FEBS Letters 580 (2006) 4430–4434

Adult neural stem cells express glucose transporters GLUT1 and GLUT3 and regulate GLUT3 expression

Martin H. Maurer*, H. Kenny Geomor, Heinrich F. Bürgers, Dominik W. Schelshorn, Wolfgang Kuschinsky

Department of Physiology and Pathophysiology, University of Heidelberg, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

Received 12 May 2006; revised 21 June 2006; accepted 4 July 2006

Available online 14 July 2006

Edited by Vladmir Skulachev

Abstract In the brain, glucose is transported by GLUT1 across the blood–brain barrier and into astrocytes, and by GLUT3 into neurons. In the present study, the expression of GLUT1 and GLUT3 mRNA and protein was determined in adult neural stem cells cultured from the subventricular zone of rats. Both mRNAs and proteins were coexpressed, GLUT1 protein being 5-fold higher than GLUT3. Stress induced by hypoxia and/or hyperglycemia increased the expression of GLUT1 and GLUT3 mRNA and of GLUT3 protein. It is concluded that adult neural stem cells can transport glucose by GLUT1 and GLUT3 and can regulate their glucose transporter densities.

Keywords: Neural stem cell; Neurosphere; Glucose transporter GLUT1; GLUT3; Rat brain subventricular zone

1. Introduction

Glucose constitutes the main substrate for the nervous tissue. In the brain, it is transported by a family of facilitative transmembrane transport proteins, the GLUT (glucose transporter)/SLC2A (solute carrier) family. Currently, 14 members of this sugar/polyol transporter protein family have been described [1], varying in their tissue of expression, and their substrate specificity and affinity. In the brain, GLUT1 is expressed at the blood–brain barrier and in astrocytes, whereas GLUT3 is responsible for the neuronal glucose uptake [2].

Only in the recent decade, neural stem cells have been identified as the source of constant cerebral plasticity lasting into adulthood [3–5]. Neural stem cells are characterized by their potential to self-renew and to differentiate into progeny of neuronal and glial phenotype [6]. Almost nothing is known about the energy metabolism of neural stem cells in the adult brain with regard to similarities and differences compared to neurons and astrocytes.

In the current study, we therefore addressed the questions, whether neural stem cells express glucose transporter molecules and if this expression can be regulated by typical pathophysiological stimuli such as hypoxia and exposure to high glucose concentrations.

2. Materials and methods

2.1. Neurosphere culture

Neural stem cells were isolated from the subventricular zone of adult rat brains as described previously [7,8]. Protocols are concordant with the policy on the use of animals, as endorsed by the National Institutes of Health, and fulfill the requirements of German law. Briefly, animals were anesthetized and sacrificed by decapitation. The subventricular zone was dissected from the brains and mechanically homogenized, washed in PBS and enzymatically digested in 10 mL of 0.01% (w/v) papain, 0.1% (w/v) dispase II (neutral protease), 0.01% (w/v) DNase I, 12.4 mM $MgSO_4$ in Hank's balanced salt solution (HBSS). The tissue was triturated by a plastic pipette tip, and incubated at room temperature for 40 min. After three times washing in 10 mL Dulbecco's Modified Eagle's Medium (DMEM)-Ham's F12 medium supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, and 2 mM L-glutamine, the cells were resuspended in 1 mL neurobasal-B27 medium (Invitrogen, Karlsdorf, Germany) and the cell number was counted. Cells were plated in 2 mL dishes at 200,000 cells in B27-neurobasal medium supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 20 ng/mL EGF, 20 ng/mL FGF-2, and 2 μg/mL heparin. About 4/5 of the medium was replaced weekly, and cells were passaged every 10–14 days. The neurospheres were cultured for 6–10 weeks in 5% CO_2 at 37 °C before use. Whereas the normal glucose concentration of the Neurobasal medium is 25 mM [9], the high glucose medium contained 50 mM glucose.

