Neuroglobin, a New Oxygen Binding Protein is Present in the Carotid Body and Increases after Chronic Intermittent Hypoxia

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1. INTRODUCTION

Neuroglobin (Ngb), a 151-amino-acid protein with a predicted molecular mass of 17 kD was recently identified as a member of the vertebrate globin family (Burmester and Hankeln, 2004; Mammen et al., 2002). Ngb, is predominantly expressed in nerve cells, particularly in the brain and in the retina (Burmester et al., 2000; Zhu et al., 2002), but is also expressed in other tissues (Burmester and Hankeln, 2004). The protein has three-on-three α-helical globin fold and are endowed with a hexa-coordinate heme-Fe atoms, which displays O_2 affinities and binds CO (Burmester & Hankeln, 2004).The physiological role of Ngb is not well understood, but it has been proposed that Ngb participates in several processes such as oxygen transport, oxygen storage, and NO detoxification (Burmester and Hankeln, 2004). Ngb as well as hemoglobin is a respiratory protein that reversibly binds gaseous ligands (NO and O_2) by means of the Fe-containing porphyrin ring. Ngb is concentrated in neuronal cellular regions that contain mitochondria, and its distribution is correlated with oxygen consumption rates (Pesce et al., 2003).

It has been proposed that Ngb enhances oxygen supply to neural components and may contribute to neuronal survival, because the level of Ngb is augmented during ischemia and hypoxia (Sun et al., 2001). Since the carotid body (CB) is the main oxygen chemoreceptor in the arterial blood and its high oxygen consumption, we tested if Ngb is present in the CB of normoxic rats and if chronic intermittent hypoxic may increase its level.

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2. METHODS

Two groups of six male Wistar rats weighting 200-250 g were used. One group was maintained at room air (FIO_2 = 21 %) and served as controls. The other group was kept for 12 days in a Plexiglas chamber in chronic intermittent hypoxia: FIO_2 10-11% for 12 hs, followed by 12 hs of normoxia. The chamber temperature and the PCO_2 level were kept between physiological ranges. The rats were anaesthetized with Nembutal (40 mg/kg, ip) and the CBs were dissected. The CB tissue was immersed overnight in ice cold 4 % paraformaldehyde in 0.1 M phosphate buffered saline (PBS). Tissues were then rinsed in 15 % sucrose PBS for 1 hr, and stored at 4 °C in 30 % sucrose PBS for 2 hrs. Histological section of 10 μm (n = 6 for each sample) were serially cut with a cryomicrotome (Reichert-Jung Frigocut 2800), thaw-mounted in microscope slides, fixed by immersion in acetone at 4°C for 5 min, and air-dried. Slides were stored at 4°C until use.

The presence of Ngb in the CB tissue was detected by immunohistochemistry using a polyclonal antibody (E-16, Santa Cruz Biotech Antibodies). Slides were preincubated in PBS for 5 min and then with an antibody for Ngb of goat origin (diluted 1:100 in PBS) for 30 min. at 37 °C. Slices were then washed twice in PBS for 5 min, and in Tris-HCl buffer, pH 7.6 for 10 min. A second antibody, rabbit antigoat IgG, was added for 10 min and slides were washed with PBS. The Ngb immunoreactivity (NGB-ir) staining was analyzed using the software package Image Pro Plus 4.5 for the densitometric analysis. Five random fields were chosen in each CB. The Ngb immunoreactivity was measured in optic integrated units.

3. RESULTS

NGB is present in the normoxic CB, presumable in the glomus cells. The intensity of the Ngb immunoreactivity increased significantly in the chronic intermittent hypoxic CBs. Fig. 1 shows the Ngb immunohistochemistry distribution in a control CB (Fig. 1 A, B) and in a hypoxic CB (Fig. 1 C, D).

The analysis of the Ngb immunoreactivity in the CB shows that the integrated optical intensity of Ngb increased significantly from 0.29± 0.067% in the control to 0.62 ± 0.086% in the hypoxic CBs ($P < 0.01$, Fig. 2).

