Electroacupuncture (EA) Speeds Up the Regulation of Hypothalamic Pituitary Adrenal Axis Dysfunction in Acute Surgical Trauma Rats: Mediated by Hypothalamic Gamma-Aminobutyric Acid (GABA)$_A$ Receptors

Liting Zhu[1#], Jing Zhu[2#], Zhejun Chen[1], Zehui Meng[1], Mingda Ju[1], Mizhen Zhang[1], Gencheng Wu[1], Zhanzhuang Tian[1*]

[1]Department of Integrative Medicine and Neurobiology, School of Basic Medical Sciences, State Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Institute of Acupuncture Research, Academy of Integrative Medicine, Fudan University, Shanghai, China
[2]Department of Anatomy, School of Basic Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China
Email: *tianvv@shmu.edu.cn

Abstract

Hypothalamic Corticotropin-releasing factor (CRF) directly activates the hypothalamic pituitary adrenal axis (HPA axis) during the surgical trauma induced stress response. Electroacupuncture (EA) has been demonstrated to have stress relieving effects in breast surgery, colorectal surgery, prostatectomy and craniotomy. This study was aimed to investigate the hypothesis that EA could regulate hypothalamic CRF in surgical trauma rats. In experiment one, Sprague-Dawley (SD) male rats were divided into intact, model (10% partial hepatectomy), sham EA and EA group. Rats from the Sham EA and EA group were stimulated at ST36-*Zusanli* and SP6-*Sanyiniiao* acupoints twice, 24 hours before the surgery and immediately after the surgery. Expressions of hypothalamic CRF and CRFR, GABA receptors, glutamate decarboxylase (GAD), serum adrenocorticotropic hormone (ACTH) and Corticosterone (CORT) were observed at 2, 4, 8 and 24 h after the surgery by radioimmunoassay (RIA), western blot, real-time PCR and immunohistochemistry. In the experiment two, SD male rats were divided into the intact, model, model + vehicle, model + L-838,417 EA and EA + L838,417 group. It was found that hypothalamus CRF, serum ACTH and CORT levels were increased in model group compared with the intact group, and those in the EA

#These two authors contributed equally.

group decreased in comparison with the model group. Compared with the model group, hypothalamus-aminobutyric acid (GABA) receptor Aα3 mRNA and protein expressions of the EA group raised strikingly. In conclusion, EA alleviated surgical stress response by improving the GABA synthesis in hypothalamus, thus enhancing GABA receptors' inhibitory regulation of the HPA axis dysfunction in rats with acute surgical trauma.

Keywords

1. Introduction

Surgical trauma leads to dysfunction of the hypothalamic pituitary adrenal axis (HPA axis), and results in hypermetabolic, organ damage or immunosuppression [1] [2]. Researchers have done many efforts to reduce endocrinal disorders from trauma, however, the mechanism still needs to be clarified as there is no significant progress [3] [4].

EA has been used clinically wildly, such as breast surgery [5], colorectal surgery [6], supratentorial craniotomy [7] and infratentorial craniotomy [8], and in experimental animal models since the 1970s [9] [10], has been adopted to relieve surgical stress since the 1980s [11]. It was found that EA suppressed surgical stress during the perioperative period by relieving pain from previous clinical trials [11]. And the anti-stress effects of EA were most likely derived from its capacity to decrease the levels of serum ACTH and Cortisol [12] and its ability to down-regulate CRF over-expression in the hypothalamus in rats undergoing surgery [13].

CRF is a peptide released by the hypothalamus, which is widely distributed in almost all brain regions, and plays a pivotal role in the endocrine, autonomic and behavioral responses to stress by interacting mostly with GABAergic neurons [14] [15]. The activation of CRF neurons in the paraventricular nucleus (PVN) is inhibitory regulated by GABAergic transmission from the peri-PVN or the bed nucleus of the stria terminalis (BnST) [16]. Γ-aminobutyric acid (GABA) receptors are divided into two types: GABA$_A$ receptors which include 19 subunits, α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3, and GABA$_B$ receptors which contain R1 and R2 subunits. However, effects of EA on the regulation of inhibitory actions of GABAergic neurons to CRF in the hypothalamus need more understanding. So hypothalamus GABA receptors were taken into consideration of the regulation functions following EA intervention in trauma rats.

In this study, serum ACTH and CORT levels, and expressions of hypothalamic CRF and CRFR, α subunits of GABA$_A$ receptor, GABA$_B$ R1 and R2, GAD67 and GAD65 will be observed, in order to probe mechanisms by which EA alleviates the surgical stress response by enhancing the GABA receptors' inhibitory

regulation of HPA axis dysfunction in rats with acute surgical trauma.