2.2. Hypoxia chamber

For hypoxia, the neurospheres were transferred to a Modular Incubator Chamber MIC-101 (Billups-Rothenberg, Del Mar, CA, USA) as described [10,11]. The chamber was flooded for 15 min with a water-saturated, 37 °C gas mixture consisting of 5% CO_2 in nitrogen. Then the chamber was sealed tightly and incubated at 37 °C for 24 h. The pO_2 and the temperature in the hypoxia chamber was continuously measured by an oxygen microoptode (Fibox3; Presens, Regensburg, Germany) [12,13].

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the neurospheres using the RNeasy kit (Qiagen, Hilden, Germany). The mRNA was reversely transcribed using the Omniscript RT kit (Qiagen, Hilden, Germany). Specific cDNA target sequences for GLUT1-4 (Table 1) were amplified by a PCR reaction mixture consisting of 1 μL cDNA template, 10 μM each primer (for sequences, see Table 1), PCR Master Mix [50 u/mL *Taq* DNA Polymerase, 400 μM dNTPs, 3 mM $MgCl_2$ and 1× reaction buffer] (Promega ReadyMix, Madison, WI, USA). The amplification protocol was 5 min initial denaturation at 94 °C, 35 amplification cycles of 1 s denaturation at 94 °C, 1 s annealing at 60 °C, and 5 s elongation at 72 °C. The protocol was completed by 5 min final elongation at 72 °C. RT-PCR products were separated in 1.2% agarose/TBE gel electrophoresis and compared to the expression of GAPDH and β-actin as housekeeping genes.

*Corresponding author. Fax: +49 6221 544561.
E-mail address: maurer@uni-hd.de (M.H. Maurer).

Abbreviations: GLUT, glucose transporter molecule

0014-5793/$32.00
doi:10.1016/j.febslet.2006.07.012

Table 1
PCR primer sequences

Name	Forward sequence (5′ → 3′)	Reverse sequence (5′ → 3′)
GLUT1	GCCTGAGACCAGTTGAAAGCAC	CTGCTTAGGTAAAGTTACAGGAG
GLUT2	TTGGCTTTCACTGTCTTCACT	CTTCCTTTTCTTTCCTCATCTC
GLUT3	AACAGAAAGGAGGAAGACCA	CGCAGCCGAGGGGAAGAACA
GLUT4	AGTGCCTGAGTCTTCTTT	TGATGTTAGCCCTGAGTAG
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	GAGGACCAGGTTGTCTCCTG	GGATGGAATTGTGAGGGAGA
β-actin (ACTB)	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA

GLUT1-4 sequences according to [17].

2.4. Western blotting

Protein extracts of neurosphere whole cell lysates were suspended in Laemmli sample buffer containing 0.5 M Tris, 10% SDS, 10% glycerol, 0.05% bromophenol blue, and 5% 2-mercaptoethanol [14], and denaturated at 65 °C for 15 min. One hundred micrograms of protein was separated in 12.5% polyacrylamide gels and transferred to 0.2 μm nitrocellulose membranes by wet blotting (300 mA for 30 min). Membranes were blocked with blocking buffer (1× TBS, 0.1% Tween-20, 5% (w/v) nonfat dry milk) for 1 h at room temperature and stained with the specific antibody for GLUT1 (0.5 μg/mL; Abcam, Cambridge, UK), or GLUT3 (0.5 μg/mL; Biogenesis, Poole, UK), respectively. The primary antibody was detected by a secondary antibody, HRP-IgG goat-anti-rabbit (1:10,000; Pierce, Rockford, IL, USA). Chemiluminescence was visualized by mixing 0.45 mM *p*-coumaric acid, 12.5 mM luminol [5-amino-2,3-dihydro-1,4-phthalazinedione] in 100 mM Tris, pH 8.5, with 0.018% H_2O_2 in 100 mM Tris, pH 8.5 and exposure on X-ray films (MRDM; Eastman Kodak, Rochester, NY, USA) for 30–60 s.

