

## Cytochrome c stimulates the superoxide anion production by cytochrome *b*<sub>5</sub> 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*<sub>5</sub> reductase (Cb<sub>5</sub>R).  $O_2^{\cdot-}$  production by Cb<sub>5</sub>R 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 Cb<sub>5</sub>R. The low dissociation constant measured for Cyt *c*/Cb<sub>5</sub>R complex ( $0.40 \pm 0.05$  and  $0.38 \pm 0.02$   $\mu$ M for soluble and membrane Cb<sub>5</sub>R, respectively), support that Cb<sub>5</sub>R 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 Cb<sub>5</sub>R upon complex formation with Cyt *c*. Participation of Cb<sub>5</sub>R in the apoptosis signalling network is suggested.

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