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# HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) IN EXPERIMENTAL BRAIN ISCHEMIA

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**INTRODUCTION.** Recently, a specific defense system against hypoxia has been described which uses the hypoxia-inducible factor-1 (HIF-1) as transcription factor. HIF-1 coordinates the response to prolonged hypoxia which pertains to glycolysis (e.g., lactate dehydrogenase = LDH-5), glucose transport, vasodilation, and angiogenesis[1]. The level of the HIF-1α subunit is oxygen-dependent, and the protein concentration is mainly regulated via degradation in the proteasome[1], involving a novel class of oxygen-sensing proline-4-hydroxylases[2]. HIF-1α combines with the constitutively expressed HIF-1β protein to form HIF-1[1]. Ischemia and hypoxia of the brain are major events in cardiac arrest and stroke. In this report, we examined changes of HIF-1α mRNA and the target gene, LDH-5, in the forebrain of rats after transient global brain ischemia[3] or in chronic oligemia[4].

**METHOD**. Global brain ischemia was induced in halothane-anesthetized, normothermic Wistar rats by using two-vessel occlusion and hypotension for 12.5 min. The recirculation periods were 30 min, 1, 3, 6 h, and 1, 2, 3, and 7 days (n = 2-5). To induce chronic oligemia, permanent bilateral common carotid artery occlusion (BCCAO) was used in Wistar rats for 1 h, 6 h, 1 day, and 7 days (n = 4-5). Rat-specific polyclonal antibodies for HIF-1α were used in immunohistochemistry[5] on vibratome sections of 4% paraformaldehyde-fixed brains. *In situ* hybridization was carried out using radioactive, rat-specific antisense probes to HIF-1α mRNA and LDH-5 mRNA[6], followed by quantitative film autoradiography.

**RESULTS.** Using a rat-HIF-1α specific polyclonal antiserum in immunohistochemical studies on control rat brains, we obtained generalized neuronal labelling in forebrain regions; astrocytes or vessels were unreactive. In parallel, *in situ* hybridization showed widespread expression of HIF-1α mRNA in control brains. HIF-1α mRNA was increased in CA1 at 1 day after global ischemia (optical density = 197 ± 14 % of control; ANOVA, $p < 0.05$) but not at earlier time points. The increased signal was clearly localized to the vulnerable CA1 neuronal layer in emulsion-coated sections. HIF-1α-like immunoreactivity slightly decreased in CA1 by 1 day, and then disappeared at 3 and 7

days due to cell death. No increase in HIF-1α mRNA or LDH-5 mRNA was found in regions with reactive glial changes. By contrast, a rapid and widespread increase of HSP70 mRNA was noted in the forebrain between 30 min and 1 day after global brain ischemia. In the oligemia model, constant levels of HIF-1α mRNA were observed, whereas LDH-5 mRNA was upregulated in the neocortex at 1 day with a columnar pattern.

**DISCUSSION.** Our study indicates that HIF-1α is constitutively expressed in forebrain neurons, implying that neurons can perform immediate regulatory responses to hypoxia and ischemia. The presently used HIF-1α antibodies indicate a high expression level in neurons, while detection of active or degraded forms of HIF-1α remains to be defined. Upregulation of HIF-1α mRNA occurred at 1 day of recirculation, and it was limited to the vulnerable CA1 neurons that are bound to die by 2–3 days after ischemia. Thus, HIF-1 regulation differs from a typical stress response gene such as HSP70 that is expressed early after ischemia and in a widespread fashion. It remains to be determined whether an additional hypoxic situation preceded cell death in CA1 neurons and whether changes in HIF-1α mRNA are in reaction to a consumption of HIF-1 protein. Oligemia per se affected the HIF-1 target gene, LDH-5, suggesting that hypoxia prevailed in the chronic carotid occlusion model.

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