Short-term hypoxia/reoxygenation activates the angiogenic pathway in rat caudate putamen

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In response to hypoxia, tissues have to implement numerous mechanisms to enhance oxygen delivery, including the activation of angiogenesis. This work investigates the angiogenic response of the hypoxic caudate putamen after several recovery times.

Adult *Wistar* rats were submitted to acute hypoxia and analysed after 0 h, 24 h and 5 days of reoxygenation. Expression of hypoxia-inducible factor-1 alfa (HIF-1α) and angiogenesis-related genes including vascular endothelial growth factor (VEGF), adrenomedullin (ADM) and transforming growth factor-beta 1 (TGF-β1) was determined by both RT-PCR and ELISA. For vessel labelling, lectin location and expression were analysed using histochemical and image processing techniques (fractal dimension).

Expression of *Hif-1α, Vegf, Adm* and *Tgf-β1* mRNA rose immediately after hypoxia and this increase persisted in some cases after 5 days post-hypoxia. While VEGF and TGF-β1 protein levels increased parallel to mRNA expression, ADM remained unaltered. The quantification of the striatal vessel network showed a significant augmentation at 24 h of reoxygenation.

These results reveal that not only short-term hypoxia, but also the subsequent reoxygenation period, up-regulate the angiogenic pathway in the rat caudate putamen as a neuroprotective mechanism to hypoxia that seeks to maintain a proper blood supply to the hypoxic tissue, thereby minimizing the adverse effects of oxygen deprivation.

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1. Introduction

Diminished oxygen delivery and tissue oxygen deprivation are the consequence as well as the cause of many neurological, cardiovascular, and respiratory disorders (Mathur *et al.* 1999; Janssens *et al.* 2000). To overcome such situations of hypoxia, cells express a variety of genes which allow adaptation to decreased oxygen availability. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that regulates the adaptive response to hypoxia. HIF-1 is a basic helix-loop-helix/PAS protein consisting of the constitutively expressed β-subunit (HIF-1β) and one of two oxygen-regulated α-subunits (HIF-1α or HIF-2α) (Wenger 2002). In hypoxia, the α/β heterodimeric HIF complex regulates a great number of target genes involved in angiogenesis, vasodilation, erythropoiesis, and glycolysis by binding to hypoxia response elements (HREs) in the promoter regions of such genes (Singh *et al.* 2012). The HIF-1-dependent target genes include vascular endothelial growth factor (VEGF), adrenomedullin (ADM), transforming growth factor-beta 1 (TGF-β1), inducible nitric oxide synthase (iNOS) and erythropoietin (EPO) (Bani Hashemi *et al.* 2008).

One potential mechanism to counterbalance tissue hypoxia is the induction of angiogenesis. Hypoxia stimulates vessel growth through the up-regulation of numerous proangiogenic pathways. In this sense, hypoxia has been shown to affect vessel patterning, maturation, and function (Cassavaugh and Lounsbury 2011; Krock *et al.* 2011). The

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most potent proangiogenic factor is VEGF, a selective endothelial mitogen and vascular permeability factor, which is generally inducible by hypoxia (Ferrara and Davis-Srnyth 1997). There are at least five different VEGF homodimeric isoforms of 206, 189, 165, 145 and 121 amino acids, termed VEGF-$A_{206, 189, 165, 145}$ and $_{121}$, which are produced by alternative splicing (Jussila and Alitalo 2002). VEGF is a direct target of HIF-1 as well as other factors related to cell division and migration, such as TGF-β. The TGF-β family of growth factors mediates vascular development and regulates endothelial responses to mechanical, inflammatory and hypoxic stress. The important role of TGF-β in vascular physiology is indicated by defective vasculogenesis and striking vascular inflammation, leading to death in mice null for TGF-β receptors (Dickson *et al.* 1995; Sanford *et al.* 1997). Particularly, TGF-β1 has been reported to modulate the activity of VEGF (Berse *et al.* 1999; Chávez *et al.* 2000; Renner *et al.* 2002). ADM is another HIF-1-dependent vasoactive target gene with an angiogenic effect, in part mediated by promoting VEGF expression. As a regulatory peptide, ADM has the capacity to dilate cerebral vessels and increase vascular permeability (Withers *et al.* 1996). ADM is up-regulated under hypoxic conditions and influences the recovery of blood flow in ischemic tissues (Iimuro *et al.* 2004; Knowles *et al.* 2004).

