39th Meeting of the
Polish Biochemical Society
Gdańsk 16–20 September 2003

SESSION 8

Cellular respiration

Organized by L. Wojtczak, J. Popinigis

372 *Lecture*

Uncoupling proteins in plants and some microorganisms

Wiesława Jarmuszkiewicz

Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, ul. Fredry 10, 61-701 Poznań

The appearance of intracellular oxidative phosphorylation at the time of acquisition of mitochondria in Eukarya was very soon accompanied by emergence of uncoupling protein that can modulate tightness of coupling between mitochondrial respiration and ATP synthesis, thereby maintaining a balance between energy supply and demand in the cell and defending cells against damaging reactive oxygen species production when electron carriers of the respiratory chain become overreduced. Uncoupling proteins, members of mitochondrial carrier family, are present in mitochondrial inner membrane and mediate free fatty acid-activated, purine-nucleotide-inhibited H^+ re-uptake. Since 1995, it has been shown that uncoupling proteins are present in many higher plants and some microorganism, including non-photosynthetic amoeboid protozoon, *A. castellanii*, non-fermentative yeast *C. parapsilosis* and slime mold *D. discoideum*. In mitochondria of these organisms, uncoupling protein activity is revealed by stimulation of state 4 respiration by free fatty acids accompanied by decrease in membrane potential and lowering ADP/O. Plant and microorganism uncoupling proteins are able to divert energy from oxidative phosphorylation, competing for proton electrochemical gradient with ATP synthase. Functional connection and physiological role of uncoupling protein and alternative oxidase, two main energy-dissipating systems in plant-type mitochondria, will be discussed.

---

373 *Lecture*

Modelling of oxidative phosphorylation in isolated mitochondria and intact cells

Bernard Korzeniewski

Institute of Molecular Biology and Biotechnology, Jagiellonian University, Kraków

It would be difficult to imagine the present physics or chemistry without quantitative theoretical models (theories). They enable, among others, to describe the analyzed system strictly and quantitatively, to explain many apparently unrelated phenomena and to predict the existence of some completely new phenomena, for example new elementary particles. On the other hand, the complexity of biological systems makes it difficult to model these systems quantitatively.

The metabolism of the living cell is too complicated to describe it in a strict analytical mathematical way. Therefore, comprehensive computer models, based on laboriously collected kinetic data and on the intuition and invention of the researcher, and at the same time well tested by confrontation with experimental results, must be used in this case. A model is only a simplified and approximate description of the reality. Many different models may be adjusted, by manipulating assumptions and free parameter values, to some simple set of experimental data. Therefore, to build a good model it is absolutely crucial to verify this model thoroughly and in many aspects. A model which has passed such a verification constitutes, in some important sense, only a systematized and quantitatively described set of experimental data obtained in different experiments by various researches. Such a model can become a very efficient and useful research tool.

The present presentation aims to demonstrate the usefulness of computer models of metabolic pathways, using as an example the model of oxidative phosphorylation in isolated mitochondria and intact cells developed by the author and co-workers.

First, this model is able to generate at least semi-quantitatively a very broad range of kinetic properties of oxidative phosphorylation and relevant parameter values. Therefore, it can be assumed that it is a good approximation of the reality, at least within the physiological range of conditions.

Second, computer simulations carried out using the discussed model have led to the conclusion that only a direct activation by some cytosolic factor of not only ATP usage and substrate dehydrogenation, but also of all oxidative phosphorylation complexes can explain very small changes in the concentration of ADP, PCr, NADH and Δp accompanying the very large changes in the respiration rate and ATP turnover during transition from rest to intensive exercise in skeletal muscle. This led to formulating the so-called parallel activation 'theory' describing the main mechanism of the regulation of oxidative phosphorylation in skeletal muscle

(and also in heart, liver and other tissues). The discussed theory constitutes significant novelty in relation to the widely known theory of the regulation of oxidative phosphorylation through a negative feedback involving ADP, proposed in the 50-ties by Chance and Williams. Thus, theoretical studies have led to postulating the existence of a completely new phenomenon that had not been discovered earlier in the experimental way. Therefore, computer models may stimulate and direct future experimental studies aimed to test the predictions resulting from computer simulations.

Third, there were investigated implications of the parallel activation mechanism for the kinetic properties of oxidative phosphorylation in intact skeletal muscle. It was found that this mechanism is able to explain a number of phenomena and properties of the system encountered in experimental studies, for which no satisfactory explanations had been found previously. These phenomena and properties comprise: (1) the fact that the maximum respiration rate in intact skeletal muscle is 2–4 times greater than in isolated muscle mitochondria; (2) short transition time between the respiration rate in rest and during exercise; (3) significant differences in the phenomenological VO2/ADP relationship between untrained muscle and trained muscle; (4) increase in the ATP/ADP homeostasis as a result of muscle training; (5) differences in several kinetic properties of oxidative phosphorylation in glycolytic skeletal muscle and oxidative skeletal muscle; (6) asymmetry in the half-transition time $t_{1/2}$ for [PCr] during rest-to-work transition and during work-to-rest transition; (7) PCr overshoot – increase of the phosphocreatine concentration above its resting level during recovery of muscle after exercise.

The discussed dynamic computer model of oxidative phosphorylation in skeletal muscle was also used in a number of other theoretical investigations, for instance in studies on the influence of changes in cytosolic pH on the bioenergetic system in the muscle cell. Summing up, this model allows better understanding of the functioning of the modelled system, enables quantitative analysis of its properties, is able to predict the existence of completely new phenomena and can help to explain a broad range of experimental data.