2.5. Immunostaining

Several drops of cell culture medium containing neurospheres and single cells were placed on poly-L-lysine-coated glass microscope slides, sealed with a cover slip and frozen at −80 °C for at least 20 min. For staining, the cover slip was removed quickly and the cells were fixed for 20 min in 4% paraformaldehyde in PBS, pH 7.4, with 0.2% Tween-20 (PBST). Slides were blocked for 30 min at room temperature in Seablock (Pierce, Rockford, IL, USA) and incubated with the respective primary antibody overnight at 4 °C in a humidified chamber using primary antibodies against GLUT1 (1:100; Abcam, Cambridge, UK), or GLUT3 (1:100; Biogenesis, Poole, UK), respectively. The next day, slides were washed 3 times for 5 min in PBST and incubated with an appropriate fluorescent secondary antibody for 1 h at room temperature. The secondary antibody was an anti-rb Cy2-, Cy3- or Texas-Red-conjugated IgG (1:100, Jackson ImmunoResearch, West Grove, PA, USA), respectively. After washing twice for 5 min each in PBST and once in PBS, nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1:5000, Molecular Probes, Eugene, OR, USA) for 10 min. Slides were mounted in Mowiol-containing fluoprotective media [15] and stored at 4 °C in the dark.

2.6. Image analysis

Images were recorded using a digital camera (DC500, Leica, Bensheim, Germany) on a fluorescent microscope (DM-R HC, Leica Microsystems, Bensheim, Germany; B×50, Olympus, Hamburg, Germany). Agarose gel images and Western blot bands as well as immunostaining were quantified using ImageJ (http://rsb.info.nih.gov/ij/) [16].

3. Results and discussion

3.1. Adult neural stem cells express for GLUT1 and GLUT3 mRNA and protein, but not GLUT2 and GLUT4

First, we evaluated the expression of the four main GLUT molecules, GLUT1-4, on the transcriptional level. RT-PCR experiments using mRNA isolated from adult rat brain neurospheres and GLUT1-4 specific oligonucleotide primers (sequences from [17]) led to the amplification of GLUT1 and GLUT3 transcripts (Fig. 1A). No amplification product was seen for GLUT2 and GLUT4. Comparing GLUT1 and GLUT3, the expression of GLUT1 appeared stronger than GLUT3 expression. We used the two "housekeeping" genes β-actin and glyceraldehyde-3-phosphate as internal standards for the RT-PCR experiments.

