J Acupunct Meridian Stud 2016;9(1):4–10

Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Hypothalamic Norepinephrine Mediates Acupunctural Effects on Hypothalamic–Pituitary–Adrenal Axis During Ethanol Withdrawal

Zheng Lin Zhao [1,†], Sang Chan Kim [2,†], Jie Zhang [1], Hong Feng Liu [1], Bong Hyo Lee [2], Eun Young Jang [2], Chul Won Lee [2], Il Je Cho [2], Won G. An [3], Chae Ha Yang [2], Young Woo Kim [2], Rong Jie Zhao [1,2,*], Yi Yan Wu [1,*]

[1] *Department of Pharmacology, Mudanjiang Medical University, Mudanjiang, China*
[2] *Medical Research Center for Globalization of Herbal Formulation, College of Oriental Medicine, Daegu Haany University, Daegu, South Korea*
[3] *Department of Pharmacology, School of Korean Medicine, Pusan National University, Yangsan, South Korea*

Available online 4 June 2015

Received: Jan 8, 2015
Revised: May 9, 2015
Accepted: May 13, 2015

KEYWORDS
acupuncture;
corticosterone;
ethanol;
hypothalamus;

Abstract
A previous study demonstrated that acupuncture at ST36 (Zu-San-Li) attenuates ethanol withdrawal (EW)-induced hyperactivation of the hypothalamic–pituitary–adrenal axis in rats. The current study investigated the involvement of hypothalamic norepinephrine (NE) in that process. Rats were intraperitoneally treated with 3 g/kg/d of ethanol or saline for 28 days. After 24 hours of EW, acupuncture was applied to rats at bilateral ST36 points or at nonacupoints (tail) for 1 minute. A high-performance liquid chromatography analysis showed that EW significantly increased both the NE and the 3-methoxy-4-hydroxy-phenylglycol (MHPG) levels in the hypothalamic paraventricular nucleus (PVN). Western blot analysis also revealed that EW markedly

* Corresponding authors. Department of Pharmacology, Mudanjiang Medical University, 3 Tongxiang Street, Aimin District, Mudanjiang 157011, China.
E-mail: zhao_rongjie@yahoo.com (R.J. Zhao), adgzfy@163.com (Y.Y. Wu).
† Z.L. Zhao and S.C. Kim contributed equally to this work.

pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2015.05.007

norepinephrine;
tyrosine hydroxylase

elevated the phosphorylation rates of tyrosine hydroxylase (TH), but spared TH protein expression in the PVN. However, acupuncture at ST36, but not at nonacupoints, greatly inhibited the increase in the hypothalamic NE, MHPG, and phosphorylation rates of TH. Additionally, post-acupuncture infusion of NE into the PVN significantly attenuated the inhibitory effects of acupuncture at ST36 on the oversecretion of plasma corticosterone during EW. These results suggest that acupuncture at ST36 inhibits EW-induced hyperactivation of the hypothalamic NEergic system to produce therapeutic effects on the hypothalamic—pituitary—adrenal axis.

1. Introduction

Disturbances of the hypothalamic—pituitary—adrenal (HPA) axis driven by ethanol withdrawal (EW) underlie EW-induced somatic and affective symptoms that are closely related to alcoholism [1]. Ethanol can not only directly affect corticotropin-releasing factor (CRF) gene expression in the hypothalamic paraventricular nucleus (PVN) but can also indirectly influence PVN CRF activity through the neurotransmitters and neuropeptides that regulate CRF secretion [2,3]. Among the neurotransmitters, norepinephrine (NE) appears to be very important. The NEergic system in the brain densely innervates the PVN, and NE acts as a key secretagogue for CRF. Evidence shows a functional change along with altered HPA activity following exposure to ethanol. Acute ethanol increases hypothalamic NE neosynthesis [4] and plasma corticosterone (CORT) secretion [5], and chronic ethanol can produce elevated blood glucocorticoid levels as well as increased activity in the sympathetic nervous system [6]. Dar and Wooles [7] have reported increased hypothalamic NE concentrations in EW mice, and we have also demonstrated that EW raises amygdaloid NE and CRF mRNA levels in rats [8,9].

