of 2.8 mM glucose. Increased glucose and pyruvate each produced a concentration-dependent mitochondrial hyperpolarization. The causal relationships between pairs of parameters – $\Delta\psi_p$ and $[Ca^{2+}]_c$, $\Delta\psi_p$ and NAD(P)H, matrix ATP and $[Ca^{2+}]_c$, and $\Delta\psi_m$ and $[Ca^{2+}]_c$ – were investigated at single cell level. It is concluded that, in these β-cells, depolarizing oscillations in $\Delta\psi_p$ are not initiated by mitochondrial bioenergetic changes. Instead, regardless of substrate, it appears that the mitochondria may simply be required to exceed a critical bioenergetic threshold to allow release of insulin. Once this threshold is exceeded an autonomous $\Delta\psi_p$ oscillatory mechanism is initiated.

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P12

Reversibly modulating cytochrome *c* oxidase inhibits blood clotting: Studies based on functional electrochemical models and respiring cells

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Hydrogen sulfide and chelating heterocycles bind reversibly to the Fe and Cu centers in a biomimetic model of cytochrome *c* oxidase (C*c*O), thereby inhibiting the 4-electron reduction of oxygen to water. The same heterocycles have been found to inhibit respiration reversibly in isolated mitochondria. Platelets are important mediators of blood coagulation that lack nuclei, but contain mitochondria. We hypothesized that the inhibition of platelet mitochondria disrupts platelet function and platelet-activated blood coagulation. Indeed, we found that the strength of mitochondrial inhibition correlates with the heterocycle's ability to deter platelet stimulation and platelet-activated blood clotting. These results suggest that for this class of molecules, inhibition of blood coagulation may be occurring through a mechanism involving mitochondrial inhibition.

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P13

Role of conformational change and lipidic ligands in the function and regulation of cytochrome *c* oxidase

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High resolution crystal structures of bacterial and mammalian cytochrome *c* oxidases (C*c*O) reveal conserved lipid and steroid binding sites [1] as well as redox-linked conformational changes [2], suggesting regulatory and gating possibilities. A bile salt, cholate, is resolved in all crystal structures of bovine C*c*O and forms a tight hydrogen bond with the carboxyl of E62, homologous to E101 in the *Rhodobacter sphaeroides* C*c*O (*Rs*C*c*O), a conserved residue important in K-path proton uptake. These positioning and kinetic studies indicate that the strong inhibitory effect of cholate in bovine C*c*O is due to blockage of the K-path.

Unexpectedly we discovered that μM levels of cholate or deoxycholate stimulate 10-fold the activity of the *Rs*C*c*O mutant, E101A, suggesting chemical rescue of the missing K-path carboxyl. Crystals of *Rs*C*c*O grown in the presence of deoxycholate show a single molecule bound close to E101, in a similar location as cholate in the bovine C*c*O. The conservation of a steroid binding site in such a key position argues for physiological significance. Further studies reveal that a limited set of amphiphilic molecules compete at the entrance of the K-path causing loss or regain of C*c*O activity at μM concentrations.

Also affecting the K-path region is an observed conformational change in the reduced crystals of *Rs*C*c*O [3], causing the loss of steroid binding and the opening of a connection between the K path and the active site. The formation of a new water chain leading into the a_3-Cu_B site indicates a role in gating of proton access. Additional changes observed in the region of heme *a* are similar to some documented by Yoshikawa and coworkers in the reduced bovine C*c*O. The precise physiological and mechanistic significance of the steroid/amphiphile binding site and redox-induced conformational changes remains to be established, but the findings point to new avenues for exploring the regulation of cytochrome *c* oxidase. (Supported by NIH GM26916 to SFM.)

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P14

ROS production and turnover of brain mitochondria — Implications for neurological diseases

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The main superoxide producing sites of brain mitochondria are the FMN of respiratory chain complex I [1] and the center 'o' of bc_1 complex [2]. The complex I formed superoxide is delivered to the mitochondrial matrix, metabolized by MnSOD to H_2O_2 and highly relevant in severe pathology of the brain. The highest rates of superoxide production from this site are detected under the conditions of a highly reduced NAD-system. In contrast, bc_1 complex formed superoxide is predominantly liberated into the intermembrane space, metabolized by Cu,ZnSOD to H_2O_2 and suggested to fulfill signaling functions. The highest rates of superoxide production from that site are detected at intermediate states of coenzyme Q pool reduction in presence of antimycin.

Brain mitochondria are not only major producers of H_2O_2, but they also considerably contribute to the removal of toxic hydrogen peroxide by the glutathione (GSH) and thioredoxin-2 (Trx2) antioxidant systems. By using the specific inhibitors auranofin and DNCB, the contribution of Trx2- and GSH-systems to ROS detoxification in rat brain mitochondria was determined to be $60 \pm 20\%$ and $20 \pm 15\%$, respectively, while catalase contributed to a non-significant extent only. In digitonin-treated hippocampal homogenates inhibition of Trx2- and GSH-systems affected mitochondrial hydrogen peroxide production rates to a much higher extent than the endogenous