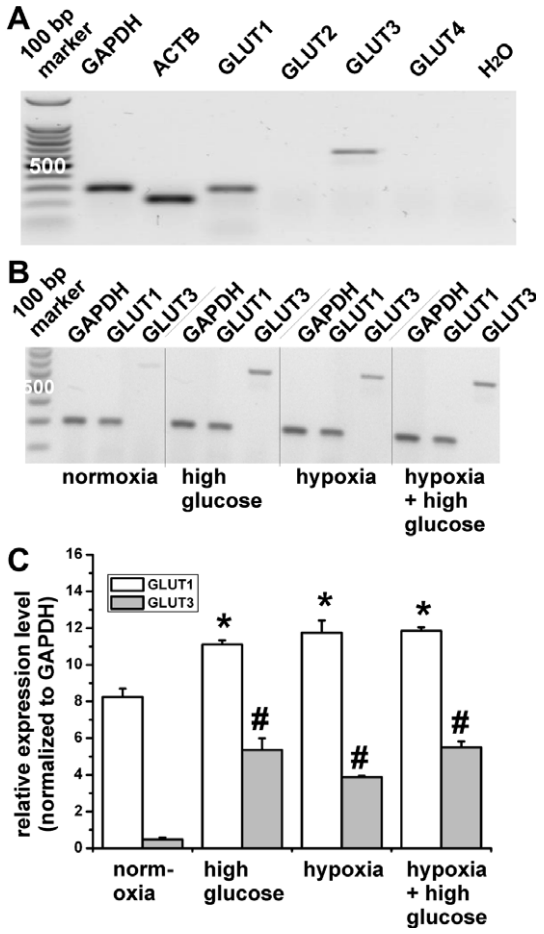

Fig. 1. Expression of GLUT1 and GLUT3 mRNA in neurospheres. (A) Agarose electropherogram of RT-PCR products. Adult neural stem cells express the mRNA encoding for GLUT1 and GLUT3, but not for GLUT2 and GLUT4. The expression of GLUT1 is stronger than GLUT3. The negative control (H_2O instead of cDNA) did not show any PCR product. (B) Agarose electropherogram of RT-PCR products. (C) Quantitation of panel (B): GLUT1 mRNA expression shows a mean increase of about 1.4-fold under hypoxia, high glucose medium, and combination of both. GLUT3 mRNA expression is weak under normoxic conditions, but its expression increases about 10-fold under hypoxia, high glucose concentration, and combination of both (means ± SD from $N = 3$ experiments each; *, $P < 0.05$, compared to GLUT1; #, $P < 0.05$, compared to GLUT3).

Second, we evaluated the expression of GLUT1 and GLUT3 proteins in neurospheres. We found immunopositive cells for both GLUT1 and GLUT3 in the adult neural stem cells (Fig. 2). To address the question whether there are distinct subpopulations within the neurospheres expressing either GLUT1 or GLUT3, we performed double-immunostaining for both molecules. We found co-expression of both GLUT1 and GLUT3 in the same cells, indicating that there are no exclusive subpopulations for either molecule (Fig. 2).

In the brain, astrocytes and endothelial cells express GLUT1, whereas neurons express GLUT3 [18,19]. In the present study, we found co-expression of both molecules in adult neural stem cells. This finding is consistent with the developmental stage of adult neurospheres, in which the cell lineage specification is not decided and which have the potential to differentiate into both neurons and astrocytes. It can be expected that the immunoreactivity for one of the glucose transporters is lost later during differentiation into either neurons or astroctyes. Whereas the timepoint during differentiation is unknown at which the expression of one of these transporters is downregulated in the adult neural stem cells, a differential regulation has been shown for GLUT1 and GLUT3 during early postnatal development [20,21]. In addition, GLUT1 levels in the brain increased in relation to glucose supply and tissue growth, whereas GLUT3 levels increased in relation to functional neuronal activity [20]. Therefore, our results appear to be compatible with the differentiation potential of neurospheres.

With regard to adult neurogenesis, the present study is the first study to describe GLUT1 and GLUT3 expression in adult neural stem cells. In embryonic stem cells of stage E10.5, GLUT1 expression has been confirmed [22], whereas GLUT3 has not been investigated.

3.2. Hypoxia and high glucose concentration increase GLUT1 and GLUT3 expression

Incubating cell cultures in low oxygen atmospheres is a common model of in vitro ischemia. With regard to the brain, interruption of the cerebral circulation results immediately in the stop of nutrient supply, including oxygen and glucose, which is followed by an irreversible detrimental cascade of events, including glutamate excitotoxicity, peri-infarct depolarizations, inflammation, and apoptosis [23]. Since neurons do not have noteworthy energy storage capacities, they depend on a constant influx of glucose. In the present study, we incubated neurospheres in an oxygen-free atmosphere consisting of 95% N_2 and 5% CO_2 for constant cell culture medium pH. The classical pO_2 electrode cannot detect pO_2 levels below a certain threshold of about 0.5% O_2 concentration, because it consumes oxygen for its function and its measurement is disturbed by CO_2 [24]. Therefore, we used an optode system based on an oxygen-catalyzed fluorometric energy transfer, which is linear in the low pO_2 range [12,13]. The oxygen concentration in the hypoxia chamber dropped within 20 min to 0 mmHg under flushing the chamber with 5% CO_2 in N_2 and was constant throughout the experiment (data not shown).

