The morphology of mitochondrial network is highly variable and there is a substantial body of evidence for close relationship between cellular physiology and mitochondrial network organization. We set out to investigate whether pathology of diabetic β-cells is reflected by the altered morphology of mitochondrial network. Conventional confocal microscopy does not provide sufficient *z* axial resolution to realistically visualize 3D mitochondrial network, and therefore we applied high resolution 4Pi microscopy with *z* axial resolution of about 100 nm. Matrix-addressed GFP was lentivirally expressed in Langerhans islets isolated from diabetic Goto Kakizaki or control wistar rats. We demonstrate that β-cells within the Langerhans islets from diabetic Goto Kakizaki rats exhibited more disintegrated mitochondrial network compared to those from control Wistar rats. Observed average diameter of mitochondrial tubule was 236 ± 27 nm in Goto Kakizaki and 214 ± 16 nm in control rats. Our next aim was to characterize organization of mt nucleoids in primary β-cells of Langerhans Islets. To visualize mt nucleoids we used immunocytochemistry or lentiviral expression of GFP-labeled TFAM protein, which is known to have a crucial role in assembling of mammalian mt DNA.

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18P.3 Mitochondrial fusion is an early and protective step of autophagy

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Autophagy is a catabolic process that allows the recycling of components of the cell under for instances conditions of nutrient depletion. Autophagy has been long regarded as an unselective process, but under some circumstances specific organelles like mitochondria are selectively engulfed by autophagosomes. We therefore explored whether mitochondrial morphological changes were associated with the onset of autophagy. Induction of autophagy led to mitochondrial elongation both *in vitro* and *in vivo*. Mitochondrial elongation correlated with increased fusion rate and required the core mitochondrial fusion proteins, as substantiated by a genetic analysis. A combination of real time imaging and the use of a pharmacological inhibitor indicated that cAMP-PKA axis mediates starvation-triggered mitochondrial elongation by blocking translocation of the pro-fission protein DRP1 to mitochondria. Elongation protected against mitophagy and was essential in the maintenance of ATP levels during periods of starvation. Ablation of the required pro-fusion genes converted mitochondria into sinks for ATP and caused starvation-induced death, showing a protective role for these morphological changes during periods of limited substrate supply. Thus, mitochondrial shape changes play an important role in the regulation of the fate of cells undergoing autophagy.

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18P.4 Changes in sympathetic and parasympathetic regulation connected with succinate dehydrogenase and α-ketoglutarate dehydrogenase activity in different physiological states of the organism

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The study was carried out by a novel cytobiochemical method highly sensitive to changes *in vivo* due to preservation *ex vivo* the native structure of mitochondrial network [1]. Succinate dehydrogenase (SDH) and α-ketoglutarate dehydrogenase (KDH) activity was measured in glass-adhered lymphocytes in smear of blood by nitro-blue-tetrazolium reduction [1]. The set of selected maximally identical, premature (6 week) male rats (6–8) was investigated simultaneously. The following states were investigated: intact state, after adrenaline (ADR) administration, under emotional immobilization stress in a box, newborn, premature and soon after maturation, depending on mother suckling, and under the action of biologically active substances. Healthy volunteers and patients with hypertension were also examined. The quiescent state in all cases is characterized by low activities of SDH and KDH well balanced with a very small prevalence of KDH over SDH. Together with increase in influence of exogenous or endogenous ADR activation of SDH per 200–400% and more is observed. This is accompanied initially by activation of KDG too, but to a lesser extent, while under more strong ADR action KDH activity falls dramatically. The state of highly active SDH without controlling action of KGL oxidation is non-stable and transforms into inhibition of both dehydrogenases under progression of influence. The moderate SDH activation is accompanied by increase in succinate-dependent Ca^{2+} accumulation in mitochondria, while hyperactivation is connected with its fall. Phases of activation and hyperactivation are respectively connected with elevation and lowering of immune function of neutrophils. Under strengthening of cholinergic regulation, related to maturation of animal, in contrast, increase in KDG activity and diminishing of SDH activation by ADR were observed. The data obtained demonstrate on a larger scale the discovery in isolated mitochondria of unification of sympathetic and parasympathetic regulation in the single system with oxidation of only the two substrates — succinate and α-ketoglutarate and explain the finding receptors also to only these two intermediates of oxidation [1].

