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Neuroscience Letters 380 (2005) 17–20
Neuroscience Letters
www.elsevier.com/locate/neulet

Changes in the expression of Hes5 and Mash1 mRNA in the adult rat dentate gyrus after transient forebrain ischemia

Takayuki Kawai, Norio Takagi, Mika Nakahara, Satoshi Takeo*

Department of Molecular and Cellular Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

Received 6 December 2004; received in revised form 29 December 2004; accepted 5 January 2005

Abstract

Accumulating evidence indicates that neurogenesis in the adult brain occurs in restricted brain regions, including the hippocampal dentate gyrus and is promoted by ischemia. The mechanism responsible for ischemia-induced neurogenesis in the adult brain, however, remains unclear. Notch pathway plays a pivotal role in the regulation of the timing for differentiation and determination of the fate of neural progenitor cells in the developing nervous system. To elucidate the mechanism underlying ischemia-induced neurogenesis, we investigated changes in the expression of mRNAs of Hes5, which is a downstream target of Notch, and Mash1, a neurogenic basic helix–loop–helix factor, which is negatively regulated by Hes5, in the adult hippocampal dentate gyrus after transient forebrain ischemia. Transient forebrain ischemia was produced by four-vessel occlusion procedure in rats. The levels of Hes5 mRNA decreased on days 1 and 3 after the start of reperfusion and the decreased levels of the mRNA returned to the basal level by 5 days after ischemia. In contrast, the level of Mash1 mRNA increased on day 1 and then returned to the basal level by 3 days after ischemia. These results suggest that an inhibition of Notch activity and subsequent expression of neurogenic basic helix–loop–helix factors, including Mash1, may, at least in part, contribute to ischemia-induced neurogenesis in the adult dentate gyrus.

Keywords: Ischemia; Neurogenesis; Adult rat dentate gyrus; Hes5; Mash1

Neurogenesis in the adult brain occurs in restricted regions, including the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus [4,5]. We previously demonstrated that neurogenesis was accelerated in the adult rat hippocampal dentate gyrus after transient forebrain ischemia and that newly generated neurons acquired biochemical features of mature neurons [9]. Although neurogenesis has been well-characterized in the embryonic brain, the mechanisms responsible for ischemia-induced cell proliferation and differentiation in the adult dentate gyrus have not been fully understood. In the developmental stages of the central nervous system, many regulatory factors control proliferation and differentiation of neural progenitor cells. It is possible that the mechanism of neurogenesis occurred in the adult brain overlaps with that in the developing nervous system.

Notch is a transmembrane receptor protein. After ligand binding to the Notch, the intracellular domain (ICD) of Notch is released by the proteolytic cleavage and translocates into the nuclei. The translocated ICD of Notch promotes the expression of target genes by binding to a transcriptional repressor CBF1/RBP-Jκ [6]. Hes1 and Hes5, which are downstream targets of Notch [11], antagonize the expression of other basic helix–loop–helix (bHLH) factors. For example, the expression of a neurogenic bHLH factor Mash1, which is an important regulator of neurogenesis in the central nervous system [1], is inhibited by Hes proteins [8]. Thus, targeted mutation of *Notch1* reduced *Hes*5 expression and up-regulated *Mash1* expression [14]. Furthermore, deletion of Hes1 and Hes5 induced premature neuronal differentiation [7,10,19]. Therefore, Notch pathway has been implicated in

* Corresponding author. Tel.: +81 426 76 4583; fax: +81 426 76 5560.
E-mail address: takeos@ps.toyaku.ac.jp (S. Takeo).

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doi:10.1016/j.neulet.2005.01.005

the regulation of the timing of differentiation and determination of the fate of neural progenitor cells. It is reported that Notch and Hes5 were expressed in the adult SVZ and SGZ where neurogenesis was observed [16]. In the present study, to determine whether Notch pathway contributes to neurogenesis in the hippocampal dentate gyrus after transient forebrain ischemia, we assessed mRNA levels of Hes5, a downstream target of Notch, and Mash1 that is negatively regulated by Hes5, in the dentate gyrus of the rat brain.

Adult male Wistar rats (8 weeks old, Charles River Japan, Atsugi, Japan) had free access to food and water according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan. The experimental protocol was approved by the Committee of Animal Care and Use of Tokyo University of Pharmacy and Life Science. Transient (15 min) forebrain ischemia was produced by four-vessel occlusion procedure as previously described [17,18]. Only rats that showed a completely flat electroencephalogram and a loss of consciousness were chosen in the present study. Sham-operated animals received exactly the same surgical procedure without arterial occlusion.

