600%. Kinetic analyses revealed that this activation occurs by increasing V_{max} but the K_M for glutamate remains unchanged (0.6 mM glutamate at 10 nM Ca^{2+}, 0.7 mM glutamate at 757 nM Ca^{2+}). In contrast the activation of α-KGDH by *intra*mitochondrial Ca^{2+} is caused by increasing affinity to the substrate (1.4 mM α-KG at 10 nM Ca^{2+}, 0.4 mM α-KG at 757 nM Ca^{2+}) but the V_{max} is not changed as is known also from earlier work [2]. With decreasing Ca^{2+} the activity of aralar diminishes and the supply of mitochondria with reducing hydrogen from NADH decreases too. Aspartate titrations revealed a large control of aralar over oxidative phosphorylation. Moreover decreasing Ca^{2+} shifts the force/flow relation of state $3_{glu/mal}$ to lower DY-values. Whereas Ca^{2+} activation of α-KGDH changes the turnover speed at low substrate concentrations, which are inside the matrix space, Ca^{2+} activation of aralar regulates the substrate supply to mitochondria. Therefore we conclude that brain mitochondria together with the cytosolic enzymes of malate aspartate shuttle act as a mitochondrial "gas pedal" with $Ca^{2+}{}_{cyt}$ as physiological controller regulating the substrate supply on demand.

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15L.7 Calcium signalling and ER-mitochondrial communication

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The ER-mitochondrial junction provides a local calcium signaling domain that is critical for both matching energy production with demand and the control of apoptosis. Here, we describe a novel genetic approach to visualize ER-mitochondrial contact sites and to monitor the localized $[Ca^{2+}]$ changes in the narrow space between ER and mitochondria ($[Ca^{2+}]_{ER\text{-}mt}$) using drug-inducible fluorescent interorganellar linkers. We show that essentially all mitochondria have contacts with the ER in both RBL-2H3 and H9c2 cells. Plasma membrane-mitochondrial contacts are less frequent because of interleaving ER stacks. Single mitochondria display discrete patches of ER contacts. Cytoplasmic and mitochondrial matrix $[Ca^{2+}]$ showed robust ER-mitochondrial Ca^{2+} transfer with considerable heterogeneity even among adjacent mitochondria. Pericam-tagged linkers revealed IP_3-induced $[Ca^{2+}]_{ER\text{-}mt}$ signals that exceeded 9 mM and were resistant to buffering bulk cytoplasmic $[Ca^{2+}]$ increases. Lengthening the linker to span the space required for the Ca^{2+} transfer machinery, positively affected the $[Ca^{2+}]_{ER\text{-}mt}$ signals. These studies provide direct evidence for the existence of high Ca^{2+} microdomains between the ER and mitochondria, and indicate their biphasic dependence on the ER-mitochondrial gap width.

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15L.8 The mitochondrial permeability transition pore: from molecular mechanism to cardioprotection

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Opening of the mitochondrial permeability transition pore (MPTP) plays a key role in mediating the damage to the heart that occurs following ischaemia and reperfusion (reperfusion injury) [1]. Reactive oxygen species (ROS) play a key role in triggering MPTP opening and new data on the involvement of cytochrome *c* in regulating ROS production will be described. Inhibition MPTP opening provides powerful protection against reperfusion injury and understanding the molecular mechanism of the MPTP is important for the design of new cardioprotective drugs. However, the true molecular identity of the MPTP remains uncertain [2]. Knockout studies have confirmed regulatory roles for cyclophilin-D (CyP-D) and the adenine nucleotide translocase (ANT). The former facilitates a conformational change in the pore forming protein within the inner membrane and is mediated by its peptidyl-prolyl cis-trans isomerase activity. The ANT mediates the inhibitory effects of adenine nucleotides on MPTP opening. Indeed, the oxidation of specific thiol groups on the ANT overcomes this inhibition and accounts, in part, for the powerful activation of MPTP opening by oxidative stress. More recently we have demonstrated a critical role for the mitochondrial phosphate carrier (PiC) in MPTP opening and shown that the PiC is the major inner membrane protein to bind CyP-D [2]. We have used knockdown of the PiC by siRNA to confirm its role in MPTP formation but these studies have raised problems. First, it is difficult routinely to achieve greater than 80% PiC knockdown. Second, the use of the calcium retention assay to measure MPTP opening in permeabilised cells is not appropriate when phosphate transport into mitochondria is prevented since calcium entry into mitochondria is dependent on coincident uptake of Pi. Progress in circumventing these technical problems to reveal whether or not the PiC is essential for MPTP formation will be described.