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Reference

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18P.5 Mitochondria from *Artemia franciscana* embryos exhibit a truncated form of ant, associated with atypical effects of its ligands on Ca^{2+} uptake capacity and unique morphology of matrix Ca^{2+} precipitates

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Mitochondria isolated from embryos of the crustacean *Artemia franciscana* lack the Ca^{2+}-induced permeability transition pore. Although the composition of the pore described in mammalian mitochondria is unknown, the impacts of several effectors on pore opening are firmly established. Notably, ADP, ATP and bongkrekic acid delay, while carboxyatractyloside hasten Ca^{2+}-induced pore opening. Here we report that adenine nucleotides decreased, while carboxyatractyloside increased Ca^{2+} uptake capacity in mitochondria isolated from *Artemia* embryos. Bongkrekic acid had no effect on either Ca^{2+} uptake or ADP–ATP exchange rate. Transmission electron microscopy imaging of Ca^{2+}-loaded *Artemia* mitochondria showed needle-like formation of electron-dense material in the absence of adenine nucleotides, and dot-like formation in the presence of ADP. Energy-filtered transmission electron microscopy identified the material to be rich in Ca^{2+} and phosphorus. Sequencing of the *Artemia* ANT protein revealed that it lacked the last ~100 amino acids from the carboxy-terminus, compared to the closest ANT homologue expressed in *Xenopus laevis*, the latter exhibiting a 78–80% homology to human ANT-1, -2, and -3 and bovine isoforms. Isolated liver mitochondria from *Xenopi* exhibited Ca^{2+}-induced permeability transition pore, sensitive to inhibition by cyclosporin A and bongkrekic acid. We propose that the atypical effects of ANT ligands on Ca^{2+} uptake capacity in mitochondria from *Artemia* embryos is a consequence of their truncated ANT isoform. This is also associated with insensitivity to bongkrekic acid and a unique pattern of Ca^{2+} precipitation in their mitochondrial matrix. We further postulate that this truncated ANT results in the absence of a Ca^{2+}-induced pore.

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18P.6 MAC induces mitochondrial fragmentation

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Accumulating literature associates mitochondrial dynamics with apoptosis, since regulation of either process has reciprocal effects. These processes seem to converge in formation of the mitochondrial apoptosis induced channel, MAC, which releases cytochrome *c* and triggers the degradation phase of apoptosis. While Bax and Bak, core components of MAC, were shown to interact with fusion and fission proteins, some studies also suggest proteins from the intermembrane space could leak to the cytosol and further promote mitochondrial fission during apoptosis. The temporal relationship between apoptosis induction, MAC formation and mitochondrial fragmentation was investigated by time lapse microscopy. MAC function was induced through staurosporine treatment and microinjection of tBid or cytochrome *c*. MAC formation and mitochondrial dynamics were monitored in Hela cells (clone 10) that stably express low levels of GFP-Bax and were transiently transfected with a pDsRed-2 plasmid. GFP-Bax relocation to mitochondria occurs during apoptosis and signals MAC formation, while pDsRed-2 expression shows mitochondrial structure as red fluorescence. Treatment with staurosporine and microinjection with tBid induced relocation of Bax and collapse of the mitochondrial network. The temporal relationship between these two events was further analyzed. Interestingly, pretreatment with iMAC2, a specific MAC blocker, protected against cell death and prevented mitochondrial fragmentation after tBid injection. Our results suggest a link exists between MAC formation and collapse of the mitochondrial network during apoptosis.

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18P.7 Reversible enhancement of succinate dehydrogenase subunit A, succinate receptor and uncoupling proteins' mRNA levels in the course of physiological stress related to the dynamics of succinate dehydrogenase activity

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The novel cytobiochemical method preserving *ex vivo* the native structure of mitochondrial network allows to reveal on a large scale the dynamics of succinate dehydrogenase (SDH) activity *ex vivo* corresponding to the magnitude of *in vivo* changes [1, 2]. Intensive adrenergic activation of the organism within the range of physiological immobilization stress (IS) created by putting rat into a box includes initial strong SDH activation after 30 min treatment and subsequent decrease in excessive activity displaying the active adaptation to excitation at 120 min. In order to penetrate the mechanism of SDH dynamics we have investigated the expression of catalytic SDH subunit (*sdha*) and major succinate receptor (*gpr91*) on mRNA levels. Also we have monitored the dynamics in expression of uncoupling proteins *ucp2* and *ucp3* under IS conditions. Total RNA fractions from rat liver and spleen were isolated by standard hot acid phenol extraction. cDNAs were generated using gene-specific primers and M-MuLV reverse transcriptase, with RNA probes as a negative control. Expression levels were monitored by qRT-PCR. For reference house-keeping genes of β-actin and 16S ribosomal RNA were used. Our results indicate that on molecular level SDH activation under IS occur even more strikingly. We have observed that during initial phase of physiological stress the mRNA levels of enzyme subunit (*sdha*) and succinate receptor (*gpr91*) were enhanced 7- and 3-fold respectively, and as animal became adopted to stress conditions, expression decreased practically to the control levels. Furthermore, 30 min immobilization led to the dramatic increase in *ucp2*-mRNA levels, up to 1000-fold in spleen and 5-fold in liver, followed by expression reduction after 120 min (on average 4-fold over control level in spleen and 2-fold in liver). The same dynamics was observed for UCP-3 expression in spleen. Uncoupling proteins can prevent excessive potential accumulation on the mitochondrial membrane under stress thus eliminating harmful consequences.

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18P.8 Visualization of mitochondrial nucleoids in HepG2 and INS-1E cells

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Mitochondria possess their own DNA (mtDNA) — in humans a 16.6 kb circular double-stranded molecule, which encodes 13 essential subunits of respiratory chain complexes and ATP synthase. mtDNA is not “naked”