ISCHEMIA-REPERFUSION INJURY EFFECTS A CHANGE IN EXPRESSION OF GnRH AND ITS RECEPTOR IN CA1 NEURONS IN RAT HIPPOCAMPUS

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ISCHEMIA-REPERFUSION INJURY EFFECTS A CHANGE IN EXPRESSION OF GnRH AND ITS RECEPTOR IN CA1 NEURONS IN RAT HIPPOCAMPUS

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Many researches on the change and protective effects of estrogen and its receptor in hippocampus with ischemia-reperfusion injury have been done in recent years; the study on the change of GnRH and its receptor in hippocampus with ischemia-reperfusion injury has not been seen yet. This study used immunohistochemistry and in situ hybridization method, together with an image analysis system to observe the change in expression of GnRH and its receptor in hippocampus with ischemia-reperfusion injury. The study found that the expression of GnRH and GnRH mRNA and the number of positive cells decreased with time after damage. Expression of GnRH receptor and GnRH receptor mRNA in single positive cell early increased and later decreased after injury; the number of positive cells decreased with time after injury. Three days after injury, rare GnRH, GnRHR immunoreactive positive cells and cells with GnRH mRNA, GnRHR mRNA hybridization signal could be found in...
the stratum pyramid of CA1 region, many cells with weak GnRH, GnRH receptor
immunoreactivity and weak GnRH mRNA, GnRH receptor mRNA hybridization
signal appeared at stratum oriens and stratum radiatum. These suggested that GnRH
may participate in the regulation of ischemia-reperfusion injury in CA1 region and
repair of brain tissue.

Keywords expression, GnRH, GnRH receptor, hippocampus, ischemia, rat

INTRODUCTION

The gonadotropin-releasing hormone (GnRH), also called luteinizing hormone-
releasing hormone (LHRH), is a decapeptide, and is mainly secreted in pulsatile
manner from hypothalamic neuron into the capillary plexus of median eminence
and reaches anterior pituitary (Fujii et al., 2001). Gonadotropin-releasing
hormone receptor (GnRH receptor) is a member of the seven-transmembrane,
G-protein-coupled receptor family. It mainly distributes on membrane of
gonadotropin cells in anterior pituitary (Sealfon et al., 1997). GnRH combined
with GnRH receptor to regulate secretion of follicle-stimulating hormone (FSH)
and luteinizing hormone (LH) and plays an important role in the reproductive
process (Weissman & Shoham, 1993).

The previous studies showed that most vertebrate species including human
have at least two forms of GnRH, namely GnRH-I and GnRH-II, and their
cognate receptors, namely GnRH-I receptor and GnRH-II receptor (Neill et
al., 2004). Recently, increasing evidence suggests that GnRH and its receptor
can also exist in other extrahypothalamic regions, such as gastrointestinal tract
(Huang et al., 2001), submaxillary gland (Yao et al., 2003), pancreas (Wang et
al., 2001), and some malignant tumor cells such as prostate cancer cells (Lau et
al., 2001). At the same time, the function of GnRH is beyond reproduction. For
examples: GnRH analogue in gastric parietal cells can regulate the secretion
of gastric acid (Chen et al., 2005); GnRH in some tumors can inhibit the
proliferation of tumor cells and induce apoptosis of tumor cells (Mizutani et al.,
1998). It has been known that GnRH and its receptor distribute broadly in brain.
Binding of GnRH agonist to the brain GnRH receptor causes a dose-dependent
increase in inositol phosphates as well as changes in intracellular Ca++ levels of
the target neurons (Jennes et al., 1997). In rat hippocampus, GnRH and GnRH
receptor exist in pyramidal neurons from CA1 to CA4 region and granule
neurons in dentate gyrus (Merchenthaler et al., 1984; Leblanc et al., 1988).
Electrophysiological study showed that hippocampal neurons had biphasic
excitatory response to GnRH (Palovcik et al., 1986; Lu et al., 1999). But the
specific function of GnRH in hippocampus remains unknown.
Global ischemia-reperfusion injury elicits selective, delayed neuronal death, and the main reason is apoptosis; pyramidal neurons of hippocampal CA1 region are particularly vulnerable (Tanaka et al., 2000; Kaplan & Miller, 2000). Many researches have showed that estrogen can protect neuron injury and neuron apoptosis in hippocampal CA1 region during ischemia, and this protection is mediated by estrogen receptors in hippocampus (Jover et al., 2002; Miller et al., 2005). GnRH, an upstream hormone of estrogen, which can regulate the secretion of estrogen, also broadly exists in hippocampus with its receptor; but there is no report about the change of expression of GnRH and its receptor in hippocampus with ischemia-reperfusion injury. The authors took research in this field in order to provide theoretic basis to the further research on the function of GnRH and its receptor in hippocampus during ischemia-reperfusion injury.

