

ESR SPIN-TRAPPING STUDY OF SUPEROXIDE GENERATION IN HUMAN EMBRYONIC KIDNEY CELLS EXPRESSING NOX4 UPON TETRACYCLINE INDUCTION

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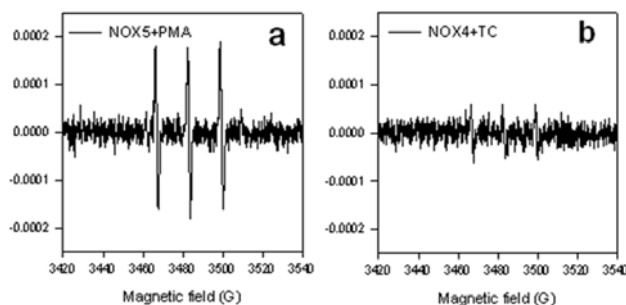
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NOX enzymes are superoxide-generating NADPH oxidases.¹ These enzymes contribute to physiologically relevant oxidative processes, but also to oxidative stress in many types of diseases.² NOX4 has a wide tissue distribution, but its pharmacology, physiological function and activation mechanisms remain largely unknown.³ Here, reactive oxygen species (ROS) formation in human embryonic kidney (HEK) cells expressing NOX4 upon tetracycline (TC) induction, and stably expressing NOX5 cells were studied. In the NOX4-transfected cells, TC induced a rapid increase in NOX4 mRNA (1 h), which was closely followed (2h) by a release of reactive oxygen species. Phorbol myristate acetate (PMA) was used to stimulate NOX5. To specifically detect superoxide radical ($O_2^{\cdot-}$) by electron spin resonance (ESR), 1-acetoxy-3-carboxy-2,2,5,5 tetramethylpyrrolidine (ACP) spin trap was used. ACP enters cells in its ester form, gets hydrolyzed by cytosolic esterases and subsequently interacts with intracellular $O_2^{\cdot-}$.^{4,5} ESR did not reveal any marked NOX4-mediated generation of $O_2^{\cdot-}$ in the cytosol. This result was also supported by dihydroethidium (DHE) fluorescence tests. Tests using nitro blue tetrazolium (NBT), an agent that may freely enter organelles, pointed to a marked generation of $O_2^{\cdot-}$. Thus, this study suggests that NOX4 most likely generates $O_2^{\cdot-}$ within intracellular compartments that are accessible to NBT, but not to DHE and ACP. These results also point to very distinct pharmacology and ROS generation pattern of NOX4.



ESR detects superoxide in NOX5-expressing HEK cells stimulated by PMA (a), but not in TC-induced NOX4-expressing HEK cells (b).

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