SERCA2b, A Ca^{2+} PUMP LINGERING IN THE E1 HIGH Ca^{2+} AFFINITY CONFORMATION

VANDECAETSBEEK I., RAEYMAEKERS L., WUYTACK F. & VANGHELUWE P.

Laboratory of Ca^{2+}-Transport ATPases, Department of Molecular Cell Biology, K.U.Leuven, B-3000 Leuven, Belgium.

The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) 2b isoform can be clearly distinguished from the other members of the SERCA family by the presence of an extended carboxyl tail (2b tail) of 49 amino-acid (a.a.) residues. The 2b-tail is responsible for the two-fold higher apparent affinity for cytosolic Ca^{2+} and a two-fold lower catalytic turnover rate of SERCA2b compared to the shorter splice-variant SERCA2a. The 2b-tail contains an 11th transmembrane segment (TM11) and a luminal extension (LE).

To investigate the function of this tail, several SERCA2b mutations and a SERCA1a-2b chimera were constructed and functionally examined by Ca^{2+}-dependent ATPase measurements. These experiments revealed that TM11 and the last 4 a.a. (-MFWS) of LE help to confer the specific enzymatic properties of SERCA2b. We hypothesized that these 4 a.a. function as a hook, interacting with more upstream luminal loops, thereby holding the pump during a longer part of its catalytic cycle in the high Ca^{2+} affinity conformation (E1). The addition of a synthetic MFWS peptide to different native SERCA isoforms inhibits their activity each with an isoform-specific K_i (2.9 mM SERCA1a, 1.1 mM SERCA2a, 1.8 mM SERCA2b). Since this peptide functions as an inhibitor it can be used in a conformation-related digestion study with proteinase K as was already shown for inhibitors like thapsigargin and DBHQ (Tadini-Buoinsegni et al., 2008). This study uncovers that the MFWS peptide indeed promotes the E1 conformation of the pump. It is therefore hypothesized that in the native SERCA2b, the tail contributes to the specific enzymatic properties of this pump by holding it for a longer fraction of time in the E1 conformation.

Tadini-Buoninsegni et al. (2008) Mol. Pharmacol, 73, 1134-1140.

Keywords: SERCA, catalytic cycle

INHIBITION OF Cx43 HEMICHANNEL RESPONSES WITH HIGH $[Ca^{2+}]_i$ IS MEDIATED BY MECHANISMS DIFFERENT FROM CALCIUM ACTIVATION OF HEMICHANNEL RESPONSES

WANG N., DE VUYST E., DE BOCK M., DECROCK E. & LEYBAERT L.

Dept. Basic Medical Sciences - Physiology Group,
Faculty of Medicine and Health Sciences, Ghent University, Ghent, B-9000, Belgium.

Previous work of our group has demonstrated that connexin (Cx) hemichannel (HC) responses can be activated by changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in both Cx32 and Cx43 expressing cells. These $[Ca^{2+}]_i$-triggered responses are characterized by a bell-shaped response curve displaying activation of the responses with moderate $[Ca^{2+}]_i$ transients (peak ~500 nM) and disappearance of the responses (further called inactivation) with $[Ca^{2+}]$i transients peaking above ~500 nM.

Here, we further explored $[Ca^{2+}]_i$-dependent inactivation of the HC responses. $[Ca^{2+}]_i$-triggered HC responses have been demonstrated to involve an intermediate step of calmodulin (CaM) and arachidonic acid (AA) signaling. Dye uptake assays with propidium iodide in C6-Cx43 cells revealed that AA-triggered responses were characterized by an S-shaped response curve with half-maximal activation occurring at ~330 µM, similar to the S-shaped curve observed for CaM activation with CALP1. AA-triggered dye uptake was inhibited by carbenoxolone and Gap 26, a Cx-mimetic peptide. The difference between S-shaped and bell-shaped response curves points to the absence of inactivation with the CaM or AA stimuli and suggests that the Ca^{2+}-dependent inactivation is mediated by other mechanisms.

