Abstract

The activity of mitochondrial cytochrome *c* oxidase (COX) can be affected by either exogenous or endogenous factors. The most efficient and in the environment abundant compound that inhibits COX is cyanide. The very frequent cause of COX deficiency in humans is represented by a defect in the *SURF1* gene.

The mechanism of cyanide inhibitory effect on COX as well as the conditions for its recovery are not yet fully explained. Three parameters of COX function, namely the transport of electrons (oxygen consumption), the transport of protons (mitochondrial membrane potential, $\Delta\psi_m$) and the enzyme affinity to oxygen (p_{50} value), were studied with regard to the inhibition by KCN and its reversal by pyruvate. The function of COX was analysed in intact isolated rat liver mitochondria, both within the respiratory chain and as a sole enzyme, using succinate or an artificial electron donor ascorbate + TMPD as a substrate. 250 μM KCN completely inhibited both electron- and proton-transporting function of COX, and this inhibition was reversible as proved with washing of mitochondria. The addition of 60 mM pyruvate induced the maximal recovery of both parameters to 60 – 80 % of original values. Using KCN in the low concentration range up to 5 μM, a profound, 30-fold decrease of COX affinity to oxygen was observed. Again, this decrease was completely reversed by washing of the mitochondria while pyruvate induced only a partial yet still significant recovery of oxygen affinity. These results demonstrate the reversible nature of inhibition of COX by cyanide and reveal the limited potential of pyruvate to act as a cyanide poisoning antidote. Importantly, it is also shown that the COX affinity to oxygen is the most sensitive indicator for the detection of toxic effect of cyanide.

The function of Surf1 protein, an assembly factor of COX, remains unclear. The influence of Surf1 defect on all three functional parameters of COX was analysed in cultivated immortalised fibroblasts originated from the *SURF1* knock-out mice. The COX content was decreased to 58 % of the control in correspondence to 38% decrease of COX activity measured spectrophotometrically and normalised to the citrate synthase activity. However, further experiments revealed that there was no change in the rate of endogenous respiration as well as in the rate of ascorbate+TMPD-dependent respiration of permeabilised cells. $\Delta\psi_m$ generated by COX achieved 92 % of maximal $\Delta\psi_m$ in the control cells, but only 73 % in the *SURF1*$^{-/-}$ cells. Therefore, the proton-pumping activity of COX was partially

impaired. Since the p_{50} value of *SURF1*$^{-/-}$ cells was approximately 2-fold increased in all metabolic states measured, the oxygen affinity was again identified as the most sensitive and the most affected functional parameter of COX. The *SURF1*$^{-/-}$ cells showed milder functional manifestations of COX impairment than the cells of Surf1-deficient patients, which indicates that the Surf1 protein is not as essential for mouse as for human.