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Abstracts

S16 Mitochondrial Ion Channels

Lectures

16L.1 Regulation of mitochondrial K_{ATP} channels

Uta C. Hoppe
University of Cologne, Department of Internal Medicine III, Cologne, Germany
E-mail: uta.hoppe@uni-koeln.de

Potassium (K^+) channels of the inner mitochondrial membrane influence cell function and survival. Increasing evidence indicates that multiple signaling pathways and pharmacological actions converge on mitochondrial adenosine triphosphate-sensitive K^+ (mitoK_{ATP}) channels and protein kinase C (PKC) as pivotal components of cytoprotection against necrotic and apoptotic cell injury. However, the molecular structure of mitoK_{ATP} channels remains unresolved, and no mitochondrial phosphoprotein has yet been identified that may mediate cytoprotection by these kinases. By patch-clamping the inner membrane of subsarcolemmal murine cardiac mitochondria we found that genetic connexin 43 (Cx43) deficiency, pharmacological connexin inhibition by carbenoxolone or Cx43 blockade by the mimetic peptide ^{43}GAP27 significantly reduces diazoxide-mediated stimulation of mitoK_{ATP} channels, explaining loss of cytoprotection in *Cx43*$^{+/-}$ mice *in vivo*. Suppression of mitochondrial Cx43 inhibited mitoK_{ATP} channel activation by PKC. MitoK_{ATP} channels of interfibrillar mitochondria, which do not contain any detectable Cx43, are completely drug- and PKC-insensitive, (i) confirming the fundamental role of Cx43 for mitoK_{ATP} channel stimulation, and (ii) indicating compartmentation of mitochondria in cell signaling. Our results define a novel molecular function of mitochondrial Cx43 and provide a link between cytoprotective stimuli and mitoK_{ATP} channel opening. Thus, mitochondrial Cx43 is an attractive target for drug development against cell injury.

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16L.2 The mitochondrial apoptosis-induced channel, MAC, and Bcl-2 family proteins are co-conspirators in a deadly plot

Kathleen W. Kinnally, Shin-Young Ryu,
Pablo M. Peixoto, Laurent M. Dejean
New York University College of Dentistry, Department of Basic Sciences, New York, USA
E-mail: kck1@nyu.edu

Apoptosis is essential to mechanisms controlling tissue homeostasis and is involved in a variety of pathologies including degenerative diseases, aging, and cancer. Bcl-2 family proteins regulate this cell death program by controlling the formation of the mitochondrial apoptosis-induced channel, or MAC. Assembly of MAC corresponds to the commitment step of apoptosis, as MAC provides the pathway across the outer membrane for the release of cytochrome *c* and other pro-apoptotic factors from mitochondria [1]. While anti-apoptotic Bcl-2 antagonizes MAC activity, oligomers of the pro-apoptotic members Bax and/or Bak are essential structural component(s) of MAC [2]. In fact, assembly of MAC from Bax or Bak was monitored in real time by directly patch-clamping mitochondria with micropipettes containing the sentinel tBid, a direct activator of Bax and Bak [3] Recently, high affinity inhibitors of MAC (iMACs) were identified by Peixoto et al. [4]. Our ability to pharmacologically open and shut MAC may provide crucial clues in mechanistic studies of apoptosis and have potential therapeutic applications.

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16L.3 Contribution of the mitochondrial potassium channel Kv1.3 to the regulation of programmed cell death

Erich Gulbins[1], Nicola Sassi[2], Heike Grassmè[1], Luigi Leanza[3], Mario Zoratti[2], Ildikò Szabò[3]
[1]*Department of Molecular Biology, University of Essen, Hufelandstrasse 5, Essen, Germany*
[2]*CNR Center for Neurosciences, viale G. Colombo 3, Padova 35122, Italy*
[3]*Department of Biology, University of Padova, viale G. Colombo 3, Padova 35122, Italy*
E-mail: ildi@civ.bio.unipd.it

Mitochondria have been shown to play a pivotal role in apoptotic signalling in various cell types. We have recently reported that in lymphocytes the voltage-gated potassium channel Kv1.3, known to reside in the plasma membrane, is active also in the inner mitochondrial membrane [1]. Upon induction of apoptosis, outer-membrane inserted Bax binds to and inhibits Kv1.3 resulting in hyperpolarization, an increase in reactive oxygen species production and cytochrome *c* release. In cells lacking Kv1.3 these events do not take place. The physiological relevance of Kv1.3 for apoptosis is illustrated by the facts that knockdown of Kv1.3 expression in human peripheral blood lymphocytes impairs apoptosis in these cells, and expression of mitochondria-targeted Kv1.3 is sufficient to sensitize to apoptotic stimuli resistant CTLL-2 T lymphocytes, which lack Kv channels [2]. Recombinant Kv1.3, when pre-incubated with Bax, prevents the actions of Bax at the level of mitochondria [3]. Data obtained with mutant Bcl-2 family proteins further point to an important role of this channel in the sequence of

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events leading to Bax-induced cytochrome *c* release. Furthermore, the presence of Kv1.3 protein in mitochondria from various cancer cells is observed, suggesting that this channel might play a role in the apoptotic signalling not only in lymphocytes but also in other cells.

