Mitochondria are the main source of reactive oxygen species in cells because 2–4% of the oxygen consumed by mitochondria is converted to superoxide anions by the electron transport chain and moreover mitochondria have restricted protection against oxidative stress. To determine the importance of mitochondrial oxygen species toxicity, we analyzed heart muscle tissue and fibroblasts from mutant mice, with deficiencies in the mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) generated in three different ways. The knockout mouse models have been produced by disruption of different regions of Sod2 gene (Li et al. (1995) Nat. Genet. 11: 376–381; Lebovitz RM et al. (1996) Proc. Natl. Acad. Sci. USA 93: 9782–9787). In agreement with literature in heart muscle we observed catalase deficiency (Melo et al. (1999) Proc. Natl. Acad. Sci. USA 96: 846–851). In comparison with wild type animals the knockout mice showed only one third of catalase activity. In contrast, the fibroblast cultures from these mice did not show any alteration of catalase activity. In the digitonin treated fibroblast the resting state of respiration was elevated, while active state respiration was not affected. Our findings demonstrate strong tissue specificity effect of MnSOD knockout.

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5P.12 Comparison of superoxide production of rat brain mitochondria analyzed with hydroethidine and MitoSOX
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The production of superoxide generation is implicated for several types of neurodegenerative diseases and aging. Despite the progress in characterising the ROS effects on cell function, the mechanisms of cellular superoxide formation are less well understood. Considerable difficulties and artefacts are observed with different methods for detection of ROS, and in particular superoxide. In this work we compared MitoSOX and Hydroethidine, the well known dyes for superoxide detection in isolated rat brain mitochondria. Hydroethidine (HET) is used to visualize superoxide localized in cytoplasm. For more targeted detection of ROS production in the mitochondria, hydroethidine is modified by conjugating this dye to triphenylphosphonium (MitoSOX). We observed that, unlike hydroethidine, MitoSOX allows to detect superoxide generation in isolated rat brain mitochondria respiring on the complex II substrate succinate. This superoxide generation, detected by MitoSOX, was sensitive to uncoupler and rotenone. This indicates that it is due to reversed electron flow caused ROS generation by Complex I. Similarly, rotenone increased the superoxide generation detected by MitoSOX but not HET in the presence of the complex I substrates glutamate + malate, α-ketoglutarate and pyruvate + malate. Since the MitoSOX is assumed to be accumulated at the inner side of mitochondrial inner membrane, this indicates that Hydroethidine and MitoSOX probably detect mitochondrial superoxide production in different local compartments.

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5P.13 Tissue specific effects of MnSOD knockout in mice
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A critical role of mitochondrial dysfunction and oxidative damage has been hypothesized in both aging and neurodegenerative diseases.