available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Influence of ischemic preconditioning on levels of nerve growth factor, brain-derived neurotrophic factor and their high-affinity receptors in hippocampus following forebrain ischemia

Tsong-Hai Lee[a,*], *Jen-Tsung Yang*[a,b], *Yu-Shien Ko*[a,c], *Hiroyuki Kato*[a,d], *Yasuto Itoyama*[a,e], *Kyuya Kogure*[a,f]

[a]*Department of Neurology, Chang Gung Memorial Hospital, Linkou Medical Center, and Chang Gung University College of Medicine, Taoyuan, 333, Taiwan*
[b]*Department of Neurosurgery, Chiayi Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Chiayi, Taiwan*
[c]*Department of Cardiology, Chang Gung Memorial Hospital, Linkou Medical Center and Chang Gung University College of Medicine, Taoyuan, Taiwan*
[d]*Department of Neurology, IUHW Hospital, Nasushiobara, Japan*
[e]*Department of Neurology, Tohoku University School of Medicine, 1-1 Seiryo-Machi, Aoba-ku, Sendai 980, Japan*
[f]*Foundation for Brain and Nerve Diseases and the Institute of Neuropathology, Kumagaya, Japan*

ARTICLE INFO

Article history:
Accepted 22 September 2007
Available online 6 October 2007

Keywords:
Neurotrophin
High-affinity receptor
Ischemic preconditioning

ABSTRACT

Preconditioning of gerbil brain with a sublethal forebrain ischemia is known to protect hippocampal CA1 neurons following a subsequent lethal ischemia (the second ischemia) which usually damages neurons (ischemic tolerance). Present report using a confocal laser scanning microscope demonstrated that the hippocampal cells of sham operation gerbils contained immunofluorescent NGF and BDNF and their high-affinity receptors (TrkA and TrkB). A 2-min ischemia caused little change of these proteins (ANOVA test, $P<0.05$). After the second lethal ischemia, in the CA1 area with ischemic preconditioning (2-min ischemia), only BDNF but not NGF and their high-affinity receptors showed a transient reduction at 4 h (ANOVA test, $P<0.01$) and improved from 1 day (ANOVA test, $P<0.05$). In the CA1 area without ischemic preconditioning (sham operation), NGF and its high-affinity TrkA receptor showed a consistent reduction from 4 h to 7 days (ANOVA test, $P<0.05$); BDNF and TrkB decreased transiently from 4 h to 1 day (ANOVA test, $P<0.05$) but were recovered in the surviving neurons from 3 days. At 3 and 7 days after the second lethal ischemia, apoptotic cell injury could be seen in the CA1 area without ischemic preconditioning but was sparsely noted in the CA1 area with ischemic preconditioning. In the ischemia-resistant CA3 and dentate gyrus areas, only BDNF decreased significantly at 7 days in the CA3 area without ischemic preconditioning (ANOVA test, $P<0.01$). However, no significant change occurred in NGF, TrkA and TrkB immunofluorescence from 4 h to 7 days after the second lethal ischemia in the CA3 and dentate gyrus areas with and

* *Corresponding author*. Fax: +886 3 3288849.
E-mail address: thlee@adm.cgmh.org.tw (T.-H. Lee).

Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; TrkA, high-affinity receptor for NGF; TrkB, high-affinity receptor for BDNF

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2007.09.078

without ischemic preconditioning. Western blot study showed that in the hippocampal formation with ischemic preconditioning, preconditioning prevented the decline of these protein levels from 1 day to 7 days after the second lethal ischemia (ANOVA test, $P>0.05$). Results of this study demonstrate that ischemic preconditioning recovers the initial decline in NGF and BDNF and their corresponding receptors in the vulnerable CA1 neurons after the second lethal ischemia, suggesting that growth factors might play a role in the protective mechanism of ischemic preconditioning.

1. Introduction

Neurotrophic factors are known to be critically involved in neurite outgrowth and cell survival in the central nervous system (Barde, 1989). Among these neurotrophic factors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are present at the highest level in the hippocampal formation and cerebral cortex (Ernfors et al., 1990; Korsching et al., 1985; Leibrock et al., 1989; Levi-Montalcini, 1987; Maisonpierre et al., 1990). These neurotrophic factors may interact with their high-affinity receptors (TrkA for NGF, TrkB for BDNF and TrkC for NT-3), which act as signal-transducing receptors (Merlio et al., 1993).