We also introduced high glucose concentrations (doubled compared to the normal cell culture medium) as an additional cellular stressor. Whereas the normal glucose medium was balanced for osmolarity, we cannot rule out that the changes in the high glucose medium are influenced by hyperosmotic pressure. On the other hand, cellular morphology did not change

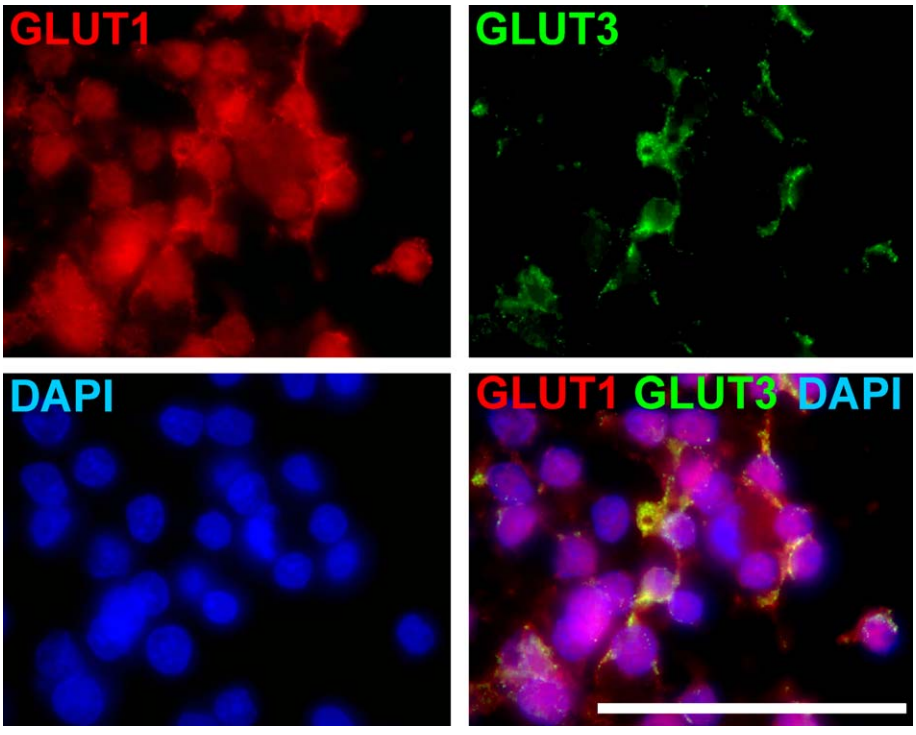

Fig. 2. Expression of GLUT1 and GLUT3 transporter proteins in neurospheres. Immunostaining shows the expression of GLUT1 (red) and GLUT3 (green) in adult neural stem cells. The overlay of GLUT1 and GLUT3 images reveals the co-expression of GLUT1 and GLUT3 in the same cells, excluding distinct subpopulations expressing either protein isoform. Moreover, the expression of GLUT1 appears to be higher than GLUT3 expression (nuclear counterstain with DAPI; Scale bar, 50 μm).