The caudate putamen is a basal ganglion of the brain that is particularly vulnerable to the hypoxic damage (Erecinska and Silver 1996). It contains a high density of dopaminergic nerve terminals and neuronal connections from the glutamatergic corticostriatal pathway. However, little is known about the angiogenic response of this brain ganglion under hypoxic conditions. In this light, the present study seeks to elucidate the effect of hypoxic challenge on the angiogenic pathway in the rat caudate putamen. It bears mentioning that this is the first time-course study which examines the behaviour of angiogenesis-related genes, as well as the vessel network in the adult rat striatum submitted to hypoxia and analysed after several reoxygenation times.

2. Methods

2.1 *Animals*

The study was performed on mature adult (4–5 months old) male albino Wistar rats kept under standard conditions of light and temperature and allowed *ad libitum* access to food and water. All the experiments were conducted according to E.U. guidelines on the use of animals for biochemical research (86/609/EU), as well as to the Guiding Principles in the Care and Use of Laboratory Animals, endorsed by the American Physiological Society.

2.2 *Experimental procedure*

The acute hypobaric hypoxia was carried out as previously published by our group (Lopez-Ramos *et al.* 2005; Rus *et al.* 2010a). Briefly, animals were placed in a chamber connected to a vacumm pump with a controlled air inflow and outflow. The acute hypobaric hypoxia was induced by reducing the barometric pressure to 225 mm Hg, resulting in a 48 mm Hg oxygen partial pressure. These conditions were maintained for 20 min. The ascent and descent speeds were kept at less than 1.000 feet/min. After the hypoxia period, animals were kept under normobaric normoxic conditions for different reoxygenation times (0 h, 24 h and 5 days), and then were sacrificed. Control animals were sacrificed after being maintained for 20 min in the chamber under normobaric normoxic conditions.

A total of 20 albino *Wistar* rats were used for the biochemical experiments (5 animals per experimental group). After the corresponding reoxygenation times, the rats were killed by cervical dislocation and the striatum was immediately removed, rinsed in saline solution, and stored at −80°C until used. For histochemistry, 20 rats (5 animals per experimental group) were anaesthetized with Ketolar (15 mg/100 g BW; Parke Davis, Madrid, Spain) and Rompun (1:5 v/v diluted in Ketolar; Bayer, Leverkusen, Germany), and then perfused in each reoxygenation time. The caudate putamen was removed, rinsed in saline solution, and fixated.

2.3 *Quantitative Real-Time Polymerase Chain Reaction (RT-PCR) for Hif-1α (hypoxia-inducible factor-1 alfa), Vegf (vascular endothelial growth factor), Adm (adrenomedullin) and Tgf-β1 (transforming growth factor-beta 1)*

The caudate putamen was homogenized in sterile PBS buffer (1:3 w/v) with a homogenator (Pellet Pestle Motor Cordless, Kontes, USA), and total RNA was directly isolated using PeqGold Microspin Total RNA kit (PeqLab, Erlangen, Germany) according to the manufacturer's protocol. cDNA was synthesized from 1.5 µg total RNA using iScript cDNA Synthesis Kit (Bio-Rad), also following the manufacturer's instructions.

FAM-labelled rat *Hif-1α* (Assay ID: Rn00577560_m1), *Vegf-a* (Assay ID: Rn00582935_m1), *Adm* (Assay ID: Rn00562327_m1), and *Tgf-β1* (Assay ID: Rn99999016_m1) TaqMan gene expression assays were purchased from Applied Biosystems. VIC labelled endogenous reference gene *18S* ribosomal RNA (Assay ID: Hs99999901_s1) TaqMan gene expression assay was also purchased from Applied Biosystems. *18S* ribosomal RNA has been reported to be the most appropriate housekeeping gene for hypoxia experiments (Nagelkerke *et al.* 2010).

RT-PCR reactions were carried out in the CFX-96™ thermal cycler (Bio-Rad) according to Applied Biosystems

amplification conditions, and following the manufacturer's protocol for absolute quantification. For each sample, expression levels for the transcripts of interest were normalized to that of the endogenous *18S* ribosomal RNA, and data were calculated as fold expression relative to the average of the control group. The relative expression of *Hif-1α, Vegf-a, Adm,* and *Tgf-β1* was calculated by the 2[–ΔΔC(T)] method (Li *et al.* 2006).