2. Materials and Methods

2.1. Animals and Partial Hepatectomy

Adult male Sprague-Dawley rats of 180 - 200 g were purchased from the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). All rats were maintained on a 12:12 h light-dark cycle with free access to food and water. They were acclimated to the experimental conditions for one week before the experiment to reduce non-specific influences. All experimental procedures involving the use of animals were conducted in accordance with NIH Guidelines (NIH Publications No. 8023, revised 1978) and were reviewed and approved by the Animal Care and Use Committee of Fudan University.

Rats of the model, sham EA and EA groups were subjected to a 10% hepatectomy under 10% chloral hydrate (400 mg·kg^{-1}). The operation incision was 7 - 8 cm long from the symphysis pubis to the cartilago ensiformis along the linea alba. The left lobe was excised after the abdominal viscera were exposed. All rats were kept warm during and after the hepatectomy.

2.2. EA Treatment

For the EA treatment, rats were moderately restrained. The rats were allowed to acclimate for 30 min before their EA treatment. ST36-*Zusanli* acupoint and SP6-*Sanyiniia* acupoint of the right side were selected. Stainless steel needles of 0.3 mm diameter were inserted to a depth of 5 mm into the acupoints. ST36-*Zusanli* acupoint was connected to the positive pole and SP6-*Sanyiniiao* acupoint was connected to the negative pole of the electrical stimulator (HANS LH202H, Huawei Industry Development Limited Company, Beijing, China). Alternating strings of dense sparse frequencies (2 Hz for 1.05 s and 15 Hz for 2.85 s, alternating) were selected. The intensity was adjusted to induce a slight twitch of the right hindlimb (1 mA, 12 volts) with the intensity lasting for 30 min. The sham EA group only was only given insertion without electrical stimulation. According to the theory of preventive treatment of disease in Chinese medicine, a prophylactic EA treatment and sham EA were given 24 h before the surgery. After completing the surgery, EA or sham EA treatment was given again.

2.3. L-838,417 Administration

L-838,417 solution contained 5 mg of L-838,417 (Tocris Co., Ltd, UK) that mixed with 1 mL DMSO (Sigma Co., Ltd, USA), which was dissolved in 9 mL of 0.01 M PBS buffer with the final concentration of 0.5 mg·mL^{-1}. Vehicle group was treated with 10% DMSO that was dissolved by 0.01 M PBS buffer. Once finished partial hepatectomy, rats of model + vehicle and Model+ L-838,417, EA+ L-838,417 groups were administered by 10% DMSO (dose of 4 mL·kg^{-1}) or L-838,417 solution (dose of 2 mg·kg^{-1}) intraperitoneally, respectively.

2.4. Tissue Collection

Tissues were collected immediately upon sacrifice from rats that were euthanized at 2, 4, 8 and 24 hours after surgery (n = 12 in each group of a timepoint). Each rat was sacrificed by decapitation, and the brain was immediately removed and dissected to collect the hypothalamus. The sample, which was split equally along the third ventricle, was snap-frozen in liquid nitrogen and then stored at −80°C until Rt-PCR or WB.

Rats (n = 12 in each group) sacrificed after 4 hours were perfused with 0.01 M PBS followed by 4% paraformaldehyde. After post-fixed at 4°C overnight, rat brains were cryoprotected in 20% and 30% sucrose for 2 nights. The brains were sliced and stored at −20°C until immunohistochemistry.

2.5. Hormone Assay by Radioimmunoassay (RIA)

The concentrations of ACTH and CORT were determined by double-antibody RIA kits purchased from the Beijing Sinouk Institute of Biological Technology (Beijing, China). Blood samples from all of the rats were collected by decapitation at the time of sacrifice. The serum was separated by centrifugation and stored at −80°C until assayed. The samples were assayed in duplicate, and all of the subjects' samples were assayed together. The peptide was extracted from the serum by addition of three volumes of absolute alcohol. After precipitation and centrifugation, the samples were dried under nitrogen flow and were redissolved in assay buffer before RIA determination. The sensitivity of the ACTH kit was less than 5 pg·mL^{-1}, and the intra- and inter-assay coefficients were less than 4.1% and 8.4%, respectively. The sensitivity of the CORT kit was 1 pg·mL^{-1}, and the intra- and inter-assay coefficients of variation were 7.5% and 9.5%, respectively.