We further performed experiments in which dye uptake through HC was stimulated with AA combined with simultaneous exposure to various concentrations of the Ca^{2+}-ionophore A23187 to induce $[Ca^{2+}]_i$ changes. A23187 concentrations in the range of 6 to 12 µM were used to incrementally induce Ca^{2+} transients peaking in the micromolar range. Dye uptake experiments revealed that AA-triggered HC responses were suppressed by a high $[Ca^{2+}]_i$ in a dose-dependent manner, indicating that the bell-shaped $[Ca^{2+}]_i$-HC response curve is composed of an activation phase that involves CaM-AA signaling and an inactivation phase that is related to direct actions of Ca^{2+} on a yet unknown target.

Keywords: connexins, hemichannels, calmodulin, arachidonic acid

A PLASTID PROTEIN CRUCIAL FOR Ca^{2+}-REGULATED STOMATAL RESPONSES

WEINL S., HELD K., SCHLÜCKING K., STEINHORST L., KUHLGERT S., HIPPLER M. & KUDLA J.

Molekulare Entwicklungsbiologie der Pflanzen, Institut für Botanik und Botanischer Garten, Universität Münster, Schlossplatz 4, D-48149 Münster, Germany.

Guard cell movements are regulated by environmental cues including, for example, elevations in extracellular Ca^{2+} concentration.

Here, the subcellular localization and physiological function of the Ca^{2+}-sensing receptor (CAS) protein was investigated. CAS protein localization was ascertained by microscopic analyses of green fluorescent protein (GFP) fusion proteins and biochemical fractionation assays. Comparative guard cell movement investigations were performed in wild-type and CAS loss-of-function mutant lines of Arabidopsis thaliana. Cytoplasmic Ca^{2+} dynamics were addressed in plants expressing the yellow cameleon reporter protein YC3.6. This study identified CAS as a chloroplast-localized protein that is crucial for proper stomatal regulation in response to elevations of external Ca^{2+}. CAS fulfils this role through modulation of the cytoplasmic Ca^{2+} concentration. This work reveals a novel role of the chloroplast in cellular Ca^{2+} signal transduction.

Keywords: Ca^{2+} signaling, chloroplast, guard cell, Arabidopsis

IN VIVO IMAGING OF NUCLEAR Ca^{2+} SIGNALS IN THE FRESHWATER POLYP HYDRA VULGARIS USING CODON-OPTIMISED RECOMBINANT Ca^{2+} INDICATORS

WEISLOGEL J.M., SCHLÜTER J., NAKAMURA Y., HOLSTEIN T. & BADING H.

Institut of Neurobiolgy, Im Neuenheimer Feld 364, University of Heidelberg, Germany.

The divalent cation, Ca^{2+}, is a highly versatile intracellular messenger and is essentially involved in a diversity of cellular mechanisms such as proliferation, differentiation and survival. In the mammalian nervous system, specifically nuclear Ca^{2+} plays an important role in signal transduction as well as changes in gene expression induced by electrical activity. The freshwater polyp hydra belongs to the Cnidaria phylum and has one of the simplest nervous systems described in the animal kingdom. Many elements of the Ca^{2+}-signalling toolkit are highly conserved from cnidarians to mammals. Therefore, hydra is of particular interest for understanding the evolutionary origin of Ca^{2+}-signalling pathways.

Here, we visualised for the first time in vivo changes in intracellular Ca^{2+} concentration in the freshwater polyp H. vulgaris. Since we are particularly interested in Ca^{2+} signalling in the nucleus, we have generated several transgenic lines expressing a nuclear localised, hydra codon optimised version of the recombinant Ca^{2+} indicator, GCaMP 1.3 (Nakai et al., 2001) under control of a hydra actin promoter. In addition, to differentiate between changes in fluorescence evoked by increases in Ca^{2+} concentration and those caused purely by movement artefacts, a hydra optimised red fluorescent protein (mRFPmars) was fused to the Ca^{2+} indicator hyGCaMP NLS (Müller-Taubenberger et al., 2006). The in vivo imaging data obtained with the transgenic hydra line expressing hyGCaMP NLS mRFP will be discussed.

Müller-Taubenberger et al. (2006) Eur. J. Cell Biol. 85, 1119-1129.
Nakai et al. (2001) Nat. Biotechn. 19, 137-141.