In traditional Chinese medicine, acupuncture exerts its therapeutic effects on alcoholism by restoring the homeostasis of the body via rectifying the disturbed biochemical balance [8]. The PVN CRF plays a critical role in the neuroendocrinological mediation of homeostasis in the body, and its activity is regulated by catecholamines. Therefore, it is conceivable that the PVN CRF is an important target of acupuncture treatment, which may be mediated by modulating catecholamines in the PVN. Indeed, catecholamines in the brain are often targeted by acupunctural interventions to produce therapeutic effects. Acupuncture at HT7 attenuates morphine-induced behavioral hyperactivity by reducing accumbal dopamine release [10], and acupuncture at SP6 alleviates morphine withdrawal-induced depression-like behavior via modulation of the NEergic system in the rat brain stem [11].

Recently, we reported that acupuncture at ST36 inhibited EW-induced hyperactivation of the HPA axis via the modulation of hypothalamic CRF [12]. Given the important role of hypothalamic NE in the regulation of the HPA response to ethanol, the present study evaluated the possible involvement of the hypothalamic NE system in the acupunctural modulation of HPA activity during EW.

2. Materials and methods

2.1. Animals and experimental design

Adult male Sprague—Dawley rats (250—270 g) were obtained from the Laboratory Animal Center at Mudanjiang Medical University (Mudanjiang, China). The rats were given food and water *ad libitum* and maintained on a 12-hour light/12-hour dark cycle throughout the course of the study. All animal procedures were conducted in accordance with the National Institutes of Health guidelines concerning the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Mudanjiang Medical University.

The rats were treated with 3 g/kg/d ethanol (20% w/v) or saline by intraperitoneal injection for 28 days. After the final dose of ethanol, the rats underwent EW for 24 hours. The acupuncture groups were subjected to acupuncture at either acupoint ST36 or the nonacupoint (tail; Fig. 1A) for 1 minute.

For purposes of acupuncture stimulation, stainless steel needles (0.2 mm in diameter) were inserted into two identical ST36 acupoints (or tail nonacupoints) located on the left and right sides of the animal (depth of 2—3 mm), and manipulated with the method of reduction and reinforcement. The anatomical locations of the stimulated acupuncture points in the rats were equivalent to the acupoints in humans. In the sham acupuncture treatment, two groups of rats were also treated with ethanol (ethanol-treated control rats) or saline (saline-treated control rats) and were then held for 1 minute without the insertion of acupuncture needles to obtain the same immobilization as with the acupuncture-treated rats.

Five minutes after the acupuncture or sham treatment, the rats were euthanized and decapitated, and the entire brain was removed and stored at −80°C for further biochemical analyses.

2.2. High-performance liquid chromatographic analysis of NE and its metabolites

Tissue samples of the PVN were punched out from the stored brains according to the coordinates of the PVN (anterior—posterior = −2.0 mm, medial—lateral = 0.3 mm, dorsal—ventral = −7.4 mm, based on the Paxinos and Watson rat brain atlas [13]; Fig. 1B) and divided into two parts, one for high performance liquid chromatographic (HPLC) analysis and the other for Western blot analysis.

The frozen PVN tissues were sonicated in 1.0 mL of 0.1 mol/L $HClO_4$ and centrifuged at 26,000*g* and 4°C for 15 minutes. Then, a 20-μL supernatant aliquot was injected directly into the HPLC with an electrochemical detector (Coulochem II; ESA, Bedford, MA, USA). The HPLC system consists of a C-18 reverse-phase column (5 μm ODS; Altex, Ann Arbor, MI, USA) and an electrochemical transducer with a glassy carbon electrode set at 350 mV. The mobile phase consisted of 0.163 mol/L citric acid, 0.02 mmol/L EDTA, 0.69 mmol/L

