Presence of Neuroglobin in Cultured Astrocytes

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ABSTRACT Neuroglobin (Ngb), a recently discovered intracellular respiratory globin in neurons, may play a crucial role in oxygen homeostasis in the brain. We report preliminary findings indicating the presence of functional neuroglobin in primary cultures of cerebral cortical astrocytes. Reverse transcription real-time polymerase chain reaction (RRT–PCR) and immunostaining confirmed such presence in cultured astrocytes isolated from newborn mouse brain. Ngb antisense treatment increased apoptosis in ischemic astrocytes. The discovery of Ngb in astrocytes may provide some insight into how oxygen homeostasis is regulated in the brain.© 2005 Wiley-Liss, Inc.

Astrocytes are the most abundant glial cells in the brain. In humans, they exceed neurons by a ratio of 10–50 astrocytes to every neuron. In recent years, astrocytes have been shown to play important roles in the central nervous system, including signal transduction (e.g., by calcium wave) and cellular communication (e.g., via gap junction), and in controlling synapse numbers (Kast, 2001; Hansson and Ronnback, 2003; Parri and Crunelli, 2003). In the present report, we outline the presence of neuroglobin transcript and protein in cultured astrocytes, suggesting further involvement of astrocytes in regulating brain function.

Neuroglobin (Ngb), named for its initial discovery in neuronal cells in the brain, is a small protein of 151 amino acids found to be expressed in the brains of a variety of species (Reuss et al., 2002; Zhang et al., 2002). The protein is highly conserved among vertebrates and has been classified as a tissue hemoglobin, a group of proteins that bind oxygen and mediate oxygen transport that also includes myoglobin (Mb) and hemoglobin (Hb). Despite the level of interest generated by the discovery of Ngb, the focus of research has been primarily on its roles in neurons. In fact, whether functional Ngb is expressed in glial cells has been questioned previously (Reuss et al., 2002). To address such question, we provide some preliminary data in this report to indicate the presence of functional Ngb in cultured astrocytes, the predominant glial cells in the brain. Ngb in astrocytes may be involved in the regulation of oxygen homeostasis in the brain.

The presence of the Ngb transcript in cultured astrocytes was examined by reverse transcription real-time PCR (RRT-PCR) with Ngb-specific primers and probe. More than 95% of the cultured astrocytes prepared from newborn ICR mice were positive for glial fibrillary acidic protein (GFAP) and 95% purity of the culture was consistently reproducible (Yu et al., 2003). Positive reporter signals for Ngb were observed for neurons and astrocytes (Fig. 1A), but not for Cos-7 cells (Fig. 1A, Ngb). The reporter signals for 18S rRNA for all tested cells were positive.

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indicating the general presence of transcripts in all tested samples (Fig. 1A, 18S RNA). Therefore the negative Ngb signal displayed by Cos-7 cells was not due to the absence of transcript in the Cos-7 sample. The real-time PCR results indicate the presence of Ngb transcript in astrocytes, but of a much less
amount when compared with neurons. DNA sequencing confirmed that the amplified product from Ngb-specific primers matched to Ngb.

After showing the presence of Ngb transcript in cultured astrocytes, immunostaining technique (according to conditions described in Chen et al., 2003) was used to...
determine if this transcribed product gave rise to the Ngb protein. Ngb immunostaining experiments were accomplished using a rabbit polyclonal antibody raised against a rat Ngb fusion protein (Zhang et al., 2002). The specificity of this rabbit polyclonal antibody was examined by transfecting cultured astrocytes with a Ngb-GFP construct (Fig. 1B). The overlapping patterns of GFP fluorescence (green) and Ngb staining (red) on one representative transfected astrocyte indicate the specificity of this anti-rat Ngb antibody to mouse Ngb. Cross-reactivity of the antibody to rat and mouse Ngb is anticipated, as rat and mouse Ngb share 96% amino acid similarity (Zhang et al., 2002). With confirmed specificity of the Ngb antibody, endogenous Ngb protein in regularly cultured astrocytes was detected by immunostaining (procedures as previously described in Chen et al., 2003), followed by confocal microscopy analysis. All astrocytes in culture were stained positive for Ngb and GFAP (green and red respectively; Fig. 1C), both of which were co-localized to the same cells in the merged image (Fig. 1C).

Since Ngb has been demonstrated to be upregulated in neurons by hypoxia-ischemia injury (Sun et al., 2001), the functional role of Ngb in astrocytes during ischemia was examined using the anti-sense technique. An anti-sense oligonucleotide (ODN) (5′-tgacgccggcactcgcG-3′) acting against the initial coding region of Ngb was designed based on the GenBank mouse Ngb sequence, NM 022414. This Ngb anti-sense ODN is similar to the ODN used previously (Sun et al., 2001), with demonstrated efficiency in blocking Ngb protein production (Sun et al., 2001). A corresponding Ngb sense ODN was used as control to examine the specific effect of the anti-sense ODN. Cultured astrocytes were transfected with the anti-sense (or sense) Ngb ODN, after which cells were subjected to 5 h of ischemic treatment. Untransfected cultured astrocytes were used as controls. Apoptosis in astrocytes was assessed by Hoechst 33342 nuclear staining as previously described (Jiang et al., 2002). In cultured astrocytes transfected with the sense Ngb ODN, only a few apoptotic astrocytes were visible after 5 h of ischemia (Fig. 2A, top, arrowheads). However, anti-sense Ngb ODN transfection resulted in a higher number of apoptotic cells (Fig. 2A, bottom, arrowheads). The percentage of apoptotic cells to total cells in the untransfected controls and sense-transfected cultures was approximately 10% after 5 h of ischemic incubation, while transfection of anti-sense Ngb ODN led to a 2.5-fold increase in apoptotic cells (~25%, Fig. 2B) after 5 h of ischemic treatment. These results suggest a possible protective role for Ngb in astrocytes against ischemic insult.

Although the human brain consists of only 2% of the total body weight, it utilizes 20% of in-taken oxygen. Therefore, it is reasonable to assume that some intracellular respiratory proteins exist to regulate oxygen balance in the brain, much like Mb for muscle (Merx et al., 2001). The recently discovered Ngb is expressed mainly in the brain (Burmester et al., 2000; Zhang et al., 2002; Geuens et al., 2003) and binds oxygen reversibly with a higher oxygen-binding affinity than Hb.
Thus, it is likely that Ngb plays a critical role in regulating oxygen homeostasis in the brain (Burmester et al., 2000; Trent et al., 2001; Kriegl et al., 2002). However, how Ngb might facilitate oxygen transport and diffusion into the brain cells remains largely unknown. The discovery of Ngb in astrocytes may provide some insight into how oxygen homeostasis is regulated in the brain through regulated expression of Ngb in neurons and astrocytes.

REFERENCES