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].

References

[1] Azarashvili *et al.* (2009) *Am. J. Physiol. Cell. Physiol.* **296**: C1428-1439.
[2] Galvita *et al.* (2009) *J. Neurochem.* **109**: 1701-1713.
[3] Azarashvili, Stricker, Reiser (2010) *Biol. Chem.* in press.

doi:10.1016/j.bbabio.2010.04.363

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

R. Brooks Robey[1,2]
[1]*White River Junction VA Medical Center, R&D Service, USA*
[2]*Dartmouth Medical School, Departments of Medicine & Physiology, USA*
E-mail: R.Brooks.Robey@Dartmouth.edu

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.

doi:10.1016/j.bbabio.2010.04.364

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*

Reto J. Strasser[1], Merope Tsimilli-Michael[2],
Sheng Qiang[1], Vasilij Goltsev[3]
[1]*Weed Research Laboratory, Nanjing Agricultural University, Nanjing 210095, China*
[2]*Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy/Geneva, Switzerland*
[3]*Department of Biophysics and Radiobiology, Faculty of Biology, St. Kliment Ohridski University of Sofia, 1164 Sofia, Bulgaria*
E-mail: tsimicha@spidernet.com.cy

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.

doi:10.1016/j.bbabio.2010.04.365

Posters

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

Nathalie Leducq-Alet, Valerie Vin, Pierre Savi, Françoise Bono
Sanofi-Aventis Research, Early to Candidate Department, Toulouse, France
E-mail: nathalie.alet@sanofiventis.com

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

tissues, heart, liver and kidney, the PBR is also known to be highly expressed in blood cells. In this work, we demonstrate by flow cytometry experiments, that SSR180575, at low concentrations, is able to protect polymorphonuclear leukocytes (PMNs) against TNFα-induced apoptosis in whole blood. Thus, in a new context, SSR180575 again shows potent anti-apoptotic properties. Moreover, TNFα-induced PMN apoptosis appears to be a good surrogate marker for determining SSR180575 blood availability and activity in treated patients.

References

[1] Veenman L *et al.* (2006) *Pharmacol. Ther.* **110:** 503-524.
[2] Leducq N *et al.* (2003) *J. Pharmacol. Exp. Ther.* **306:** 828-837.
[3] Kunduzova OR *et al.* (2004) *J. Am. Soc. Nephrol.* **15** 2152-2160.
[4] Ferzaz B *et al.* (2002) *J. Pharmacol. Exp. Ther.* 3011067-3011078.

doi:10.1016/j.bbabio.2010.04.366

15P.2 The dynamics of mitochondrial Ca^{2+} fluxes monitored with targeted aequorin

Sergio de la Fuente, Pablo Montenegro, Rosalba I. Fonteriz, Alfredo Moreno, Carmen D. Lobatón, Mayte Montero, Javier Alvarez
Institute of Biology and Molecular Genetics (IBGM), Deptartment of Biochemistry, Molecular Biology and Physiology, Faculty of Medicine, University of Valladolid and CSIC, Ramón y Cajal, 7, E-47005 Valladolid, Spain
E-mail: jalvarez@ibgm.uva.es