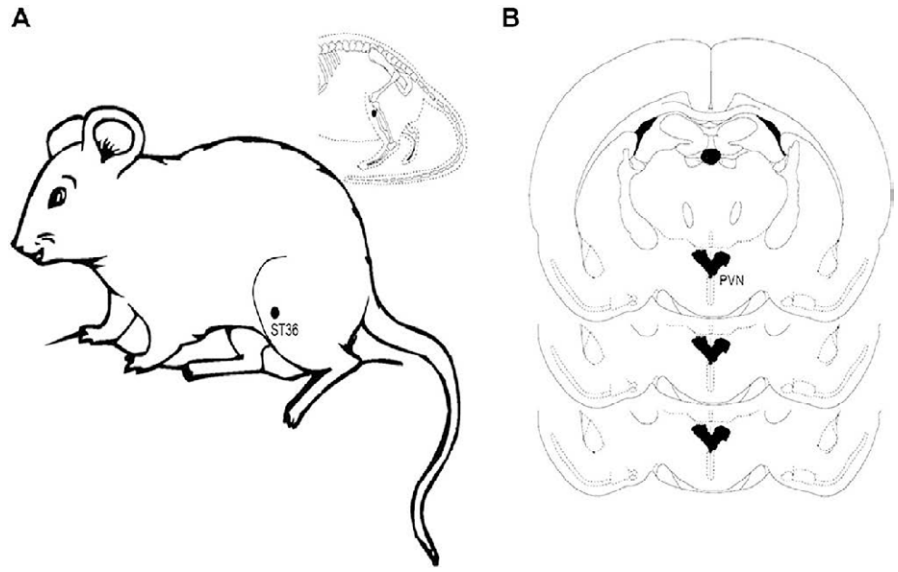

Figure 1 (A) Diagram of the acupoint ST36 and the nonacupoint located in the tail in a rat. (B) Diagrammatic representation of coronal sections showing the paraventricular nucleus of hypothalamus. PVN, paraventricular nucleus.

sodium octanesulfonic acid as an ion-pairing reagent, 4% (v/v) acetonitrile, and 1.7% (v/v) tetrahydrofurane and was titered to pH 3.0 by H_3PO_4. The peaks and values of NE and 3-methoxy-4-hydroxy-phenylglycol (MHPG) in the samples were identified and calculated by comparing their retention times and peak heights with those of standards (Sigma-Aldrich, St. Louis, MO, USA). Results are reported as ng/mg protein. The protein concentrations in brain homogenates were determined using the bicinchoninic acid protein assay.

2.3. Western blot analysis

The frozen PVN tissues were homogenized in lysis buffer [20 mM Tris, 5 mM EDTA, 1% Nonidet P-40 (v/v), and protease and phosphatase inhibitors], incubated for 20 minutes on ice, and then centrifuged at 13,000*g* for 20 minutes at 4°C. The supernatants were resolved by electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel for the separation of proteins. The following antibodies were used for the Western blotting assays: primary antibodies, a rabbit polyclonal antibody to tyrosine hydroxylase (TH; Abcam, Cambridge, MA, USA) and a rabbit polyclonal antibody to phosphorylated TH at serine 40 (anti-pTH ser40; Santa Cruz Biotechnology, Santa Cruz, CA, USA); a secondary antibody, IRDye 800CW goat anti-rabbit immunoglobulin G (Li-Cor Bioscience, Lincoln, NE, USA). β-Actin was used as a loading control and detected with a rabbit polyclonal antibody (Abcam). The ODYSSEY infrared Imaging System (Li-Cor Biosciences) was used to detect signals according to the manufacturer's manual.

2.4. Microinjection of NE into the PVN

To determine whether NE in the PVN mediates the inhibitory effect of acupuncture on the EW-induced oversecretion of plasma CORT, NE 20 nmol/100 nL/side was dissolved in modified Ringer's solution (MRS; 150 mM NaCl, 3.0 mM KCl, 1.4 mM $CaCl_2$, and 0.8 mM $MgCl_2$ in 10 mM phosphate buffer at pH 7.4) and bilaterally microinjected into the PVN 5 minutes after acupuncture treatment. Radioimmunoassay (RIA) was performed after another 5 minutes to measure the plasma level of CORT. For the intra-PVN infusion of NE, a cohort of rats were bilaterally implanted with stainless steel guide cannulae (15 mm; 23-gauge) into the PVN under anesthesia (sodium pentobarbital, 50 mg/kg, intraperitoneally) using a stereotaxic instrument with the cannula tips 2 mm above the PVN. After 7 days of recovery following surgery, the rats were subjected to the same EW and acupuncture treatment as the aforementioned schedule. The intra-PVN infusion of NE was accomplished using motorized syringe pumps (Sage Instruments, Boston, MA, USA) over 30 seconds.

