Acupuncture Stimulation to HT8 Enhances Cell Proliferation in Hippocampus on an Epilepsy Mouse Model

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Abstract

Purpose: The systemic corticosteroid dose in HT8 rat hippocampal tissue injection was investigated in a rat experimental model of epilepsy. The KA (Kainic acid) administration at 3 and 6 hours after injection increased the proliferation rate of hippocampal neuroblasts. The KA administration during the first 6 hours after injection increased the proliferation rate of hippocampal neuroblasts. The KA administration during the first 6 hours after injection increased the proliferation rate of hippocampal neuroblasts.

Methods: Saline (n=8), KA (n=8), KA+Acu (n=8) were injected into the hippocampus of the HT8 rat. The proliferation rate of hippocampal neuroblasts was measured by BrdU labeling. The proliferation rate of hippocampal neuroblasts was increased by the administration of KA, KA+Acu, and neuropeptide Y (NPY).

Results: The proliferation rate of hippocampal neuroblasts was increased by the administration of KA, KA+Acu, and neuropeptide Y (NPY).

Conclusion: The administration of KA, KA+Acu, and neuropeptide Y (NPY) increased the proliferation rate of hippocampal neuroblasts.

Key words: acupuncture, bromodeoxyuridine, cell proliferation, neuropeptide Y, hippocampus, dentate gyrus

Introduction

Acupuncture, a technique of needling into specific locations in the body, has been used for therapeutic purposes including pain, neurological disorders and gastroenteric...
disorders in East Asia over 2000 years. Over the past two decades, the acupuncture therapy has also been used in Europe and the United States to help various types of patients\(^1\).

Neurogenesis, the process of new neuronal birth, consists of cell proliferation, survival, migration and neuronal differentiation. In adult brain, it occurs in only subventricular zone and dentate gyrus (DG) of hippocampus\(^2\). DG is known to have a crucial role in learning, memory, stress and some neurological disorders\(^3\). Various physiological, pathological and pharmacological methods have been studied to enhance the adult neurogenesis in the DG\(^4\).

Acupoints in the Heart Meridian (HT) have been used to treat psychopathic or neurological disorders such as epilepsy. Among them, HT8 (Sobu) is traditionally known as a representative acupoint for balancing homeostasis by regulating the excitatory or inhibitory functions in the body.

We previously reported that acupuncture to the acupoint HT8 has neuroprotective effects against kainic acid (KA)-induced cell death and seizure behavior by decreasing the KA-induced FBJ osteosarcoma oncogene (Fos) and Jun expressions and enhancing glutamate decarboxylase 67 in the hippocampus\(^5\). In the present study, we examined whether acupuncture stimulation may enhance the cell proliferation in mouse hippocampus.

II. Materials and Methods

1. Animals and Grouping

This study was approved by the ethics committee of Acupuncture and Meridian Science Research Center and all efforts were taken to minimize the number of animals and their suffering in accordance with current guidelines for animal research, the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985). Male ICR mice (20–25 g, Orientbio Inc., Gyeonggi-do, Korea) were housed at room temperature (22 ± 3°C) under a standard 12 hour light/dark cycle (lights on at 07:00 a.m.) and were given unlimited access to food and water. After 1-week–adaptation, the mice were randomly assigned to one of three groups (n=8 at each group): saline, KA or KA+Acu. Mice in the saline group would be injected with normal saline and not receive acupuncture stimulation. Those in the KA group (n=8), which would be injected with KA and not receive acupuncture stimulation, and those in KA+Acu group would be injected with KA and receive acupuncture stimulation bilaterally to acupuncture point HT8.

2. Bromodeoxyuridine Injection
Three days before KA injection, we began to give 5-bromo-2'-deoxyuridine (BrdU; 50 mg/kg in normal saline; Sigma, MO, USA) to all mice by intraperitoneally injection. The mice underwent the injection once a day (total 3 times).