---

374 *Lecture*

Thresholds and excess capacities of respiratory chain enzymes in human brain and skeletal muscle

Wolfram Kunz[1], Alexei Kudin[1], Stefan Vielhaber[2]

1 – Div. Neurochemistry, Dept. Epileptology, University Bonn Medical Center, Sigmund-Freud-Str. 25, Bonn, Germany,
2 – Dept. Neurology, University Magdeburg Medical Center, Leipziger Str. 44, Magdeburg, Germany

To evaluate tissue-specific metabolic consequences of mild respiratory chain enzyme deficiencies we determined the flux control and the metabolic reserve capacity of NADH:CoQ oxidoreductase and cytochrome *c* oxidase (COX) in human saponin-permeabilised muscle fibers and digitonin-treated parahippocampal homogenates. In these tissue preparations it is possible to investigate mitochondrial function under conditions which are close to the *in vivo* situation. In the presence of NAD-dependent substrates flux control coefficient of NADH:CoQ oxidoreductase and COX of 0.08 ± 0.04 and 0.42 ± 0.10, as well as 2.2 ± 0.6- and 1.9 ± 0.2-fold excess capacities, respectively, were observed in human skeletal muscle fibers. In human parahippocampal gyrus we determined under similar conditions flux control coefficients of NADH:CoQ oxidoreductase and COX of 0.37 ± 0.09 and 0.12 ± 0.05 as well as 1.4 ± 0.3- and 3.9 ± 0.6-fold excess capacities, respectively. The observed differences in metabolic control can be attributed to activity differences of NADH:CoQ oxidoreductase and COX in human brain and muscle mitochondria. Our results predict possible stronger metabolic effects of mild NADH:CoQ oxidoreductase activity deficits in human brain tissue and can serve as explanation for the tissue specificity of the 3 different homoplasmic LHON mutations affecting the mitochondrial-encoded subunits of NADH:CoQ oxidoreductase ND2 (C4640A), ND4 (G11778A) and ND6 (T14484C). All mutations caused in patients and non-affected carriers of the mutation an almost identical decrease of citrate synthase-normalized activities of complex I being most severe for the ND6, less severe ND4 and close to the detection limit for the ND2 mutation. This enzyme activity change was observed to be responsible for decreased respiration rates with NAD-dependent substrates detected in saponin-permeabilized muscle fibers, isolated skeletal muscle mitochondria and digitonin-treated brain homogenates (for the ND2 mutation). Summarising, our data support the point of view that the tissue specific effects of homoplasmic mitochondrial DNA point mutations are caused by tissue specific differences of flux control and metabolic reserve capacities of affected enzymes.

375 ***Lecture***

Mitochondrial complex I: physiological and pathological aspects

Giorgio Lenaz

Department of Biochemistry, University of Bologna, 40126 Bologna, Italy

The most accepted view of the mitochondrial respiratory chain consists of a series of enzymes independently dissolved in the inner membrane and connected through mobile components by means of random diffusion of small connecting molecules, Coenzyme Q (CoQ) and cytochrome *c*. Mitochondrial DNA encodes for 13 subunits belonging to the 4 complexes involved in proton translocation, in particular 7 subunits for Cmplex I, 1 for Complex III, 3 for Complex IV and 2 for ATP synthase.

The special features of mtDNA have been the reason for the postulation of the mitochondrial theory of aging. Mitochondria are known to be at the same time strong producers of Reactive Oxygen Species (ROS) and particularly susceptible to damage by their action on lipids, proteins and DNA. In particular, damage to mtDNA would induce damage to the polypeptides encoded by mtDNA in the respiratory complexes, with consequent decrease of electron transfer, leading to further production of ROS, and thus establishing a vicious circle of oxidative stress and energetic decline. This fall of mitochondrial energetic capacity is considered the cause of aging and age-related degenerative diseases.

Evidence for super-complexes. According to the mitochondrial theory of aging, Complex I would be the enzyme most affected by ROS, since it contains the highest level (seven) of subunits encoded by mtDNA. Complex I catalyzes the oxidation of matrix NADH by CoQ dissolved in the bilayer and its molecular organization has not been solved yet. It contains 46 protein subunits and a large number of redox prosthetic groups: FMN, 7 iron-sulfur clusters, and some molecules of bound CoQ. Several inhibitors interrupt electron transfer at different levels in the complex and are used to dissect the electron path within the enzyme.

An eventual change of Complex I activity is significant only if the enzyme controls the entire OXPHOS system. The extent of control of an enzyme on a pathway is studied by flux control analysis. The flux control coefficient represents the fractional change of total activity of a pathway induced by a fractional change of the individual enzyme in the pathway. We have applied flux control analysis on each segment of the respiratory chain using specific inhibitors of each Complex. In open bovine heart submitochondrial particles where the system is simplified by the absence of membrane potential, ATP synthesis and carrier systems for substrates, we surprisingly found that both Complex I and Complex III are almost completely controlling aerobic NADH oxidation. The most obvious explanation is that Complex I and Complex III behave as a single enzyme, forming a super-complex with metabolic channeling of the connecting intermediate, CoQ. This is contrary to the common view of electron transfer, however is in line with recent structural findings. Only Complex I and III appear to form a super-complex, while Complex IV seems to behave independently and not to be rate limiting. On the other hand, Complex II is rate limiting over succinate oxidation, but there appears to be no channeling between Complexes II and III.