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15L.9 Novel protein partners in the control of the brain mitochondrial permeability transition: 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and $p42^{IP4}$ (centaurin α1; ADAP 1)

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Increase of permeability of the inner mitochondrial membrane in response to threshold calcium concentration or oxidative stress leads to the formation of an unselective permeability transition pore (PTP) complex. In spite of intense studies of the PTP phenomenon, the structure of the pore complex is still unknown. Earlier accepted involvement of voltage-dependent anion channel and adenine

nucleotide translocase as structural elements of PTP was questioned after gene knockout experiments. Moreover, the peripheral benzodiazepine receptor (PBR), now designated the 18 kDa translocator protein (TSPO) of the outer membrane, seems to take part in PTP regulation. We present data on evidence how ligands of TSPO or PBR (PK11195, Ro5-4864, protoporphyrin and diazepam binding inhibitor) are able to modulate the induction of Ca^{2+}-induced PTP in rat brain mitochondria. In addition, we summarize the newly revealed contribution of two novel proteins, 2',3'-cyclic nucleotide 3'-phosphodiesterase [1] and $p42^{IP4}$ [2], to Ca^{2+} efflux from rat brain mitochondria loaded by threshold [Ca^{2+}] and thus to induction of PTP. In conclusion, the mitochondria permeability transition pore complex in brain with its interacting proteins presents a promising target for protection in neurodegenerative diseases [3].

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15L.10 Warburg and tumor metabolism revisited: roles for mitochondrial hexokinases and metabolic crosstalk

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More than three-quarters of a century have elapsed since Warburg and colleagues first applied contemporary manometric techniques to the biochemical characterization of cancer metabolism. Their studies identified several cardinal features of tumor metabolism, most notably increased glucose-derived lactate generation in the presence, as well as the absence, of O_2 – or so-called aerobic glycolysis. Recent advances in our understanding of the relationship between metabolism and cell survival and resurgent interest in targeting cancer metabolism for therapeutic benefit have refocused attention on the characteristic features of cancer that Warburg described, as well as their mechanistic underpinnings. Hexokinases, which catalyze the first committed step of glucose metabolism, are overexpressed in cancer and have recently emerged as important mediators of the anti-apoptotic effects of growth factors and Akt. They are also major contributors to the signature glycolytic phenotype of tumors. The ability of hexokinases to prevent apoptosis is mediated, in part, by direct physical and functional interaction with mitochondria and competition with pro-apoptotic Bcl-2 proteins for binding to common mitochondrial target sites. Bound hexokinases also promote the open state of voltage dependent anion channels and the associated exchange of adenine nucleotides and other metabolites into and out of mitochondria, thereby contributing to mitochondrial integrity and directly coupling the first committed step of glucose metabolism in the cytosol to its terminal oxidation and oxidative phosphorylation within mitochondria. This and closely related forms of metabolic crosstalk play important roles in the coordination and control of intra- and extramitochondrial amphibolic metabolism and contribute to the characteristic proliferative and metabolic phenotypes of cancer cells. As such, they constitute attractive potential targets for therapeutic cancer intervention.

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15L.11 Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820 nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*

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A new instrument (M-PEA), which measures simultaneously kinetics of prompt fluorescence (PF), delayed fluorescence (DF) and modulated light reflection at 820 nm (MR), was used to screen dark-adapted leaves of the resurrection plant *Haberlea rhodopensis* during their progressive drying, down to 1% relative water content (RWC), and after their rewatering. This is the first investigation using M-PEA, which employs alternations of actinic light (627 nm peak, 5000 µmol photons m^{2} s^{1}) and dark intervals, where PF-MR and DF kinetics are respectively recorded, with the added advantages: (a) all kinetics are recorded with high time resolution (starting from 0.01 ms), (b) the dark intervals' duration can be as short as 0.1 ms, (c) actinic illumination can be interrupted at different times during the PF transient (recorded up to 300 s), with the earliest interruption at 0.3 ms. Analysis of the simultaneous measurements at different water-content-states of *H. rhodopensis* leaves allowed the comparison and correlation of complementary information on the structure/function of the photosynthetic machinery, which is not destroyed but only inactivated (reversibly) at different degrees; the comparison and correlation helped also to test current interpretations of each signal and advance their understanding. Our results suggest that the desiccation-tolerance of the photosynthetic machinery in *H. rhodopensis* is mainly based on mechanism(s) that lead to inactivation of photosystem II reaction centres (transformation to heat sinks), triggered already by a small RWC decrease.

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Posters

15P.1 Protective effect of SSR180575, a potent and selective peripheral benzodiazepine ligand, on TNF-α induced PMN apoptosis in whole human blood

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The peripheral benzodiazepine receptor (PBR) has been shown to play a key role in the regulation of the mitochondrial process leading to apoptosis (for review, see [1]). Despite much controversy in the literature on this subject, PBR synthetic ligands (and specifically agonists such as Ro5-4864 and SSR180575) are described as presenting potent anti-apoptotic effect against oxidative stress, TNFα- and tamoxifen-induced apoptosis when the PBR ligand is administrated at a low dose, close to the affinity range of the ligand to its receptor [2]. Such anti-apoptotic activity has already been correlated with a protective effect of PBR ligands against ischemia-reperfusion induced tissue dysfunction. Previously, we had shown that SSR180575 is a specific and high affinity PBR ligand of potential interest in pathological cardiovascular [2], renal [3] and neurodegenerative indications [4]. Beyond its expression in steroid-producing