2.4 *Enzyme-linked immunosorbent assay (ELISA) for VEGF (vascular endothelial growth factor), ADM (adrenomedullin) and TGF-β1 (transforming growth factor-beta 1)*

The caudate putamen was homogenized in PBS buffer (1:3 w/v) with a homogenator (Pellet Pestle Motor Cordless, Kontes, USA). Homogenates were centrifuged at 40.000 rpm during 30 min and the supernatant was collected. VEGF, TGF-β1, and ADM protein concentration was determined using Rat VEGF-A ELISA kit (Ab Frontier, Cat. No. LF-EK50417), Rat TGF-β1 ELISA kit (Ab Frontier, Cat. No. LF-EK50357) and ELISA kit for rat Adrenomedullin (Uscn Life, Cat. No. E90220Ra) respectively, according to the manufacturer's protocol. Total protein concentrations were determined by the Bradford method (Bradford 1976), using bovine serum albumin as the standard. Final VEGF, ADM and TGF-β1 values were referred to the total protein concentration in the initial extracts.

2.5 *Histological procedures for vessel labelling*

Biotinylated lectin from Lycopersicon esculentum specific for *N*-acetyl-glucosamine and *N*-acetyl-polylactosamine oligomers is used as the best marker for brain endothelium (Mazzetti *et al.* 2004). Deeply anaesthetized animals were perfused through the left ventricle with 50 mL of 0.01 M phosphate-buffered saline (PBS; pH 7.4), and then with 250 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The caudate putamen was removed and then post-fixed for a further 4 h in the same fixative at room temperature. Each sample was then cryoprotected by immersion overnight at 4°C in 0.1 M PB containing 30% sucrose. After that, the caudate putamen was embedded in O.C.T medium and frozen in 2-methylbutane pre-chilled in liquid nitrogen. Serial rostro-caudal sections (15 µm) were cut using a cryostat (Leica CM1950, Germany).

Free-floating sections were washed in Tris-HCl (1 M) and incubated in 1:100 Lycopersicon esculentum Lectin (10 µg/mL, Sigma-Aldrich, Ref. L0651) diluted in Tris-HCl buffer (1 M) overnight at 4°C. After the incubation, sections were washed in PBS buffer (0.01 M, pH 7.4), and then incubated in Cy3-Streptavidin (Sigma-Aldrich, Ref. S6402) solution diluted in PBS buffer (1:50) for 30 min.

2.6 *Quantification of fractal dimension of vascular surface area*

Vascular surface area was quantified by computerized-assisted image analysis using ImageJ (an NIH image analysis and processing software downloaded free from *http://rsbweb.nih.gov/ij/*). One random 1.56 mm^2 field (image 10×) on each section, and five random sections (from rostral to caudal striatum) for each rat, were digitally captured from a fluorescence microscope (Olympus BX51). The fractal dimension was estimated using the box-counting method as previously described (Di Ieva *et al.* 2007), since it makes it possible to estimate the global complexity of a set of irregularly shaped objects, like two-dimensional vascularity (Abu-Eid and Landini 2003).

2.7 *Statistical analysis*

Data were expressed as mean±SD (standard deviation). The statistical treatment to evaluate significant differences between groups was performed with SPSS 17.0 software. The data followed neither a normal distribution (tested with Kolmogorov-Smirnov test; α-value=0.05), nor the principle of homoscedasticity (tested with Levene test; α-value=0.05); therefore they were tested using the Kruskal Wallis test. The degree of statistical significance was established by applying the U Mann Whitney test to compare differences between means. The statistically significant differences *vs*. the control group were expressed as *$p<0.05$; **$p<0.001$.

3. Results

3.1 *mRNA expression of hypoxia-inducible genes*

The expression of *Hif-1α, Vegf, Adm* and *Tgf-β1* was determined by RT-PCR using TaqMan technology. The mRNA expression of these hypoxia-inducible genes was significantly increased immediately after the hypoxic stimuli. Particularly, *Hif-1α* mRNA expression (figure 1) augmented at 0 h ($p<0.05$) and 5 days post-hypoxia ($p<0.05$) in comparison to the control group. The quantitative analysis of *Vegf* mRNA expression (figure 2) showed a statistically significant increase throughout the reoxygenation period (0 h, 24 h and 5 days: $p<0.05$). Finally, while *Adm* mRNA levels (figure 3) followed the same pattern as *Hif-1α* mRNA (0 h, 5 days: $p<0.05$), *Tgf-β1* (figure 4) only rose immediately after hypoxia (0 h: $p<0.05$).

