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BRAIN RESEARCH

Research Report

Regional expression of NO synthase, NAD(P)H oxidase and superoxide dismutase in the rat brain

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ABSTRACT

Nitric oxide (NO) derived from the endothelial NO synthase (eNOS) contributes to regulation of cerebral circulation, whereas that produced by neuronal NOS (nNOS) participates in the regulation of brain function. In particular, NO plays an important role in modulation of sympathetic activity and hence central regulation of arterial pressure. Superoxide derived from NAD(P)H oxidase avidly reacts with and inactivates NO and, thereby, modulates its bioavailability. Calmodulin (CM) is required for activation of NOS and soluble guanylate cyclase (sGC) serves as a NO receptor. Superoxide is dismutated to H_2O_2 by superoxide dismutase (SOD) and H_2O_2 is converted to H_2O by catalase or glutathione peroxidase (GPX). Given the importance of NO in the regulation of brain perfusion and function, we undertook the present study to determine the relative expressions of immunodetectable nNOS, eNOS, CM, sGC, NAD(P)H oxidase and SOD by Western analysis in different regions of the normal rat brain. nNOS was abundantly expressed in the pons cerebellum and hypothalamus and less so in the cortex and medulla. sGC abundance was highest in the hypothalamus and pons, and lowest in the cerebellum and medulla. eNOS and calmodulin were equally abundant in all regions. NAD(P)H oxide was most abundant in the pons compared to other regions. Cytoplasmic SOD was equally distributed among different regions but catalase and GPX were more abundant in pons, hypothalamus and medulla and less so in the cortex and cerebellum. Thus, the study documented regional distributions of NOS, NAD(P)H oxidase, antioxidant enzymes, sGC and calmodulin which collectively regulate production and biological activities of NO and superoxide, the two important small molecular size signaling molecules.

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1. Introduction

Nitric oxide (NO) is a ubiquitous messenger molecule which participates in a wide array of diverse biological functions including neurotransmission, immune regulation, renal and vascular homeostasis, cell adhesion, proliferation and apoptosis among others (Furchgott and Vanhoutte, 1989; Ignarro and Murad, 1995; Moncada et al., 1991).

NO is produced from oxidation of L-arginine by a family of homodimeric enzymes known as NO synthases (NOS). Three distinct isoforms of NOS have, thus far, been identified; NOS-I (neuronal NOS), NOS-II (inducible NOS) and NOS-III (endothelial NOS). Activation of all NOS isoforms depends on calmodulin binding. Calmodulin binding and hence enzymatic activities of nNOS and eNOS, but not iNOS, are regulated by modulations of cytosolic Ca^{2+} concentration. In contrast, calmodulin binding and hence enzymatic activity of iNOS is calcium-independent. NO produced by a given cell can exert either an autocrine action on the cell itself, a paracrine action on the adjacent cells (via direct diffusion or S-nitrosothiol formation) or an endocrine action on distant tissues (via S-nitroso-albumin or -hemoglobin formation). Many of the biological actions of NO are mediated by cyclic guanosine monophosphate (cGMP) which is produced from GTP following activation of soluble guanylate cyclase (sGC). The availability of biologically active NO in a given tissue depends not only on the rate of its production, but also the rate of its inactivation by superoxide ($NO + O_2^{\cdot -} \rightarrow ONOO^-$).

NAD(P)H oxidase has been recognized for many years as the primary source of reactive oxygen species (ROS) production by the activated phagocytes (Babior, 1999). The phagocytic (phox), as well as several closely related isoforms of NAD(P)H oxidase (NOX) are widely expressed in many other tissues including neuronal tissues, kidney and cardiovascular system among others. NAD(P)H oxidases consist of two membrane-associated ($gp91^{phox} + p22^{phox}$) and 3 cytoplasmic subunits ($p47^{phox}$, $p67^{phox}$ and rac1). Activation of the prototypical phagocytic NAD(P)H oxidase is dependent on assembly of the subunits which requires phosphorylation of the regulatory $P47^{phox}$ subunit. NAD(P)H oxidase catalyzes the reduction of molecular oxygen to superoxide ($O_2 + e^{\cdot -} \rightarrow O_2^-$) which is converted to H_2O_2 by the superoxide dismutase (SOD) family of enzymes. H_2O_2 is, in turn, converted to water by catalase or glutathione peroxidase. Normally, small amounts of ROS produced by NAD(P)H oxidases serve biologically important functions as signaling molecules (Droge, 2001; Fridovich, 1997; Thannickal and Fanburg, 2000). However, excess production of ROS by either the tissue-specific isoforms and/or that derived from the infiltrating phagocytes and/or resident cells results in oxidative stress, functional NO deficiency, cytotoxicity and tissue damage.

