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ORIGINAL ARTICLE

Effects of high temperature and high humidity stress on the negative feedback regulation of hippocampus on HPA axis in rats

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Abstract

OBJECTIVE: To investigate the negative feedback regulation from rat hippocampus on hypothalamic-pituitary-adrenal (HPA) axis under high temperature and high humidity stress.

METHODS: Thirty (30) SD male rats were randomly divided into three groups: control group, high temperature and high humidity group, drug intervention group. The rats in control group were kept in the environment with temperature of 24 ± 1°C and humidity of 50 ± 5%, without any stimulation. The rats in the other groups were exposed to high temperature and high humidity environment for 4 h each day, with temperature of 35±1 °C and humidity of 85±5%. The rats in drug intervention group were intragastrically administered with the glucocorticoid receptor antagonist mifepristone. The administration was continued for 3 weeks. After 3 weeks, the serum levels of corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone (CORT) were detected by ELISA.The protein and mRNA levels of corticosteroid receptors (MR), glucocorticoid receptors (GR) and inducible nitric oxide synthase (iNOS), transient receptor potential vanilloid 1 (TRPV1) and 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) in hippocampus were determined by immunohistochemistry and in situ hybridization, respectively. The apoptosis of hippocampal cells was examined with TUNEL apoptosis staining.

RESULTS: After stimulation with high temperature and high humidity stress for 3 weeks, the serum levels of CRH, ACTH and CORT in the high temperature and high humidity group were significantly increased compared to that of control group; the levels of these indicators in drug intervention group were decreased compared to that of high temperature and high humidity group ($P<0.05$). In high temperature and high humidity group, the protein and mRNA levels of MR, GR, iNOS in hippocampus of rats were significantly increased compared with that

of control group (p<0.05); and the levels of these indicators in drug intervention group were lower than that of high temperature and high humidity group (p<0.05). In addition, compared with the control group, the TRPV1 protein level in hippocampus of rats in high temperature and high humidity group was not significantly changed (p>0.05), while the TRPV1 mRNA level was significantly increased (p<0.05). Neither the protein nor mRNA levels of 11β-HSD1 showed significant difference compared to control group (p>0.05). The apoptosis of hippocampus cells in the high temperature and high humidity group was significantly increased compared with that of control group (p<0.05); and it was lower in the drug intervention group than that of in high temperature and high humidity group while the result was not significant (p>0.05). **CONCLUSION:** High temperature and high humidity stress may up-regulate the local expression of iNOS in hippocampus and decrease the activity of glucocorticoids (GC) receptor, then the effective binding of GR-GC would be decreased and the negative feedback regulation of hippocampus on HPA axis would be inhibited. The glucocorticoid receptor antagonist can improve the negative feedback regulation of hippocampus on HPA axis in rat.

Abbreviations:

GC	- glucocorticoid
ACTH	- adrenocorticotrophic hormone
SD	- sprague dawley
CRH	- corticotropin releasing hormone
CORT	- corticosterone
HPA	- hypothalamic-pituitary-adrenal
GR	- glucocorticoid receptor
mRNA	- messenger ribonucleic acid
NO	- nitric oxide
iNOS	- inducible nitric oxide synthase
MR	- mineralcorticoid receptor
ELISA	- enzyme linked immunosorbent assay
TRPV1	- transient receptor potential vanilloid 1
11β-HSD1	- 11βhydroxysteroid dehydrogenase 1
TUNEL	- terminal deoxynuleotidyl-transferase-mediated d-UTP nick end labeling