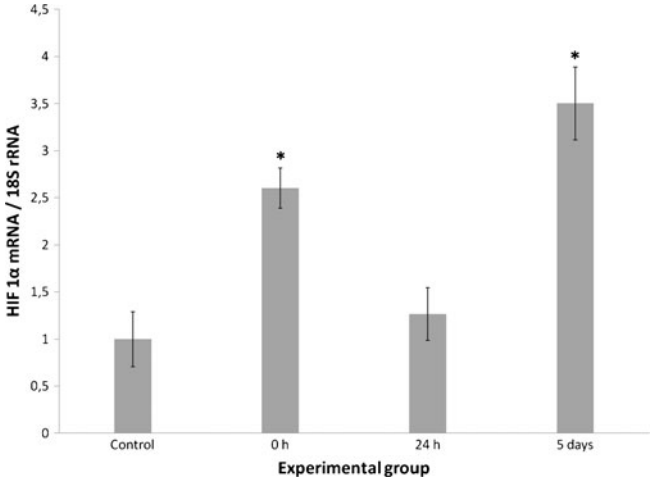

Figure 1. Hypoxia-inducible factor-1 alfa (*Hif-1α*) mRNA expression in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results were expressed as arbitrary units. Results are mean values of three independent experiments and five animals per group. All experiments were performed in triplicates, and the values were used to calculate the ratio of *Hif-1α* to *18S* ribosomal RNA, with a value of 1 used as the control. The statistically significant differences *vs.* the control group were expressed as *p<0.05.

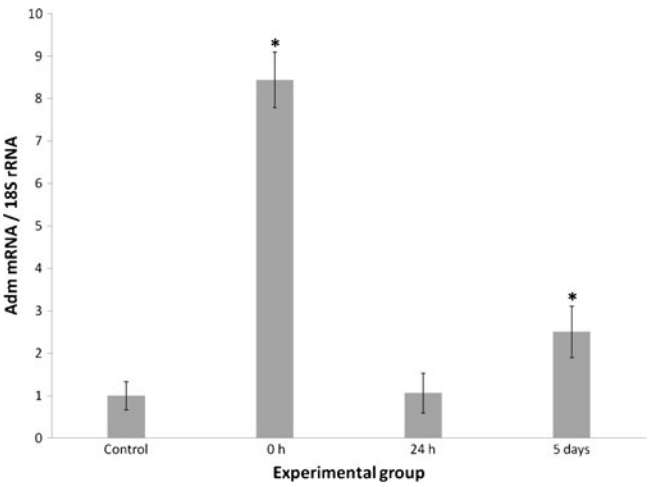

Figure 3. Adrenomedullin (*Adm*) mRNA expression in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results were expressed as arbitrary units. Results are mean values of three independent experiments and five animals per group. All experiments were performed in triplicates, and the values were used to calculate the ratio of *Adm* to *18S* ribosomal RNA, with a value of 1 used as the control. The statistically significant differences *vs.* the control group were expressed as *p<0.05.

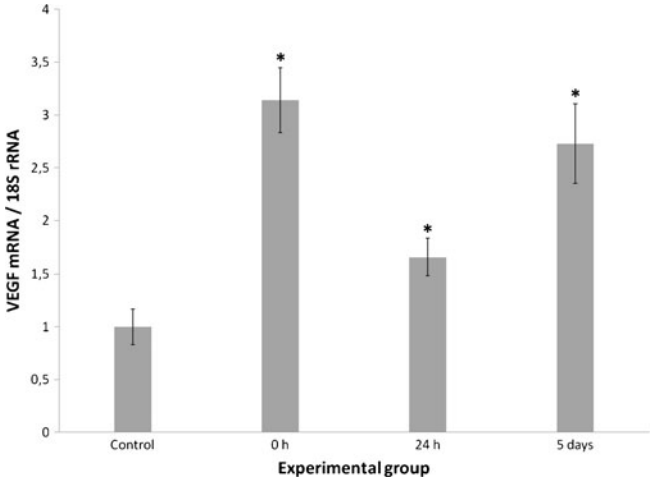

Figure 2. Vascular endothelial growth factor (*Vegf*) mRNA expression in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results were expressed as arbitrary units. Results are mean values of three independent experiments and five animals per group. All experiments were performed in triplicates, and the values were used to calculate the ratio of *Vegf* to *18S* ribosomal RNA, with a value of 1 used as the control. The statistically significant differences *vs.* the control group were expressed as *p<0.05.

