Electroacupuncture to Shenmen Ameliorates Behavioral and Biochemical Responses to Restraint Stress in Rats

Hyun Soo Shim[1,2,3], Hyun Jung Park[1,2,3], Kyung Soo Kim[1,2], Dae Hyun Hahm[3], Hye Jung Lee[3], Insop Shim[3]*

1 : Department of Integrative Medicine, 2 : Research Center of Behavioral Medicine, College of Medicine, The Catholic University, 3 : Acupuncture and Meridian Science Research Center, Kyung Hee University

Acupuncture has been used for the treatment and prevention of stress-related disorders. In the present study, the effect of electroacupuncture on the behavioral and biochemical responses to restraint stress was evaluated in rats. Sprague-Dawley male rats underwent to immobilization stress for 21 days (6 hours/day). Electroacupuncture (2 Hz, 2 mA, and 10 minutes) was applied either to the acupuncture point HT7 (Shenmen) or the nonacupuncture point in the tail for the last 7 days. Rats were randomly divided into four groups: the normal group (n=10, without the restraint stress), the stress group (n=10, with restraint stress), the HT7 group (n=10, with restraint stress and electroacupuncture to HT7), and the NA group (n=10, with restraint stress and electroacupuncture to the nonacupuncture point). The anxiety-related behavior was tested using the elevated plus maze and the Vogel test on day 22. The expression of tyrosine hydroxylase in the locus coeruleus was measured by immunohistochemistry. R maze and the Vogecreased the response of the anxiety-related behavior. The number of tyrosine hydroxylase-immunoreactive cells were also increased. The HT7 group showed a significant decrease of anxiety-related behavioral response, compared to the stress group. The tyrosine hydroxylase-immunoreactive expression was also decreased in the HT7 group. These findings suggestthat electroacupuncture to HT7 might play a role in reducing the stress-related responses, which may be helpful for the treatment of stress-related disorders.

Key words : electroacupuncture, Shenmen (HT7), stress, tyrosine hydroxylase (TH)

Introduction

Stress means any physical or psychological stimulus that disturbs homeostasis. Exposure to stress induced various mood-related disorders, such as depression and anxiety[1]. Stress stimulates the norepinephrine biosynthesis in the sympathetic ganglia and the locus coeruleus[2]. Especially, restraint stress induces the release of norepinephrine in the brain[3].

Acupuncture has been widely used in East Asia for the treatment and prevention of neurological disorders[4]. The therapeutic effects of acupuncture on depressive disorder have been researched in human and animal studies[5-8]. HT7 (Shenmen) has been known one of the major sedating points. It can be used to treat mental disorder, to calm spirit and to pacify the heart[5]. In recent, several studies have reported the effect of HT7 in animal models. Park et al. and Lim et al. reported that acupuncture treatment at acupoint HT7 was effective on anxiety and neuropeptide Y expression in the brain of maternally separated rats[7,9].

In the present study, we evaluated the effects of electroacupuncture to HT7 on the behavioral and biochemical responses induced by restraint stress. Stress-related behavioral changes were evaluated using the elevated plus maze and the Vogel test, and the biochemical responses were also measured by protein expression of tyrosine hydroxylase in the locus coeruleus.

* To whom correspondence should be addressed at : Insop Shim, Acupuncture and Meridian Science Research Center, Kyung Hee University, 1 Hoegidong, Dongdaemengu, Seoul 130-701, Korea
· E-mail : ishim@khu.ac.kr, · Tel : 02-961-0975
· Received : 2010/02/02 · Revised : 2010/03/15 · Accepted : 2010/03/31

Materials and Methods

1. Animals

Adult male Sprague-Dawley rats weighing 230-250 g were obtained from Samtaco Corp. (Gyeonggi-do, Korea). All rats were kept in groups of three and freely provided with food and water ad libitum. Rats were maintained on a 12 hour light/dark cycle regulated at 23℃ room temperature. Rats were acclimatized to their new environment for at least 7 days

before the experiments started. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Catholic University Medical College Institutional Animal Care.