3. Acupuncture Stimulation

From 10:00 to 10:30 a.m., the mice in the KA+Acu group were lightly immobilized, and acupuncture needles (0.18 × 8 mm, Dongbang Acupuncture Inc., Gyeonggi-do, Korea) were inserted to the bilateral acupuncture point HT8. HT8 have been used to treat not only balancing homeostasis by regulating the excitatory and inhibitory functions in the body but also psychopathic and neurological disorders such as epilepsy. The point was located on the palmar surface of the forelimbs, between the fourth and fifth metacarpal bones[7], and the point in mice corresponded anatomically to the point in humans. The depth of needle insertion was 1 mm. The needles were turned at a rate of two spins per second for 15 seconds and removed immediately afterward. The entire stimulation lasted for 30 seconds, and repeated 3 times (2 days before, 1 days before and immediately after the KA injection). The mice of the saline and the KA groups were also lightly immobilized as those in the KA+Acu group for 30 seconds, but they didn't have the acupuncture stimulation.

4. Kainic Acid Injection

KA (Sigma, MO, USA) was injected intracerebroventricularly at bregma with a 50 μl Hamilton microsyringe fitted with a 26-gauge needle, which was inserted to a depth of 2.4 mm according to Laursen and Belknap’s method[9]. The injection volume was 5μl (0.02 μg/μl) in the KA and KA + Acu groups. The mice in the saline group underwent the same procedure, except that normal saline was injected intracerebroventricularly instead of KA.

5. Immunohistochemistry

Three hours after KA injection, the animals were perfused with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. The brains were sectioned coronally (40 μm) on a freezing microtome. Hippocampal sections (1.82 to 2.06 mm from the bregma) from each animal were stained. The sections were incubated with antibody against neuropeptide Y (NPY) (1:2000, Zymed Laboratories Inc., CA, USA) staining and then with biotinylated secondary antibody (Vector Laboratories, CA, USA). After incubation with a Vector Elite ABC Kit (Vector Laboratories, CA, USA), the antibody biotinavidinperoxidase complex was visualized with diaminobenzidine (DAB). After the DAB reaction, the tissues were rinsed with PBS, mounted on gelatin
coated slides, air-dried, dehydrated and coverslipped. For BrdU staining, sections incubated in 0.5% Triton X-100 at room temperature, pre-treated in 50% formamide 2 × standard saline citrate at 65°C for 2 hours, denatured in 2N HCl for 30 minutes at 37°C, and washed. Next procedures were same as above-mentioned immunostaining, but mouse antiBrdU antibody (Roche, Germany) was used as primary antibody. The histological pictures were taken using a bright field BX51 microscope (Olympus, Japan) and DP70 camera (Olympus, Japan). The number of BrdU or NPY positive cells in the DG was manually counted from each section exhaustively at 200 × magnification, and all counts were done in a blinded fashion. For Nissl staining, the DG tissues were mounted on gelatin-coated slides, dried for 1 hour at room temperature, and stained with 0.5% cresyl violet.

Statistical Analysis
All the data were expressed as the mean ± SEM, and the data were analyzed by one-way analysis of variance with the Newman-Keuls post hoc multiple comparison test. In all the analyses, differences were considered statistically significant at p<0.05.

III. Results
1. Acupuncture Prevents KA-induced Cell Death in the Dentate Gyrus

To observe the KA-induced cell death in the DG, we stained hippocampal sections with cresyl violet. The KA + Acu group showed a substantial protective effect against the KA-induced cell death in the DG compared to the KA group (Fig. 1).

![Fig. 1. Nissl staining in the dentate gyrus (DG) of hippocampus. Saline, saline-injected group; KA, kainic acid (KA)-injected group; KA+Acu: KA-injected and acupuncture stimulated group. Scale bar represents 100 μm.](image-url)

2. The Increase of 5′-bromo-2′deoxyuridine-positive Cells in the Dentate Gyrus after Acupuncture Stimulation

The number of BrdU-positive cells of DG in the KA + Acu group (19.4 ± 1.6) was significantly increased comparing to both the saline group (10.7 ± 1.5, p<0.05) and the KA group (13.2 ± 1.7, p<0.05). However, the number of BrdU-positive cells in the KA group was not significantly different
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compared to the Saline group (Fig. 2).