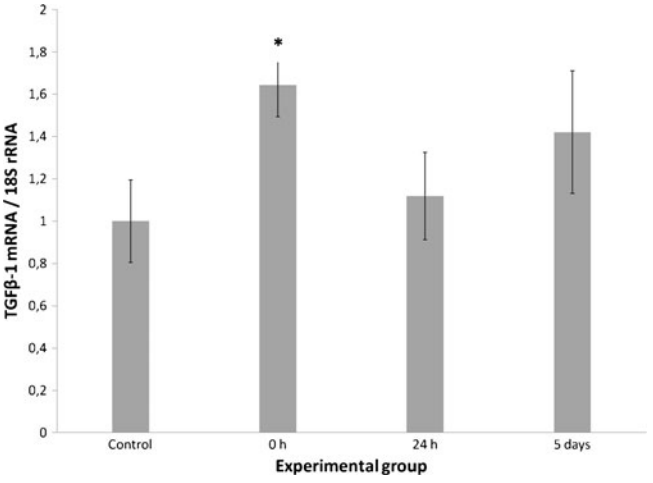

Figure 4. Transforming growth factor-beta 1 (*Tgf-β1*) mRNA expression in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results were expressed as arbitrary units. Results are mean values of three independent experiments and five animals per group. All experiments were performed in triplicates, and the values were used to calculate the ratio of *Tgf-β1* to *18S* ribosomal RNA, with a value of 1 used as the control. The statistically significant differences *vs.* the control group were expressed as *p<0.05.

3.2 *Protein expression of proangiogenic genes*

The VEGF protein level (figure 5) significantly rose from 0 h to 5 days of reoxygenation ($p<0.001$), showing a trend similar to that of *Vegf* mRNA expression. However, ADM protein expression remained unaltered in the hypoxic groups *vs*. control (figure 6). In parallel with the greater *Tgf-β1* mRNA expression, the TGF-β1 protein level (figure 7) increased just after the hypoxic insult (0 h: $p<0.05$).

3.3 *Location and quantification of vascular surface area*

For vessel labelling, lectin location and expression were analysed using both histochemical and image processing techniques, including the box-counting method for estimating the fractal dimension. No labelling was detected in the negative controls when lectin was omitted. The microphotographs showed that the blood vessels were homogeneously distributed throughout the rat caudate putamen (figure 8). The cylindrical shape of the vessels is visible, as is the circular or elliptical lumen. The quantification of the vessel network in this brain ganglion (figure 9) showed a significant increase at 24 h post-hypoxia ($p<0.05$).

4. Discussion

Hypoxia is a common cause of cell death and is involved in many disease processes. The activation of angiogenesis is a potential mechanism to counterbalance tissue hypoxia. Striatal neurons are highly vulnerable to hypoxia (Erecinska and Silver 1996), but few works have investigated the response of this brain ganglion to situations of oxygen deficiency. Therefore, the angiogenic response of the hypoxic caudate putamen remains unknown, despite the importance of these situations, which we have formerly investigated in several vital organs (central nervous system, CNS: Martínez-Romero *et al.* 2006;

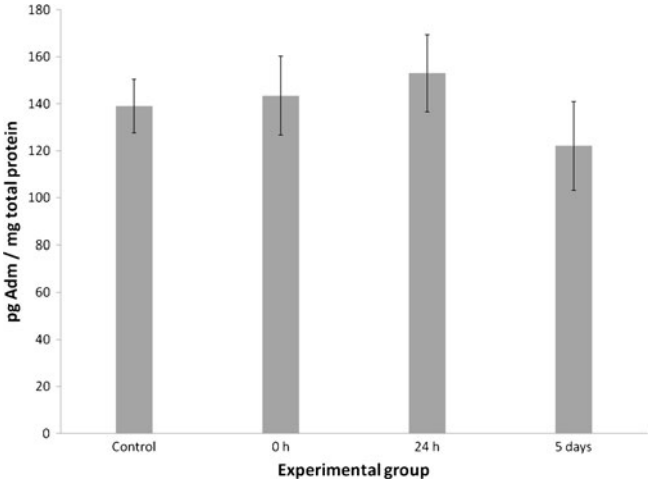

Figure 6. Adrenomedullin (ADM) protein expression (ELISA) in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results are mean values of three independent experiments with five animals per group. There were no statistically significant differences *vs*. the control group.