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16L.4 MIMIK: The mitochondrial inner membrane intermediate conductance K^+-selective Ca^{2+}-activated channel

Mario Zoratti[1,2], Umberto De Marchi[3], Nicola Sassi[2], Bernard Fioretti[4], Ildikò Szabò[5]
[1]*CNR Institute of Neuroscience, Padova, Italy*
[2]*University of Padova, Department of Biomedical Sciences, Italy*
[3]*University of Geneva, Department of Cellular Physiology and Metabolism, Switzerland*
[4]*University of Perugia, Department of Cellular and Environmental Biology, Italy*
[5]*University of Padova, Department of Biology, Italy*
E-mail: zoratti@bio.unipd.it

A channel often observed in patch-clamp experiments on the inner membrane of mitochondria isolated from Human Colon Tumor 116 (HCT116) cells has been identified as the intermediate conductance K^+-selective Ca^{2+}-activated channel $K_{Ca}3.1$ (MIMIK) on the basis of its biophysical and pharmacological properties. The channel can exhibit different conductance states and kinetic modes, possibly reflecting post-translational modifications. As for the other known mitochondrial K^+ pores, MIMIK represents a population of a channel also present in the plasma membrane. Its mitochondrial location has been demonstrated by electrophysiological experiments on mitoplasts expressing a mito-targeted fluorescent protein and by a biochemical approach using specific markers of mitochondrial and contaminating membranes. In a limited survey of $K_{Ca}3.1$-expressing cells MIMIK has also been found in the mitochondria of HeLa cells and of a line of mouse embryonic fibroblasts, but not in those of two other colon tumour-derived cells, Caco-2 and C-26. Its presence in mitochondria thus appears to be regulated. The channel is predicted to have a role in mitochondrial physiology: moderate increases (K_{50} is about 300 nM) in matrix Ca^{2+} will cause its activation, leading to K^+ influx and depolarization in response to a Ca^{2+} signal. We are exploring the possibility that the channel may also be involved in cellular processes such as proliferation or death.

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Posters

16P.1 Voltage-gated potassium channel in hippocampus mitochondria

Piotr Bednarczyk[1,2], Joanna E. Kowalczyk[3], Krzysztof Dołowy[2], Adam Szewczyk[1], Barbara Zabłocka[3]
[1]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
[2]*Warsaw University of Life Sciences - SGGW, Department of Biophysics, Warsaw, Poland*
[3]*Mossakowski Medical Research Institute, Molecular Biology Unit, Warsaw, Poland*
E-mail: p.bednarczyk@nencki.gov.pl

Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP regulated or Ca^{2+} regulated large conductance potassium channels that can prevent or delay neuronal injury. However, molecular mechanism of this effect remains unclear. In this study, a single channel activity was measured with patch-clamp of the mitoplasts isolated from gerbil hippocampus. In 70% of all the patches, a potassium selective current with properties of the voltage-gated potassium channel (Kv type channel) was recorded with mean conductance 109 ± 6 pS in symmetrical 150 mM KCl solution. Detected channel was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 channel inhibitor. The inhibition by MgTx was irreversible. We observed that ATP/Mg^{2+} complex or Ca^{2+} ions had no effects on observed activity of ion channel. Additionally, we showed that agitoxin-2 (AgTx-2), potent inhibitor of the voltage-gated potassium channels, was without effect on channel activity. This observation suggests that mitochondrial voltage-gated potassium channel can represent different molecular structures without affinity to AgTx-2 in comparison to surface membrane channels. Also, Western blot analysis of mitochondria isolated from gerbil hippocampus and immunohistochemistry on gerbil brain sections confirm the expression of Kv1.3 protein in mitochondria. All together, we conclude that gerbil hippocampal mitochondria contain voltage-gated potassium channel (mitoKv1.3 channel) with properties similar to the surface membrane Kv1.3 channel which can influence function of mitochondria in physiological and pathological conditions.

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16P.2 Molecular dynamics of the mitochondrial protein translocase TIM22: Structure–function correlations of the channel's partakers

Pablo Peixoto[1,2], Lauro González-Fernández[1], Patricia Rojo[1], Jorge Bermejo[1], María Luisa Campo[1]
[1]*University of Extremadura, Department of Biochemistry and Molecular Biology, Cáceres, Spain*
[2]*New York University, College of Dentistry, New York, USA*
E-mail: mlcampo@unex.es

Mitochondrial translocases convey the precise relocation of cytoplasmic encoded proteins to one of the four discrete compartments enfolded by their two membranes. Among their multiple subunits, those forming the aqueous channels have proven essential for the functioning of TOM andTIM23 translocases. Previously we have reported the conditions to uncover *in organello* the channel activity of TIM22, the translocase mediating the insertion of multispaning proteins into the inner membrane. Only cargo proteins facing the intermembrane space trigger the activity of this otherwise silent channel. Three membrane proteins: Tim22p, Tim54p and Tim18p partake TIM22. We have performed the molecular dissection of TIM22 present in mitochondria of eight yeast strains with different expression levels of its defined components. These results combined with those of the native complex and those of patch-clamping the inner membranes of their mitochondria, outline the biogenesis of the complex and the role played by each component. Our results indicate that Tim22p is present in a complex of about 380 kDa also containing Tim18p and Tim54p. The biogenesis of this complex depends on the simultaneous presence of the three membrane proteins. Tim54p