2.5. Plasma CORT assays

To measure plasma levels of CORT, the rats were euthanized and decapitated 5 minutes after NE administration, 1.0 mL of blood was collected in a chilled tube containing EDTA (20 mg/mL, 20 μL), and the brain was removed for histological verification of cannula placements.

The blood was centrifuged at 1,000*g* for 10 minutes at 4°C, and the plasma was separated for measuring CORT. The CORT levels in the plasma were measured using the ImmuChem Double Antibody ^{125}I RIA kit from MP Biomedicals (Orangeburg, NY, USA); the values are expressed in ng/mL.

2.6. Statistical analysis

All data were expressed as means ± standard errors of the mean and statistically analyzed with one-way analysis of variance followed by the Newman–Keuls multiple-comparison test using the commercially available GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). A p value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Effect of acupuncture at ST36 on EW-induced alteration of NE and MHPG levels in the PVN

As shown in Fig. 2, we found significantly higher NE and MHPG levels in the PVN in ethanol-treated control rats compared with saline-treated control rats [NE: $F_{3,24} = 6.51$, $p < 0.01$, ethanol ($n = 7$) *vs.* saline ($n = 7$), $p < 0.05$; MHPG: $F_{3,24} = 11.48$, $p < 0.001$, ethanol ($n = 7$) *vs.* saline ($n = 7$), $p < 0.01$], indicating that hyperproduction of NE in the PVN occurred during the early stage of EW (24 hours after the final dose of ethanol), accompanied by high utilization. However, the hyperproduction and high utilization of NE was greatly inhibited by acupuncture treatment at ST36 [NE: ethanol *vs.* ethanol + ST36 ($n = 7$), $p < 0.05$; MHPG: ethanol *vs.* ethanol + ST36 ($n = 7$), $p < 0.01$] but not at the nonacupoint (tail) [NE: ethanol *vs.* ethanol + Tail ($n = 7$), $p > 0.05$; MHPG: ethanol *vs.* ethanol + Tail ($n = 7$), $p > 0.05$; Fig. 2].

3.2. Effect of acupuncture at ST36 on EW-induced functional changes of TH in the PVN

To investigate a possible link between the hyperproduction of NE and the functional states of TH in the PVN during EW, the TH protein expression and its phosphorylation rate at serine 40 in the PVN were examined using Western blot analyses. The analyses revealed no significant difference between the saline-treated control group and the ethanol-treated control group in TH protein (total TH) levels 24 hours after the final dose of ethanol; however, we observed a significantly higher pTH/TH ratio in the ethanol-treated control group relative to the saline-treated control group [$F_{3,16} = 6.72$, $p < 0.01$, ethanol ($n = 5$) *vs.* saline ($n = 5$), $p < 0.01$], indicating increased phosphorylation of TH in the PVN during EW. Similar to the effect of acupuncture at ST36 on NE production and utilization, acupuncture at ST36, but not at the nonacupoint, restored the elevated ratio of pTH/TH [ethanol *vs.* ethanol + ST36 ($n = 5$), $p < 0.05$; ethanol *vs.* ethanol + Tail ($n = 5$), $p > 0.05$; Fig. 3].

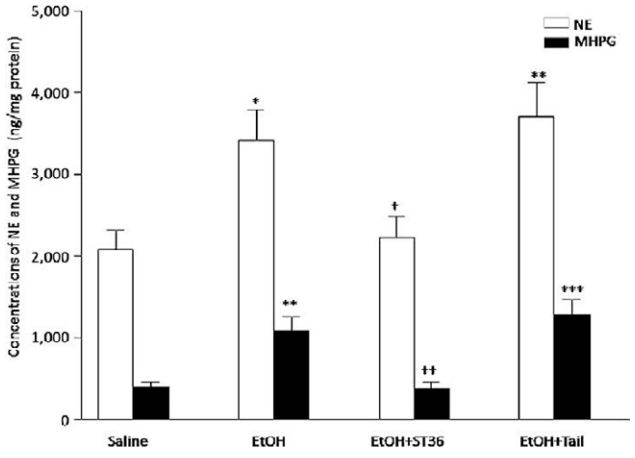

Figure 2 Acupuncture at the ST36 but not at the nonacupoint significantly reduced EW-induced increased levels of hypothalamic NE and MHPG. Data are expressed as mean ± standard error of the mean of the levels of NE or MHPG. EtOH = ethanol; EW = ethanol withdrawal; NE = norepinephrine; MHPG = 3-methoxy-4-hydroxy-phenylglycol; ST36 = acupuncture at the acupoint ST36; Tail = acupuncture at the nonacupoint located in the tail. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with saline-treated control group; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, compared with EtOH-treated control group.