**MATERIALS AND METHODS**

**Animal and Group**

Thirty male adult Sprague-Dawley rats, weighting about 280–300 kg, were purchased from the laboratory animal center of the authors’ university. Rats were divided into 5 experiment groups according to the time duration after ischemia-reperfusion injury (3 h, 6 h, 12 h, 24 h, and 3 d after injury), and a sham operation group as control group. There were 6 rats in each group; 3 were used to make immunohistochemistry stain, and the other 3 rats were used to make *in situ* hybridization.

**Ischemia Operation and Tissue Section**

For all experimental procedures, the study adhered strictly to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Models of middle cerebral artery occlusion (MCAO) were made in experiment groups by using the method described by Longa (Longa et al., 1989). Briefly, the rat was anesthetized and a cervical middle incision was made, right common carotid artery and external carotid artery were exposed and ligated. Nylon thread (3–0, Ethicon Inc, Japan), which was burned with round point, was inserted into the right internal carotid artery about 17–18 mm, then the right internal carotid artery was ligated for 2 h and the thread was pulled out to make reperfusion. Rats in the sham operation group only exposed right common carotid artery, external carotid artery, and internal carotid artery. Animals were deeply anesthetized.
with overdosed pentobarbital and were killed by transcardiac perfusion with 4% paraformaldehyde in PBS (0.1M, pH 7.4) at 3 h, 6 h, 12 h, 24 h, and 3 d after injury and 24 h after sham operation, respectively. Brains were quickly removed and immersed into 30% sucrose (4°C for 48 h). Coronal sections (30 µm) were cut through the entire dorsal hippocampus (Bregma -2.3 to -4.5 mm) with Leica1000 cryotome. All sections were divided into 4 groups, two groups were under immunostain, and remained two groups were under in situ hybridization.

**Immunohistochemistry Stains of GnRH and GnRH Receptor**

Sections were first incubated with 1% H2O2 for 30 min to block the endogenous peroxidase activity, washed in 0.01 M phosphate-buffered saline (PBS) containing 0.4% TritonX (PBST) and incubated with 5% normal goat serum for 30 min. Then the sections were incubated with rabbit anti-GnRH antibody (Boster Company, China; at 1:100) and rabbit anti-GnRH idiotypic antibody (Huang et al., 1994) (made by department of histology and embryology of our university; at 1:300) for 24 h at room temperature respectively; incubated with biotinylated goat anti-rabbit IgG (Sigma company, America; at 1:300) for 4 h at room temperature and incubated with ABC complex (Sigma company, America; at 1:300) for 2 h at room temperature. Sections were washed in PBST after each step and finally visualized by diaminobenzidine (DAB, Sigma company, America). Sections used as negative control were incubated with PBS instead of primary antibody.

