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Research Report

Brainstem monoamine pathology of neonatal hypoxic–ischemic brain damage: A model of acute stage of neonatal asphyxia

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ABSTRACT

Neonatal hypoxic–ischemic encephalopathy (HIE) is one of the most severe perinatal diseases and leads to high mortality and sometimes severe neurological sequelae. At the acute stage of HIE, it is thought to be the damage of catecholaminergic system in the brainstem. And then, HIE reflects mental development throughout the norepinephrine and serotonin systems, which mainly originates in the brainstem. Therefore, we studied both systems in the brainstem of neonatal HIE model rats with tyrosine hydroxylase (TH) and tryptophan hydroxylase (TpH) immunohistochemistry and a high-performance liquid column (HPLC) to measure norepinephrine and serotonin and their metabolism. As a result, the TH-positive and TpH-positive cell numbers significantly decreased 2 days after hypoxic–ischemic (HI) insult ($n=10$). However, 7 days after insult ($n=10$), the TH-positive and TpH-positive cell numbers had recovered in most regions. HPLC demonstrated a significant difference in the norepinephrine concentration 2 days after HI insult, but not in the other monoamines.

1. Introduction

Neonatal hypoxic–ischemic encephalopathy (HIE) induced by birth asphyxia is one of the most frequent perinatal problems and sometimes leads to severe neurological sequelae (Lipper et al., 1986; Asakura et al., 2000). There have not been any treatments that are proven to be effective, and we have taken only supportive therapy. The neonatal mortality rate depends on recovery from the acute stage of birth asphyxia. At the acute stage of HIE, a temporary increase of brain catecholamine is well known (Hedner and Lundborg, 1979, 1980a). This phenomenon is thought to damage the catecholaminergic system in the brainstem (Lagercrantz and Bistoletti, 1977).

In the neonatal period, the autonomic system is extremely vulnerable and greatly influenced by dynamic alteration from the internal to external uterine environment. More than 50% of all norepinephrine (NE) neurons in the brain are located in the locus ceruleus (LC) (Bremner et al., 1996; Ordway et al.,

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Abbreviations: HIE, hypoxic–ischemic encephalopathy; TH, tyrosine hydroxylase; TpH, tryptophan hydroxylase; NE, norepinephrine; 5-HT, serotonin; HVA, homovanillic acid; 5HIAA, 5-hydroxyindoleacetic acid; HPLC, high-performance liquid column; GFAP, glial fibrillary acidic protein; TUNEL, in situ DNA end labeling

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1997; Boulton and Eisenhofer, 1998; Nestler et al., 2001a), in which the neurons project extensively to the cerebral cortex, amygdala, hippocampus, brainstem and spinal cord (Bremner et al., 1996; Ordway et al., 1997; Boulton and Eisenhofer, 1998; Nestler et al., 2001a). This NE system is implicated in several important functions, such as regulation of the sleep–wake cycle and working memory (Bremner et al., 1996; Ordway et al., 1997; Boulton and Eisenhofer, 1998; Nestler et al., 2001a). The serotonin (5-HT) neurons are mainly located in the raphe nuclei complex of the brainstem, and project to cortices (Brunner and Hen, 1997; Rueter et al., 1997; Nestler et al., 2001b). The relationship of the nuclei and its projected region is well known. The dorsal raphe (DR) preferentially innervates the cerebral cortex, thalamus, striatum and substantia nigra (Brunner and Hen, 1997; Rueter et al., 1997; Nestler et al., 2001b), and the median raphe innervates the limbic system, including the hippocampus and septum (Brunner and Hen, 1997; Rueter et al., 1997; Nestler et al., 2001b). The 5-HT system is thought to play an important role in neuropsychiatric conditions and to be easily influenced by mood alteration or various stress (Brunner and Hen, 1997; Rueter et al., 1997). At the acute stage of HIE, within 6 h after insult, the NE and 5-HT concentrations increase transiently. However, the catecholamine and 5-HT conditions in the HI-insulted brainstem are unknown.

To understand neurological sequelae, it is necessary to know the pathophysiology of the acute stage. Recently, autistic behavior or learning disability may be caused by NE and/or 5-HT system maldevelopment (Takase et al., 2005). In the present study, we investigated these neurotransmitter systems' alteration of the brainstem in the acute stage of neonatal HIE.