2.6. Western Blot

The expression of CRF, GABA$_A$ receptor α3 subunits was investigated by Western blot using a standard procedure. For total protein extraction, each hypothalamus was homogenized in 300 μL RIPA lysis buffer (Beyotime, China) with protease inhibitors (phenylmethanesulfonyl fluoride, Beyotime, China) using a polytron homogenizer. The tissue lysates were cleared by centrifugation at 13,000 rpm for 15 min at 4°C. The protein concentration was determined using the BCA method (Thermo Fisher; MA, USA). The samples were boiled with loading buffer (65.8 mM Tris-HCl, pH 6.8, 2.1% SDS, 26.3% (w/v) glycerol, 5% β-mercaptoethanol and 0.01% bromophenol blue) for 10 min and stored at −80°C until use.

The protein aliquots were separated on 10% SDS polyacrylamide gels. Next, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, MA, USA) using a Trans-Blot apparatus (Bio-Rad, CA, USA) for 100 min at 300 mA. The PVDF membranes were blocked for 2 h in blocking buffer (TBS with 0.1% Tween 20, TBST, and 5% BSA) at room temperature. The

membranes were then incubated overnight at 4˚C with one of the following primary antibodies: rabbit anti-CRF antibody, diluted 1:200 (H-104, sc-10718; Santa Cruz, TEX, USA); mouse anti-GABA$_A$$\alpha$3 antibody, diluted 1:200 (AB5594; Millipore; MA, US). After the membranes were washed extensively in washing buffer (TBS-0.1% Tween 20), the membranes were incubated with either HRP-conjugated goat anti-rabbit IgG (ab136817 Abcam; Cambridge, UK) or HRP-conjugated goat anti-mouse IgG (H+L) (SA00001-1; Proteintech Group, Inc, Chicago, USA). The membranes were incubated in the solution for 3 h at 4˚C. The membranes were washed again several times in washing buffer to remove unbound secondary antibody, and the signal was detected using an ECL detection kit (GE Healthcare, Buckinghamshire, UK). Then, the membranes were exposed in an Image Quant LAS 4000 mini (GE Healthcare, Buckinghamshire, UK), and the signals for each protein were determined using Quantity One, Version 4.6.2 (Bio-Rad, CA, USA). The results for signal intensity are expressed in arbitrary densitometric units, after normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Sigma, MO, USA) as an internal standard.

2.7. Real-Time Reverse Transcriptase-PCR (RT-PCR)

To analyze the mRNA expression of CRF, CRFR, GAD and the GABA receptors, total hypothalamic RNA was extracted using TRIzol (Invitrogen Corp., CA, USA), according to the manufacturer's instructions. The purity and integrity of the RNA was confirmed spectrophotometrically prior to analysis.

To obtain cDNAs, 2.0 μg of the total RNA was reverse-transcribed by the Go-Script™ Reverse Transcription System (Promega, WI, USA), according to the manufacturer's instructions. The total RNA was digested with RQ1 RNase-free DNase (Promega, WI, USA) before real-time reverse transcriptase-PCR was conducted.

The primers used to analyze the mRNA levels of CRF, CRFR, GAD and GABA receptors were designed and synthesized with HPLC purification by Invitrogen. Quantitative Real-Time PCR was performed using the IQ5 Real-time PCR detection system (Bio-Rad, CA, USA). The linearity ranges for the PCR assay were previously established for the cDNA for each gene to determine the sensitivity and efficiency of the amplification. The reactions contained 10 μLSYBR Green Real Master Mix (Promega, WI, USA), 1.6 μL primer mixture (200 nM) (Sangon Biotech, Shanghai, China) and 1.6 μLcDNA template. The thermal cycling conditions were as follows: 95˚C for 3 min for denaturation, followed by 40 cycles of 95˚C for 10 s, 58˚C for 30 s, and 72˚C for 30 s. After the cycles, a melting curve analysis was performed to ensure the purity of the PCR products.

All real-time experiments were run in triplicate, and a mean value was used to determine the mRNA levels. The relative mRNA expression of CRF, CRFR, GAD and GABA receptors were determined by the $2^{-\Delta\Delta Ct}$ method and norma-

lized to β-act in mRNA. The target primers used for PCR are listed in Table 1.

2.8. Immunohistochemistry

The brain slices were incubated in a 0.1% BSA solution for 1 hour at 37˚C, then incubated with sheep anti-CRF primary antibody (NB110-81721, 1:50, NOVUSBIO) for 24 hours, then washed them in 0.01 M PBS for 5 times, and incubated them in biotinylated anti-sheep IgG secondary, thereafter incubated them in peroxidase solution. And the immunoreactivity was visualized using a microscope.