NGF and BDNF, but not NT-3, can enhance the survival and differentiation of cultured embryonic basal forebrain cells (Alderson et al., 1989; Knusel et al., 1991). Following cerebral ischemia or hypoglycemic coma, NGF and BDNF increase but NT-3 decreases in the hippocampal neurons (Lindvall et al., 1992; Merlio et al., 1993; Takeda et al., 1993). However, following cerebral ischemia or hypoglycemic coma, only TrkB, but not TrkA and TrkC, increase in the hippocampal formation (Merlio et al., 1993). Hippocampal kindling-induced seizure can also cause a transient increase in the levels of TrkB mRNA and protein, but not of TrkA and TrkC, in the hippocampal formation (Merlio et al., 1993). These data reveal that under pathophysiological conditions, each neurotrophin/receptor system may play a distinct role in the central nervous system.

Preconditioning of the rodent brain with sublethal cerebral ischemia is known to generate resistance to subsequent lethal period of ischemia (Kato et al., 1991, 1994; Kirino et al., 1996). Our previous studies (Kato et al., 1991, 1994) have shown that a 2-min period of cerebral ischemia in gerbils produces no appreciable neuronal damage in the hippocampal formation. However, preconditioning of the gerbil brain with this period of ischemia, followed by 1–7 days of reperfusion, protects against neuronal damage following subsequent longer periods of ischemia that usually kills hippocampal CA1 neurons (Kato et al., 1994). This phenomenon has been termed ischemic tolerance, and its mechanism remains to be defined.

The NGF and BDNF have been reported to protect the hippocampal CA1 neurons against ischemic cell damage (Beck et al., 1994; Shigeno et al., 1991; Tanaka et al., 1994). Neurotrophic factors, such as NGF and BDNF, might play a role in the mechanism of ischemic tolerance. This work examined NGF and BDNF and their corresponding receptor levels following a ischemic preconditioning model in gerbils.

2. Results

The TUNEL method was used to detect apoptotic cell injury; the DAPI method was adopted to stain all cells, and the H&E stain was used to examine the normal cells. The CA1 area with sham operation (Fig. 1A) and with 2-min ischemia (Fig. 1D) had no TUNEL-positive cell, and their DAPI-positive cell densities were 2.66±0.15 and 2.58±0.15 cells/μm (ANOVA test, $P>0.05$), respectively. The CA1 area with ischemic preconditioning (2-min ischemia) had sparse TUNEL-positive cell at 3 days (Fig. 1E, DAPI-positive cell density=2.21±0.06, ANOVA test, $P>0.05$) and at 7 days (Fig. 1F, DAPI-positive cell density=2.18±0.32, ANOVA test, $P>0.05$) after the second lethal ischemia. However, the CA1 area without ischemic preconditioning (sham operation) contained some TUNEL-positive cells at 3 days (Fig. 1B, DAPI-positive cell density=1.68±0.53, ANOVA test, $P>0.05$) and remarkable TUNEL-positive cells at 7 days (Fig. 1C, DAPI-positive cell density=0.95±0.80, ANOVA test, $P<0.05$). The H&E stain showed a similar result to DAPI stain.

The immunofluorescence of NGF, TrkA, BDNF and TrkB was normally present in the hippocampal neurons with sham operation and with 2-min ischemia (Figs. 2 and 3A and E). At 4 h after the second lethal ischemia, only BDNF decreased significantly (Fig. 4A, ANOVA test, $P<0.05$). At 1 day, all four proteins decreased significantly in the CA1 area without ischemic preconditioning (Figs. 2B and 4A, ANOVA test, $P<0.05$) but did not change in the CA1 area with ischemic preconditioning (Figs. 2F and 4A, ANOVA test, $P>0.05$). At 3 days, the levels of BDNF and TrkB improved, but that of NGF and TrkA decreased consistently (Figs. 2G and 4A, ANOVA test, $P<0.01$) in the CA1 area without ischemic preconditioning. At 7 days after

Fig. 1 – The detection of apoptosis using the TUNEL method and the detection of normal cells using DAPI staining show no TUNEL-positive cell in the CA1 area with sham operation (A) and with 2-min ischemia (D). The DAPI-positive cell density is 2.66±0.15 and 2.58±0.15 cells/μm (ANOVA test, $P>0.05$), respectively. The CA1 area with ischemic preconditioning (2-min ischemia) shows sparse TUNEL-positive cell at 3 days (E, DAPI-positive cell density=2.21±0.06, ANOVA test, $P>0.05$) and at 7 days after the second lethal ischemia (F, DAPI-positive cell density=2.18±0.32, ANOVA test, $P>0.05$). However, the CA1 area without ischemic preconditioning (sham operation) exhibits some TUNEL-positive cells at 3 days (B, DAPI-positive cell density=1.68±0.53, ANOVA test, $P>0.05$) and many TUNEL-positive cells at 7 days (C, DAPI-positive cell density=0.95±0.80, ANOVA test, $P<0.05$). The arrowhead indicates the CA1 area, and the arrow indicates the dentate gyrus area. The scale bar in panel A indicates 100 μm. $^{*}P<0.05$, $^{\#}P<0.01$.