INTRODUCTION

High temperature and high humidity are inevitable climate phenomena during producing, living and working (Inoue *et al.* 2016). Stress refers to the comprehensive response state of the body under the stimulation from the dramatically changed external environment (Herman *et al.* 2015; Zu *et al.* 2019). Chronic stress generally refers to continuous or repeated stress over 24 hours, mainly accompanied by changes in nerve, spirit, endocrine, immunity and behavior, affecting the balance of the body's environment, leading to dysfunction of the body's neuro-endocrine-immune network system, and even structural changes (Srivastava *et al.* 1991; Giessing *et al.* 2020). According to the relationship between the ambient temperature and the human body heat balance, the living environment above 35 °C and the working environment above 32°C are generally regarded as high temperature environments; the environment with a relative humidity of 60% or more is called the high humidity environment (Huang *et al.* 2016). High temperature and high humidity environments are common in practical work and life. The degree of stress in such environment would be obviously increased, which was characterized by fatigue, irritability, and inefficient work. Exposed to such circumstances for a long time would suffer from serious physical and psychological effects, neuroendocrine system dysfunction, even lead to serious accidents and unpredictable disasters (Ravanelli *et al.* 2015; Chmura *et al.* 2017; Luo *et al.* 2019).

Therefore, investigating the effects of high temperature and high humidity environment on the neuroendocrine function of the body was significant, as well as clarifying the potential mechanism. Because it was conductive to improve the work efficiency and life quality, as well as reducing the incidence of diseases and accidents in special circumstances. The main feature of the stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The hippocampus is the high-level regulation center of stress response, which is sensitive to stress but also vulnerable. In this study, rats were exposed to high temperature and high humidity for inducing stress. Then, the regulation effects of negative feedback from rat hippocampus on HPA axis under high temperature and high humidity stress were explored.

MATERIAL AND METHODS

All experiments were approved by the Ethical Committee of the General Hospital of Western Theater Command, PLA and were in agreement with the Guidelines of the Animal Protection Law of the People's Republic of China and Guidelines for the treatment of laboratory animals of Chengdu University of Traditional Chinese Medicine.

Animals

Thirty (30) healthy male Sprague Dawley (SD) rats weighing 200±10 g were purchased from Chengdu Dashuo Experimental Animal Co., Ltd., with the animal license number SCXK (chuan) 2008-24. The animals were raised in the SPF animal room of Chengdu University of Traditional Chinese Medicine. The experiment was conducted after adaptive feeding for 1 week.

Groups and treatment

Thirty (30) rats were randomly divided into three groups: control group, high temperature and high humidity group, drug intervention group, with 10 rats in each group. The rats in control group were kept in an environment with temperature of 24±1°C and humidity of 50±5%, without any stimulation. The other two groups were stimulated for 4 h in a high temperature

Tab. 1. Sequences of target gene mRNA

Genes	Sequences
MR	5′-ACAGT CTCCC TGAAG GCCTA GACAT GGAAA-3′ 5′-AGCTG AGTCC ATGGG TTTAT ATATG GATTC-3′ 5′-GAGGA CAAAT TACAT CAAAG AACTG AGGAA-3′
GR	5′-AGGTT TCTGC GTCTT CACCC TCACT GGCTG-3′ 5′-AAGAG CAGTG GAAGG ACAGC ACAAT TACCT-3′ 5′-AGCTG AAATC ATCAC CAATC AGATA CCAAA-3′
iNOS	5′-GGAAA AGGAC ATTAA CAACA ACGTG GAGAA-3′ 5′-GACCA GAGGA CCCAG AGACA AGCCC ACCCC-3′ 5′-CTTCC AGCTC AAGAG CCAGA AACGT TATCA-3′
TRPV1	5′-ACGTT GCCCG GAAGA CAGAC AGCCT GAAGC AGTTT GTCAA-3′ 5′-GGGAC AGATT TGTCA AGCGC ATCTT CTACT TCAAC TTCTT-3′ 5′-GAGAT AGACA TGCCA CCCAG CAGGA AGAAG TTCAA CTGAA-3′
11β-HSD1	5′-CAGAC CAGAA ATGCT CCAGG GGAAG AAAGT GATTG-3′ 5′-ATGGG AGCCC ATGTG GTATT GACTG CAAGG TCGGA-3′ 5′-TCCAC GATGA TATCC ACTCT GTGCG AAGAA GCATG-3′

and high humidity environment, with temperature of 35±1°C and humidity of 85±5%. The environment was simulated by artificial climate chamber (SHH250GS, Chongqing Yingbo Experimental Instrument Co., Ltd.; Yadu ultrasonic humidifier YC-D209, Beijing Yadu Indoor Environmental Protection Technology Co., Ltd.). The drug intervention group was intragastrically administered with mifepristone (25 mg, batch number 43170108, China Resources Zizhu Pharmaceutical Co., Ltd.), with the dosage of 25 mg/kg in 1 ml/100 g. The administration was continued for 3 weeks.