2. Repeated restraint stress and electroacupucture stimulation

Rats were randomly divided into four groups: the normal group (n=10, without the restraint stress), the stress group (n=10, with restraint stress), the HT7 group (n=10, with restraint stress and electroacupuncture to HT7), and the NA group (n=10, with restraint stress and electroacupuncture to the nonacupuncture point). The rats underwent to repeated restraint stress for 21 days in a prone position (6 hours/day). Excess space around each restrained rat was minimized using tape, allowing only slight movements within a cone[10]. Electroacupuncture was applied either to the acupuncture point HT7 or the nonacupuncture point in the tail for the last 7 days. The acupuncture needle (26 gauge, stainless steel) was inserted around 3 mm deep into left and right HT7. The HT7 is located near the inferior lateral wall of the triangular fossa. The acupuncture needles were connected to the output terminals of a stimulator that delivered square waves of 2 Hz and 0.3 ms pulse width for 10 minute per day for 7 days. The intensity of electroacupuncture stimulation was 2 mA[11]. The same parameter of electroacupuncture was applied to rats in the NA group.

3. Elevated plus maze test

The plus maze apparatus was made of black wood. It consisted of two open arms and two closed arms (each arm extended from central space of 50 × 10 × 40 cm). The apparatus was elevated 50 cm above the floor. The animals were individually placed on the central platform facing the closed arm and were allowed to explore the maze for 5 minutes. The following data were recorded: (1) the duration of time spent in the open and the closed arm, and (2) the total number of entries to each compartment of the maze[12].

4. Vogel test

The Vogel test was conducted in polycarbonate cages (32 × 25 × 30 cm) with a grid floor and a spout of a water bottle located 6 cm above the grid floor. Both the grid and the spout were connected to an anxiometer (Columbus Instruments, OH, USA) which delivered electrical shocks and recorded the number of licks. In brief, the rats were d Irived of water for 24 hours and put briethe test cage for adaptation, to be allowed to freely lick the water for 9 minutes. After that, electric shocks were applied through the drinking spout every 20 licks during a 5 minute test period[13].

5. Immunohistochemistry of tyrosine hydroxylase

The rats were transcardially perfused with PBS, then with chilled 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were sectioned coronally (30 μm) on a freezing microtome. The sections were incubated with antibody against tyrosine hydroxylase (1:2000, Zymed Laboratories Inc., CA, USA), and then with biotinylated secondary antibody (Vector Laboratories, CA, USA). After incubation with a Vector Elite ABC Kit (Vector Laboratories), the antibody biotin–avidin–peroxidase complex was visualized with diaminobenzidine. For counting the cells, a microrectangular grid (200 × 200 mm) was placed according to the atlas of Paxinos and Watson[14] under the light microscope.

6. Statistical analysis

The data were analyzed using the one-way ANOVA followed by the Tukey's post hoc comparison. All of the results are represented as mean ± SEM. Significant difference was defined as $p<0.05$ The analysis was performed using SPSS statistical software (version 15.0 for Windows).

Results

1. Effect of electroacupuncture on elevated plus maze test

In Fig. 1 anxiety-like behaviors were altered significantly within groups (spent time in open arms, $F_{(3,19)}=34.060$, $p<0.001$; spent time in closed arms, $F_{(3,19)}=51.509$, $p<0.001$; the number of entries in to arms, $F_{(3,36)}=25.584$, $p<0.001$). The normal group ($p<0.001$), the HT7 group ($p<0.01$) and the NA group ($p<0.05$) spent more time in the open arms and less time in the closed arms, compared to the stress group(Fig. 1A). When the normal group and the stress group were compared, the number of entries was significantly lower in the stress group ($p<0.001$). The HT7 group showed a significantly higher number of entries compared to the stress group($p<0.001$, Fig. 1B).

However, these changes of the HT7 group were not observed in the NA group.

2. Effect of electroacupuncture on Vogel test

The result of the Vogel test is illustrated in Fig. 2 Anxiety-like behavior was altered significantly within groups ($F_{(3,19)}=4.457$, $p<0.05$). When the normal group and the stress group were compared, the number of licks was significantly lower in the stress group ($p<0.05$). The HT7 group showed a significantly higher number of licks compared to the stress group ($p<0.05$). However, the NAgroup did not show an

significantly effect on the number of licks.

