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Research Report

Does neuroglobin protect neurons from ischemic insult? A quantitative investigation of neuroglobin expression following transient MCAo in spontaneously hypertensive rats

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ABSTRACT

Neuroglobin (NGB) is a recently characterized heme globin expressed primarily in retinal nerve cells and at very low levels in endocrine-active regions of vertebrate brains. When artificially over-expressed, NGB reduces the infarct size observed after transient Middle Cerebral Artery occlusion (tMCAo) in rats. This study addresses the post-ischemic NGB expression in vivo. Ten Spontaneously Hypertensive Rats (SHRs) were randomized to tMCAo ($n = 6$) or sham ($n = 4$), and euthanized 24 h later. NGB mRNA expression was determined by means of quantitative Reverse Transcription Polymerase Reaction (qRT-PCR). Thirteen animals subjected to either 90 min tMCAo ($n = 7$) or sham ($n = 6$) surgery, were euthanized 1 week after surgery. Post-ischemic expression of NGB and the neuronal marker NeuN was studied using free-floating immunohistochemistry. Design-based stereological quantification of NGB- and NeuN-positive cells in the striatum was performed using the optical fractionator. Significantly less NGB mRNA was expressed in the ischemic hemispheres of tMCAo animals after 24 h ($P \leq 0.002$). At the protein level, we found a significantly lower number of NGB- and NeuN-positive striatal neurons in tMCAo rats ($P \leq 0.004$). NGB expression was mainly confined to the hypothalamus and amygdala. Less than one out of every two thousand neurons expressed NGB in the striatum. In the ischemic territory we did not observe selective sparing of NGB expressing neurons. No significant change in the NGB/NeuN ratio was observed. Our data indicate that endogenous expressed NGB does not provide protection against ischemic injury induced by tMCAo in SHRs.

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Abbreviations:
ABC, Avidin–Biotin–peroxidase Complex
BPM, Beats Per Minute
CCA, Common Carotid Artery
cDNA, complimentary DeoxyriboNucleic Acid
DWI, Diffusion Weighted Imaging
ECA, External Carotid Artery
Hb, Hemoglobin
HR, Heart Rate
ICA, Internal Carotid Artery
LA, Lingual Artery
MA, Maxillary Artery
MABP, Mean Arterial Blood Pressure
Mb, Myoglobin
MCA, Middle Cerebral Artery
mRNA, messenger RiboNucleic Acid
MRI, Magnetic Resonance Imaging
NeuN, Neuronal Nuclei
NGB, Neuroglobin
PBS, Phosphate Buffered Saline
qRT-PCR, quantitative Reverse Transcriptase Polymerase Chain Reaction
SHRs, Spontaneously Hypertensive Rats
tMCAo, transient Middle Cerebral Artery occlusion

1. Introduction

Neuroglobin (NGB) is a recently characterized heme globin (Burmester et al., 2000) expressed primarily in retinal nerve cells (Schmidt et al., 2003) and at very low levels in vertebrate brain tissues. NGB has the same overall globin fold as hemoglobin (Hb) and myoglobin (Mb) (De Sanctis et al., 2004; Pesce et al., 2003), but differs radically from Hb and Mb in that its heme is hexacoordinated (Dewilde et al., 2001; Pesce et al., 2002). Phylogenetically, it resembles the nerve hemoglobin from the invertebrate Aphrodite aculeata more closely than it resembles Hb and Mb (Dewilde et al., 1996). Unlike the well-characterized invertebrate nerve hemoglobins, the function of NGB is largely unknown. NGB has, however, been proposed to act as: (a) an intracellular oxygen store, (b) an oxygen sensor, (c) a scavenger of reactive oxygen or nitrogen species, (d) a NO converting enzyme due to its dioxygenase activity, and (e) a terminal oxidase facilitating ATP production under hypoxia (Burmester et al., 2000; Hankeln et al., 2005; Moens and Dewilde, 2000; Wakasugi et al., 2005).

NGB was found to be up-regulated in cultured hippocampal neurons from mice exposed to hypoxia (Sun et al., 2001), but not in in vivo studies where mice were exposed to different time regimes and severities of hypoxia (Fordel et al., 2004; Hundahl et al., 2005; Mammen et al., 2002). In addition, over-expression of NGB in rat brains reduced the infarct size following transient middle cerebral artery occlusion (tMCAo) (Sun et al., 2003). This exciting observation indicates that modulation of NGB expression may offer a significant potential in stroke treatment strategies (Garry and Mammen, 2003; Hankeln et al., 2005). Unfortunately, the terms cerebral hypoxia and ischemia have not been clearly defined in most of the published NGB studies. Despite the suggested roles of NGB, data on the in vivo expression pattern of NGB in neurons after transient cerebral ischemia are lacking. In particular, no studies have until now focused on NGB expression after matured ischemic brain damage. Based on previous reports (Sun et al., 2001, 2003), we hypothesized that an up-regulation of NGB would be observed at the mRNA and protein levels following an ischemic challenge.

In the current study, we used a transient thread occlusion model of the middle cerebral artery in spontaneously hypertensive rats (SHRs) (Longa et al., 1989; Zarow et al., 1997). The expression of NGB at mRNA level 24 h after tMCAo was examined using quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR). Further, we quantified the number of NGB and NeuN expressing neurons in the striatum 1 week after the ischemic event using the optical fractionator. To our knowledge, this is the first study where the total number of NGB-positive neurons is estimated in a specific brain region. Surprisingly, less than one out of two thousand neurons expressed NGB in the striatum. Further NGB expression evidenced by immunohistochemistry was primarily restricted to the limbic system. Contrary to the claimed neuroprotective role of NGB, we found no evidence for an up-regulation of NGB after tMCAo. Nor did we see any protection of neurons expressing NGB at naturally occurring levels.