We have applied flux control analysis to Complex I in aerobic respiration in coupled liver mitochondria from young and aged rats of 32 months. In this system there are other steps that partly control total respiration, such as the substrate carriers and the ATP synthesis, so that the control coefficients for Complex I are lower than in SMP. Complex I has little control in young rats but very high control in the old animals. The alteration of Complex I is also documented by the small albeit significant decrease of rotenone sensitivity of the enzyme and of whole respiration, documented by the increase of I_{50}, the inhibitor concentration inducing half inhibition of the activities

Complex I in platelets in aged individuals. We have also investigated Complex I activity in human platelets from young and aged individuals. In this case the most striking result was the decrease of rotenone sensitivity. We have also investigated the steady-state level of mtRNA transcripts of subunits ND1 and ND5 of Complex I and COX I and COX III of Complex IV using Real Time RT PCR. The level of transcripts was not the same, and in particular the level of COX I was double that of COX III: this is unexpected since mtDNA transcription is polycystronic. The level of Complex I transcripts, but not of Complex IV transcripts, was increased in aging. The different levels of different transcripts can only be explained by different stability of the RNAs once transcribed. The unexpected increase in aging may be considered as a compensatory mechanism to cope with the functional alteration of the enzyme, due to its rate control over the OXPHOS system

ROS production by Complex I. It is calculated that and up to 4-5% of oxygen in mitochondria is not converted to water by Complex IV but is reduced by a single electron transfer to superoxide radical by Complex I and Complex III. We have studied the role of Complex I in superoxide production in bovine heart SMP. In order to functionally isolate Complex I from the rest of the chain we inhibited Complex III with mucidin. Mucidin

enhances superoxide formation over the control, as expected by the block of the chain resulting in a more reduced state of the electron carriers; *p*-chloro-mercuribenzoate, that inhibits at the level of FeS clusters, decreases superoxide production, whereas all inhibitors acting at three different levels at the CoQ reduction sites, either singly or in combination, enhance the formation of the radical. Different quinones added in presence of mucidn elicit a large increase of superoxide production. This also means that under normal conditions of assay of Complex I, there is production of superoxide radical! The effect is further potentiated by rotenone and other Complex I inhibitors. Idebenone is shown to be the most powerful enhancer of superoxide production, reaching levels 20-fold or more the basal ones. This finding suggests caution and further studies *in vivo* before considering safe the use of this compound in clinics. The results overall suggest that the site of superoxide production is located between the PCMB and the rotenone inhibition sites, thus excluding bound CoQ10 as the source of the radical. We have produced further evidence by studying superoxide production by CoQ-extracted and reconstituted particles. In all conditions studied, either in presence or absence of inhibitors and short chain quinones, the two types of particles behave similarly with a conspicuous superoxide production, indicating that the endogenous CoQ10 has no part in the univalent reduction of oxygen.

The main electron donor to oxygen is FeS cluster N2, in view of its proximity to the water phase and its role of reducing bound CoQ in the Complex. In addition to direct reaction with oxygen, N2 may give one electron to a water-soluble quinone, and the semiquinone thus formed would reduce molecular oxygen. Bound CoQ10 does not appear to have any role in ROS production in Complex I.

The effect of rotenone on ROS production in intact cultured cells is not univocal: in fact in some cells (HL60) rotenone decreases ROS formation, while in others (papillary thyroid carcinoma) it enhances ROS formation. The difference may well be explained by the sidedness of ROS formation by Complex I (matrix) and Complex III (inter-membrane space), the level of matrix antioxidant enzymes, and the relative level of ROS formation by Complex III (inhibited by rotenone) and Complex I (enhanced by rotenone). Thus many contradictory data in the literature may be easily explained.

---

376 *Lecture*

Regulation and role of alternative oxidase in higher plant respiration

Anna Rychter, Izabela Juszczuk

Zakład Bioenergetyki Roślin, Instytut Biologii Eksperymentalnej Roślin, Uniwersytet Warszawski, ul. Miecznikowa 1, 02-096 Warszawa

Alternative oxidase is the terminal oxidase, which branches in plant respiratory chain from ubiquinone. Transfer of the electrons from ubiquinone to oxygen by alternative oxidase has non-protonmotive character and bypassing of two sites of ATP formation (in the complexes III and IV) lowers the energy efficiency of respiration. We review the theoretical and experimental studies about the structure and possible function of the alternative oxidase. The evidence for specific gene expression dependent on the physiological, developmental and environmental conditions is also described. We underline the physiological role of the alternative oxidase as the "survival" protein that allows plants to cope with the stressful environment.

---

377 *Lecture*

Mitochondrial oxidative function in human skeletal muscle

Kent Sahlin, Michail Tonkonogi

Biochemistry, Univ., Odense, Denmark

Mitochondria have a central role in muscle energetics and muscle metabolism. The control of oxidative phosphorylation is exerted both by feed-back and feed-forward mechanisms. Increases in the products of ATP hydrolysis (ADP and Pi) appear to be the major feed-back signals in skeletal muscle. The response is modulated by the redox drive, which is dependent on the availability of tricarboxylic acid substrate and probably feed-forward Ca activation. In oxidative muscle fibres there is an additional role of the creatine shuttle, which amplifies the control by adenine nucleotides. Isolated mitochondria have a high sensitivity towards

