

Cytochrome c stimulates the superoxide anion production by cytochrome *b*₅ reductase in neuronal synaptic plasma membrane vesicles.

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ABSTRACT

The plasma membrane NADH oxidase activity of synaptosomes and cerebellar granule neurons releases superoxide anion ($O_2^{\cdot-}$) as a collateral product. In this work, we have measured the effect of cytochrome *c* (Cyt *c*) on the NADH-dependent $O_2^{\cdot-}$ production by rat neuronal synaptic plasma membrane vesicles. In these membranes, the Cyt *c* stimulated $O_2^{\cdot-}$ production was inhibited by antibodies against cytochrome *b*₅ reductase (Cb₅R). $O_2^{\cdot-}$ production by Cb₅R was confirmed using purified soluble and membrane recombinant enzymes. Addition of Cyt *c* to the assay induced a burst of $O_2^{\cdot-}$ concomitant to the reduction of Cyt *c* by Cb₅R. The low dissociation constant measured for Cyt *c*/Cb₅R complex (0.40 ± 0.05 and 0.38 ± 0.02 μ M for soluble and membrane Cb₅R, respectively), support that Cb₅R is a main molecular partner of Cyt *c* upon its release from mitochondria during apoptosis. $O_2^{\cdot-}$ production and Cyt *c* reduction are consequences of the stimulated NADH consumption by Cb₅R upon complex formation with Cyt *c*. Participation of Cb₅R in the apoptosis signalling network is suggested.

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