Serum biomarker

The serum levels of adrenocorticotropic hormone (ACTH), corticosterone (CORT) and corticotropin releasing hormone (CRH) were determined with ELISA. The Rat ACTH ELISA KIT(XL-Er0004), Rat CRH ELISA KIT (XL-Er0077) and Rat CORT ELISA KIT(XL-Er0551) were purchased from Abcam. The ELISA was performed according to manufacture's instructions: the samples were added to wells pre-coated with the ACTH, CORT, and CRH monoclonal antibodies. In wells with standard controls, 50μL standard and 50μL HRP-streptomycin was added; in wells with samples, 40μL sample, 10μL anti-ACTH, CORT or CRH antibody, and 50μL HRP-streptomycin was added, incubating at 37°C for 1 h. Then, the wells were washed for 5 times with diluted washing buffer concentrate. After washing, 50μL of each of the developers A and B was added to each well, and the mixture was shaken and incubated at 37°C for 10 min. Finally, 50μL stop solution was added to each well to stop the reaction. The absorbance (OD value) of each well at 450 nm was measured sequentially using a microplate reader (Multlskan Mk3, Thermo Fisher). The standard curve was plotted with concentration as ordinate and OD value as horizontal axis. The concentration of samples was calculated according to the standard curve and the dilution factor.

Immunohistochemistry and In situ hybridization

The protein levels of corticosteroid receptors (MR), glucocorticoid receptors (GR)and inducible nitric oxide synthase (iNOS), transient receptor potential vanilloid 1 (TRPV1) and 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) in hippocampus were detected. The fixed tissues were dehydrated with automatic dehydrator (TSJ-II automatic closed tissue dewatering machine, Zhongwei Electronic Instrument Factory, Changzhou), embedded (BMJ-III type embedding machine, Zhongwei Electronic Instrument Factory, Changzhou) and sliced (Leica-2016 rotary slicer, Germany). Then the slices were processed with bleaching oven (PHY-III pathological tissue bleaching oven, Zhongwei Electronic Instrument Factory, Changzhou) according to the following steps:the dewaxed slices were firstly placed in a dyeing tank and incubated with 3% methanol in hydrogen peroxide for 10 min at room temperature; then the slices were washed with PBS for 3 times, 5 min each time; the slices were immersed in 0.01 M citrate buffer (pH 6.0), heated to boiling in a microwave oven, repeated once after 5 min and washed with PBS for 2 times after cooling, 5 min each time. The slices were blocked with goat serumblocking solution (normal goat serum, ZLI-9021, Beijing Zhongshan Jinqiao Biological Co., Ltd.) at room temperature for 20 min; primary antibody was added (GR rabbit polyclonal antibody, 1:100, ab217006, Abcam; MR mouse monoclonal antibody, 1:100, ab2774, Abcam; iNOS rabbit polyclonal antibody, 1:100, AF6270, Affinity; TRPV1rabbit polyclonal antibody, 1:400, ab31895, Abcam; 11β-HSD1rabbit polyclonal antibody, 1:100, DF3972, Affinity) and incubated overnight at 4°C; biotinylated secondary antibody (Beijing Zhongshang Jinqiao Biological Co., Ltd.) was added and incubated for 30 min at 37°C.The slices were then washed with PBS for 3 times, 5 min each time. The color development of slices was performed with DAB kit (concentrated DAB kit, K135925C, Beijing Zhongshan Jinqiao Biological Co., Ltd.). The slices were stained

Tab. 2. Serum CRH, ACTH and CORT levels in rats

Group	N	CRH(ng/mL)	ACTH(ng/mL)	CORT(ng/mL)
Control group	10	5.89±0.66	376.83±39.11	76.77±8.07
High temperature and high humidity group	10	7.27±0.80*	533.15±67.40*	110.09±18.12*
Drug intervention group	10	6.47±0.64△	425.53±68.90△	94.98±15.02△

Note: "*" indicated $p<0.05$ compared with the control group; "△" indicated $P<0.05$ compared with the high temperature and high humidity group.