2.9. Data Analysis

All data are presented as the means ± standard error of mean (SEM), and p values less than 0.05 were considered significant. The statistical analyses were performed using SPSS 17.0 (IBM, Chicago, IL, US) to generate a two-way analysis of variance (ANOVA) in group × time interaction. Normally distributed data that contained more than two groups were analyzed by one-way ANOVA.

3. Results

3.1. Effects of EA on Serum ACTH and CORT Expressions

A two-way ANOVA of the ACTH ($p = 0.001$) and CORT ($p = 0.000$) total score showed significant group × time interactions. Two hours after surgery, neither the model group nor the EA group showed any differences in their serum ACTH levels compared with the intact group, respectively ($p = 0.646$ and 0.571, respectively), while 4 hours after surgery, serum ACTH in the model and sham EA group showed an increase when compared with the intact group ($p = 0.024$ and 0.002, respectively). Serum ACTH levels in the model group were elevated in comparison with the intact group at 24 hours after surgery ($p = 0.001$), while ACTH in EA group was lower than model group ($p = 0.024$) (Figure 1(a)).

The serum CORT levels in the model group were consistently higher than those of the intact group at 2, 4, 8 and 24 h ($p = 0.000$, 0.035, 0.004 and 0.000, respectively) (Figure 1). The serum CORT levels in the EA group stayed the same as that in the model group at 2 hours and 4 hours after surgery ($p = 0.847$, $p = 0.652$), and dropped below that of the model group at 24 hours after surgery ($p = 0.000$) (Figure 1(b)).

3.2. Effects of EA on the Expressions of Hypothalamic CRF and CRFR

Effects of EA on the expression of hypothalamic CRF and CRF receptors after hepatectomy were studied by means of real-time PCR, Western blot and immunohistochemistry. A two-way ANOVA of the CRF ($p = 0.000$), CRFR1 ($p = 0.000$) and CRFR2 ($p = 0.000$) mRNA total score showed significant group × time interactions.

Two hours after surgery, CRF, CRFR1 and CRFR2 mRNA expression were higher in the hypothalamus of the model group than the intact group 2 hours

Table 1. The sequences of target primers.

Oligo Name		Sequence (5' to 3')
CRF	Forward	CAG CAA CCT CAG CCG ATT CT
	Reverse	CCC CAG GCG GAG GAA GTA
CRFR1	Forward	TGG AAC CTC ATC TCG GCT TT
	Reverse	GTG AGC TGG ACC ACA AAC CA
CRFR2	Forward	TTC CTG CTG CAA CTC ATC GA
	Reverse	GCG GCA CCA GAC CTC ATT
GABAR$_A$ α1	Forward	AGG GTA AGG TGA GGC TGT CAT TGT
	Reverse	TCT GTG TTA GGC TGG CTC ATC TCC
GABAR$_A$ α2	Forward	GAG GAT GGG CTT GGG ATG GAA AG
	Reverse	GCT GGC TTG TTC TCT GGC TTC TT
GABAR$_A$ α3	Forward	TCC TGC TGA GAC CAA GAC CTA CAA
	Reverse	GGT TGC TGT GCT GCC ACT ATT ATC T
GABAR$_A$ α4	Forward	AGA TGT CAA CAG CAG AAC TGA GGT G
	Reverse	GCG ATG CGG CAG ACG AAA GA
GABAR$_A$ α5	Forward	GGG ACT GGG AAT GCT GTG GGT A
	Reverse	CGA TCT TGC TGA TGC TGT TGT AGG T
GABAR$_B$ 1	Forward	GTG ACC ATG ATC CTT TCC AG
	Reverse	AAC AGT CGG GAC TTC TCT T
GABAR$_B$ 2	Forward	ACT GGG GTT CTG TAT GGG GA
	Reverse	TCT GTA GGG TCT TGG CGA TG
GAD67	Forward	GGC TAA CCA TCT CGC AAG CAA CT
	Reverse	GCC ATC CAT CAT CCA TCC ATT CAG G
GAD65	Forward	TGC GAG TTC TGG AAG ACA ATG AAG A
	Reverse	GAG ATG ACC ATG CGG AAG AAG TTG A
β-actin	Forward	GAC CTC TAT GCC AAC ACA GTG C
	Reverse	CTG CTG GAA GGT GGA CAG TGA

after surgery ($p = 0.010$, $p = 0.000$ and $p = 0.014$, respectively) and 4 hours ($p = 0.000$, $p = 0.000$ and $p = 0.027$, respectively) and 24 hours after surgery ($p = 0.018$, $p = 0.003$ and $p = 0.041$, respectively). CRF, CRFR1 and CRFR2mRNA level in the EA group was decreased compared with the model group at 4 ($p = 0.000$, $p = 0.000$ and $p = 0.000$, respectively) and 24 hours ($p = 0.001$, $p = 0.047$ and $p = 0.022$, respectively) (Figure 2(a)).