**In Situ Hybridization of GnRH and GnRH Receptor**

*In situ* hybridization kits of GnRH and GnRH receptor (Boster Company, China) were used, in which oligonucleotide probes were labeled by digoxin. Sections were first incubated with 1% H2O2 for 30 min to block the endogenous peroxidase activity, washed in distilled water, digested by 3% citric acid-diluted pepsin, and washed in 0.05 M PBS. Then sections were incubated with pre-hybridization solution for 2 h at 37°C and incubated with GnRH and GnRH mRNA hybridization solution for 48 h at 37°C; washed in 2 × SSC, 0.5 × SSC, 0.2 × SSC in order; incubated with block solution for 30 min; incubated with biotinylated mouse anti-digoxin for 1 h at 37°C; incubated with SABC for 20 min at 37°C; incubated with biotinylated peroxidase for 20 min at 37°C; washed in PBS and finally visualized by DAB. Sections used as negative control were incubated with PBS instead of hybridization solution.
Image Analysis and Statistic Analysis
Visualized sections were observed under Zeiss microscope and photographs were taken (Kodak CCD), software of Image-pro was used to measure the mean grey level of immunoreactive positive product and hybridization signal (mean ± SD; 0 = black, 255 = white; the bigger number, the more content; the smaller number, the less content)(Brookes & Kaufman, 2005), the number of positive cells (cell number/mm², mean ± SD) of GnRH and GnRH receptor in stratum pyramidale of hippocampal CA1 region in each group was also been measured. Five sections in each group were randomly chosen; 3 different fields in CA1 region were measured in each section. Analysis of variance (ANOVA) and LSD-t test were used to analysis the statistical significance.

RESULT
Immunohistochemistry Stain of GnRH and GnRH Receptor
Immunohistochemistry showed GnRH and GnRH receptor positive cells as round shape, dark brown cells against light yellow or unstained cells. The immunoreactive cells were easily identified. The controls showed no immunoreactivity. Positive products distributed in cytoplasm and axon of neuron, nucleus was negative (Figures 1 and 2).

In hippocampal CA1 region of rats in sham operation group, GnRH and GnRH receptor immunoreactive positive pyramidal neurons were strongly stained, distributed in banded shape, and arranged tightly (Figures 1A and 2a). From 3 h to 6 h after ischemia-reperfusion injury, color of stained GnRH immunoreactive positive neurons in CA1 region gradually became weak, positive cells decreased and arranged sparsely (Figures 1B and 1C); from 12 h to 24 h after injury, color of stained positive neurons clearly became weaker, positive cells remarkably decreased. The neurons swelled and the intercellular gap gradually became broader (Figures 1D and 1E).

From 3 h to 12 h after ischemia-reperfusion injury, GnRH receptor immunoreactive positive pyramidal neurons in CA1 region decreased gradually, swelled, and arranged sparsely. However, GnRH receptor immunoreactivity in single positive neurons gradually became stronger and stained stronger (Figures 2b, 2c, 2d). At 24 h after injury, positive neurons sharply decreased, the intercellular gap became broader, the immunoreactivity in single positive neurons became weaker and stained weaker (Figure 2e).

Three days after ischemia-reperfusion injury, almost no GnRH and GnRH receptor immunoreactive positive neurons could be found in stratum pyramidale
Figure 1. Immunostain of GnRH in CA1 region. GnRH positive pyramidal neurons in rats of sham operation group were strongly stained (1A). From 3 h to 6 h after ischemia-reperfusion injury, GnRH positive neurons decreased and weakly stained (1B, 1C); from 12 h to 24 h after injury, GnRH positive neurons remarkably decreased and stained weaker (1D, 1E). Three days after injury, almost no GnRH positive neurons could be found in stratum pyramidale (SP); many cells with weak GnRH immunoreactivity (arrow) appeared in stratum oriens (SO) and stratum radiatum (SR) (1F). Bar = 200 µm.

In CA1 region except some cell contour remained. At the same time, many cells with tiny dendrite and relatively small shape appeared in stratum oriens and stratum radiatum, showed weak GnRH and GnRH receptor immunoreactivity (Figures 1F and 2f).

In Situ Hybridization of GnRH and GnRH Receptor

Cells with GnRH and GnRH receptor mRNA hybridization signal were round shape, dark brown, and easily identified. The controls showed no positive signal (Figures 3 and 4).

In hippocampal CA1 region of rats in sham operation group, pyramidal neurons with strong GnRH and GnRH receptor mRNA hybridization signal
Figure 2. Immunostain of GnRH receptor in CA1 region. GnRH receptor positive pyramidal neurons in rats of sham operation group were strongly stained (2a). From 3 h to 12 h after injury, GnRH receptor positive neurons decreased but strongly stained (2b, 2c, 2d); at 24 h after injury, GnRH receptor positive neurons remarkably decreased and stained weak (2e). Three days after injury, almost no GnRH receptor positive neurons could be found in stratum pyramida; many cells with weak GnRH receptor immunoreactivity (arrow) appeared in stratum oriens and stratum radiatum (2f). Bar = 200 µm.

