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BRAIN RESEARCH

Short Communication

Induction of CITED2 expression in the rat hippocampus following transient global ischemia

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ARTICLE INFO

Article history:
Accepted 4 December 2005
Available online 24 January 2006

Keywords:
CITED2
HIF-1
Transient forebrain ischemia
Gene expression
Dentate gyrus

Abbreviations:
CITED2, cAMP-responsive element binding protein (CBP)/p300-interacting transactivators with glutamic acid (E) and aspartic acid (D)-rich tail 2
HIF-1, hypoxia inducible factor-1
DG, dentate gyrus

ABSTRACT

CITED2 is implicated in the modulating the activity of HIF-1 which is a major transcription factor involved in ischemia-related gene expression. Following transient forebrain ischemia, we found that CITED2 was induced in a subset of brain regions including dentate gyrus of the hippocampal formation and piriform cortex. Because CITED2 was not induced in cultured neurons exposed to oxygen–glucose deprivation, we concluded that hypoxia is not sufficient to trigger its induction.

Hypoxia is a major cause of brain damage in stroke, heart failure, and related diseases. The early induction of transcriptional modulators under hypoxic condition may influence subsequent hypoxia-induced effector gene expression. A well-characterized transcription factor implicated in hypoxia-induced gene expression is hypoxia inducible factor-1 (HIF-1) (Kietzmann et al., 2001; Semenza, 2001). The role of HIF-1 in hypoxia-induced neuronal death appears to be dependent on the degree of hypoxia and the cellular milieu, because it both protects and enhances

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doi:10.1016/j.brainres.2005.12.016

ischemia-induced neuronal death (Bergeron et al., 2000; Chavez and LaManna, 2002; Kietzmann et al., 2001). These results suggest that other hypoxia-dependent transcriptional modulators and their interactions are involved in hypoxia-dependent downstream events. Recently, a negative regulator of HIF-1, CITED2 [cAMP-responsive element binding protein (CBP)/p300-interacting transactivators with glutamic acid (E) and aspartic acid (D)-rich tail 2 (also called MRG-1, MSG-1, and p35srj) has been identified (Bhattacharya et al., 1999). However, there is no direct evidence that the CITED2 is involved in regulating brain hypoxia-related gene expression. To begin to address this issue, we examined the expression of CITED2 in the rat brain following transient global ischemia.

Only marginal levels of CITED2 mRNA were detected in the normal adult brain. Following transient global ischemia, there was a rapid induction of CITED2 mRNA in the dentate gyrus (DG) of the hippocampal formation, and in the piriform cortex (PIR). Induction of CITED2 mRNA in the DG was apparent at 3 h; it was sustained until 12 h, and returned to basal level by 48 h (Fig. 1). Induction was also observed in the PIR by 3 h after ischemia. However, no induction was observed in other brain regions including other hippocampal subfields (CA1–CA3) until 48 h after ischemia, although most forebrain regions were exposed to hypoxic conditions in our experimental condition (Kietzmann et al., 2001). In contrast, expression of HIF1 was selectively induced in the CA1 region of the hippocampal formation 12–48 h after ischemia. On the other hand, neither CITED2 nor HIF-1 mRNA expressions appeared to be altered in CA3 subfield. Quantitative analysis of CITED2 and HIF-1 gene expression in the CA1 and DG regions is presented in Fig. 2. By 72 h, expression of HIF-1 had fallen in the CA1 region, most likely due to the massive loss of CA1 neurons by this time (Fig. 1). Because we found limited induction of CITED2 in the DG in the face of global hypoxia, we tested whether hypoxic stress was sufficient to induce CITED2 in cultured cerebral cortex neurons under oxygen–glucose deprivation (OGD) (Fig. 2). Following a 60-min OGD treatment, there was rapid induction of HIF-1 mRNA. On the other hand, CITED2 expression was not induced, suggesting that its expression was not directly regulated under hypoxic conditions.

We have shown that the transcription factor, CITED2, is selectively expressed in the DG of the hippocampal formation during reperfusion following transient global ischemia. Because induction of CITED2 was not observed in cultured cerebral cortex neurons after OGD treatment, we reasoned that hypoxia is not sufficient to induce CITED2. Expression of CITED2 is reported to be induced by several cytokines, including GM-CSF, PDGF, interferon γ, and lipopolysaccharides (Sun et al., 1998). Hence it is possible that the induction of CITED2 following ischemia is mediated by paracrine factors that are released from non-neuronal cells and are absent from our neuronal cultures. Recently, we found that CITED2 is also induced by depolarization of neurons in vitro (unpublished observations). Because neuronal depolarization evokes the secretion of neurotrophic factors/neurotransmitters from

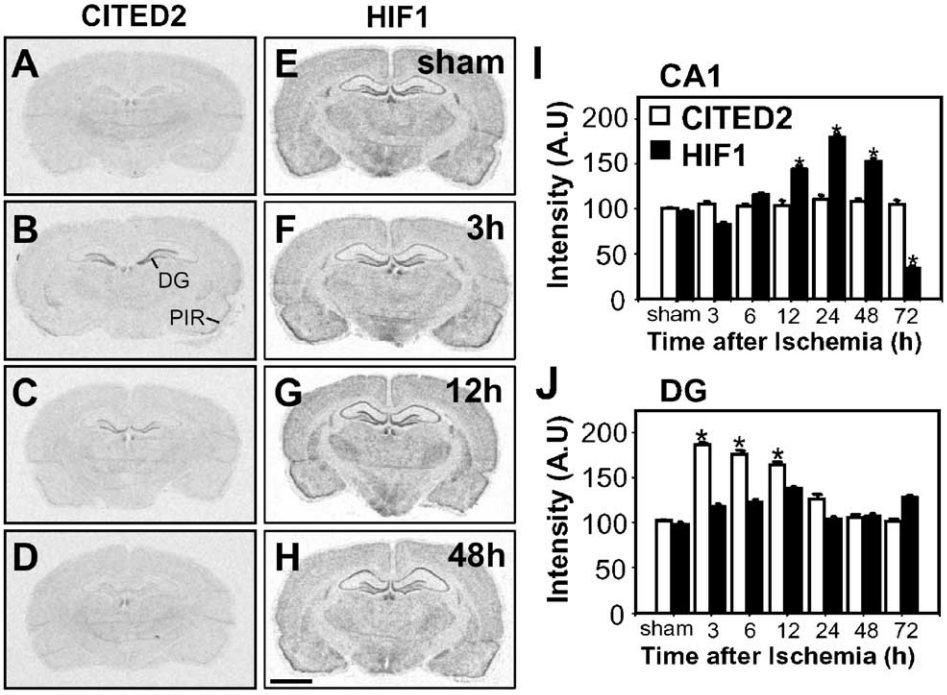

Fig. 1 – Expression of CITED2 (A–D) and HIF-1 (E–H) in sham controls (A, E), and 3 h (B, F), 12 h (C, G) and 48 h (D, H) after global ischemia. DG, dentate gyrus; PIR, piriform cortex. Scale Bar: 3.2 mm. (I–J): Quantification of CITED2 (open bars) and HIF1 (closed bars) expression in the CA1 (I) and DG (J) of the hippocampal formation. Three independent animals were analyzed at each time point. Data are means ± SE. *$P < 0.05$ relative to the sham control group by one way ANOVA followed by scheffe test.