In protein levels, hypothalamic CRF expression showed a significant increase in the model group compared with the intact group ($p = 0.032$), while in the EA group it was lower than the model group at two hours after surgery ($p = 0.014$) (Figure 2(b)).

We further examined CRF activity in hypothalamus PVN using immunohistochemically analysis. The number of CRF positive neurons in the PVN was increase compared with the intact group ($p = 0.000$) and decreased in the EA group compared with the model group ($p = 0.000$) (Figure 2(c)).
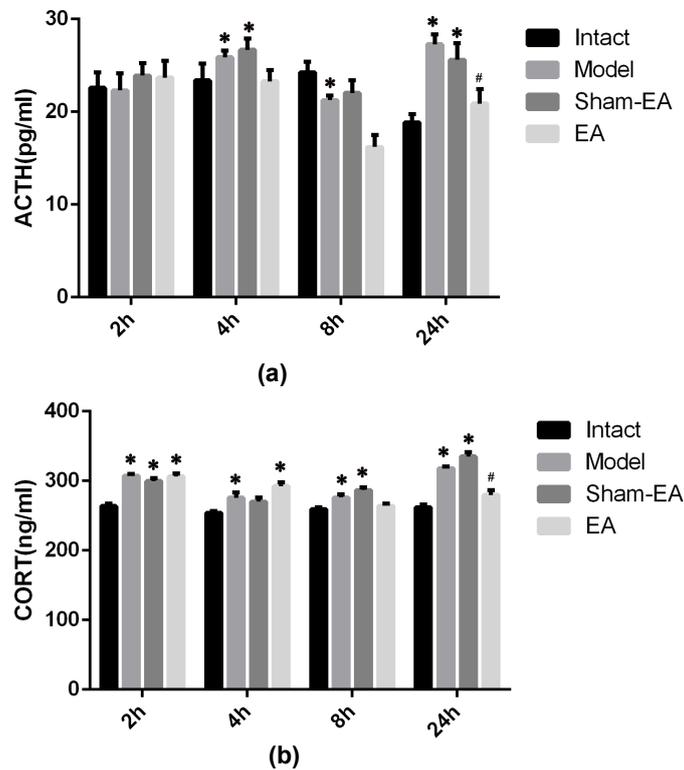
Figure 1. Serum ACTH and CORT among intact, model, sham-EA and EA groups. Serum ACTH (a) and CORT (b) detected by RIA in the intact, model, sham-EA and EA groups at 2 hours, 4 hours, 8 hours and 24 hours after hepatectomy. Data presented as mean ± SEM, n = 7 - 13 in each group of each time point. *: versus the intact group; #: versus the model group.

3.3. Effects of EA on GABA Receptor Expression in the Hypothalamus

To identify the mechanism of EA in improving the hyperactivity of HPA axis, we observed the involvement of GABA receptor in surgical trauma induced dysfunction of the HPA axis. GABARAα3 ($p = 0.000$), GABARAα4 ($p = 0.000$) and GAD67 ($p = 0.000$) mRNA was found increase at four hours after surgery in EA group compared with the model group (**Figure 3(a)**). Besides, the protein level of GABARAα3 was increase in the sham EA ($p = 0.000$) and EA group ($p = 0.000$) at four hours after surgery (**Figure 3(b)**).