Tab. 3. The percentage of apoptosis in rat hippocampus ($\bar{x}\pm sd$)

Group	N	Apoptosis (%)
Control group	10	0.07±0.14
High temperature and high humidity group	10	0.37±0.55*
Drug intervention group	10	0.10±0.16

Note: "*" indicated $p<0.05$ compared with the control group; "△" indicated $P<0.05$ compared with the high temperature and high humidity group.

for 2 min at room temperature, which was controlled under the microscope. The slices were mildly counterstained with hematoxylin, dehydrated, transparent, and sealed.The images were acquired with digital trinocular imaging microscopy system (BA400Digital, McAudio Group Co., Ltd.). A 400-foldmicroscopic image was taken from 3 fields of view. The OD and area of all acquired images were determined with Image-Pro Plus 6.0 image analysis system, and the average OD value of each image was calculated.

The mRNA levels of MR, GR, iNOS, TRPV1 and 11β-HSD1 in hippocampus were detected.MR *in situ* hybridization detection kit (MK2166-r), GR *in situ* hybridization detection kit (MK1449-r), iNOS *in situ* hybridization detection kit (MK1059-r), TRPV1 *in situ* hybridization detection Kit (MK10468-r) and 11β-HSD1 *in situ* hybridization assay kit (MK2573-r) were purchased from Boster Bioengineering Co., Ltd. (Wuhan, China). In the kit, there were pepsin (X10), pre-hybridization solution, oligonucleotide probe hybridization solution (corresponding to the target gene), blocking solution, biotinylated mouse anti-digoxigenin, SABC-POD, and biotinylated peroxidase. The target gene sequences were summarized in Table 1 (Tab. 1).

The frozen slices were placed into the dyeing tank and fixed with 4% paraformaldehyde (containing 1/1000 DEPC) at room temperature for 20–30 min, then the slices were incubated in 3% hydrogen peroxide at room temperature for 30 min and washed with distilled water for 3 times, 5 min each time. The mRNA nucleic acid fragments were exposed by adding pepsin freshly diluted in 3% citric acid on slices. Then, the slices were fixed for 10 min with 1% paraformaldehyde (containing 1/1000 DEPC) and washed with distilled water for 3 times, 5 min each time. Pre-hybridization solution was added to each slice and kept at 38-42°C overnight. Then the slices were washed at 37°C, twice with 2XSSC, 5 min each time; once with 0.5XSSC, 15 min each time; once with 0.2XSSC, 15 min each time. The slices were then blocked with blocking solution for 0.5 h at 37°C, incubated with biotinylated mouse anti-digoxigenin for 1h at 37°C and washed with PBS for 4 times, 5 min each time. The SABC (strept avidin-biotin complex) was added, incubated for 20 min at 37°C and washed with PBS for 3 times, 5 min each time. The biotinylated peroxidasewas added, incubated for 20 min at 37°C and washed with PBS for 4 times, 5 min each time. The color development was also performed with DAB kit. The protocols were consistent with above description. The images were acquired with digital trinocular imaging microscopy system (BA400Digital, McAudio Group Co., Ltd.). A 400-foldmicroscopic image was taken from 1 fields of view according to the tissue size and expression profile.

