our results show that HCN channels are involved in the nitrergic modulation observed in magnocellular neurons of the supraoptic nucleus acting through s-nitrosylation mechanism.

3841-Pos Board B569

Identification of a Pseudotetrameric K^+-Selective CNG Channel in *Amphioxus*

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A K^+-selective cyclic nucleotide-gated (CNGK) channel is key for chemotaxis in sea urchin sperm; it translates the second messenger signal after chemoattractant binding into an electrical response. We identified genes homologous to the sea urchin channel in the genome of different species - from invertebrates to vertebrates.

We cloned the CNGK channel from the testis of *Amphioxus*; *Amphioxus*, a chordateis considered as evolutionary link between invertebrates and vertebrates. We raised polyclonal antibodies to localize the channel in tissue and identified the channel in the flagellum of *Amphioxus* sperm. We analyzed the properties of the channel by functional expression and its physiological function by motility experiments of swimming sperm cells. Surprisingly, in contrast to the sea urchin CNGK channel, the *Amphioxus* CNGK channel seems to prefer cAMP over cGMP. In conclusion, signal transduction schemes in different sperm species display considerable diversity.

3842-Pos Board B570

A Family of HCN Channel Homologs in Bacteria

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HCN channels belong to the superfamily of six transmembrane domain voltage-gated ion channels. Functionally, these channels activate upon membrane hyperpolarization and carry an inward current that is weakly selective between potassium and sodium and can be modulated by cyclic nucleotides. At the structural level, insight into these channels is limited to a crystal structure of the intracellular C-linker and cyclic nucleotide binding domain, which assembles into a tetramer. To advance our structural understanding of HCN channels, we investigated bacterial homologs of HCN channels as candidates for large-scale expression and future structural studies. Using a BLAST search of the bacterial genome database we identified different homologs, which have a sequence identity of 24-32% with the human HCN2 channel. Using a C-terminal fusion with green fluorescent protein (GFP) we investigated whether the bacterial HCN homologs could be expressed in E. coli. We identified homologs that could be expressed at a whole-cell fluorescence level of ~40% compared to KcsA-GFP as a positive control. Next, we conducted detergent screening using fluorescence size exclusion chromatography (FSEC). We found that dodecylmaltoside or undecylmaltoside are suitable detergents to solubilize bacterial HCN channels in a tetrameric monodisperse state.

In parallel, we investigated the functional properties of the bacterial HCN channel homologs using expression of C-terminal GFP fusions in Xenopus oocytes. Using cell-attached patch recordings we observed in a minority of patches a current that may closely resemble the kinetics of the invertebrate spHCN channel as clear current inactivation is observed at most hyperpolarizing potentials. Confocal microscopy demonstrates marked fluorescence just below the oocyte membrane, indicating that trafficking to the oocyte cell membrane may be compromised and limits our success in obtaining patches for detailed functional characterization.

Together, our biochemical characterization paves the way for large-scale production and crystallization screening.

Intracellular Channels

3843-Pos Board B571

Functional Coupling of the Mitochondrial BKCa Channel to the Respiratory Chain

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Potassium channels as present in the plasma membrane of various cells have also been found in the inner mitochondrial membrane. Potassium channels have been proposed to regulate the mitochondrial membrane potential, respiration, matrix volume and Ca^{2+} ion homeostasis. Also, it has been suggested that mitochondrial potassium channels participate in ischemic preconditioning and neurodegenerative disorders.

In our study single channel activity of a large conductance Ca^{2+}-regulated potassium channel was measured by patch-clamp of mitoplasts isolated from an astrocytoma cell line. Mitoplast were prepared by addition to a hypotonic solution causing unfolding of the cristae of the inner membrane and consequently breaking of the outer membrane. Isotonicity was restored by adding a hypertonic solution. A potassium selective current was recorded with a mean conductance of 290 pS in symmetrical 150 mM KCl solution. The channel was activated by Ca^{2+} at micromolar concentrations and inhibited irreversibly by iberiotoxin, an selective inhibitor of the BKCa channel. Additionally, we showed that substrates of the respiratory chain like succinate decrease the activity of the channel. The effect was abolished by cyanide and antimycine, being inhibitors of respiratory chain.

Our findings indicate that mitochondrial large conductance Ca^{2+}-regulated potassium channels with properties similar to the surface membrane BKCa channel are present in human astrocytoma mitochondria and can be stimulated by redox status of the respiratory chain.

More details: Bednarczyk et al., (2013) PLoS ONE 8(6): e68125. doi:10.1371/journal.pone.0068125.

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3844-Pos Board B572

Electrophysiological Characterization of the Activity and Regulation of the Mitochondrial Calcium Uniporter

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Mitochondrial calcium uptake is present in all eukaryotic living organisms and represents a critical point because it is implicated in highly sophisticated processes that have multiple consequences for the cells' survival or death.

Ca^{2+} uptake by energized isolated mitochondria was directly measured for the first time half a century ago (Vasington and Murphy, 1962), but the molecular identity of the pore-forming subunit of the Ca^{2+} mitochondrial uptake protein complex MCU (mitochondrial calcium uniport), became available only in 2011, thank to the combination of bioinformatics, cell biology and the application of planar lipid bilayers experiments using recombinant MCU protein (De Stefani *et al*., 2011). Today, it is clear that the channel responsible for Ca^{2+} uptake is an inner mitochondrial membrane protein that works as a highly selective channel which can be inhibited by ruthenium red and gadolinium (Kirichok *et al*., 2004, De Stefani *et al*., 2011).

The application of electrophysiology in addition to cell biology techniques opens new perspectives to elucidate the physiological and pathological relevance of MCU and to directly study the role of numerous putative regulators/additional components of the MCU. For example, recent evidence shows that MCU is able to form hetero-oligomers with a protein, called MCUb, which acts as a dominant-negative pore forming subunit (Raffaello *et al*., 2013). Here we report further biophysical characterization of recombinant MCU studied in BLM experiments where possible regulatory factors are not present. Furthermore, we describe our studies using molecules, shown to affect calcium uptake in intact cells, like MICU1 (Perocchi *et al.,* 2013). Given that regulation of MCU activity in intact cells by MICU1 however is controversial (Mallilankaraman *et al*., 2012a/b; *Csordas et al., 2013*), our results provide an important, direct evidence in favor of direct regulation of channel activity.

3845-Pos Board B573

The Open State of Human VDAC Isoforms Compared through MD Simulations

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Voltage dependent anion channel (VDAC) is the pore-forming protein of outer mitochondrial membrane. In mammals three isoforms exist: VDAC1, VDAC2, VDAC3 (1). The VDAC1 is the most abundant (2) and studied isoform, the only one whose 3D structure was solved at high resolution. All-atom MD