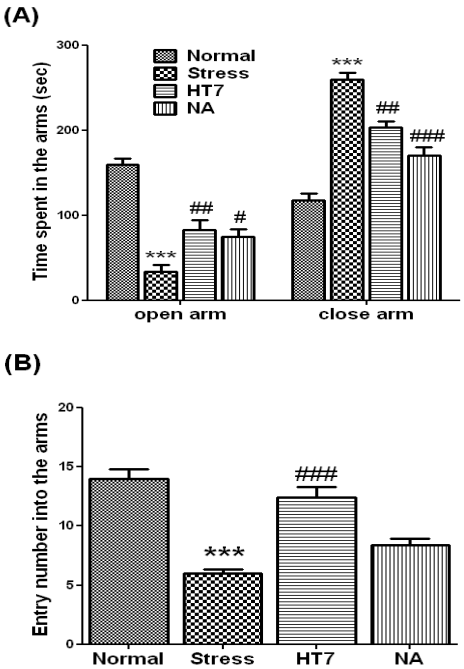

Fig. 1. Behavioral response after electroacupuncture stimulation in the elevated plus maze. (A) The time spent in the open and closed arms of the maze (seconds). (B) the total number of entries (5 minutes). Normal (n=10), the group without restraint stress; Stress (n=10), the group with restraint stress; HT7 (n=10), the group with restraint stress and electroacupuncture (2 Hz, 2 mA, and 10 minutes) to the acupuncture point HT7; NA (n=10), the group with restraint stress and electroacupuncture to the nonacupuncture point (tail). Values are presented as mean ± SEM. ***p<0.001, compared to the normal group; ###p<0.001, ##p<0.01 and #p<0.05, compared to the stress group

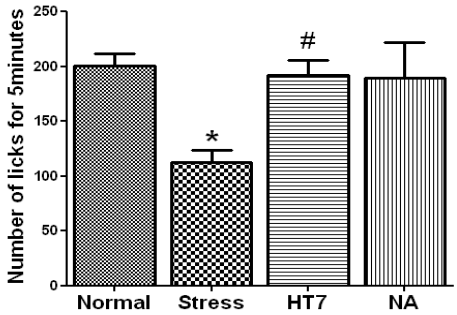

Fig. 2. Behavioral response after electroacupuncture stimulation in the Vogel test. The total number of licks was measured during the 5 minute test period. Normal (n=10), the group without restraint stress; Stress (n=10), the group with restraint stress; HT7 (n=10), the group with restraint stress and electroacupuncture (2 Hz, 2 mA, and 10 minutes) to the acupuncture point HT7; NA (n=10), the group with restraint stress and electroacupuncture to the nonacupuncture point (tail). Values are presented as mean ± SEM. *p<0.05, compared to the normal group; #p<0.05, compared to the stress group

3. Effect of electroacupuncture on tyrosine hydroxylase expression in locus coeruleus

In Fig. 3 the result of tyrosine hydroxylase expression in the locus coeruleus is shown. The expression of tyrosine hydroxylase was altered significantly within groups ($F_{(3,51)}$=52.506, p<0.001). When the normal group and the stress group were compared, the number of tyrosine hydroxylase was significantly higher in the stress group (p<0.001). The HT7 group showed a significantly lower number of tyrosine hydroxylase compared to the stress group (p<0.001).

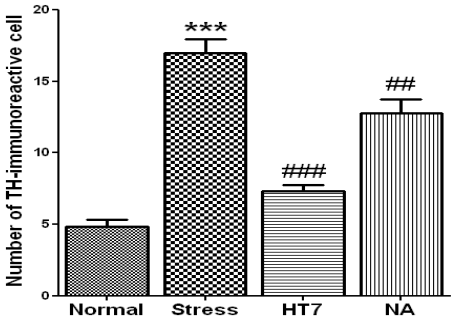

Fig. 3. Expression of tyrosine hydroxylase-immunoreactive cells in the locus coeruleus. The number of tyrosine hydroxylase-immunoreactive cells in the locus coeruleus was measured. Normal (n=10), the group without restraint stress; Stress (n=10), the group with restraint stress; HT7 (n=10), the group with restraint stress and electroacupuncture (2 Hz, 2 mA, and 10 minutes) to the acupuncture point HT7; NA (n=10), the group with restraint stress and electroacupuncture to the nonacupuncture point (tail). Values are presented as mean ± SEM. ***p<0.001, compared to the normal group; ###p<0.001 and ##p<0.01, compared to the stress group