Mitochondrial Ca^{2+} fluxes play a very important role in cell physiology. In spite of the large negative potential of the mitochondrial matrix, mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_M$) is low under resting conditions because of the operation of systems able to extrude Ca^{2+} from mitochondria in exchange by Na^+ or H^+. During cell activation, the increase in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) triggers the opening of the mitochondrial Ca^{2+} uniporter and large amounts of Ca^{2+} flow through this channel into the mitochondrial matrix. We have used here low-Ca^{2+}-affinity aequorin to investigate in permeabilized cells the behaviour of mitochondrial Ca^{2+} fluxes under different conditions of $[Ca^{2+}]_c$, $[Na^+]$ and temperature to obtain a clearer picture of mitochondrial $[Ca^{2+}]$ homeostasis. The rate of Ca^{2+} release from mitochondria increased linearly with mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_M$). Na^+-dependent Ca^{2+} release was predominant al low $[Ca^{2+}]_M$ but saturated at $[Ca^{2+}]_M$ around 400mM, while Na^+-independent Ca^{2+} release was very slow at $[Ca^{2+}]_M$ below 200 mM, and then increased at higher $[Ca^{2+}]_M$, perhaps through the opening of a new pathway. Half-maximal activation of Na^+-dependent Ca^{2+} release occurred at 5-10 mM $[Na^+]$, within the physiological range of cytosolic $[Na^+]$. Ca^{2+} entry rates were comparable in size to Ca^{2+} exit rates at cytosolic $[Ca^{2+}]_c$ below 7 mM, but the rate of uptake was dramatically accelerated at higher $[Ca^{2+}]_c$. As a consequence, the presence of $[Na^+]$ considerably reduced the rate of $[Ca^{2+}]_M$ increase at $[Ca^{2+}]_c$ below 7 mM, but its effect was hardly appreciable at 10 mM $[Ca^{2+}]_c$. Exit rates were more dependent on the temperature than uptake rates, thus making the $[Ca^{2+}]_M$ transients to be much more prolonged at lower temperature. Our kinetic data suggest that mitochondria have little high affinity Ca^{2+} buffering. Comparison of our results with previous data on total mitochondrial Ca^{2+} fluxes indicate that the mitochondrial Ca^{2+} bound/Ca^{2+} free ratio is around 100 for most of the observed $[Ca^{2+}]_M$ range and suggest that massive phosphate precipitation can only occur when $[Ca^{2+}]_M$ reaches the millimolar range. In conclusion, our data reveal mitochondria as a highly dynamic compartment in terms of Ca^{2+} homeostasis, able to take up and release Ca^{2+} fast enough to follow the cytosolic Ca^{2+} transients, and also able to undergo reversible variations in $[Ca^{2+}]_M$ that could span up to four orders of magnitude, from 100 nM to 1 mM.

doi:10.1016/j.bbabio.2010.04.367

15P.3 Is nitric oxide synthase present in mitochondria?

Wendy H.Y. Cheng, Kate J. Heesom, Andrew P. Halestrap, Elinor J. Griffiths
University of Bristol, Department of Biochemistry and Bristol Heart Institute, UK
E-mail: wendy.cheng@bristol.ac.uk

In addition to the three known forms of nitric oxide synthase (NOS) in the heart, it has been proposed that NOS is also present in mitochondria. However, studies are controversial due to the possibility of contamination by non-mitochondrial NOS, and because none of the known forms of NOS contain a mitochondrial targeting sequence. We investigated whether NOS was present in isolated mitochondria using antibodies against all 3 forms of NOS (endothelial NOS, inducible NOS and neuronal NOS). Crude fractions of heart and liver mitochondria were obtained by differential centrifugation, and 35% Percoll used to obtain highly-purified mitochondria, as tested using antibodies against subcellular marker proteins: cyclophilin D — mitochondrial marker; monocarboxylate transporter-1 — plasma membrane marker; ryanodine receptor — sarcoplasmic reticulum marker (heart mitochondria only); and catalase — peroxisomal marker (liver only). Western blotting using antibodies against eNOS and iNOS revealed that these isoforms were not present in either heart or liver purified mitochondria (whereas whole heart or liver lysate tested positive). We used 5 different antibodies against nNOS, and again failed to detect anything in purified heart mitochondria. In purified liver mitochondria one of the nNOS antibodies revealed the presence of a band at the correct molecular weight. However, subsequent analysis by mass spectrometry revealed that this was the enzyme carbamoyl phosphate synthase, and not an isoform of NOS. Although our results show that mitochondria do not contain a specific form of NOS, it remains possible that NO from one of the known forms of NOS can regulate mitochondrial function.

This study was supported by the BBSRC and NiCOx.

doi:10.1016/j.bbabio.2010.04.368

15P.4 Effect of long-term exercise training on the sensitivity of calcium-induced mitochondrial permeability transition pore opening and uncoupling protein 3 expression

Snizhana Chorna, Nataliia Strutynska, Galyna Vavilova, Anatoliy Kotsuruba, Vadim Sagach
Bogomoletz Institute of Physiology, Department of Blood Circulation Physiology, Ukraine
E-mail: snizhana-chorna@inbox.ru

Exercise training improves cardiovascular capacity and reduces the risk of cardiovascular heart diseases. Exercise has the potential to reduce apoptosis through upregulation of protective stress-sensitive proteins including nuclear factor kappa-B, insulin-like growth factor, and heat shock proteins [1]. Mitochondrial permeability transition pore (MPTP) opening plays a significant role in the transition of mitochondria from a physiological condition to induction of cell death [2]. In the heart, MPTP opening was shown to occur during reperfusion after ischemia and to be involved in contractile dysfunction and tissue injury [3]. However, the mechanisms by which