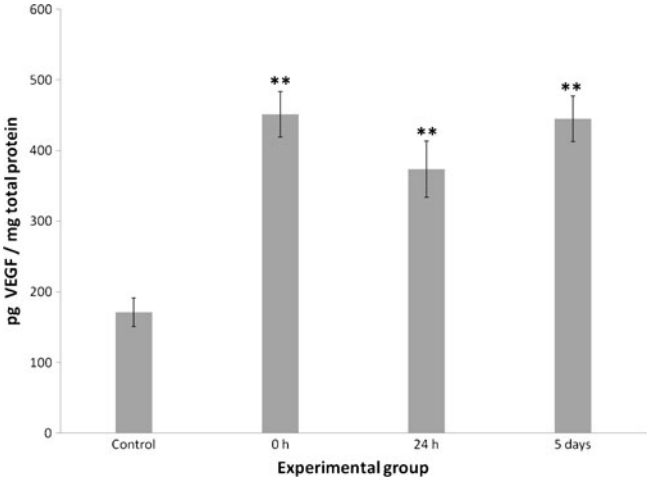

Figure 5. Vascular endothelial growth factor (VEGF) protein expression (ELISA) in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results are mean values of three independent experiments with five animals per group. The statistically significant differences *vs*. the control group were expressed as $**p<0.001$.

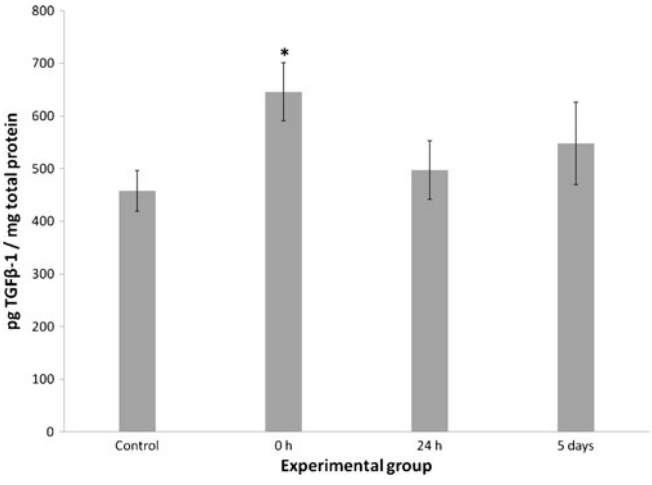

Figure 7. Transforming growth factor-beta 1 (TGF-β1) protein expression (ELISA) in the rat caudate putamen. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results are mean values of three independent experiments with five animals per group. The statistically significant differences *vs*. the control group were expressed as $*p<0.05$.

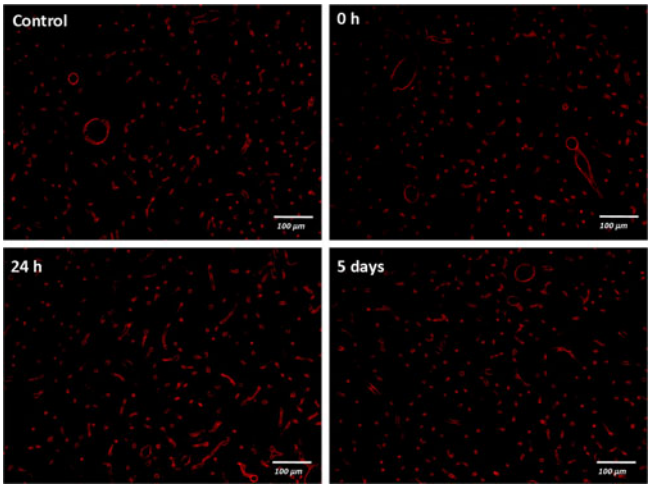

Figure 8. Representative microphotographs of histological sections of rat caudate putamen stained for lectin histochemistry. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. The blood vessels are homogeneously distributed throughout the rat caudate putamen in all the experimental groups. The cylindrical shape of the vessels is visible, as is the circular or elliptical lumen.

Cañuelo *et al.* 2007; lung: Rus *et al.* 2010b; heart: Rus *et al.* 2011).