3.4. Serum Corticosterone Levels after L-838,417 Administration

In the experiment, partial hepatectomy was performed on rats of the model, model + vehicle, model+L-838,417, EA and EA+L-838,417 groups. Compared with the intact group, serum CORT increased in the model ($p = 0.045$) and model + vehicle ($p = 0.038$) group. Serum CORT in the EA group decreased compared with that in the model group ($p = 0.018$). Besides, compared with the EA group, serum CORT in the EA + L838417 group increased ($p = 0.000$) (**Figure 4**).
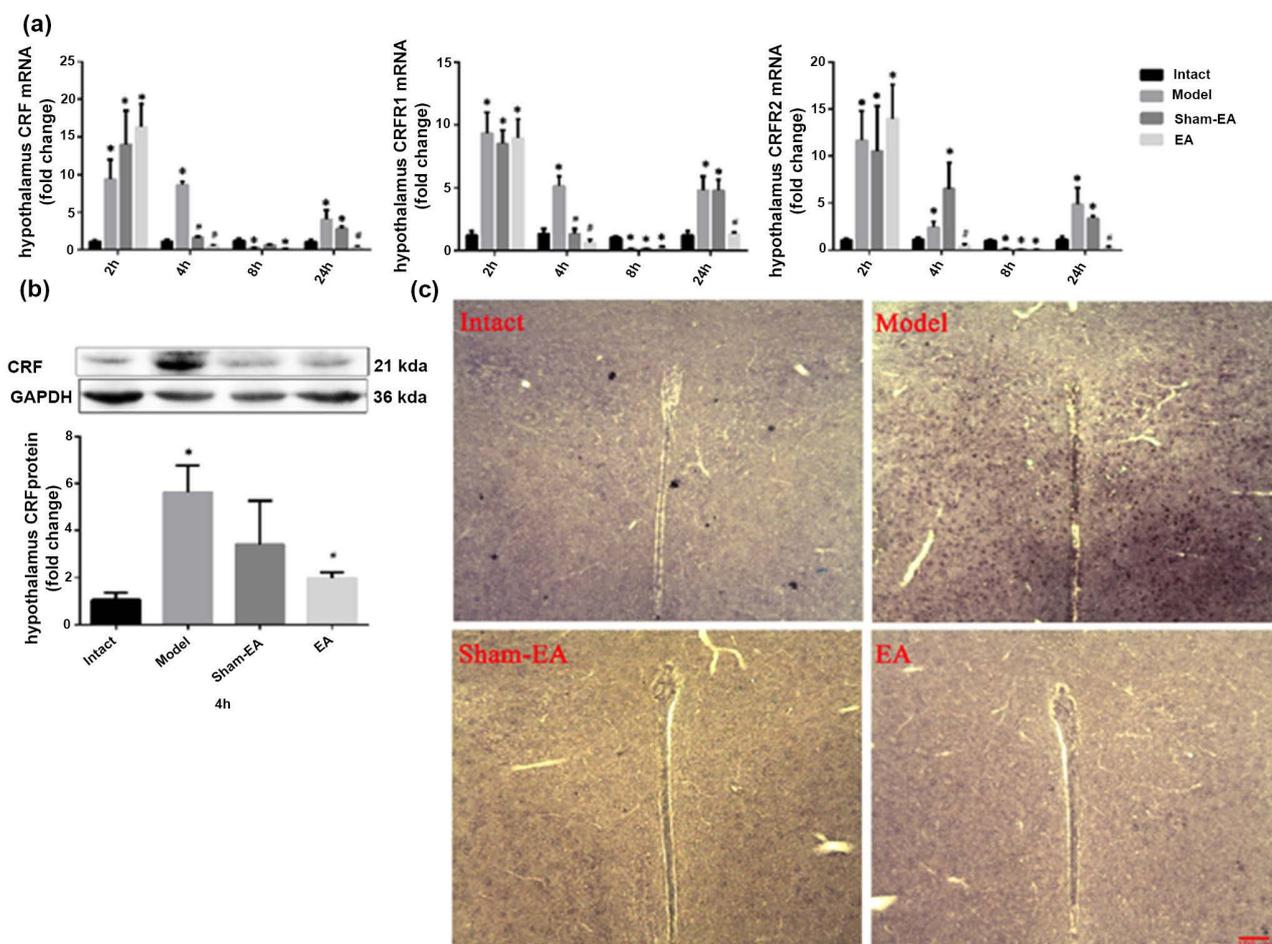
Figure 2. Hypothalamus CRF among intact, model, sham-EA and EA groups. Hypothalamus CRF, CRFR1 and CRFR1 mRNA detected by rt-PCR in the intact, model, sham-EA and EA groups at 2 hours, 4 hours, 8 hours and 24 hours after hepatectomy (a). Data presented as mean ± SEM, n = 12 in each group. *: versus the intact group; #: versus the model group. Representative band and relative quantitation of hypothalamus CRF protein level in the intact, model, sham-EA and EA groups at 4 hours after hepatectomy detected by WB (b) and representative immunohistochemistry image IHC of CRF in the hypothalamus PVN (C), bar = 100 μm. Data presented as mean ± SEM, n = 12. *: versus the intact group; #: versus the model group.