ADP, with a K_m close to 20 μM. However, measurements in permeabilied fibres demonstrate that K_m is 3-4 fold higher, which is more compatible with the calculated cellular free ADP. The impact of acidosis on mitochondrial function is unclear. Maximal respiration is not affected by acidosis in isolated mitochondria, when acidosis is induced in active mitochondria. However, permeabilized fibres have a reduced stimulation by creatine during acidosis. Furthermore, *in vivo* measurements with 31-P NMR indicate a decreased maximal rate of respiration during acidosis in human skeletal muscle. The adaptation to training involves both quantitative and qualitative changes. An unexpected finding is that the sensitivity towards ADP appears to decrease with training at the single mitochondrial level although the increase in mitochondrial density compensate for this. Part of the oxygen consumption is due to back-leakage of protons into mitochondrial matrix (non-coupled respiration). The mechanism for this proton leakage is unclear but a role of uncoupling proteins (UCP3) has been discussed. Non-coupled respiration is stimulated by fatty acids and it has been shown that the degree of stimulation is augmented after endurance training. Non-coupled respiration in skeletal muscle may play a role in the thermogenic response and in weight control.

378 *Lecture*

Two faces of cytochrome *c*

Lech Wojtczak

Zakład Biochemii Komórki, Instytut Biologii Doświadczalnej PAN im. M. Nenckiego, ul. Pasteura 3, 02-093 Warszawa

This lecture will outline the history of research on cytochrome *c* from its discovery by C.A. MacMunn and re-discovery by David Keilin, through classic investigations on cytochrome *c* as respiratory chain mediator, up to recent findings on its role in initiation and generation of programmed cell death (apoptosis). Some results from the author's laboratory on the mechanism of cytochrome *c* liberation from mitochondria in the initial step of apoptosis will also be presented.

379 *Lecture*

Oxygen uptake kinetics at the onset of exercise and the VO2/power output relationship in humans

Jerzy Zoladz

Department of Muscle Physiology, AWF-Kraków, Al. Jana Pawla II 78, Kraków

The adjustment of the rate of ATP supply to the rate of its utilization in the working muscles plays the most important role in resisting fatigue (for review see e.g. Sahlin *et al.*, 1998). During normal daily human activity creatine kinase reaction and oxidative phosphorylation play the most important role in maintaining the appropriate energy status in the working muscles cells.

Studies on the dynamics of the VO2 at the onset of exercise has attracted researches already since the beginning of this century (see Krogh & Lindhard, 1913; Hill and Lupton, 1923). Recently, on the basis of the early studies (see Henry, 1951; Whipp *et al.*, 1982) mathematical models of description of VO2 kinetics have been developed (see Barstow *et al.*, 1996), which allows to measure and interpret various parameters of VO2 kinetics.

In the rest-to-work transition three phases in VO2 kinetics were identified and quantified: phase one was called the cardiodynamic component, phase two the primary component and phase three the slow component (see e.g. Barstow, 1996). Phase one and two are present at moderate exercise intensities (below the lactate threshold). At high power outputs (above the lactate threshold) the slow component of VO2 kinetics is present as well (see Whipp *et al.*, 2002).

It should be stressed that in the current research in this area most attention is paid to the origin and functional importance of the primary and the slow component of VO2 kinetics in humans. The functional significance of the primary component of the VO2 kinetics is important due to the fact that shortening of its duration decreases the magnitude of oxygen debt at the onset of exercise and the contribution of anaerobic energetic pathways (creatine kinase reaction and anaerobic glycolysis) to the ATP supply at the onset of exercise.

We have postulated that the presence of the slow component of VO2 kinetics is responsible for the non-linear increase in the VO2 / power output relationship occurring during incremental power output exercise tests (see Zoladz *et al.*, 1995; Zoladz *et al.*, 1998) illustrating

occurrence of a substantial decrease in the muscle mechanical efficiency.

In the recent years more evidence has been provided showing that in the physiological conditions, not the oxygen transport, but the local factors in the working muscle cells, especially the relative activity of oxidative phosphorylation, determine the rate of oxygen uptake at the onset of exercise (see e.g. Grassi *et al.*, 1998; Bangsbo *et al.*, 2000). However, the quantitative significance of different factors that control the rate of VO2 at the onset of exercise remains to be established (see also Korzeniewski & Zoladz, 2002; Korzeniewski & Zoladz, 2003.

Similarly, little is know about the physiological mechanism(s) underlying the slow component of VO2 kinetics. A number of factors including: increase of the cost of pulmonary ventilation, blood lactate and H^+ accumulation, hypercatecholaminemia, increase of muscle temperature, recruitment of type II muscle fibers etc. has been suggested as a possible cause of the slow component of VO2 kinetics (for review see Whipp, 1995; Zoladz & Korzeniewski, 2001); however, the exact mechanism responsible for this phenomena is still not fully understood.

In this lecture the current state of knowledge on the factors affecting the kinetics of the VO2 at the onset of exercise as well as the VO2/power output relationship in humans will be presented.