Hypoxia-inducible factor-1 (HIF-1) is a transcriptional regulator of oxygen homeostasis and a key factor to generate adaptive responses through the up-regulation of various target genes involved in angiogenesis, including vascular endothelial growth factor (VEGF), adrenomedullin (ADM) and transforming growth factor-beta 1 (TGF-β1). Our results show an increase in *Hif-1α* expression in the caudate putamen of rats submitted to acute hypoxia/reoxygenation, suggesting that such situations may induce the implementation of a number of cellular mechanisms to boost oxygen delivery to the hypoxic striatum. Similar results have previously been described in the hypoxic brain (Wiener *et al.* 1996) and cerebellum (Kaur *et al.* 2006).

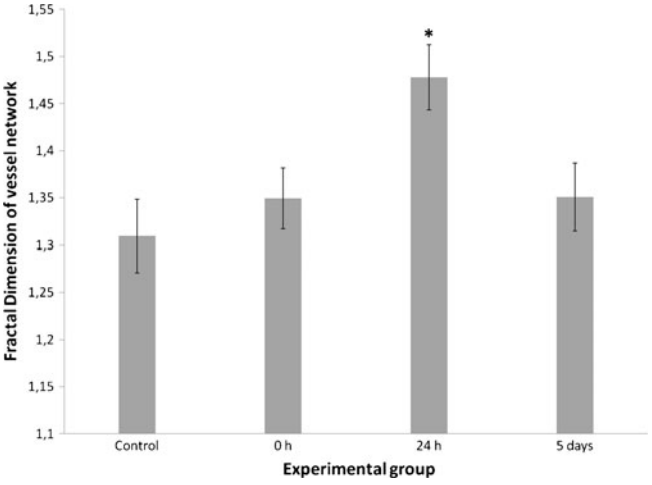

Figure 9. Quantitative data from image analysis (fractal dimension of vascular surface area) of histological sections of rat caudate putamen stained for lectin histochemistry. Experimental groups: Control and 0 h, 24 h and 5 days post-hypoxia. Results are mean values of 25 microphotographs (five microphotographs per animal and five animals per group). The statistically significant differences *vs*. the control group were expressed as *$p<0.05$.

Regarding the proangiogenic genes, the expression of *Vegf, Adm* and *Tgf-β1* followed the same pattern of *Hif-1α* and increased after short-term hypoxia. In this sense, the up-regulation of these vasoactive target genes may indicate the functional activation of HIF-1. Particularly, *Vegf* mRNA expression significantly rose throughout the reoxygenation period until 5 days post-hypoxia, suggesting that this HIF-1-target gene may play an important role in the response of the striatum to acute hypoxia. The upregulation of *Vegf* under hypoxic conditions agrees with other works performed in rat brain (Bani Hashemi *et al.* 2008), rat cerebellum (Kaur *et al.* 2006), and mouse brain (Kuo, *et al.* 1999). Furthermore, VEGF protein expression increased parallel to *Vegf* gene expression, implying an effective coordination between transcription and translation in order to develop a successful angiogenic response in the hypoxic caudate putamen. In this context, increased VEGF protein expression has previously been reported after hypoxia in the striatum (Mammen *et al.* 2011), brain (Kuo *et al.* 1999), and cerebellum (Kaur *et al.* 2006).

Similarly, *Adm* gene expression was significantly activated in the rat caudate putamen at 0 h and 5 days post-hypoxia. Previous studies confirm the induction of *Adm* in the CNS after hypoxia (Serrano *et al.* 2008; Bani Hashemi *et al.* 2008). However, this greater mRNA expression did not correspond to a higher ADM protein level. In agreement, Peebles *et al.* did not detect enhanced ADM protein expression in the CNS after hypoxia (Peebles *et al.* 2008). Nevertheless, increases in both ADM gene and protein expression have been reported in the hypoxic CNS (Nakanishi *et al.* 2004; Serrano *et al.* 2008). This discrepancy may be due to the different hypoxia models used, as well as to the organs or tissues studied. Based on these results, we propose that the stress induced by our hypoxia/reoxygenation model is not sufficiently strong or prolonged to cause the translation of the *Adm* mRNA. This hypothesis may be confirmed by previous results, which corroborated that the activation of ADM gene and protein depends on the time of exposure to hypoxia, so that the greatest increases occur after long exposure to hypoxia (Kitamuro *et al.* 2000). Furthermore, it has been suggested that hypoxia may decrease the percentage of translatable *Adm* mRNA (Hwang *et al.* 2007). In this context, the lack of a change in ADM level despite an