On various days after the start of reperfusion, animals were sacrificed by decapitation. The hippocampal dentate gyrus was dissected on ice and flash frozen in liquid nitrogen. The total RNA was isolated using the ISOGEN solution (Nippon gene, Tokyo, Japan) according to the manufacturer's protocol. The total RNA (1 μg) was converted into cDNA using a Reverse Transcription System (Promega, Madison, WI). The following primers were used: Hes5-forward: 5′-CGCATCAACAGCAGCATTGAG-3′; Hes5-reverse: 5′-TGGAAGTGGTAAAGCAGCTTC-3′ [21]; Mash1-forward: 5′-AGCAGCTGCTGGACGAGCA-3′; Mash1-reverse: 5′-CCTGCTTCCAAAGTCCATTC-3′ [13]. All samples were normalized by β-actin control bands.

The results are expressed as mean ± S.E. Statistical analysis was performed using ANOVA followed by Fisher's protected least significant difference test.

The expression of Hes5 mRNA in the dentate gyrus was decreased on days 1 and 3 after transient forebrain ischemia. The mean percentages of the expression of Hes5 mRNA on days 1 and 3 after ischemia were 17.5 ± 4.8% and 25.7 ± 5.1% of naïve control animals, respectively (Fig. 1). The decreased Hes5 mRNA returned to the level of naïve control animals on day 5 after ischemia (Fig. 1). In contrast, the expression of Mash1 mRNA in the dentate gyrus was significantly increased on day 1 after ischemia (Fig. 2). The mean percentage of the expression of Mash1 mRNA was 273.2 ± 36.8% of naïve control animals. The increased expression of Mash1 mRNA returned to the level of naïve control animals on day 3 after ischemia (Fig. 2). The levels of Hes5 and Mash1 mRNAs in the dentate gyrus of sham-operated animals were comparable to those of naïve control animals throughout the experiment (Figs. 1 and 2). We next examined the expression of Mash1 mRNA in the hippocampal CA1 region on day 1 after ischemia, to determine whether the increased expression of Mash1 mRNA occurs only in

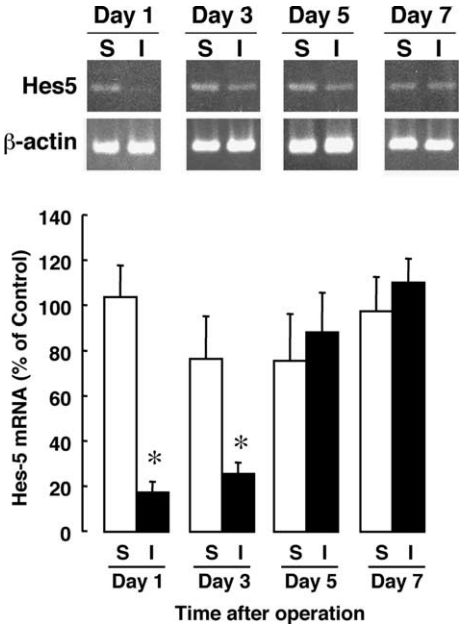

Fig. 1. Effect of transient forebrain ischemia on expression of Hes5 mRNA in the dentate gyrus of adult rats. The upper panels indicated representative RT-PCR of Hes5 and β-actin in the hippocampal dentate gyrus of the sham-operated (S) and ischemic (I) rats on days 1, 3, 5, and 7 after surgery. Semiquantified data for the level of Hes5 mRNA are shown in the lower panel. Each value was normalized against the level of β-actin mRNA and expressed as a percentage vs. the level of non-operated control animals. Values are mean ± S.E., $n = 4$ each. Significantly different from the corresponding sham-operated group ($^{*}P < 0.05$).

the restricted brain area where transient forebrain ischemia-induced neurogenesis is detected. The level of Mash1 mRNA in the CA1 region of ischemic animal was not significantly different from those of naïve control and sham-operated animals (Fig. 3).

We demonstrated that Hes5 mRNA levels were decreased on days 1 and 3 after ischemia, suggesting that the Notch signaling may be inhibited after ischemia. Hes5 proteins negatively regulate the expression of Mash1, which is known to express in neural progenitor cells and positively regulates the initial differentiation of progenitor cells into neurons [1]. Therefore, ischemia-induced decreases in Hes5 mRNA levels raise the possibility that the expression of Mash1 mRNA could be enhanced. In agreement with this assumption, transient forebrain ischemia increased the level of Mash1 mRNA in the dentate gyrus on day 1. It is noteworthy that status epilepticus that accelerates neurogenesis reduced the expression of Hes5 mRNA and promoted the expression of Mash1 mRNA in the proliferative cells located in the SGZ [3]. This finding supports the idea that decreased Hes5 and increased Mash1 mRNAs contribute to the regulation of neurogenesis in the adult dentate gyrus after ischemia. In the adult injured spinal cord tissues, the expression of Notch was enhanced, whereas neither the expression of neurogenic bHLH factors including Mash1 nor subsequent neurogenesis could be detected [20]. Furthermore, activation of Notch signaling in vitro inhibited differentiation of adult neural progenitors,