References:
1. Bangsbo J, Krustrup P, Gonzalez-Alonso J, Boushel R, Saltin B (2000) Am J Physiol, 279: R899–R906.
2. Barstow TJ (1996) Med Sci Sports Exerc, 26: 1327–1334.
3. Barstow TJ, Jones AM, Nguyen PH, Casaburi R (1996) J Appl Physiol, 81: 1642–1650.
4. Grassi B, Gladden LB, Samaja M, Stary CM, Hogan MC (1998) J Appl Physiol, 85: 1394-1403.
5. Henry FM (1951) J Appl Physiol, 3: 427–438.
6. Hill AV, Lupton H (1923) Q J Med, 16: 135–171.
7. Korzeniewski B, Zoladz JA (2002) Biochem J, 365: 249–258.
8. Korzeniewski B, Zoladz JA (2003) Biochem J, 373: 1–4.
9. Krogh A, Lindhard J (1913) J Physiol London, 47: 112–136.
10. Sahlin K, Tonkonogi M, Soderlund K (1998) Acta Physiol Scand, 162: 261–266. Review.
11. Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K (1982) J Appl Physiol, 52: 1506–1513.
12. Whipp BJ, Rossiter HB, Ward SA (2002) Biochem Soc Trans, 30: 237–247. Review.
13. Zoladz JA, Rademaker AC, Sargeant AJ (1995) J Physiol (London), 488: 211–217.
14. Zoladz JA, Duda K, Majerczak J (1998) Eur J Appl Physiol, 77: 445–451.
15. Zoladz JA, Korzeniewski B (2001) J Physiol Pharmacol, 52: 167–184.

380

Oral Presentation

Nonesterified fatty acids as protonophores and openers of mitochondrial permeability transition pore

Dorota Dymkowska, Lech Wojtczak

Zakład Biochemii Komórki, Instytut Biologii Doświadczalnej PAN im. M. Nenckiego, ul. Pasteura 3, 02-093 Warszawa

It is known that nonesterified fatty acids have a significant effect on mitochondrial energy coupling. They are good respiratory substrates for oxidative phosphorylation but they also act as uncouplers due to their protonophoric effect [1]. In addition, various fatty acids can cause opening of the mitochondrial permeability transition pore (MPTP) [2,3]. Both the protonophoric effect and the MPTP opening result in dissipation of the mitochondrial membrane potential and stimulation of mitochondrial resting state respiration. The aim of the present work was to assess the role of these two mechanisms in energy dissipation by fatty acids in tightly coupled rat liver mitochondria. Mitochondria were either suspended in calcium-free medium (in the presence of EGTA) or were posed with small amounts of Ca^{2+} in order to make them sensitive to the pore-opening action of fatty acids [2]. As an additional tool cyclosporin A, a known blocker of MPTP, was also used. We found that participation of the protonophoric and MPTP-opening effects depended on the chain length and the degree of unsaturation of various fatty acids. Myristic (C-14:0) and arachidonic (C-20:4) acids appeared to produce the highest dissipation of the membrane potential and activation of resting state respiration and their action was to the highest proportion due to MPTP opening. In contrast, saturated fatty acids of shorter chain length, decanoic (C-10:0) and lauric (C-12:0), as well as those of longer chain length, e.g. stearic (C-18:0), were less uncoupling and their action was mostly due to the protonophoric effect. High uncoupling effect of oleic acid (C-18:1) was also mostly due to the protonophoric action.

References:
1. Wojtczak L, Schönfeld P (1993) Biochim Biophys Acta, 1183: 41–57.
2. Więckowski MR, Wojtczak L (1998) FEBS Lett, 423: 339–342.
3. Wojtczak L, Więckowski MR, Schönfeld P (1998) Arch Biochem Biophys, 357: 76–84.

381 *Oral Presentation*

The VDAC channel modulating activity in the intermembrane space of *Acanthamoeba castellanii* mitochondria

Hanna Kmita, Magdalena Świderska, Małgorzata Sciłba, Małgorzata Budzińska, Lilla Hryniewiecka

Zakład Bioenergetyki, Uniwersytet im. A. Mickiewicza, ul. Fredry 10, 61-701 Poznań

The VDAC channel (the voltage dependent anion selective channel), known also as mitochondrial porin, provides the primary permeability pathway through the mitochondrial outer membrane for metabolites crucial to mitochondrial physiology. A highly conserved and important property of the channel, observed in reconstituted systems and lately also with intact mitochondria, is voltage dependence. It is clear that cellular protein(s) able to modulate the voltage-dependent regulation of the VDAC channel could contribute importantly to the mitochondrial outer membrane permeability, which could be used to control mitochondrial functions. The VDAC channel modulating activities, often referred to as the VDAC channel modulator, were proved to exist in the intermembrane space of mitochondria of animal, plant and fungal species but responsible protein(s) has not been identified yet. In reconstituted systems, the VDAC channel modulator increases the voltage dependence of VDAC channels in three ways: it increases the rate of channel closure, decreases the rate of opening and induces the channels to assume lower conductance closed states. With intact mitochondria, the VDAC channel modulator was shown to inhibit transport of adenine nucleotides as well as external NADH across the outer membrane.

The amoeba *A. castellanii* is a simple protozoan of a very interesting position in the molecular philogenetic tree, where it is located at the divergence point for plants, animals and fungi. Thus, the amoeba VDAC channel and the VDAC channel modulator should share properties with representatives of the above kingdoms. Here we report that electrophysiological properties of the VDAC channel isolated from *A. castellanii* mitochondria are very conserved. Further, the intermembrane space of amoeba mitochondria contains protein(s) displaying typical activity of the VDAC channel modulator as measured in the reconstituted system and with intact amoeba mitochondria. Finally, the modulator crossreacts with the VDAC channel from *S. cerevisiae* i.e. is structurally and functionally conserved.

---

382 *Oral Presentation*

Effect of ciprofloxacin on mitochondrial energy metabolism, calcium signaling and viability of Jurkat T-cells

Rafał Kozieł, Krzysztof Zabłocki, Jerzy Duszyński

Zakład Biochemii Komórki, Instytut Biologii Doświadczalnej PAN im. M. Nenckiego, ul. Pasteura 3, 02-093 Warszawa

Ciprofloxacin belongs to a class of antibiotics that inhibit mitochondrial topoisomerase II, an enzyme required during the alteration of mtDNA topography. At high concentrations, this compound inhibits the growth of cultured mammalian cells. This results from decreased mtDNA replication and transcription level and from a gradual loss of mtDNA and proteins encoded by this molecule.