From 3 h to 6 h after ischemia-reperfusion injury, GnRH mRNA hybridization signal in pyramidal neurons of CA1 region gradually became weak. Neurons with positive signal gradually stained weak, decreased and arranged sparsely (Figures 3B and 3C); from 12 h to 24 h after injury, hybridization signal in neurons clearly became weaker. The neurons with positive signal clearly stained weaker, remarkably decreased, swelled, and the intercellular gap gradually became broader (Figures 3D and 3E).

From 3 h to 12 h after ischemia-reperfusion injury, GnRH receptor mRNA hybridization signal in single positive pyramidal neuron of CA1 region gradually became strong. However, the neurons with positive signal gradually decreased, swelled, and arranged sparsely (Figures 4b, 4c, and 4d); at 24 h after injury,
Figure 3. In situ hybridization of GnRH in CA1 region. Pyramidal neurons with strong GnRH mRNA hybridization signal in rats of sham operation group were strongly stained (3A). From 3 h to 6 h after injury, neurons with positive signal decreased and weakly stained (3B, 3C); from 12 h to 24 h after injury, neurons with positive signal remarkably decreased and stained weaker (3D, 3E). Three days after injury, almost no neurons with positive signal could be found in stratum pyramidale; many cells with weak GnRH mRNA hybridization signal (arrow) appeared in stratum oriens and stratum radiatum (3F). Bar = 200 µm.

Analysis of Mean Grey Level in Positive Cells

Compared with that of the sham operation group, mean grey level of GnRH immunoreactive neurons in CA1 region gradually increased from 3 h to 6 h.
Figure 4. *In situ* hybridization of GnRH receptor in CA1 region. Pyramidal neurons with strong GnRH receptor mRNA hybridization signal in rats of sham operation group were strongly stained (4a). From 3 h to 12 h after injury, neurons with positive signal decreased but strongly stained (4b, 4c, 4d); at 24 h after injury, neurons with positive signal remarkably decreased and stained weak (4e). Three days after injury, almost no neurons with positive signal could be found in stratum pyramidale; many cells with weak GnRH receptor mRNA hybridization signal (arrow) appeared in stratum oriens and stratum radiatum (4f). Bar = 200 µm.

after injury and increased remarkably from 12 h to 3 d after injury. The dates showed statistical significance (ANOVA, $p < .01$; LSD-t test, $p < .05$, except 6 h group). Compared with that of the sham operation group, mean grey level of GnRH receptor immunoreactive neurons in CA1 region gradually decreased from 3 h to 12 h after injury and increased from 24 h to 3 d after injury. The dates showed statistical significance (ANOVA, $p < .01$; LSD-t test, $p < .05$, except 6 h group; Figure 5).

Compared with that of the sham operation group, mean grey level of GnRH mRNA hybridization signal in neurons of CA1 region gradually increased from 3 h to 6 h after injury and increased remarkably from 12 h to 3 d after injury. The dates showed statistical significance (ANOVA, $p < .01$; LSD-t test, $p < .05$, except 3 h group). Compared with that of the sham operation group, mean grey level of GnRH receptor mRNA hybridization signal in neurons of CA1
Figure 5. Mean grey level of GnRH and GnRH receptor immunoreactive positive pyramidal neurons in CA1 region. Compared with that of sham operation group, mean grey level of GnRH immunoreactive neurons gradually increased from 3 h to 6 h after injury and increased remarkably from 12 h to 3 d after injury. Mean grey level of GnRH receptor immunoreactive neurons gradually decreased from 3 h to 12 h after injury and increased from 24 h to 3 d after injury (*LSD-t test: $p < .05$ versus sham group).

Figure 6. Mean grey level of GnRH and GnRH receptor immunoreactive positive pyramidal neurons in CA1 region. Compared with that of sham operation group, mean grey level of GnRH immunoreactive neurons gradually increased from 3 h to 6 h after injury and increased remarkably from 12 h to 3 d after injury. Mean grey level of GnRH receptor immunoreactive neurons gradually decreased from 3 h to 12 h after injury and increased from 24 h to 3 d after injury (*LSD-t test: $p < .05$ versus sham group).