3.3. Abolition of the inhibitory effect of acupuncture on EW-induced oversecretion of plasma CORT by the intra-PVN infusion of NE

In a previous study, acupuncture at ST36 attenuated EW-induced hyperactivation of the HPA. In the present study, RIA consistently showed a significant increase in plasma CORT levels during EW [$F_{3,20} = 6.95$, $p < 0.01$, ethanol ($n = 6$) *vs.* saline ($n = 6$), $p < 0.01$], which was significantly inhibited by acupuncture at ST36 [ethanol *vs.* ethanol + ST36 + MRS ($n = 6$), $p < 0.01$]. However, RIA also revealed a significant increase in plasma CORT secretion in NE-infused rats compared with MRS-infused rats [ethanol + ST36 + NE ($n = 6$) *vs.* ethanol + ST36 + MRS, $p < 0.05$], and the level of CORT in the NE-infused group did not differ from that in the ethanol-treated control group (ethanol + ST36 + NE *vs.* ethanol, $p > 0.05$ group). These results indicate that the postacupuncture infusion of NE into the PVN greatly diminished the inhibitory effect of acupuncture on the hyperactivation of the HPA during EW (Fig. 4).

4. Discussion

In the previous study, we demonstrated that acupuncture at acupoint ST36 attenuated EW-induced hyperactivation of the HPA via modulation of hypothalamic CRF [12]. In the present study, we found markedly increased NE and MHPG levels in the PVN 24 hours after the termination of 28 days of ethanol administration; this was prevented by acupuncture treatment at ST36 but not at the nonacupoint. Additionally, the EW did not produce significant alteration in TH protein expression, but it did generate an increase in the TH phosphorylation rate at serine 40 in the PVN, which was also blocked by acupuncture treatment at ST36 but not at the nonacupoint. Moreover, the postacupuncture infusion of NE into the PVN led to a significant abatement of the inhibitory effect of acupuncture at ST36 on EW-induced oversecretion of plasma CORT. These results suggest that the attenuating effect of acupuncture at ST36 on EW-induced hyperactivation of the HPA is mediated through the NE system in the PVN.

Generally, the levels of both NE and CRF in the PVN fluctuate during EW depending on the amount of ethanol and the duration of EW. Nonetheless, it is now widely acknowledged that central NEergic activity increases during withdrawal from drugs of abuse, at least during the early stage of withdrawal, which elevates the activity of the stress system and promotes relapse after periods of abstinence [14,15]. Recently, Skelly and Weiner [16] reported that the selective NEergic alpha1 receptor antagonist prazosin concurrently blocks the anxiety and the increase in