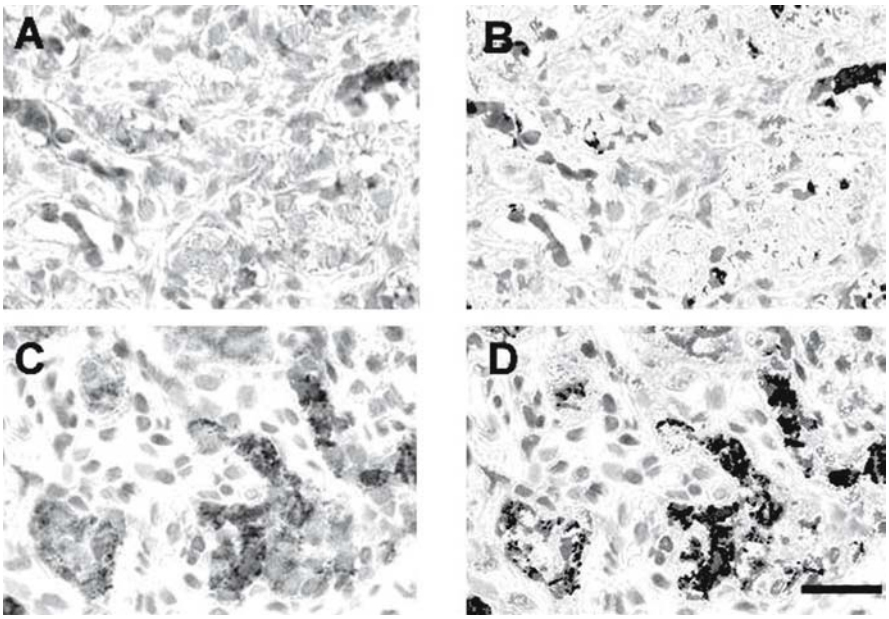

Figure 1. Immunohistochemical analysis of NGB in the rat CB, A and B, control CB. C and D, hypoxic CB. B and D, after having distinguished the NGB expression by the image processing (Magnification: 400 x).

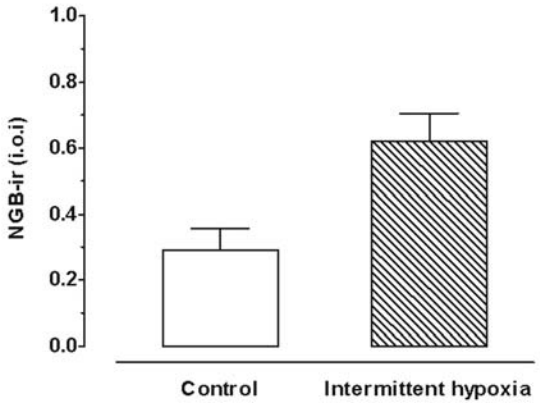

Figure 2. Integrated optical intensity of Ngb immunoreactivity in 6 control and 6 hypoxic CBs. The difference between both groups was statistically significant ($P < 0.01$).

4. DISCUSSION

To our knowledge, this is the first study showing that Ngb is expressed in the CB tissue under normoxic condition, and its expression increased during hypoxia. Regardless the small size, the CB has the highest blood flow reported for any organ and high oxygen consumption (Gonzalez et al., 1994). Therefore, it is likely that Ngb may work in the CB as a "neuronal globin protein", providing oxygen to the respiratory chain of CB cells. In addition, Ngb also may act as a sensor to detect cellular oxygen concentration, with an affinity comparable to myoglobin (P_{50} of 1-2 Torr; Burmester & Hankeln, 2004).

Although, Ngb may storage and transport oxygen, it has been proposed that Ngb participates in several processes. Hypoxia upregulates the expression of Ngb in neurons, suggesting that Ngb protects neurons against hypoxic damage. In fact, Sun et al. (2001) found that hypoxia stimulates Ngb transcription in cultured neurons, and antisense inhibition of Ngb expression increases hypoxic neuronal injury, whereas overexpression of Ngb provides resistance to hypoxia. These findings are consistent with a role for Ngb in promoting neuronal survival after hypoxic insults. Ngb may also be involved in the detoxification of nitric oxide and other reactive oxygen species (ROS) that probably are generated under hypoxic conditions. Thus, the presence of Ngb as “a respiratory protein” in CB may have important physiological effects such as NO and ROS detoxication.

A prominent feature of cell adaptation to hypoxia is the increased expression of hypoxic-inducible proteins (Bunn & Poyton, 1996; Lahiri et al., 2002). In the CB, it is well known that hypoxia stimulates the production of hypoxia-inducible factor-1, nitric oxide synthase, and tyrosine hydroxilase (Di Giulio et al., 2003). All of these proteins may exert protective effects through diverse mechanisms, but their hypoxic-responsiveness depends on O_2-binding proteins such as Ngb (Dewilde et al., 2001), which can sense hypoxia and trigger appropriate cell adaptative responses (Burmester & Hankeln, 2004).

In summary, in addition to an oxygen-storage role for Ngb in the CB, we cannot excluded that Ngb may act as an oxygen sensor to detect tissue oxygen levels, or has a protective role for neuronal and glomus cell survive. Further studies are needed to establish the role of Ngb in the CB.

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