Keywords: Ca^{2+} signalling, nucleus, Hydra, invertebrates

STRUCTURAL AND FUNCTIONAL REMODELING OF THE COUPLING BETWEEN SARCOLEMMAL Ca^{2+} CHANNELS AND THE RYANODINE RECEPTOR IN A MODEL OF ATRIAL FIBRILLATION

LENAERTS I., BITO V., HOLEMANS P., HEIDBÜCHEL H., SIPIDO K.R. & WILLEMS R.

Laboratory of Experimental Cardiology, Department of Cardiovascular Diseases, University of Leuven, O&N Herestraat 49, 3000 Leuven, Belgium.

Background: Atrial fibrillation (AF) in humans has been associated with structural and electrical remodelling. Data on contractile remodelling and control of sarcoplasmic reticulum (SR) Ca^{2+} release via the ryanodine receptor (RyR) in AF are limited.

Methods: Ewes were atrially paced at 600 bmp for a median of 23 weeks resulting in AF (mean of 89.6±6 days, N=13) and compared to matched, non-instrumented control animals (CTRL, N=17). Atrial myocytes were isolated and cell shortening (field stimulation), membrane currents (whole cell voltage clamp) and $[Ca^{2+}]_i$ (Fluo-3) were measured. Protein expression was analyzed by immunoblotting. T-tubule density was quantified from confocal Z-stack images of cells stained with di-8-ANEPPS. Data are shown as mean±SEM.

Results: Myocyte shortening and underlying $[Ca^{2+}]_i$ transients were reduced in AF (by 54.8% and 62 %, $p<0.01$). This reduced cell shortening could be corrected by increasing $[Ca^{2+}]_i$ during caffeine-induced Ca^{2+} release from the SR (L/L_0 9.7±0.5% in AF vs. 10.8±0.5 in CTRL). SR Ca^{2+} content (integrated Na^+/Ca^{2+} exchange current during caffeine) was not different (CTRL 1.9±0.3 pC/pF vs. AF 2±0.4 pC/pF), but calculated fractional SR Ca^{2+} release during a depolarizing step to +10 mV was reduced in AF (by 20.6%, $p<0.05$). Peak Ca^{2+} current density was modestly decreased (at +10 mV by 23.9%, $p<0.01$). T-tubules were present in the CTRL atrial myocytes. Their density was lower than in ventricular cells (11.4% vs. 20.6%, $p<0.05$). T-tubule density was reduced in AF vs. CTRL (by 45%, $p<0.01$) with a reduction of myocyte surface:volume ratio (by 26%, $p<0.01$). The organization of RyR was unchanged but protein expression was reduced (by 18.6%, $p<0.05$).

Conclusion: In AF, reduced SR Ca^{2+} release is a major factor in the reduced cell contraction. Loss of T-tubules contributes to uncoupling of sarcolemmal Ca^{2+} channels to RyR and reduced fractional SR Ca^{2+} release despite preserved SR Ca^{2+} content.

Keywords: atrial fibrillation, contractility, ryanodine receptor, cardiomyocytes

IP_3 RECEPTORS AND SERCA PUMPS ARE BOTH TARGETS OF THE FAMILIAL ALZHEIMER'S DISEASE MUTANT PRESENILIN-2-T122R

ZAMPESE E., BRUNELLO L., FLOREAN C., POZZAN T., FASOLATO C. & PIZZO P.*

Department of Biomedical Sciences, University of Padua, Padua, Italy.

The mutations in presenilin-1 (PS1) M146L and P117L or presenilin-2 (PS2) M239I and T122R cause a reduced instead of an exaggerated calcium release from intracellular stores of primary fibroblasts obtained from Familial Alzheimer's Disease (FAD) patients (Zatti et al., 2004 & 2006; Giacomello et al. 2005). Those and other PS mutants (PS1-A246E, PS1-D385A, PS1-H163R, PS2-N141I, PS2-D366A), as well as the wild type (wt) form of these proteins, share the same feature when transiently over-expressed in different cell lines and rat primary neuronal cultures. Such effect is more dramatic for PS2 variants which significantly lower the steady-state calcium concentration at the level of endoplasmic reticulum (ER) and Golgi apparatus, as demonstrated in HeLa and SH-SY5Y cells by organelle-targeted aequorins (Zatti et al., 2006).