In non-excitable cells intracellular Ca^{2+} concentration ($[Ca^{2+}]c$) is regulated by both calcium release from the endoplasmic reticulum (ER) and Ca^{2+} influx *via* Store Operated Channels (SOC) located in the plasma membrane. Depletion of ER stores activates of SOC. An increase in $[Ca^{2+}]c$ due to calcium influx into the cell leads to a feed-back inhibition of SOC and thereby reduces the rate of calcium entry. Mitochondria can take up the excess of calcium in a close proximity of SOC and therefore delay this inhibition.

The aim of this study was to investigate the effect of ciprofloxacin on the mitochondrial energy status and the rate of calcium entry into Jurkat T cells. The latter process was measured in Jurkat cells with ER depleted by thapsigargin. The cells were maintained in the regular medium supplemented with ciprofloxacin (25 μg/ml). Every 2-3 days, the cell growth, the rate of cellular respiration, mitochondrial membrane potential, and mitochondrial DNA content were determined. A gradual decrease in all these parameters was observed. It is noteworthy that these changes were also in a good correlation with the inhibition of calcium influx into the cells. Despite inhibition of many mitochondrial functions, ciprofloxacin does not influence the cellular ATP content until the seventh day of the treatment, because of active glycolysis. These results suggest that the ciproflaxin-induced decrease in mitochondrial energy status may lead to an impairment of calcium signaling and thereby to the reduced viability of the cells.

383

Poster

Diverse effects of tempol in rats subjected to endurance training

Tomasz Borkowski[1], Tomasz Wierzba[1], Wiesław Ziółkowski[2], Łukasz Nowakowski[1], Jan Kaczor[2], Jerzy Popinigis[2]

1 – Department of Physiology, Medical University of Gdańsk, ul. Debinki 1, Gdańsk, 2 – Department of Bioenergetics, Jedrzej Sniadecki University School of Physical Education and Sport, ul. Wiejska 1, 80-336 Gdańsk

Ischemia and reperfusion can easily occur during strenuous exercise, promoting excessive ROS generation and oxidative tissue damage. Hence rationale for ROS elimination. In turn, substantial signalling role of ROS in the development of functional adaptation to physical training can not be overruled.

Aim: The study was performed to evaluate the effect of oxyradical scavenger tempol on endurance capacity (EC), basal hemodynamics, and mitochondrial function in rats subjected to endurance training.

Methods: Male Wistar rats (270–340 g, N=28) divided into sedentary (SED) and trained (TR) subsets (6 wk running cycles on treadmill with increasing intensity up to estimated 85% VO2max) were treated with tempol (200 mmol/kg) or given vehicle instead. EC was obtained by the time of running to exhaustion. Systolic blood pressure (SBP) and heart rate (HR) was measured with a plethysmographic tail-cuff method. Mitochondrial respiration rate (MRR) was assayed with a Clark electrode. Mitochondrial nitrite concentration was obtained with DAN method. To assess peroxidative damage, mitochondrial protein SH groups were examined.

Results: Training resulted in an increase in EC in control rats (by 398%), and those given tempol (+478%), with no effect on SBP, while HR was reduced. Tempol reduced SBP in SED (–13 mmHg) and TR rats (–16 mm Hg). During training HR decreased in SED (–23 beats/min) and accelerated (+10 beats/min) in TR. This may indicate that the regulatory effect of training is predominant upon the reported cardioinhibitory action of tempol. MRR tested with succinate was lower in the hearts from TR and even more in gastrocnemius muscle (MG), suggesting overtraining. MRR was improved by tempol (+40%) in MG of SED. In case of pyruvate and malate substrates, MRR was higher in the hearts taken from TR (+20%), but lower in the MG (–9%). Tempol evoked almost 50% increase of both MG and heart MRR in SED, but not in TR. Training resulted in an increase in the protein SH groups (+ 24%, and +12% in SED and TR, respectively) and nitrite content in the heart mitochondria. The latter was decreased in tempol TR.

Conclusion: Our data show that elimination of ROS by use of a oxyradical scavenger, such as tempol, improved endurance capacity in rats challenged with intense running training, but not in those remained inactive. The reason for the observed effect is more likely attributed to some improvement of cardiovascular reflex regulation, than to the impact on function of mitochondria.

384

Poster

Oxidative stress induced by 3-hours lasting ischaemia is more evident in white than in red — skeletal muscles of rat

Jan Kaczor[1], Aleksandra Kawecka[2], Wiesław Ziółkowski[1], Jerzy Lipiński[2], Jerzy Popinigis[1]

1 – Department of Bioenergetics, Jedrzej Sniadecki University School of Physical Education and Sport, ul. Wiejska 1, 80-336 Gdańsk, 2 – Department and Clinic of Trauma Surgery, Medical University of Gdańsk, ul. Dębinki 1, 80-211 Gdańsk

Introduction: Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly toxic molecules that can damage essential cell components such as DNA, proteins, and lipids. Oxidative damage, due to elevated generation of reactive oxygen and nitrogen species, has been implicated as a potential cause in numerous chronic diseases.

The aim of this work was to check whether 3 h lasting ischaemia of leg would exert different damaging effect on protein structure and enzyme activities in two different types of rat leg skeletal muscles, the fast and the slow.