Apoptosis

Apoptosis of rat hippocampus was detected by terminal deoxynuleotidyl-transferase-mediated d-UTP nick end labeling (TUNEL). Tunel kit (*In situ* cell death detection kit-POD, 10279600) was purchased from Roche (Switzerland), involving No.1 Enzyme Solution, No.2 Label Solution and No.3 Converter-POD: fluorescence antibody labeled HRP. The kit was performed according to the instructions. The slide-proof was performed by firstly soaked with APES and then placed in an oven at 60°C for 1 h to make the slices tightly adhered. The slices were routinely dewaxed to water; the tissue was treated with trypsin K working solution (No. 1245680100, Merck Millipore, USA) for 25 min at 37°C and rinsed with PBS for 3 times. The TUNEL reaction mixture of No.1 and No.2 solutions was prepared and added to the slices after it was dry. The slices were incubated in dark humid chamber for 1 h at 37°C and washed with PBS for 3 times. The No.3 solution was added, incubated for 30 min at 37°C

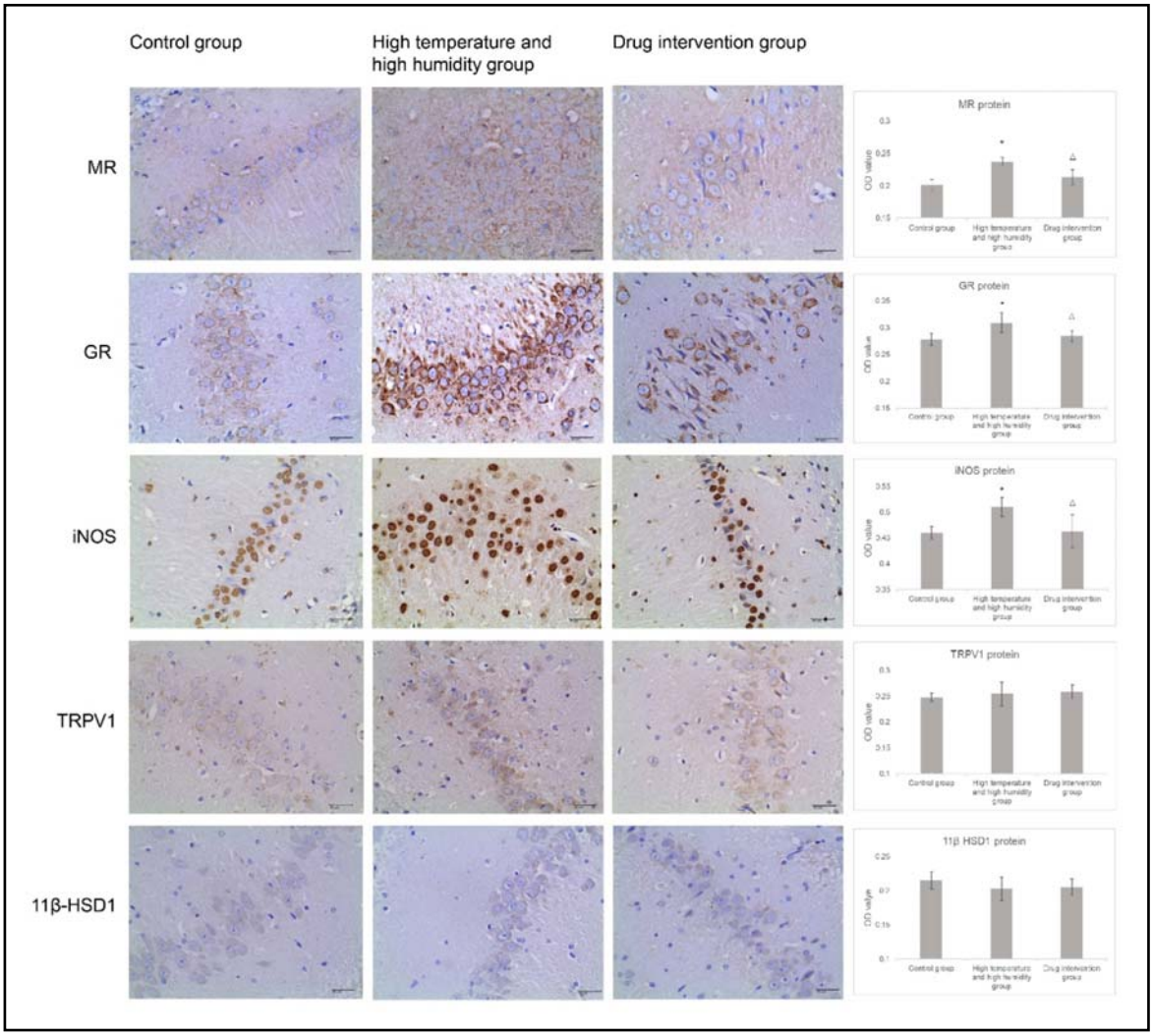

Fig. 1. Effects of high temperature and high humidity stress on the protein levels of MR, GR, iNOS, TRPV1 and 11β-HSD1 in rat hippocampus, determined with immunohistochemistry. The results were observed and captured with digital trinocular imaging microscopy system (magnification: *400). The optical density analysis of the images was applied for quantifying the protein level and expressed as $\bar{x}\pm sd$. Note: "*" indicated $P<0.05$ compared with the control group; "△" indicated $p<0.05$ compared with the high temperature and high humidity group. Scale bar: 40μm.

