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