4. Discussion

Surgical trauma presents a series of pathologic physiological reactions after surgery by over-activating the HPA axis [1] [2] which reflects the level of recovery for patients who underwent operations [17]. However, there is not an exact and effective method that is supported by evidence-based medicine or multi-center clinical trials of management for dysfunction of the HPA axis after surgery. Thus, experimental trials remain a feasible way to explore the mechanism of surgical trauma [2]. Previous studies have demonstrated that EA may speed up homeostasis by promoting extragonadal aromatization and increasing the circulating estradiol (E2) and hypothalamic CRF in ovariectomized rats [13] [18]. The current study attempted to investigate the role of EA in regulating the HPA axis in surgical trauma.

Our findings suggest that EA can effectively normalize the over-expression of hypothalamic CRF and CRFR. In addition, the results showed that the GAD65 in
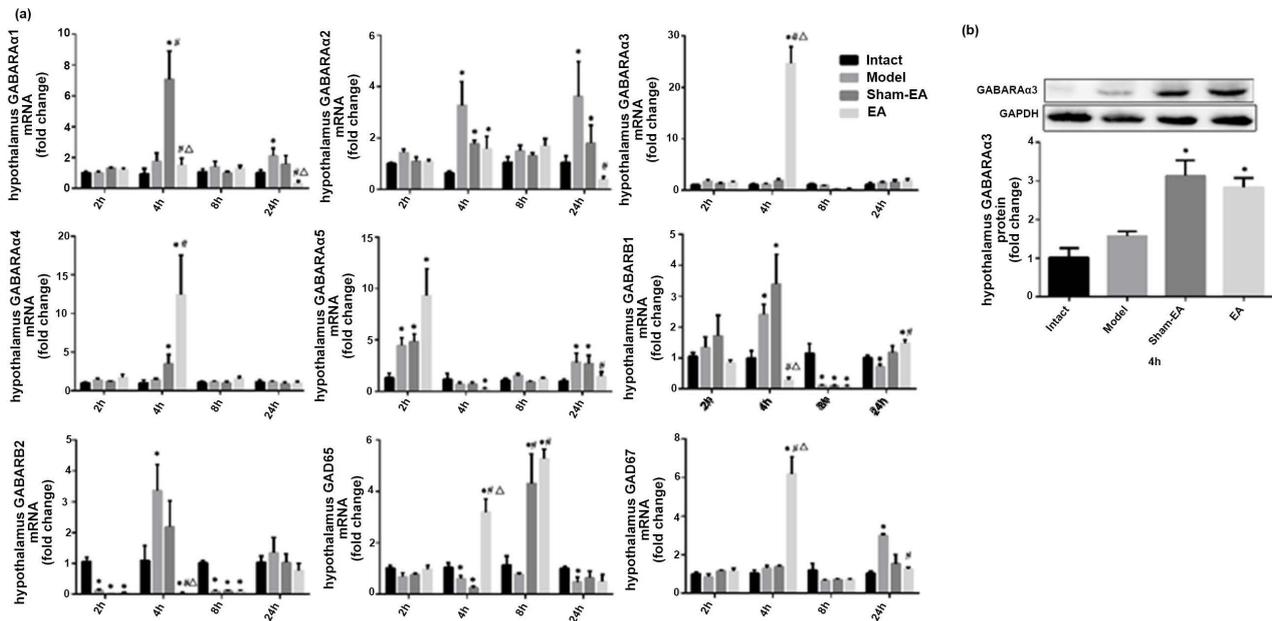
Figure 3. Effects of EA on GABA receptor expression in the hypothalamus after hepatectomy. Hypothalamus GABARA and GABAB receptors mRNA level in the intact, model, sham-EA and EA groups at 2 hours, 4 hours, 8 hours and 24 hours after hepatectomy (a). GABARA*a*1 mRNA expression increased in the Sham-EA and EA groups at 4 h, and fell in the EA group at 24 h. GABARA*a*2 mRNA expression climbed in the Model, Sham-EA and EA groups both at 4 h and 24 h. GABARA*a*3 mRNA expression skyrocketed in the EA group at 4 h. GABARA*a*4 mRNA expression went up in the Sham-EA and EA groups at 4 h. GABARA*a*5 mRNA expression came up in the Model, Sham-EA and EA groups at 2 h and 24 h. GABARB1 mRNA expression raise in the Model and Sham-EA groups while decreased in the EA group at 4 h, and went down in the three groups at 8 h, while increased in the EA group at 24 h. GABARB1 mRNA expression came down in the Model, Sham-EA and EA groups at 2 h and 8 h, and ascended in the Model group at 4h and fell off in the EA group. GAD65 mRNA expression dropped in the Model and Sham-EA group while climbed up in the EA group at 4 h, and went up in both the Sham-EA and EA groups at 8 h, and decreased in the Model group at 24 h. GAD67 mRNA expression boosted in the EA group at 4h in the Model group at 24 h, and went down at 24 h. Representative band and relative quantitation of GABARAα3 protein level at 4 hours after hepatectomy (b). Data presented as mean ± SEM, n = 12 in each group of each time point. *: versus the intact group; #: versus the model group; Δ: versus the sham-EA group.