2. Results

2.1. *Evaluation of cell damage in the brainstem of neonatal HIE*

A small number of TUNEL-positive cells were observed in the brainstem on post-HI day 2 ($n=10$) and day 7 ($n=10$) (Figs. 1A–D), although many in situ DNA end labeling (TUNEL)-positive cells were seen in the cerebral cortex and striatum (data not shown). On the other hand, more glial fibrillary acidic protein (GFAP)-positive cells and fibers were widely distributed in the pontine tegmentum on post-HI day 2 than on day 7 (Figs. 1E, F), but there were a few GFAP-positive cells and fibers in the medulla oblongata on post-HI day 2 and day 7 (Figs. 1G, H). After HI insult, necrotic cell death was not definitive, but an astrocytic reaction appeared in the midbrain at least until post-HI day 2.

2.2. *Alterative distribution of TH- and TpH-positive cells in brainstem of neonatal HIE*

The number of TH-positive cells widely decreased in the neonatal HIE. On post-HI day 2, the number significantly decreased in the LC, raphe obligata (RO) and solitary nucleus (NS) (Figs. 2A, B, E, F, 3A). The % TH-positive cells were 64.3±1.3 (average±standard deviation) of neonatal HIE and 71.6±1.8 of control in the LC ($p<0.00002$) (Fig. 3A). In the RO and NS, 8.8±0.6 and 4.4±0.9 of neonatal HIE significantly decreased ($p=0.002$, $p=0.005$), compared with 12.1±0.6 and 6.7±0.9 of control, respectively (Fig. 3A). However, on post-HI day 7, the number of TH-positive cells recovered in most regions, except in the medulla oblongata (Figs. 2C, D, G, H, 3B). The % TH-

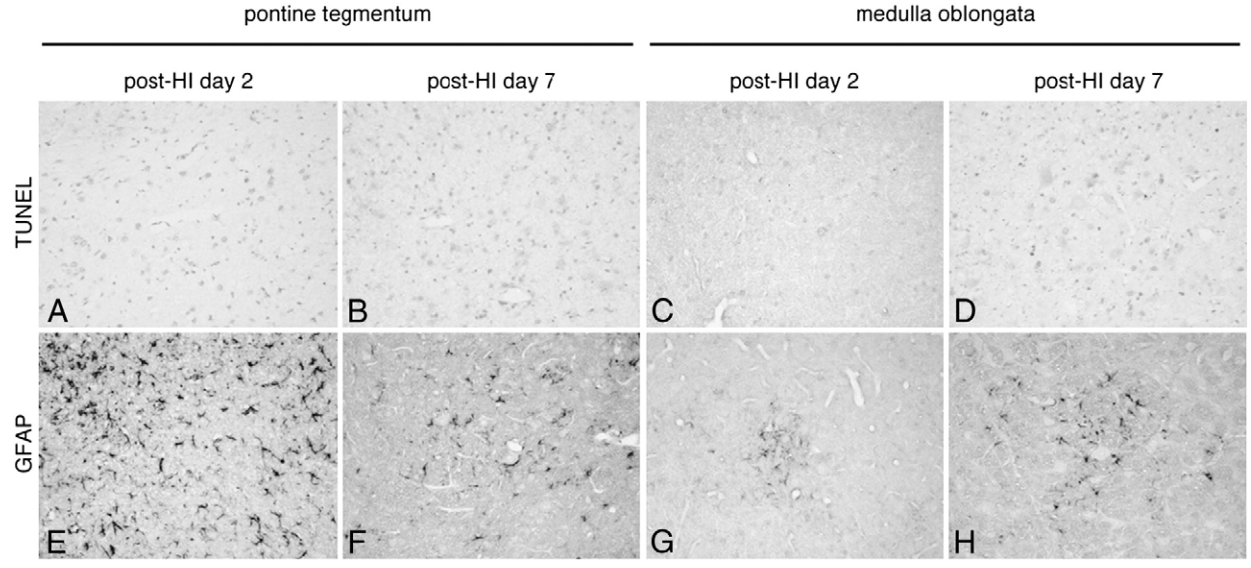

Fig. 1 – Cell damage in the brainstem of neonatal HIE rats. A small number of TUNEL-positive cells were observed on both post-HI day 2 and day 7 (A–D). A and B show no TUNEL-positive cell in the pontine tegmentum on post-HI day 2 and post-HI day 7, respectively. C and D are the dorsolateral region of the medulla oblongata post-HI day 2 and post-HI day 7, respectively. More GFAP-positive cells and fibers distribute in the pontine tegmentum on post-HI day 2 (E) than on day 7 (F). On the other hand, GFAP-positive cells and fibers show no significant difference in the medulla oblongata on post-HI day 2 (G) and day 7 (H). A–D: TUNEL reaction, ×200 of original magnification. E–H: GFAP immunohistochemistry, ×200 of original magnification.