![Fig. 2. Photographs (A) and changes (B) of 5'-bromo-2'-deoxyuridine (BrdU)-positive cells in the dentate gyrus (DG) of hippocampus.
Saline, saline-injected group; KA, kainic acid (KA)-injected group; KA+Acu, KA-injected and acupuncture stimulated group. The number of BrdU-positive cells in the DG. Data in the graph are presented as means ± SEM. * p<0.05 compared to the KA group; # p<0.05 compared to the Saline group. Scale bar represents 100 μm.](image)

3. The Increase of NPY-positive Cells in the Dentate Gyrus after Acupuncture Stimulation

Acupuncture stimulation to HT8 induced significantly to be increase the number of NPY-positive cells (55.4 ± 2.5) in the DG compared to the KA group (46.3 ± 2.3, p<0.01). However, there was no significant difference on the number of NPY-positive cells between the Saline (43.3 ± 1.9) and KA group (Fig. 3).

![Fig. 3. Photographs (A) and changes (B) of neuropeptide Y (NPY)-positive cells in the dentate gyrus (DG) of hippocampus.
Saline, saline-injected group; KA, kainic acid (KA)-injected group; KA+Acu, KA-injected and acupuncture stimulated group. The number of NPY-positive cells in the DG. Data in the graph are presented as means ± SEM. ** p<0.01 compared to the KA group; ## p<0.01 compared to the Saline group. Scale bar represents 100 μm.](image)

IV. Discussion

This study demonstrates that acupuncture stimulation to HT8 significantly increases BrdU and NPY expressions in the DG of KA-injected mouse hippocampus.

It has been known that cell proliferation in the DG was increased after KA injection\textsuperscript{5,10}. However, since the duration of the cell
cycle of dividing cells in the DG of mice has been estimated to be around 20 hours\textsuperscript{11}, it is unlikely that the proliferation was increased within 3 hours after KA injection. Indeed, Ledergerber et al. showed that there were no differences between vehicle and KA injection in the hippocampus after 24 hours\textsuperscript{22}. Consistently, in our result, the number of BrdU-positive cells in the DG of KA-injected mice without acupuncture stimulation was not increased. Therefore, the result indicated that KA didn’t have influence on the increase of cell proliferation in the present study. Meanwhile, since acupuncture stimulations were performed 51 hours and 27 hours before the sample preparation for immunohistochemical staining, it is likely that the increase in the number of BrdU-positive cells in the DG of KA-injected mice may be associated with acupuncture stimulation to HT8.

There were several reports on the correlation between acupuncture and cell proliferation. Although some reports proposed that acupuncture decreased the cell proliferation in stroke rat\textsuperscript{31} or gerbil\textsuperscript{19} model, mounting evidence suggested that acupuncture stimulation can enhance cell proliferation in the DG of cognitive deficient mice\textsuperscript{14}, chronic unphaiatable stressah\textsuperscript{19}, streptozocin-induced diabetic\textsuperscript{19} or maternally separated rats\textsuperscript{17}. Moreover, it has been shown that ischemic gerbils with acupuncture stimulation had more BrdU-positive cells than sham-operated gerbils in the DG\textsuperscript{19}. Altogether, these results may support that the increase in the number of BrdU-positive cells in the DG of KA-injected mice in this study could be due to acupuncture stimulation to HT8.

NPY, a widely expressed peptide in the central and peripheral nervous system, is involved in various brain diseases including seizure, depression, anxiety and drug addiction\textsuperscript{29}, and it is also important to promote cell proliferation in the DG of hippocampus\textsuperscript{20,21}. In addition, it was reported that the substance was increased by acupuncture stimulation for a few days in the rat hippocampus against the NPY reduction by maternal separation\textsuperscript{22} or streptozocin injection\textsuperscript{19}. Therefore, the increased NPY-positive cells in the DG by acupuncture stimulation to HT8 may possibly mediate the cell proliferation under the KA-injected condition. However, since the present study only demonstrates that acupuncture stimulation to HT8 increased the number of BrdU-positive cells and NPY-positive cells significantly under the KA-injected condition, it remains to be tested whether acupuncture stimulation to HT8 could exert similar effects on cell proliferation and NPY expression under normal conditions.

In conclusion, we show that acupuncture stimulation to HT8 increased the population of both BrdU-positive cells and NPY-positive cells in the DG of KA-injected mice. These
results may suggest that acupuncture stimulation to HT8 can enhance the cell proliferation, probably through upregulating NPY expression in the DG of KA-injected mice.

References