Discussion

In this study, the electroacupuncture to acupuncture point HT7 had significant anxiolytic effects: the HT7 group showed a significant decrease of anxiety-related behavioral response both in elevated plus maze and Vogel test compared to the stress group. In addition, the number of tyrosine hydroxylase-immunoreactive cells were also decreased in the HT7 group compared to the stress group. Our study revealed that the rats in the stress group exhibited more anxiety-like behaviors than those in normal group in the elevated plus maze and Vogel tests after restraint stress for 21 days. The effects of electroacupuncture had point-specificity; the electroacupuncture to nonacupoint did not alter the anxiety-like behaviors induced by long-term immobilization stress. This result supports previous findings of significant increases of anxiety-like behaviors due to the repeated restraint stress in the rats[15]. However electroacupuncture stimulation to HT7 significantly reduced anxiety-like behaviors, compared to the stress group. Chae et al. also showed that a medicinal effect of acupuncture stimulation on nicotine withdrawal in terms of anxiety-like behaviors[16]. They suggested that the acupuncture therapy could have an anxiolytic effect in a variety of animal models.

Norepinephrine-containing neurons in the brain are concentrated in the locus coeruleus[18]. These neurons are part

of a wide network that extend through out the neuroaxis and accounts for about 70% of all norepinephrine in primate's brain[19]. Activation of the locus coeruleus produces intense anxiety, hypervigilance, and suppression of exploratory behavior[17,20,21]. Based on these findings, it has been proposed that clinical anxiety or depression may be the result of alterations of the activities of noradrenergic systemin the locus coeruleus. The locus coeruleus is considered as a crucial site of central nervous system stress response. Numerous reports revealed that corticotrophin-releasing factor could modulate the discharge rate of norepinephrine and the tyrosine hydroxylase activity in the locus coeruleus neurons[22-24]. In this study, repeated restraint stress caused a 350% increase in tyrosine hydroxylase- immunoreactive cells in the locus coeruleus. However, electroacupuncture stimulation to HT7 significantly reduced the expected increases of tyrosine hydroxylase-ir cells in the locus coeruleus. These results suggest that electroacupuncture stimulation to HT7 may have an effect on anxiety-like behavior by decreasing the number of tyrosine hydroxylase-immunoreactive neurons in the locus coeruleus.

Taken together, our experimental results suggest that electroacupuncture stimulation to HT7 could reduce anxiety-like behavior induced by stress.

Acknowledgements

This study was supported by the Korean Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No.R11-2005-014).