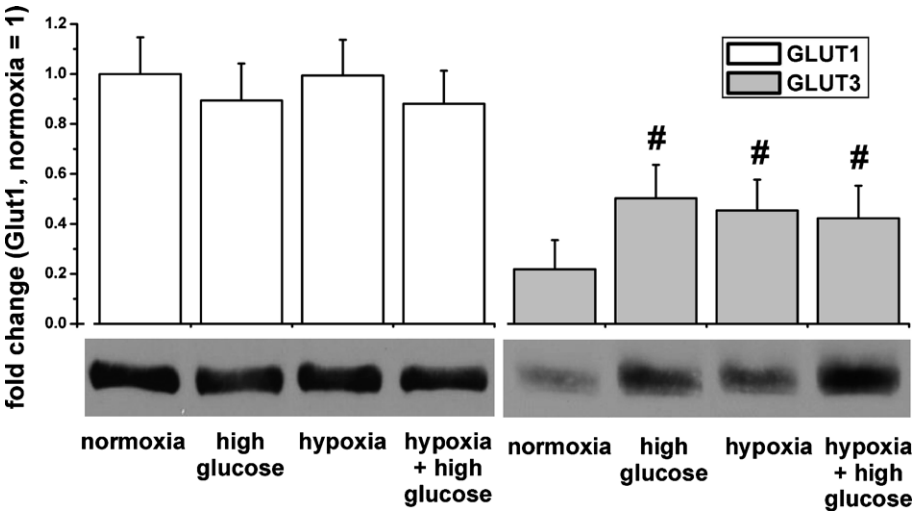

Fig. 3. Expression of GLUT1 and GLUT3 proteins in hypoxia and high glucose medium. Western blotting of neurosphere protein lysates did not show an increase in GLUT1 concentration, whereas GLUT3 increased under hypoxia, high glucose concentration, and the combination thereof (means ± SD from $N = 3$ experiments normalized to GLUT1 expression in normoxia; #, $P < 0.05$, compared to GLUT3 expression in normoxia).

in either medium in the present study, indicating only a minor influence of osmolarity. Of note, the glucose concentration in the Neurobasal cell culture medium (25 mM) is higher than the *in vivo* physiological glucose concentration in blood plasma (5 mM), although the medium used in the present study is the recommended one for neural stem cells [25]. Therefore, we cannot rule out that the *in vivo* situation of GLUT1 and GLUT3 expression in the brain environment is different from the *in vitro* experimental setup of this study.

Both hypoxia and high glucose concentrations increased GLUT1 and GLUT3 expression on the mRNA and protein level (Figs. 1 and 3). Whereas GLUT1 mRNA concentrations increased moderately (about 1.4-fold), GLUT3 mRNA expression increased strongly (about 10-fold) in hypoxia, high glucose medium and when both were combined. With regard to protein concentrations, we did not detect changes in GLUT1 expression under these conditions (Fig. 3). This result may be explained by its high baseline expression level under normal conditions. On the other hand, GLUT3 protein concentration was increased about 2-fold (Fig. 3) under all three conditions, starting from a low expression level.

So far, the effects of hypoxia and hyperglycemia had not been measured in adult neural stem cells before. Effects compatible with those of the present study have been found earlier in neuronal and astroglial cell cultures, in which both GLUT1 and GLUT3 transcripts were found to be up-regulated by hypoxia [26]. On the other hand, effects of hyperglycemia were divergent depending on the experimental models [27]. In the present study, the expression of GLUT1 and GLUT3 in neurospheres indicates cell biological properties similar to those of a combination of neurons and astrocytes, which appears plausible when taking into consideration that the adult neural stem cells have the potential to differentiate into neurons and astrocytes.

In summary, we found the expression of GLUT1 and GLUT3 mRNA and protein in neurospheres from the adult rat subventricular zone. GLUT1 mRNA and protein showed higher expression levels than GLUT3. GLUT1 and GLUT3 mRNA and GLUT3 protein expression was up-regulated by hypoxia, high glucose concentrations, or combination of both. However, GLUT1 remained the dominant glucose transporter.

Acknowledgements: We thank Mrs. Tilly Lorenz and Mrs. Maria Harlacher for technical assistance. This work was supported by the German Ministry of Education and Research (BMBF) within the National Genome Research Network NGFN-2 (01GS0496, to M.H.M. and W.K.), the German Research Foundation (DFG, MA 2492/2-2, to M.H.M. and W.K.) and the Estate of Friedrich Fischer (to M.H.M.).