Cell Count

Rats in the sham operation group had the largest number of GnRH and GnRH receptor immunoreactive positive cells in stratum pyramidale of CA1 region. The number of GnRH positive cells began to decrease from 3 h after injury and decreased sharply from 6 h after injury. The number of GnRH receptor positive cells began to decrease from 3 h to 6 h after injury and decreased sharply from 12 h after injury. From 24 h to 3 d, almost no GnRH and GnRH receptor positive cells could be found in stratum pyramidale (ANOVA, $p < .01$; LSD-t test, $p < .05$, except 3 h group; Figure 6).
Figure 6. Mean grey level of pyramidal neurons with GnRH and GnRH receptor mRNA hybridization signal in CA1 region. Compared with that of sham operation group, mean grey level of GnRH mRNA hybridization signal in neurons of CA1 region gradually increased from 3 h to 6 h after injury and increased remarkably from 12 h to 3 d after injury. Mean grey level of GnRH receptor mRNA hybridization signal in neurons of CA1 region gradually decreased from 3 h to 12 h after injury and increased from 24 h to 3 d after injury (\(^*\)LSD-t test: \(p < .05\) versus sham group).

Figure 7. Number of GnRH and GnRH receptor immunoreactive positive pyramidal neurons in CA1 region. Rats in sham operation group had the largest number of GnRH and GnRH receptor positive pyramidal neurons. The number of GnRH positive neurons began to decrease from 3 h after injury and decreased sharply from 6 h after injury. The number of GnRH receptor positive neurons began to decrease from 3 h to 6 h after injury and decreased sharply from 12 h after injury. From 24 h to 3 d, almost no GnRH and GnRH receptor positive neurons could be found in stratum pyramidale (\(^*\)LSD-t test: \(p < .05\) versus sham group).
Figure 8. Number of pyramidal neurons with GnRH and GnRH receptor hybridization signal in CA1 region. Rats in sham operation group had the largest number of pyramidal neurons with GnRH and GnRH receptor mRNA hybridization signal in CA1 region. The number of GnRH mRNA positive neurons began to decrease from 3 h after injury and decreased sharply from 6 h after injury. The number of GnRH receptor mRNA positive neurons began to decrease from 3 h to 6 h after injury and decreased sharply from 12 h after injury. From 24 h to 3 d, almost no GnRH and GnRH receptor mRNA positive neurons could be found in stratum pyramidale (*LSD-t test: $p < .05$ versus sham group).

no GnRH and GnRH receptor mRNA positive cells could be found in stratum pyramidale (ANOVA, $p < .01$; LSD-t test, $p < .05$; Figure 8).

DISCUSSION

Researches in recent years showed that GnRH, GnRH receptor, and GnRH receptor mRNA exist in pyramidal neurons from CA1 to CA4 region and granule neurons in dentate gyrus in hippocampus (Merchenthaler et al., 1984; Leblanc et al., 1988; Jennes et al., 1995). The present study found that pyramidal neurons from CA1 to CA4 region and granule neurons in dentate gyrus in hippocampus showed both GnRH immunoreactivity and GnRH mRNA hybridization signal, which suggested that rat hippocampus can express GnRH. At the same time, the study also found these hippocampal neurons showed both GnRH receptor immunoreactivity and GnRH receptor mRNA hybridization signal, which suggested that rat hippocampus can also express GnRH receptor.

Global ischemia-reperfusion injury elicits selective, delayed neuronal death, and the main reason is apoptosis; pyramidal neurons of hippocampal CA1 region are particularly vulnerable (Tanaka et al., 2000; Kaplan & Miller, 2000).
The study found that the number of GnRH immunoreactive positive cells and the cells with GnRH mRNA hybridization signal in CA1 region decreased with time after damage. The number of GnRH receptor immunoreactive positive cells and the cells with GnRH receptor mRNA hybridization signal in CA1 region also decreased with time after damage. Three days after injury, rare GnRH, GnRHR immunoreactive positive cells and the cells with GnRH mRNA, GnRH receptor mRNA hybridization signal could be found in stratum pyramidale of CA1 region. These suggested that pyramidal neurons in CA1 region start apoptosis and the number of neurons under apoptosis increased during the three days after injury.