and washed with PBS for 3 times. The color development was also performed with DAB kit. The protocols were consistent with above description. The apoptotic rate of nerve cells was counted under a microscope, and one region was selected for image acquisition at 400-fold.

Statistical analysis

Statistical analysis was performed using SPSS 16.0.The count variable was expressed with frequency and percentage; the measurement variable was expressed with mean ± standard deviation (SD). The normal distribution was tested with Kolmogorov–Smirnov test. The comparison between groups was performed with LSD-t test for the normal distribution, and it was performed with Mann-Whitney if the normal distribution index was not followed, and the p value was calibrated using the Bonferroni method. $p<0.05$ was considered as statistically significant difference.

RESULTS

Effects of high temperature and high humidity stress on serum CRH, ACTH and CORT in rats

After treating with high temperature and high humidity for 3 weeks, the serum levels of CRH, ACTH and CORT in high temperature and high humidity group were significantly increased ($p<0.05$) compared to control group; while the levels of these markers were significantly decreased in drug intervention group ($p<0.05$) (Tab. 2).

Effects of high temperature and high humidity stress on the protein levels of MR, GR, iNOS, TRPV1 and 11β-HSD1 in hippocampus of rats

The protein levels of MR, GR and iNOS in hippocampus of rats in high temperature and high humidity group was significantly higher than that of in control group ($p<0.05$). The levels of MR, GR and iNOS protein