References

[1] Joost, H.G. et al. (2002) Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am. J. Physiol. Endocrinol. Metab. 282, E974–E976.
[2] Duelli, R. and Kuschinsky, W. (2001) Brain glucose transporters: relationship to local energy demand. News Physiol. Sci. 16, 71–76.
[3] Maurer, M.H. and Kuschinsky, W. (2006) Towards a molecular signature of neural stem cells in: Neural Stem Cell Research (Greer, E.V., Ed.), Nova Science Publishers., Hauppauge, NY.
[4] Taupin, P. (2005) Adult neurogenesis in the mammalian central nervous system: functionality and potential clinical interest. Med. Sci. Monit. 11, RA247–RA252.
[5] Lie, D.C., Song, H., Colamarino, S.A., Ming, G.L. and Gage, F.H. (2004) Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu. Rev. Pharmacol. Toxicol. 44, 399–421.
[6] Gage, F.H. (2000) Mammalian neural stem cells. Science 287, 1433–1438.
[7] Maurer, M.H., Feldmann Jr., R.E., Fütterer, C.D. and Kuschinsky, W. (2003) The proteome of neural stem cells from adult rat hippocampus. Proteome Sci. 1, 4.
[8] Maurer, M.H., Feldmann, R.E., Fütterer, C.D., Butlin, J. and Kuschinsky, W. (2004) Comprehensive proteome expression profiling of undifferentiated vs. differentiated neural stem cells from adult rat hippocampus. Neurochem. Res. 29, 1129–1144.
[9] Brewer, G.J., Torricelli, J.R., Evege, E.K. and Price, P.J. (1993) Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J. Neurosci. Res. 35, 567–576.
[10] Potrovita, I. et al. (2004) Tumor necrosis factor-like weak inducer of apoptosis-induced neurodegeneration. J. Neurosci. 24, 8237–8244.
[11] Maurer, M.H., Tripps, W.K.C., Feldmann, R.E. and Kuschinsky, W. (2003) Expression of vascular endothelial growth

factor and its receptors in rat neural stem cells. Neurosci. Lett. 344, 165–168.
[12] Holst, G., Glud, R.N., Kühl, M. and Klimant, I. (1997) A microoptode array for fine-scale measurement of oxygen distribution. Sensor. Actuator. B 38–39, 122–129.
[13] Klimant, I., Kühl, M., Glud, R.N. and Holst, G. (1997) Optical measurement of oxygen and temperature in microscale: strategies and biological applications. Sensor. Actuator. B 38–39, 29–37.
[14] Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
[15] Osborn, M. and Weber, K. (1982) Immunofluorescence and immunocytochemical procedures with affinity purified antibodies: tubulin containing structures. Meth. Cell. Biol. 24, 97–132.
[16] Girish, V. and Vijayalakshmi, A. (2004) Affordable image analysis using NIH Image/Image J. Indian J. Cancer 41, 47.
[17] Merriman-Smith, R., Donaldson, P. and Kistler, J. (1999) Differential expression of facilitative glucose transporters GLUT1 and GLUT3 in the lens. Invest Ophthalmol Vis. Sci. 40, 3224–3230.
[18] Vannucci, S.J., Maher, F. and Simpson, I.A. (1997) Glucose transporter molecules in brain: delivery of glucose to neurons and glia. Glia 21, 2–21.
[19] Maher, F., Vannucci, S.J. and Simpson, I.A. (1994) Glucose transporter proteins in brain. FASEB J. 8, 1003–1011.
[20] Vannucci, S.J. (1994) Developmental expression of GLUT1 and GLUT3 glucose transporters in rat brain. J. Neurochem. 62, 240–246.
[21] Zeller, K., Vogel, J. and Kuschinsky, W. (1996) Postnatal distribution of Glut1 glucose transporter and relative capillary density in blood-brain barrier structures and circumventricular organs during development. Develop. Brain Res. 91, 200–208.
[22] Cai, J., Cheng, A., Luo, Y., Lu, C., Mattson, M.P., Rao, M.S. and Furukawa, K. (2004) Membrane properties of rat embryonic multipotent neural stem cells. J. Neurochem. 88, 212–226.
[23] Dirnagl, U., Iadecola, C. and Moskowitz, M.A. (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 22, 391–397.
[24] Clark, L.C. (1956) Monitor and control of blood and oxygen in tissue. Trans. Am. Soc. Artif. Intern. Organs 2, 41–48.
[25] Wachs, F.P. et al. (2003) High efficacy of clonal growth and expansion of adult neural stem cells. Lab. Invest. 83, 949–962.
[26] Bruckner, B.A., Ammini, C.V., Otal, M.P., Raizada, M.K. and Stacpoole, P.W. (1999) Regulation of brain glucose transporters by glucose and oxygen deprivation. Metabolism 48, 422–431.
[27] Simpson, I.A. et al. (1999) Blood–brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. J. Neurochem. 72, 238–247.