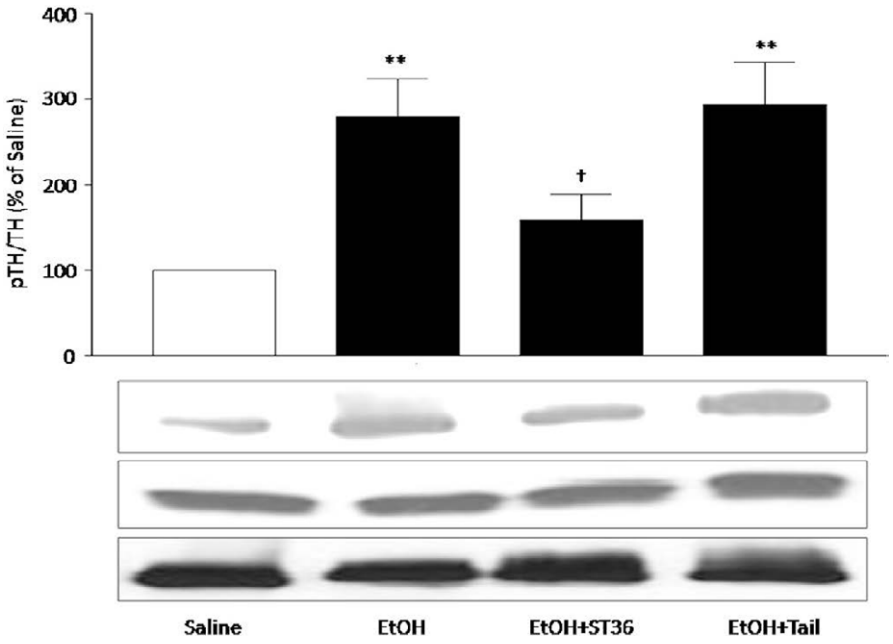

Figure 3 Acupuncture at the ST36 but not at the nonacupoint inhibited EW-induced increased TH phosphorylation rates in the PVN. Data are expressed as mean ± standard error of the mean of the percentage of saline-treated control group. EtOH = ethanol; ST36 = acupuncture at the acupoint ST36; Tail = acupuncture at the non-acupoint located in the tail. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with saline-treated control group; $^{\dagger}p < 0.05$, compared with EtOH-treated control group. EtOH = ethanol; EW = ethanol withdrawal; PVN = paraventricular nucleus; TH = tyrosine hydroxylase.

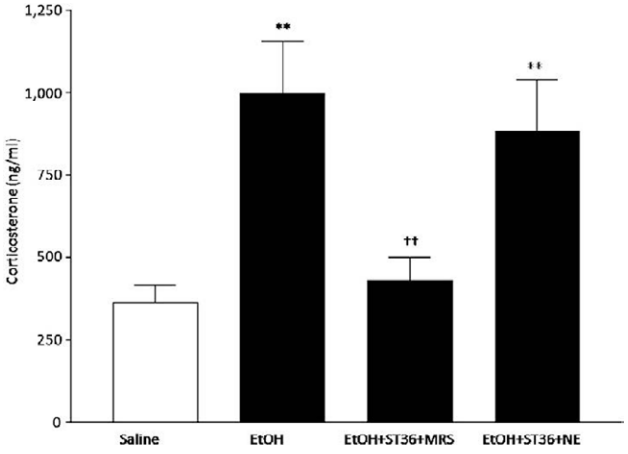

Figure 4 The postacupuncture infusion of norepinephrine (NE) into the PVN diminished the inhibitory effect of acupuncture on EW-induced oversecretion of plasma corticosterone. Data are expressed as mean ± standard error of the mean of the levels of plasma corticosterone. $^{**}p < 0.01$, compared with saline-treated control group; $^{\dagger\dagger}p < 0.01$, compared with EtOH-treated control group. EtOH = ethanol; EW = ethanol withdrawal; MRS = modified Ringer's solution; PVN = paraventricular nucleus; ST36 = acupuncture at the acupoint ST36; Tail = acupuncture at the nonacupoint located in the tail.

ethanol intake induced by EW, reflecting the positive correlation between the NEergic system and the HPA in the regulation of ethanol consumption. The simultaneous hyperactivation of NE and CRF in the central nervous system during EW has also been demonstrated in our previous studies showing elevated activation in both amygdaloid NE and CRF during EW [8,9]. Additionally, in a recently published study, we also reported increased CRF protein expression in the rat PVN 24 hours after cessation of ethanol administration [12]. In the present study, we consistently observed markedly increased concentrations of NE and MHPG in the PVN during EW. These results indicate increased NEergic activity along with enhanced CRF functioning in the PVN during EW. The present study also found that the increase in NE levels and utilization was effectively inhibited by prior acupuncture treatment at ST36. This result is consistent with the inhibitory effect of acupuncture at ST36 on the hyperactivation of the HPA during EW shown by the recent study [12]. Given that NE is a major secretagogue for CRF in the PVN, it can be concluded that acupuncture treatment at ST36 attenuates the EW-induced hyperactivation of the HPA axis triggered by increased PVN CRF activity via inhibiting the PVN NEergic activity.