Materials and Methods: The experiments were carried out on 12 male Wistar rats, (400 — 450 g body mass). Total restriction of blood flow was induced by unilateral ligation of the right common iliac artery and than by cut of all muscle groups near the inguinal ligament. Animals were killed by decapitation and skeletal muscle (m. EDL, Extensor Digitorum Longus — white, m. Soleus — red) were collected, frozen and kept at — 80°C until analyzed. Then the muscles were homogenized with a Potter-Elvehjem homogenizer in ice-cold 50 mM phosphate buffer, pH 7.4, containing 1.0 mM

EDTA, 0.1 mM BHT, 1.15% KCl and 0.05% Triton X-100. The soluble fraction was then prepared by centrifugation (600 x *g*, 4°C, 10 min) and used for the enzyme activities assay or for further centrifugation (10000 x *g*, 4°C, 10 min) to measured the level of carbonyl and SH-groups contents in supernatant.

Results and Discussion: The level of the carbonyl groups, the SH-groups and the activities of catalase (Cat), and lactate dehydrogenase (LDH) were estimated in both, the ischaemic and in contralateral muscles, using the extracts from m. Soleus and m. EDL as red and white skeletal muscles, respectively.

After ischaemia the level of carbonyl contents increased 26% in m. EDL, but not in m. Soleus. Also the concentration of SH-groups decreased 17% in m. EDL, but not in m. Soleus. 3-hours of ischaemia did not change the activities of LDH in both muscles. In comparison to the contralateral muscle, the activity of catalase seems to increase (not statistically) in m. EDL (25%) but not in m. Soleus.

Our results indicate that the estimation of the level of carbonyl groups, as well as, the SH-groups, may be useful parameters to asses the degree of the damage of different fiber types of skeletal muscle, induces by oxidative stress.

---

385 *Poster*

Effect of growth at low temperature on the uncoupling protein activity and expression in *Acanthamoeba castellanii* mitochondria

Magdalena Kowalczyk, Maciej Behrendt, Aleksandra Świda, Wiesława Jarmuszkiewicz

Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, ul. Fredry 10, 61-701 Poznań

Mitochondria of amoeba *Acanthamoeba castellanii*, a non-photosynthetic soil amoeboid protozoon, possess an uncoupling protein located in the inner mitochondrial membrane that mediates free fatty acid-activated, purine nucleotide-inhibited proton re-uptake dissipating the proton electrochemical gradient built up by respiration and produces heat instead of ATP. In mitochondria isolated from amoeba batch culture grown temporarily at low temperature (6^{o}C), at fixed linoleic acid (LA) concentration, higher LA-induced respiration was accompanied by lower coupling parameters as compared to control culture (grown at 28^{o}C). Increased contribution of UCP activity to total mitochondrial phosphorylating respiration in low-temperature-grown amoeba cells was confirmed by calculation of its contribution using ADP/O measurements. Furthermore, in mitochondria from low-temperature-grown cells the content of the uncoupling protein was increased and correlated with the increase in the LA-stimulated UCP-mediated respiration (higher V_{max} value). A possible physiological role of higher activity of uncoupling protein as response to growth at a low temperature in unicellular organisms, such as amoeba, will be discussed.

---

386 *Poster*

Glycerol-1-phosphate — supported respiratory activity of porcine adrenal cortex mitochondria

Jerzy Popinigis[1], Jędrzej Antosiewicz[1], Jan Kaczor[1], Hana Rauchova[2], Giorgio Lenaz[3]

1 – Department of Bioenergetics, Jedrzej Sniadecki University School of Physical Education and Sport, ul. Wiejska 1, 80-336 Gdańsk, 2 – Center for Experimental Cardiovascular Research, Czech Academy of Sciences, Prague, Czech Republic, 3 – Department of Biochemistry, University of Bologna, 40126 Bologna, Italy

Aim: To investigate possible role of glycerol-1-phosphate as respiratory substrate used for two processes, the oxidative phosphorylation, and the 11-β-hydroxylation of deoxycorticosterone to corticosterone, in adrenal cortex mitochondria.

Materials and Methods: Mitochondria were isolated from porcine or bovine adrenal cortex as described earlier [1, 2]. Oxygen uptake was measured with a Clark oxygen electrode in a Gilson Polarograph (USA). The reaction medium (pH 7.2) contained in a 2 ml volume: 0.2 M sucrose, 50 mM Tris/HCl, 15 mM KCl, 5 mM potassium phosphate buffer, 2 mM EDTA and 0.2% BSA. RCI and ADP:O were calculated from oxygen electrode traces. 11- β-Hydroxylase activity was measured as transient increase in oxygen uptake above resting value after introduction of deoxycorticosterone. Mitochon-

drial glycerol-1-phosphate dehydrogenase activity was measured with method of Swierczynski *et al.* [3].

Results: Glycerol-1-phosphate supported both, oxidative phosphorylation (ADP:O = 0.8 ± 0.1 for n=8), and 11-β-hydroxylation, exclusively in mitochondria from porcine adrenal cortex. The mean respiratory rates were: 37.7 ± 2.6 na atom O per min per mg of mitochondrial protein, in the presence of ADP, and 30.7 6 na atom O per min per mg of mitochondrial protein in the presence of deoxycorticosterone (n=8). Glycerol-1-phosphate dehydrogenasee activity in mitochondrial fraction of porcine adrenal cortex was 33.4 ± 4.3 nanomol of DCIP reduced per min per mg of mitochondrial protein (n=9) The activity of the same enzyme in beef adrenal cortex mitochondria was only 8.2 ± 2.5 (n=3).