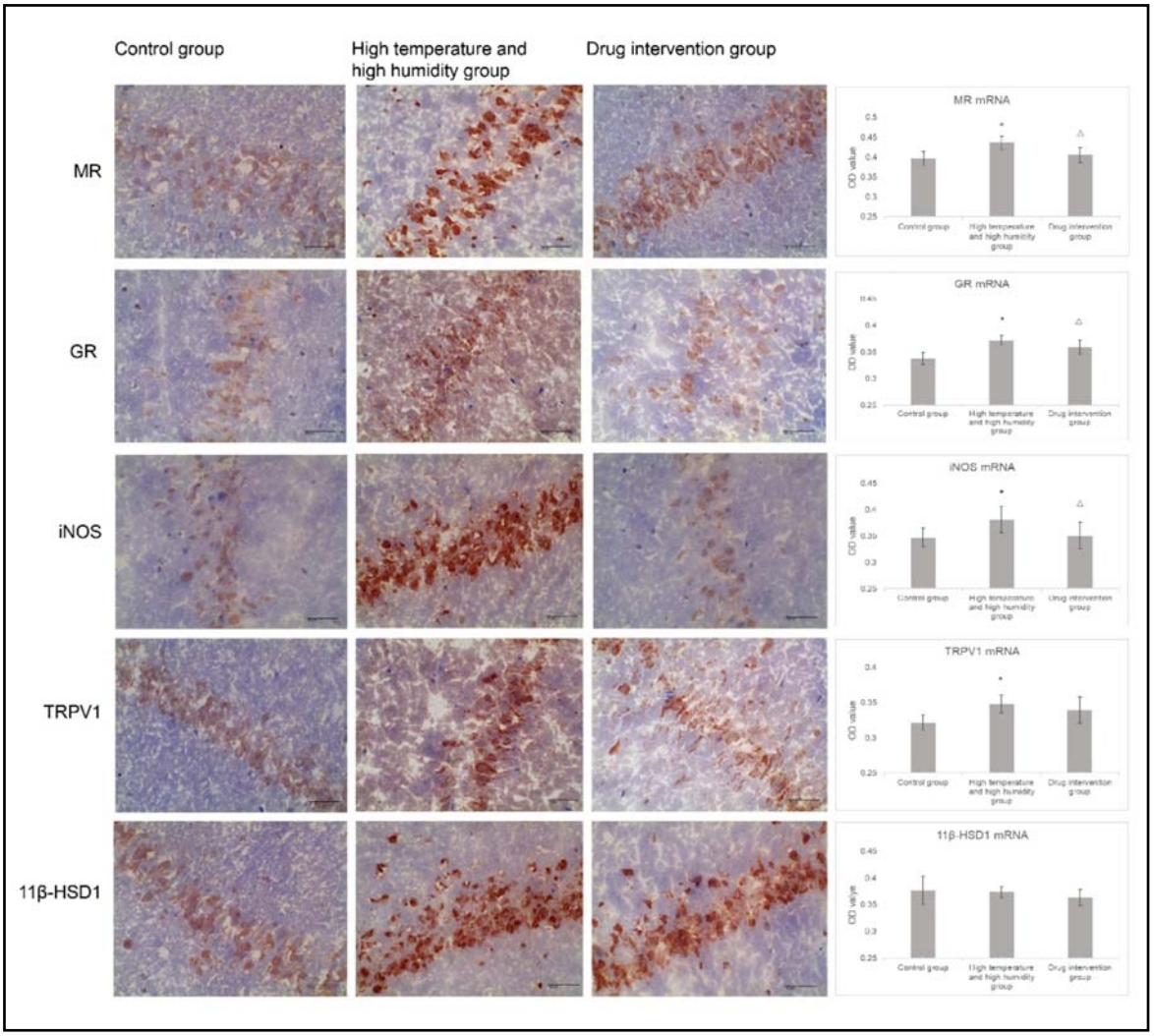

Fig. 2. Effects of high temperature and high humidity stress on the mRNA levels of MR, GR, iNOS, TRPV1 and 11β-HSD1 in hippocampus of rats, determined with in situ hybridization. The results were observed and captured with digital trinocular imaging microscopy system (magnification: *400). The optical density analysis of the images was applied for quantifying the mRNA level and expressed as $\bar{x}\pm sd$. Note: "*" indicated $p<0.05$ compared with the control group; "△" indicated $p<0.05$ compared with the high temperature and high humidity group. Scale bar: 40μm.

in drug intervention group was significantly lower than that of in high temperature and high humidity group ($p<0.05$). (Fig. 1).

For the levels of TRPV1 and 11β-HSD1 proteins in rat hippocampus, there was no statistically significant difference between groups ($p>0.05$) (Fig. 1).

Effects of high temperature and high humidity stress on the mRNA levels of MR, GR, iNOS, TRPV1 and 11β-HSD1 in hippocampus of rats

The levels of MR, GR and iNOS mRNA in hippocampus of rats in high temperature and high humidity group was significantly higher than that of in control group ($p<0.05$). The levels of MR, GR and iNOS mRNA in drug intervention group was significantly lower than that in high temperature and high humidity group ($P<0.05$). (Fig. 2).

The TRPV1 mRNA level in rat hippocampus of high temperature and high humidity group was significantly higher than that of in control group ($p<0.05$). However, there was no significant difference inTRPV1 mRNA level between high temperature and high humidity group and drug intervention group ($p>0.05$) (Fig. 2).

For the level of 11β-HSD1 mRNA in rat hippocampus, there was no significant difference between groups ($p>0.05$) (Fig. 2).