The production of NE is regulated primarily by TH, the rate-limiting enzyme in the synthesis of catecholamines. Various stressors, including withdrawal from drugs of abuse, elevate the TH protein and mRNA expressions in both the somatic and terminal loci within a NEergic neuron. For example, morphine withdrawal results in an increase in TH functioning in both the locus coeruleus (LC) and PVN, which contributes to the adverse effects of withdrawal [17,18]. Upregulation of norepinephrinergic (NEergic) functioning in the PVN can be caused by increased TH protein expression and/or activities. TH activity is regulated by hierarchical phosphorylation at serine residues 19, 31, and 40, and evidence shows that phosphorylation at serine 40 is the most critical in enhancing TH activity [19,20]. In the present

study, Western blot analyses revealed that TH protein levels in the PVN remained unchanged, whereas increased phosphorylation rates during EW were observed. These results indicate that the elevated NEergic functioning in the PVN during EW resulted from the promoted TH phoshporylation at serine 40 rather than from an increase in TH protein expression. Furthermore, the Western blot analyses also showed that acupuncture treatment at ST36 significantly attenuated the increased TH phosphorylation rate. Taken together, these results suggest that acupuncture at ST36 can normalize the hyperphosphorylated state of hypothalamic TH at serine 40 to inhibit the hyperactivation of the NEergic system during EW.

Moreover, in the present study, the involvement of hypothalamic NE in the acupunctural modulation of the HPA during EW was further identified by the bilateral infusion of NE into the PVN after acupuncture treatment. The RIA results showed that the postacupuncture infusion of NE greatly diminished the inhibitory effect of acupuncture at ST36 on the oversecretion of plasma CORT during EW. These results pharmacologically support that acupuncture at ST36 attenuates the hyperactivation of the HPA during EW via modulation of the hypothalamic NE system.

ST36 is the He-Sea point in the "stomach" meridian (ST) and plays an important role in maintaining energy and humoral homeostasis in the body. The hypothalamus is the center for maintaining internal homeostasis in the body, and chronic ethanol interferes with hypothalamic NEergic transmissions to induce internal instability. Therefore, it is reasonable that acupuncture at ST36 would modulate the hypothalamic NEergic system to produce therapeutic effects on EW symptoms. In traditional Chinese medicine, acupuncture corrects the unbalanced Yin and Yang via the facilitation of stagnant Qi to treat the relevant disease. As phosphorylated protein can be considered the Yang (activated) form of a protein, we can infer that acupuncture rectifies the disturbed molecular signaling underlying a disease by restoring the balance between non-phosphorylated (Yin) and phosphorylated (Yang) proteins. In the present study, this notion was supported by the result that acupuncture at ST36 effectively inhibited the EW-induced increased phosphorylation of TH to normalize the disturbed hypothalamic NEergic function.

In summary, the present study showed significantly increased hypothalamic NE activity 24 hours after the termination of ethanol administration. This was caused by the increase in TH phosphorylation, and acupuncture at ST36—but not at the nonacupoint—significantly blocked this increase. Moreover, in the present study, the post-acupuncture microinfusion of NE into the PVN greatly attenuated the inhibitory effect of acupuncture at ST36 on the hyperactivation of the HPA induced by EW. These findings suggest that the therapeutic effect of acupuncture at ST36 on the hyperactivation of the HPA during EW is mediated through the modulation of NEergic system in the PVN.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

Acknowledgments

This study was supported by the Research Foundation for Overseas & Returned Scholars from Educational Commission of Heilongjiang Province (1154h24), the Natural Science Foundation of Heilongjiang Province (H201495), China, and the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP; No. 2011-0030124).