Conclusion: In our earlier papers [1, 2] we have already described differences in glutamate oxidation between beef and porcine adrenal cortex mitochondria. Findings presented here indicate that these differences can be also observed for another substrate, the glycerol-1-phosphate.

References:
1. Litwińska D, Szczęsna-Kaczmarek A, Popinigis J (1984) Int J Biochem, 16: 943–946.
2. Popinigis J, Antosiewicz J, Mazzanti L, Bertoli E, Lenaz G, Cambria A (1990) Biochem Int, 21: 441–451.
3. Swierczynski J, Scislowski PW, Aleksandrowicz Z, Żydowo MM (1983) Biochim Biophys Acta, 756: 271–278.

387 *Poster*

Effect of immobilization on cytochrome *c* oxidase activity in human gluteus medius muscle

Wojciech Skrobot[1], Jan Kaczor[2], Wiesław Ziółkowski[2], Włodzimierz Ziółkowski[1], Jerzy Popinigis[2]

1 – Orthopaedic Clinic, Medical University of Gdańsk, Gdańsk, 2 – Department of Bioenergetics, Jedrzej Sniadecki University School of Physical Education and Sport, ul. Wiejska 1, 80-336 Gdańsk

Introduction: It is well known that legs immobilization, handling unloaded, or muscular disuses, lead to the muscle wasting. It has been postulated that these dystrophic changes in muscles are the product of atrophy of skeletal muscle fibers which occurs *via* increased protein degradation, It is also postulated, that the "oxidative stress" may play an important role in this process. The immobilization, the state which is completely opposite to physical activity, is supposed to increase the rate of production of reactive oxygen and nitrogen species (RONS). In this work we attempted to study the response of human skeletal muscle (the gluteus medius) to conditions of prolonged limited activity, caused by the state of the disease, the coxarthrosis.

The main aim of this work was to measure the effect of immobilization on activity of one of the most important mitochondrial enzyme — cytochrome *c* oxidase (COX) in human skeletal muscle.

Materials and Methods: The experiment was performed with written permission from Local Ethical Commission at Medical University of Gdańsk. The samples of gluteus medius muscle (15–50 mg) were obtained from 14 women (age from 53 to 75) who underwent surgery because of coxarthrosis (immobilized group) and 4 women (age 69 and 77) who underwent surgery because of break of the neck of the femoral bone (control group). The medium used for muscle homogenization consisted of: phosphate buffer 50 mM, pH 7.4 + 0.1 mM BHT + 5mM EDTA + 150 mM KCl + 0.05% BSA. After centrifugation at 600 *g* for 10 min in the supernatant measured were: cytochrome *c* oxidase activity and total protein content.

Results and Discussion: COX activity in the control group was: 19.37 ± 1.7 nmoles/min/mg of protein, and in the immobilized group: 12.17 ± 6.0 nmoles/min/mg of protein.

The results presented here indicate that higher degree of hypokinesia (induced by legs immobilization) caused decrease of cytochrome *c* oxidase activity in human skeletal muscle.

388 *Poster*

A channel-forming activity of the isolated TOM complex of *Acanthamoeba castellanii* mitochondria

Małgorzata Wojtkowska[1], Hanna Kmita[2], Joanna Pieńkowska[3], Olgierd Stobienia[1], Lilla Hryniewiecka[2]

1 – Department of Bioenergetics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, ul. Fredry 10, 61-701 Poznań, 2 – Zakład Bioenergetyki, Uniwersytet im. A. Mickiewicza, ul. Fredry 10, 61-701 Poznań, 3 – Department of Animal Physiology, Institute of Experimental Biology, Adam Mickiewicz University, ul. Fredry 10, 61-701 Poznań

It is well known that the TOM complex (the translocase of the outer membrane) is essential for protein import into mitochondria. Most of the knowledge about the TOM complex was obtained by studying *Saccha-*

romyces cerevisiae and *Neurospora crassa* mitochondria but there are also some data concerning the TOM complexes from mammal and plant mitochondria pointing at their similarities in overall structure to *S. cerevisiae* and *N. crassa* complexes. The TOM complex consists of surface receptors for the specific recognition of imported proteins and a general import/insertion pore (GIP), termed also the TOM complex channel, which mediates translocation of all nuclear encoded mitochondrial preproteins into or across the outer membrane.

Acanthamoeba castellanii mitochondria constitute a simple model system very useful in comparative studies on processes characteristic for plant or animal mitochondria. We have already determined that the TOM complex from *A. castellanii* mitochondria recognises targeting signals of mitochondrial proteins of other species, e.g. *S. cerevisiae* and *N. crassa,* and has M_m of approximately 500 kDa. Here we report that the TOM complex of *A. castellanii* separated from the VDAC channel by ion-exchange chromatography and reconstituted into black membrane bilayers made of asolectin displays channel-forming activity. In the presence of a membrane potential of ± 20 mV at cis side, an average conductance for the studied channel of about 2 nS (for 80 single insertions) in 1 M KCl was calculated which corresponds to the dominance of a fully open state of the TOM complex channel. Further, the fusion protein pSu9-DHFR reduces the observed channel open state probability in a voltage dependent manner when added in chemical amounts to the cis site at positive voltages but not at negative voltages. All the data are consistent with those concerning the TOM complex channels from *S. cerevisiae* and *N. crassa* mitochondria.