control ratios (RCR) were measured using respiratory medias containing [K^+] of 15, 37, 81, 111 and 146 mM. In all measurements, the media contained (in mM): 1 EGTA, 20 HEPES, 5 $MgCl_2$, 5 KPO_4^- and 1 g/l bovine serum albumin. pH was adjusted to 7.4 and the osmolarity to 330 mOsm/kg H_2O using a 1:3 ratio of sucrose and mannitol. The RCR of kidney cortex mitochondria decreased when the [K^+] was elevated compared to the media containing 15 mM K^+ (5.2 ± 0.2 vs. 2.5 ± 0.2, 3.7 ± 0.2, 3.9 ± 0.2, and 3.0 ± 0.1, respectively). However, RCR of heart mitochondria was lowest at 37 mM (3.9 ± 0.3) and was highest at 146 mM K^+ (10.1 ± 0.45). A two-way ANOVA showed that kidney cortex mitochondria have a different sensitivity towards K^+ compared to heart mitochondria (interaction $p < 0.05$, treatment $p < 0.05$, and group $p < 0.05$). Glibenclamide (100 μM), an inhibitor of the ATP-sensitive K^+ channel, increased RCR in kidney cortex mitochondria at 15 mM K^+ (+32%), but significantly more at 146 mM K^+ (+47%). Blockade of the voltage-gated K^+ channel by 4-aminopyridine (4-AP, 1 mM) together with glibenclamide improved RCR by +73% at 146 mM K^+. Neither of the applied K^+-channel blockers had any effect on the RCR of heart mitochondria. Mitochondria swelling at increasing [K^+] were observed in kidney cortex mitochondria, measured as loss of absorbance at 540 nm. Kidney cortex mitochondria in K^+-based media are non-functional in [K^+] ranging from 37 to 146 mM. Heart mitochondria do not display K^+-sensitivity to the same degree, but rather increase respiratory function with increasing [K^+]. Furthermore, we demonstrated that a tissue specific difference in mitochondria K^+-channels may explain these differences. The present study therefore demonstrates the importance of choosing a correct *in vitro* media to ensure a high quality of mitochondria research.

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16P.10 Novel assay and regulation of the mitochondrial K_{ATP} channel

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The mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$) protects the heart from damage induced by ischemia–reperfusion injury. Despite its central role in cardioprotection, the **mo**lecular identity of mitoK$_{ATP}$ remains controversial, and the validity of current methods to assay mitoK$_{ATP}$ activity is disputed. We sought to apply new and updated methods to investigate properties of the K^+ transporting component of mitoK$_{ATP}$ and its role in IPC. Using a thallium (Tl^+) sensitive fluorophore, a novel assay was developed to measure Tl^+ flux through mitoK$_{ATP}$. With both this assay and the classical mitoK$_{ATP}$ osmotic swelling assay, four key observations were made: (i) The IC_{50} for ATP-dependent channel inhibition was 4.5 μM. (ii) The EC_{50} for UDP-dependent channel activation was 20 μM. (iii) In isolated mitochondria, mitoK$_{ATP}$ activity rapidly degraded with time, and this channel "run-down" was reversed by phosphatidylinositol-4,5-bisphosphate (PIP_2). (iv) The antidepressant fluoxetine (Prozac™) both inhibited mitoK$_{ATP}$ (IC_{50} 2.4 μM) and blocked mitoK$_{ATP}$-dependent cardioprotection, while the related drug zimelidine was without effect. These findings are consistent with the hypothesis that the pore-forming subunit of mitoK$_{ATP}$ is an inward rectifying K^+ channel (Kir), likely Kir6.2. The ability of PIP_2 to reverse channel run-down is the first demonstration of modulation of a mitochondrial ion channel by PIP_2, and provides a mechanism to extend the assayable lifetime of mitoK$_{ATP}$ activity. The effect of fluoxetine on mitoK$_{ATP}$-dependent cardioprotection has implications for the widespread use of antidepressants in cardiovascular disease patients.

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16P.11 Potassium channel opener CGS7184 modulates activity of mitochondria by Ca^{2+} release through ryanodine receptor

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Large-conductance Ca^{2+}-activated potassium channel (BK_{Ca} channel) opener ethyl-1-{[(4-chlorophenyl)amino]oxo}-2-hydroxy-6-trifluoromethyl-1H-indole-3-carboxylate (CGS7184) acts on endothelium in the aorta and coronary circulation, NO production, calcium homeostasis, and mitochondrial function, especially on mitochondrial membrane potential, and mitochondrial respiration in cultured endothelial cells. All effects may be triggered by the CGS7184-induced modulation of intracellular Ca^{2+} homeostasis. To find the source of Ca^{2+} we studied the calcium homeostasis in H9C2 and C2C12 cell lines, and isolated sarcoplasmic reticulum (SR). The effects of CGS7184 on calcium homeostasis in C2C12 and H9C2 cell lines were measured with fura-2 fluorescence. Calcium uptake and Ca^{2+}-ATPase activity in isolated SR vesicles from rat skeletal muscles were applied. Single channel properties of calcium RYR channel were studied in bilayer lipid membrane (BLM). The BK_{Ca} channel opener CGS7184 has a profound effect on release of calcium from internal stores in the concentration-dependent manner. Data demonstrate that potassium channel opener CGS7184 modulates cytosolic Ca^{2+} concentration in H9C2, C1C12, and EA.hy 926 cell lines due to SR modulation. CGS7184 (5 μM) activated the cardiac RYR channel from both cytosolic and luminal sides, when the open probability of the control channel was >0.01. The large conductance potassium channel opener CGS7184 affects intracellular calcium homeostasis by interaction with RYR receptor.

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