References

[1] Becker HC. Effects of alcohol dependence and withdrawal on stress responsiveness and alcohol consumption. *Alcohol Res*. 2012;34:448—458.
[2] Rivier C. Role of hypothalamic corticotropin-releasing factor in mediating alcohol-induced activation of the rat hypothalamic—pituitary—adrenal axis. *Front Neuroendocrinol*. 2014; 35:221—233.
[3] Li Z, Kang SS, Lee S, Rivier C. Effect of ethanol on the regulation of corticotropin- releasing factor (CRF) gene expression. *Mol Cell Neurosci*. 2005;29:345—354.
[4] Pohorecky LA, Jaffe LS. Noradrenergic involvement in the acute effects of ethanol. *Res Commun Chem Pathol Pharmacol*. 1975;12:433—447.
[5] Pastor R, Sanchis-Segura C, Aragon CM. Brain catalase activity inhibition as well as opioid receptor antagonism increases ethanol-induced HPA axis activation. *Alcohol Clin Exp Res*. 2004;28:1898—1906.
[6] Patterson-Buckendahl P, Kubovcakova L, Krizanova O, Pohorecky LA, Kvetnansky R. Ethanol consumption increases rat stress hormones and adrenomedullary gene expression. *Alcohol*. 2005;37:157—166.
[7] Dar MS, Wooles WR. Striatal and hypothalamic neurotransmitter changes during ethanol withdrawal in mice. *Alcohol*. 1984;1:453—458.
[8] Zhao ZL, Zhao GW, Li HZ, Yang XD, Wu YY, Lin F, et al. Acupuncture attenuates anxiety-like behavior by normalizing amygdaloid catecholamines during ethanol withdrawal in rats. *Evid Based Complement Alternat Med*. 2011;2011:429843.
[9] Zhao Z, Jin X, Wu Y, Yang X, Xu Y, Jiang JZ, et al. Amygdaloid corticotropin-releasing factor is involved in the anxiolytic effect of acupuncture during ethanol withdrawal in rats. *J Acupunct Meridian Stud*. 2013;6:234—240.
[10] Kim MR, Kim SJ, Lyu YS, Kim SH, Lee YK, Kim TH, et al. Effect of acupuncture on behavioral hyperactivity and dopamine release in the nucleus accumbens in rats sensitized to morphine. *Neurosci Lett*. 2005;387:17—21.
[11] Lee B, Sur BJ, Shim I, Hahm DH, Lee H. Acupuncture stimulation attenuates impaired emotional-like behaviors and activation of the noradrenergic system during protracted abstinence following chronic morphine exposure in rats. *Evid Based Complement Alternat Med*. 2014;2014:216503.
[12] Zhao Z, Lee BH, Lin F, Guo Y, Wu Y, In S, et al. Effects of acupuncture at Zu-San-Li (ST36) on the activity of the hypothalamic—pituitary—adrenal axis during ethanol withdrawal in rats. *J Acupunct Meridian Stud*. 2014;7:225—230.
[13] Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press; 1988.
[14] Fuertes G, Laorden ML, Milanés MV. Noradrenergic and dopaminergic activity in the hypothalamic paraventricular nucleus after naloxone-induced morphine withdrawal. *Neuroendocrinology*. 2000;71:60—67.
[15] Laorden ML, Castells MT, Milanés MV. Effects of morphine and morphine withdrawal on brainstem neurons innervating hypothalamic nuclei that control the pituitary—adrenocortical axis in rats. *Br J Pharmacol*. 2002;136:67—75.

[16] Skelly MJ, Weiner JL. Chronic treatment with prazosin or duloxetine lessens concurrent anxiety-like behavior and alcohol intake: evidence of disrupted noradrenergic signaling in anxiety-related alcohol use. *Brain Behav*. 2014;4:468—483.

[17] Benavides M, Laorden ML, Marín MT, Milanés MV. Role of PKC-alpha, gamma isoforms in regulation of c-Fos and TH expression after naloxone-induced morphine withdrawal in the hypothalamic PVN and medulla oblongata catecholaminergic cell groups. *J Neurochem*. 2005;95:1249—1258.

[18] Lee B, Sur B, Yeom M, Shim I, Lee H, Hahm DH. Effect of berberine on depression- and anxiety-like behaviors and activation of the noradrenergic system induced by development of morphine dependence in rats. *Korean J Physiol Pharmacol*. 2012;16:379—386.

[19] Dunkley PR, Bobrovskaya L, Graham ME, von Nagy-Felsobuki EI, Dickson PW. Tyrosine hydroxylase phosphorylation: regulation and consequences. *J Neurochem*. 2004;91:1025—1043.

[20] Harada, Wu J, Haycock JW, Goldstein M. Regulation of L-DOPA biosynthesis by site-specific phosphorylation of tyrosine hydroxylase in AtT-20 cells expressing wild-type and serine 40-substituted enzyme. *J Neurochem*. 1996;67:629—635.