The study found that the expression of GnRH and its mRNA in positive cells remarkably decreased with time after injury and finally disappeared. From 3 h to 12 h after injury, although the number of GnRH receptor immunoreactive positive cells and the cells with GnRH receptor mRNA hybridization signal decreased, the expression of GnRH receptor and its mRNA increased in single positive cell. It might be the cell up-regulate the expression of GnRH receptor and its mRNA due to the feedback of decreased expression of GnRH and its mRNA. But the expression of GnRH receptor and its mRNA disappeared in the end because of neuron apoptosis.

GnRH in some tumors can inhibit the proliferation of tumor cells and induce apoptosis of tumor cells (Mizutani et al., 1998). Using GnRH analogue in chemotherapy can prevent toxic effects on testis (Krause & Pfluger, 1989; Kangasniemi et al., 1995), ovary (Sugiyama et al., 2003; Imai et al., 2006), kidney, and gastrointestinal tract (Nomura et al., 1995). These indicate that GnRH has the function of organism protection. The result of the present research indicated that the expression of GnRH and its receptor decreased in line with neuron death and apoptosis in CA1 region after ischemia-reperfusion injury, which suggested that GnRH in rat hippocampus might participate in the regulation of ischemia-reperfusion injury and neuron function through paracrine or autocrine. It was supposed that GnRH in central nervous system might have the similar neuron protective function as that of estrogen, and the function might be mediated by GnRH receptor.

Takahashi et al. (2004) studied ischemia injury of monkey hippocampus. They found that normal pyramidal neurons in CA1 region can express estrogen receptor β; three days after brain ischemia, estrogen receptor β immunoreactive positive neurons in CA1 region disappeared; at the same time, many estrogen receptor β positive astrocytes and microglia appeared at stratum oriens and stratum radiatum. In the present research, three days after ischemia-reperfusion injury, not only GnRH receptor positive cells but also GnRH positive cells appeared at stratum oriens and stratum radiatum. It was
supposed that these positive cells might be glial cells. They might be activated
during injury and might participate in neuron regulation and brain tissue repair.
All these suppositions should be tested in further research through functional
experiments.

REFERENCES

extrasplenic microvasculature and lymphatics in the rat in vivo. *Journal of
Physiology, 565*(Pt 1), 269–277.

Chen, L., Sun, X. D., Zhao, J., Yang, A. G., & Huang, W. Q. (2005). Distribution,
cloning and sequencing of GnRH, its receptor, and effects of gastric acid secretion

administration of gonadotrophin-releasing hormone agonist during the luteal phase
in IVF. *Human Reproduction, 16*(8), 1671–1675.


Huang, W., Yao, B., Sun, L., Pu, R., Wang, L., & Zhang, R. (2001). Immunohistochem-
ical and in situ hybridization studies of gonadotropin releasing hormone (GnRH)

by a gonadotropin-releasing hormone analog from doxorubicin-induced granulosa
cell damage. *Gynecol Obstet Invest, 63*(2), 102–106.

of hippocampal gonadotropin releasing hormone (GnRH) receptor mRNA and
GnRH-stimulated inositol phosphate production by gonadal steroid hormones.
*Brain Research Mol Brain Research, 33*(1), 104–110.

releasing hormone receptors: Localization and regulation. *Recent Progress in
Hormone Research, 52*, 475–490.

Jover, T., Tanaka, H., Calderone, A., Oguro, K., Bennett, M. V., Eigen, A. M., &
Zukin, R. S. (2002). Estrogen against global ischemia-induced neuronal death
and prevents activation of apoptotic signaling cascades in the hippocampal CA1.
*Journal of Neuroscience, 22*(6), 2115–2124.

against procarbazine-induced testicular damage by GnRH-agonist and antiandro-
gen treatment in the rat. *Endocrinology, 136*(8), 3677–3680.


