Molecular characterization of a superoxide-generating NAD(P)H oxidase in the ventilatory muscles.

Javeshghani D, Magder SA, Barreiro E, Quinn MT, Hussain SN.

Erratum in

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Abstract

The molecular sources of reactive oxygen species (ROS) in skeletal muscles are not well understood. We hypothesized that nonphagocyte NAD(P)H oxidase could be a source of ROS in muscle fibers. We thus investigated the existence, structure, and contribution of nonphagocyte NAD(P)H oxidase to ROS production in rat skeletal muscles. ROS production and NAD(P)H oxidase activity were evaluated by lucigenin-enhanced chemiluminescence and NADH consumption rate, whereas enzyme composition was monitored by reverse transcription-polymerase chain reaction and immunoblotting. Basal O(-)(2) production in muscle strips from normal rats averaged 1.4 nmol/mg per 10 min and increased to approximately 18 nmol/mg per 10 min in the presence of NADH. Muscle O(-)(2) production and NADH consumption were inhibited by Tiron, superoxide dismutase, apocynin, and diphenyleneiodonium but not by inhibitors of cyclo-oxygenases, xanthine oxidase, nitric oxide synthases (NOS), and mitochondrial enzymes. We detected mRNA and proteins of p22(phox), gp91(phox), p47(phox), and p67(phox) subunits in normal rat muscles. These subunits were localized in close proximity to the sarcolemma. Induction of sepsis in rats doubled muscle O(-)(2) production with no major changes in muscle NADPH oxide subunit expression. In lipopolysaccharide-treated but not in control muscles, O(-)(2) production was increased significantly by NOS inhibition. We conclude that a constitutively active NAD(P)H oxidase enzyme complex exists in normal skeletal muscle fibers and contributes to ROS production. In septic rats, this production is increased but measurable O(-)(2) is reduced by enhanced NO production.