For several years, a number of laboratories have explored the role of oxidative stress and the associated interactions between NO and ROS in the pathogenesis of hereditary and acquired hypertension in experimental animals (Campese et al., 2004; Glowinski and Iversen, 1966; Somers et al., 2000; Swei et al., 1999; Vaziri et al., 2004; Vaziri and Rodriguez-Itrube, 2006; Vaziri et al., 1999; Vaziri et al., 2000). In particular, we have focused on the interplay of NO and ROS in the kidney, cardiovascular system, as well as brain, organs which play a critical role in regulation of blood pressure. Given the critical role of the central sympathetic activity and its modulation by NO in regulation of blood pressure, we sought to determine normal distributions of NOS and related proteins (CM and sGC), NAD(P)H oxidase subunits and key antioxidant enzymes, i.e. superoxide dismutase, catalase and glutathione peroxidase in the regions of the brain involved with the regulation of blood pressure. In addition, cerebral cortex and cerebellum

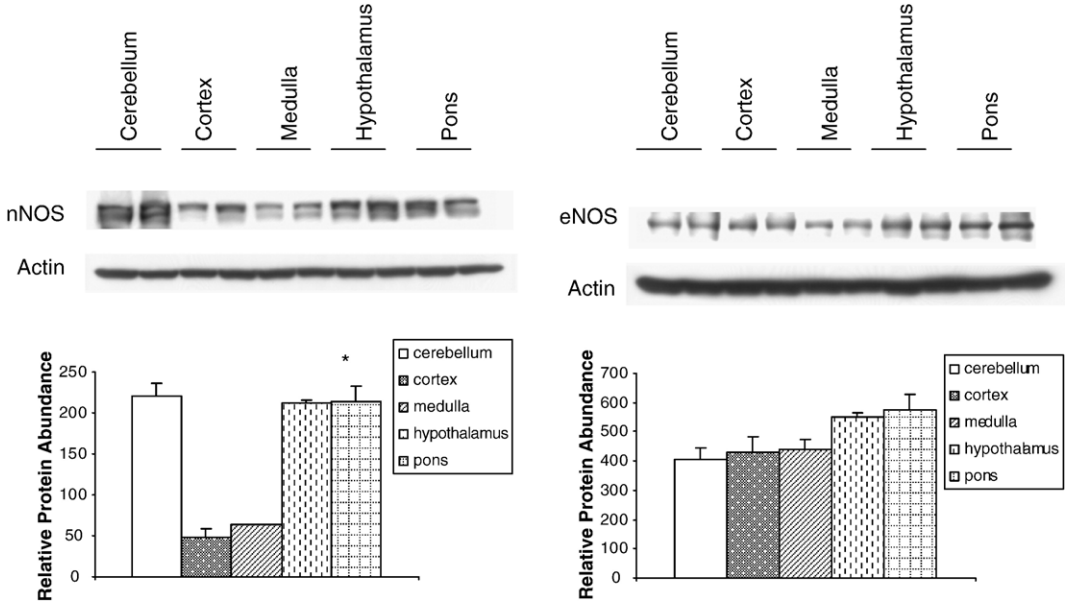

Fig. 1 – Representative Western blots and group data depicting relative abundance of nNOS, eNOS and β actin in various regions of the normal rat brain. *N*=4 animals. **P*≤0.05 ANOVA.