increase in *Adm* mRNA has been shown after blockade of glycolysis with 2-deoxyglucose (Autelitano *et al.* 1999). This metabolic alteration has been proposed to mimic several features of hypoxia (Yuan *et al.* 1994; Bright *et al.* 1995). The 2-deoxyglucose mediated inhibition of ADM secretion most likely results in reduced ADM receptor activation, which might lead to stimulation of *Adm* gene expression (Autelitano *et al.* 1999). Both hypoxia and 2-deoxyglucose have been reported to increase intracellular Ca^{2+} levels, which may ultimately lead to vasoconstriction (Yuan *et al.* 1994; Bright *et al.* 1995). However, whether the hypoxia or 2-deoxyglucose mediated activation of *Adm* gene expression without greater ADM level is directly dependent on intracellular Ca^{2+} concentrations is yet to be established.

TGF-β1 is another hypoxia-inducible gene, which is involved in stabilizing HIF-1α (McMahon *et al.* 2006). Parallel to *Hif-1α, Vegf* and *Adm* expression, *Tgf-β1* mRNA was induced immediately after the hypoxic insult in the rat caudate putamen. Similar results were found in the brain after hypoxia (Klempt *et al.* 1992; Huang *et al.* 2010). In addition, TGF-β1 protein expression also increased at 0 h of reoxygenation, suggesting a role for this transcription factor in the angiogenic response of the caudate putamen to hypoxia.

Finally, to test whether the upregulation of the angiogenesis-related genes led to an effective physiological change in the vasculature of the hypoxic caudate putamen, we analysed the vessel network in this brain ganglion using the glycoprotein lectin, an effective endothelial marker (Mazzetti *et al.* 2004). Our data reflected a significantly denser striatal vessel network at 24 h of reoxygenation, implying vascular adaptation after short-term hypoxia in the rat caudate putamen. In this context, brain vessel density has been reported to increase in the striatum after chronic hypoxia, leading to greater regional blood flow (LaManna *et al.* 1992; Patt *et al.* 1997). On the other hand, all the angiogenic factors analysed augmented immediately after the hypoxic episode, while the increase in the vessel network was noted at 24 h post-hypoxia. This lack of correlation may have occurred because the process of creation of blood vessels is long and complex and can last from hours to days, depending on the tissue and physiological state (Krupinski *et al.* 1994; Pettersson *et al.* 2000). On the other hand, our results also show that the blood vessel density returned to basal values 5 days after the hypoxic stimulus, although VEGF protein levels remained increased. In this sense, once the vessel network has expanded enough to re-establish the blood flow to the hypoxic tissues (24 h post-hypoxia), it may return to the baseline level. Similar data were reported by Pichiule and LaManna (2002), showing that the capillary density in the rat cerebral cortex was increased by chronic hypoxia and fell to basal levels after 7 days of normoxic recovery. Moreover, other pro-angiogenic factor, TGF-β1, was not increased from 24 h post-hypoxia in the hypoxic striatum, which may contribute partly to the regression of the vessel network thereafter.

These results may imply that striatal cells can detect situations of oxygen deficiency and respond to acute hypoxia by activating *Hif-1α*, which might initiate the angiogenic pathway by activating *Vegf, Adm* and *Tgf-β1*. As mentioned above, mRNA expression of these hypoxia-inducible genes immediately rose after hypoxia and returned to basal levels at 24 h of reoxygenation. In consequence, VEGF and TGF-β1 protein expression augmented in the rat striatum, leading to greater vessel density at 24 h post-hypoxia. Nevertheless, a VEGF-induced increase in the vessel network can generate the haemodynamic steal phenomena (Wang *et al.* 2005). That is, an increase in the vessel density without an augmentation in the blood supply can lead to reduced blood flow. Accordingly, the significant proangiogenic response detected immediately after acute hypoxia in the rat striatum may have led to a profuse vessel network capable of inducing the haemodynamic steal phenomena during the post-hypoxia period. This phenomena may thereby provoke transient hypoxia with the subsequent re-activation of the hypoxia-inducible genes (*Hif-1α, Vegf* and *Adm*), as revealed by our data at 5 days of reoxygenation.

In short, endogenous acute hypoxia-inducible mechanisms in the rat caudate putamen include the activation of the angiogenic pathway. Our results reveal that the proangiogenic factors, especially VEGF, were induced by both short-term hypoxia and the following reoxygenation period. As a result of this induction, a significant increase in the striatal vessel network was detected in this brain ganglion, in order to reduce the negative consequences of oxygen deprivation.

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