References

1. Kaufman, J., Charney, D. Effects of early stress on brain structure and function: implications for understanding the relationship between child maltreatment and depression: Dev. Psychopathol 13: 451-471, 2001.
2. Sabban, E.L., Kcetnansky, R. Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events: Trends Neurosci 24: 91-98, 2001.
3. Mravec, B., Tillinger, A., Bodnar, I., Nagy, G.M., Palkovits, M., Kvetnanský, R. The response of plasma catecholamines in rats simultaneously exposed to immobilization and painful stimuli: Ann NY Acad Sci 1148: 196-200, 2008.
4. Lee, H., Park, H.J., Park, J., Kim, M.J., Hong, M., Yang, J., Choi, S., Lee, H. Acupuncture application for neurological disorders: Neurol Res, 29(1):S49-S54, 2007.
5. Chan, J., Briscomb, D., Waterhouse, E., Candy, A.M. An uncontrolled pilot study of HT7 for 'stress': Acupunct. Med 20: 73-74, 2002.
6. Phol, A., Nordin, C. Clinical and biochemical observations during treatment of depression with electroacupuncture: a pilot study: Hum. Psychopharmacol 17: 345-348, 2002.
7. Park, H.J., Chae, Y., Jang, J., Shim, I., Lee, H., Lim, S. The effect of acupuncture on anxiety and neuropeptide Y expression in the basolateral amygdala of maternally separated rats: Neurosci. Lett 377: 179-184, 2005.
8. Kim, H., Park, H.J., Han, S.M., Hahm, D.H., Lee, H.J., Kim, K.S., Shim, I. The effects of acupuncture stimulation at PC6 (Neiguan) on chronic mild stress-induced biochemical and behavioral responses: Neurosci Lett 460: 56-60, 2009.
9. Lim, S., Ryu, Y.H., Kim, S.T., Hong, M.S., Park, H.J. Acupuncture increases neuropeptide Y expression in hippocampus of maternally-separated rats: Neurosci Lett 343: 49-52, 2003.
10. Yun, S.J., Hahm, D.H., Lee, E.H. Immobilization stress induces the expression of alphaB-crystallin in rat hippocampus: implications of glial activation in stress-mediated hippocampal degeneration: Neurosci Lett 324: 45-48, 2002.
11. Park, H.J., Lee, H.S., Lee, H.J., Yoo, Y.M., Lee, H.J., Kim, S.A., Leem, K., Kim, H.C., Seo, J.C., Kim, E.H., Lim, S., Chung, J.H. Decrease of the electroacupuncture-induced analgesic effects in nuclear factor-kappa B1 knockout mice: Neurosci Lett 319: 141-144, 2002.
12. Bessa, J.M., Mesquita, A.R., Oliveira, M., Pêgo, J.M., Cerqueira, J.J., Palha, J.A., Almeida, O.F., Sousa, N. A trans-dimensional approach to the behavioral aspects of depression: Front Behav Neurosci, 3, 1, Epub, 2009.
13. Vogel, J.R., Beer, B., Clody, D.E. A simple and reliable conflict procedure for testing anti-anxiety agents: Psychopharmacologia 21: 1-7, 1971.
14. Paxinos, G., Watson, C., Pennisi, M. Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex: Strain and weight 13: 139-143, 1985.
15. San Román, J.A., Sanz-Ruiz, R., Ortega, J.R., Pérez-Paredes, M., Rollán, M.J., Muñoz, A.C., Segura, F., Jimenez, D., Carnero, A., Pinedo, M., Arnold, R., Gómez, I., Fernández-Aviles, F. Safety and predictors of complications with a new accelerated dobutamine stress echocardiography protocol: J Am Soc Echocardiogr 21: 53-57, 2008.
16. Chae, Y., Yeom, M., Han, J.H., Park, H.J., Hahm, D.H., Shim, I., Lee, H.S., Lee, H. Effect H. Ecupuncture on anxiety-like behavior during nicotine withdrawal and relevant mechanisms: Neusci Lett 430: 98-102, 2008.
17. Liao, Y.Y., Seto, K., Saito, H., Fujita, M., Kawakami, M.

Effect of acupuncture on adrenocortical hormone production. (Ⅱ) Effect of electro-acupuncture on the response of adrenocortical hormone production to stress: Am J Chin Med 8: 160-166, 1980.

18. Dinan, T.G., Aston-Jones, G. Acute haloperidol increases impulse activity of brain noradrenergic neurons: Brain Res 307: 359-362, 1984.
19. Svensson, T.H. Stress, central neurotransmitters, and the mechanism of action of alpha 2-adrenoreceptor agonists: J Cardiovasc Pharmacol, 10, suppl 12: S88-92, 1987.
20. Redmond, D.E.Jr., Huang, Y.H. The primate locus coeruleus and effects of clonidine on opiate withdrawal: J Chin Psychiatry 43: 25-29, 1982.
21. Foote, S.L., Berridge, C.W., Adams, L.M., Pineda, J.A. Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting, and attending: Prog Brain Res 88: 521-532, 1991.
22. Valentino, R.J., Foote, S.L. Corticotrophin-releasing factor disrupts sensory responses of brain noradrenergic neurons: Neuroendocrinology 45: 28-36, 1987.
23. Valentino, R.J., Foote, S.L., Page, M.E. The locus coeruleus as a site for integrating corticotrophin-releasing factor and noradrenergic mediation of stress responses: Ann NY Acad Sci 697: 173-188, 1993.
24. Melia, K.R., Rasmussen, K., Terwilliger, R.Z., Haycock, J.W., Nestler, E.J., Duman, R.S. Coordinate regulation of the cyclic AMP system with firing rate and expression of tyrosine hydroxylase the rat locus coeruleus: Effects of chronic stress and drug treatment: J Neurochem 58: 494-502, 1992.