the hypothalamus overexpressed at eighth hour and recovered to the level of Intact group at twenty-fourth hour, conjecturing the inhibiting effects of GABA on CRF expression which need verifying in further study. More importantly, EA can enhance GABA synthesis and up-regulate the expression of the GABARA *a*3 subunit, which may significantly inhibit hypothalamic CRF synthesis and release.

EA exerts 'normalizing' effects on the dysfunction of the HPA axis, both in surgical trauma patients and experimental animals [12] [13] [19]. EA participates in neuroendocrine regulation in the upper structures of the HPA axis in rats with surgical trauma by triggering the synthesis and release of neuropeptides, modulating the gene expression of neuronal cells in the central nervous system (CNS), eliciting profound physiological effects and activating self-healing mechanisms [20].

EA treatment can attenuate the serum ACTH and CORT levels in both surgical patients and rats [12] [19]. The fluctuated ACTH and CORT levels were
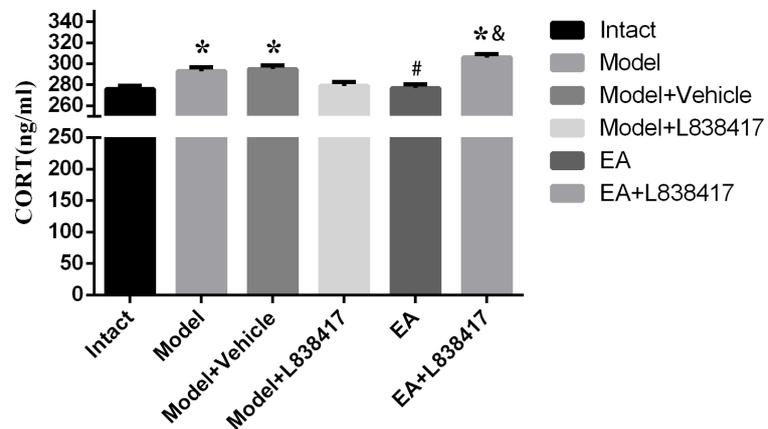
Figure 4. Serum Corticosterone levels after L-838417 administration. Serum CORT in the rats among the intact, model, model, model + vehicle, model+L-838,417 group, EA and EA+L-838,417 groups. Data presented as mean ± SEM, n = 5 in each group. *: versus the intact group; #: versus the model group; &: versus the EA group.

reported regulated by EA after surgical stress [21]. In this study, serum CORT is increased in the model animals and EA presents a long-lasting inhibitory effect, which is in accordance with a psychological stress model [22], on the over-expression of hypothalamic CRF and CRFR.

Many studies show that GABARA in the peri-PVN play an inhibitory role in psychological stress [17] [23]. A previous study found that GABAergic inhibitory synaptic contacts are increased on CRF neurons during stress, which is consistent with higher GABA receptor expression in the present study, and follows the rearrangement of inhibitory GABA-containing inputs with the increase of contacts on dendrites and decrease in contacts at the soma region of CRF neurons in the PVN [24]. The rearrangement of GABAergic input may exist in the present study to enhance the hyperresponsivity of CRF neurons in the model group, while the soma/dendrite ratio of GABAergic inputs might increase in EA. L-838,417 is a GABAA receptor α2,3,5 agonist. In this study, L-838,417 application decreased the serum CORT level in trauma rats.

GABAergic neurons were involved in the effect of EA improving chronic pain [25] and hypertension [26]. And EA was found promoting GAD transcription in this study, which indicates an enhanced GABA synthesis involved in the further suppressive effect of EA.

5. Conclusion

EA can alleviate the surgical stress response by improving the GABA synthesis, thus enhancing the GABA receptors' inhibitory regulation of the HPA axis dysfunction in rats with acute surgical trauma.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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