PSs have recently been reported to act as calcium channels per se (Tu et al., 2006) to increase SERCA pump activity (Green et al., 2008) as well as IP_3 receptor sensitivity (Cheung et al., 2008). We here demonstrate that, in SH-SY5Y cells as well as in mouse embryonic fibroblasts, either wt or devoid of endogenous PSs, expression of PS2 variants decrease the store calcium content by increasing the ER calcium leakage through IP_3 receptors and by reducing (but not increasing) SERCA pump activity. The full-length (FL) but not the N- and C-terminal dimer of presenilin-2 is directly involved in store calcium handling, an effect which seems also independent of ryanodine receptors and ribosomal translocon complexes.

Cheung et al. (2008) Neuron 58, 871-883.
Giacomello et al. (2005) Neurobiol. Disease 18, 638-648.
Green et al. (2008) J. Cell Biol.181,1107-1116.
Tu et al. (2006) Cell 126, 981-993.
Zatti et al. (2004) Neurobiol. Disease 15, 269-278.
Zatti et al. (2006) Cell Calcium 39, 539-550.

*Tel +39 049 8276067; Fax: +39 049 8276049; e-mail: paola.pizzo@unipd.it

Keywords: Alzheimer's disease, presenilins, IP_3 receptor, SERCA

TWO-PORE CHANNELS FOR INTRACELLULAR CALCIUM MOBILIZATION AND NAADP SIGNALING

ZHU M.X.[1], GALIONE A.[2], EVANS A.M.[3], MA J.[4], PARRINGTON J.[2], PAN Z.[4], CALCRAFT P.J.[3], CHENG X.[2], RUAS M.[2], RIETDORF K.[2], HAO X.[1] & XIAO R.[1]

[1] Dept Neuroscience, The Ohio State University, Columbus, OH, USA.
[2] Dept Pharmacology, University of Oxford, Oxford, UK.
[3] Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK.
[4] Dept Physiology & Biophysics, UMDNJ, Piscataway, NJ, USA.

Two-pore channels (TPCs) are novel members of the superfamily of voltage-gated ion channels. Their predicted structures indicate 2-fold symmetry with a total of 12 putative transmembrane (TM) α-helices. Sequence homology and membrane topology analyses suggest that TPCs may represent evolutionary intermediates from the single domain 6-TM architecture K^+ and non-selective cation channels to the four-repeat 24-TM structure of voltage-gated Ca^{2+} and Na^+ channels. Three genes (TPCN1-3) exist in most vertebrates but their functions remain elusive.

We now show that TPC1 and TPC3 are expressed on the membrane of different endosome populations while TPC2 is expressed on the membrane of lysosomes. Functional studies revealed that TPC2 is the target of nicotinic acid adenine dinucleotide phosphate (NAADP), a potent Ca^{2+} mobilizing messenger that evokes Ca^{2+} release from acidic organelles rather than from the sarco/endoplasmic reticulum. Microsomal membranes enriched with TPC2 exhibit high affinity, specific binding with NAADP that is comparable with that of liver preparations.

Cells overexpressing TPC2 exhibit enhanced intracellular Ca^{2+} release in response to NAADP that is blocked by disrupting the H^+ gradient of lysosomes using bafilomycin A1. Furthermore, RNAi-mediated silencing of TPC2 abolishes the NAADP response in these cells and in cultured hepatocytes.

These data demonstrate that NAADP targets TPC2 to release Ca^{2+} from lysosomal stores. Our findings provide for the first time molecular basis for further detailed characterizations of the regulatory mechanisms and physiological functions of NAADP-mediated signaling and, in addition, suggest a general role for TPCs in Ca^{2+} mobilization, Ca^{2+} homeostasis, and Ca^{2+} signaling from endosomal/lysosomal compartments of vertebrate cells, which are known to be important for diverse function in many physiological systems.

Keywords: lysosomes, NAADP receptor, Ca^{2+} release, two-pore channels