New rapid ESR assay for measurements of superoxide and hydrogen peroxide production by vascular NAD(P)H oxidase

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NAD(P)H oxidases are major sources of superoxide and hydrogen peroxide in vascular cells. The NAD(P)H oxidases have been found to be essential in the physiological response of vascular cells. The superoxide radical is an effective scavenger of NO•, producing peroxynitrite. Increased production of superoxide radicals can deplete the pool of NO• leading to impaired vasorelaxation. Paradoxically, hydrogen peroxide can stimulate NO• production by eNOS, and it is an important growth factor in smooth muscle cells. Moreover, hydrogen peroxide is much more stable molecule than superoxide, which makes it important in cell signaling systems. However, quantification of superoxide radical and hydrogen peroxide production by NAD(P)H oxidase is still very challenging. Moreover, the ratio of superoxide to hydrogen peroxide formation by the vascular NAD(P)H oxidase have not been defined.

We have developed a new assay for NAD(P)H oxidase activity using ESR spectroscopy with 1-hydroxy-3-carboxy-pyrrolidine (CPH), which provides high sensitivity quantitative measurements of superoxide radical and hydrogen peroxide. Superoxide formation can be assayed as NADPH-dependent, SOD-inhabitable formation of 3-carboxy-proxyl (1). Production of superoxide by the NADPH oxidase was measured in the membrane fraction of vascular smooth muscle and endothelial cells stimulated by serum as well as angiotensin II. Production of hydrogen peroxide by vascular NAD(P)H oxidase was measured by co-oxidation of CPH in a peroxidase-acetamidophenol reaction (2). These new ESR techniques reveal that production of hydrogen peroxide by the vascular smooth muscle NAD(P)H oxidase is more than 2-times higher than the production of superoxide, while the macrophage NADPH oxidase generates superoxide radical as a primary product, which decomposes to the corresponding amount of hydrogen peroxide. The ratio of superoxide to hydrogen peroxide formation by the vascular NAD(P)H oxidase was thus very different from that of macrophage NADPH oxidase. Our data suggest that hydrogen peroxide is major product of vascular NAD(P)H oxidase.

This new ESR assay allows quantitative measurements of both superoxide and hydrogen peroxide, and shows for the first time that hydrogen peroxide may be a major product of vascular smooth muscle NAD(P)H oxidase.

[1]. Dikalov S., Skatchkov M., Bassenge E. (1997) Spin trapping of superoxide radicals and peroxynitrite by 1-hydroxy-3-carboxy-pyrrolidine and 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine. Stability of corresponding nitroxyl radicals towards biological reductants. Biochem. Biophys. Res. Comm. 231, 701-704.
[2]. Matsuo T., Shinzawa H., Togashi H., Aoki M., Sugahara K., Saito K., Saito T., Takahashi T., Yamaguchi I., Aoyama M., Kamada H. (1998) Highly sensitive hepatitis B surface antigen detection by measuring stable nitroxide radical formation with ESR spectroscopy. Free Rad. Biol. Med. 25(8), 929-935.