Effect of high temperature and high humidity on apoptosis of rat hippocampus

After treating for 3 weeks, the percentage of apoptosis in hippocampus of rats in high temperature and high humidity group was significantly higher than that of in control group ($p<0.05$). The apoptosis of hippocampus in the drug intervention group was decreased compared to that in the high temperature and high humidity group, however, these differences did not reach significance ($p>0.05$) (Table 3).

DISCUSSION

In hippocampus, there was abundant MR and GR, which are sensitive sites towards stress damage (Wingenfeld *et al.* 2014; Murck *et al.* 2014). Under stress conditions, high levels of glucocorticoid (GC) bind to GC receptors in the hippocampus, affecting the hormone secretion of HPA axis. The excessive stress response of the HPA axis can be inhibited, or the function of HPA axis under stress state can be restored to the basal level. However, the long-term chronic stress makes the HPA axis function persistently, causing hippocampal damage, which weakens the inhibitory effects of HPA axis (Cai 2002; Pittenger *et al.* 2008). Feedback regulation from the hippocampus on the HPA axis was performed by the binding of GC with MR and GR in the hippocampal neurons. The results of our study showed that the serum levels of ACTH, CORT and CRH in rats under high temperature and high humidity stress were significantly increased. HPA continued to over-activate, hippocampal cell apoptosis was significantly increased, specifically blocking the combination of GC with MR and GR. Therefore, damage of hippocampus and HPA axis negative feedback regulation caused by high temperature and high humidity stress can be prevented.

The negative feedback regulation from hippocampus on HPA axis may be impaired by many mechanisms. Firstly, the total amount of GC receptors affects the effective binding between hormone and receptors. Many previous disease models have shown that the down-regulation of MR and GR in hippocampal local neurons is a cause of weakened hippocampal negative feedback, then leading to hyperfunction of the HPA axis (Qi *et al.* 2013; Han *et al.* 2017). Our study found that both the gene transcription and protein expression levels of GR and MR in rat hippocampus could be significantly increased by high temperature and high humidity stress. At the same time, we noticed that some studies have reported the local MR and GR upregulation in hippocampus, and some other have reported no change (Kim *et al.* 2015). Under high temperature and high humidity stress, the expression of GR and MR in rat hippocampus was significantly increased, while the HPA axis of rats was hyperactive, and the negative feedback of hippocampus did not play a role, suggesting that there may be abnormal function after the combination of GC with MR and GR receptors.

Secondly, the production of a local active ligand (hormone) affects the effective binding amount of the hormone-receptor. The role of GC and receptors also depends on the glucocorticoid metabolism enzyme, 11β-HSD1. Previous study found rich 11β-HSD1 in hippocampal neurons, which can make inactive 11-dehydrocorticosterone (rodent)/cortisone (human) convert to active corticosterone (rodent)/cortisol (human) (Bauman *et al.* 2013; Dammann *et al.* 2019; Scott *et al.* 2014). In this study, we found that the high temperature and high humidity stress did not affect the gene transcription and protein expression of 11β-HSD1, suggesting that high temperature and high humidity stress did not affect the HPA axis function by changing the bioavailability of local active GC in hippocampus.

Thirdly, receptor inactivation is an important cause of impaired negative feedback regulation of the hippocampal HPA axis. Both *in vitro* and *in vivo* studies have demonstrated that nitric oxide (NO) can inactivate the sulfhydryl group of GR and impair its binding ability to ligand (Goodwin *et al.* 2013; Ruhs *et al.* 2012). NO is an active substance in blood vessel and nerve, playing roles of both protection and toxicity in the central nervous system. It is neurotoxic when exogenous or endogenous NO is excessive. In the biosynthesis of NO, nitric oxide synthase (NOS) plays a key role. Nitric oxide synthase in the nervous system has three subtypes of isoenzymes, namely neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) expressed under normal conditions and inducible nitric oxide synthase (iNOS) induced after injury. iNOS works only after the cells are stimulated and activated, generating large amount of nitric oxide (Beheshti *et al.* 2020; Almeida *et al.* 2019). Thus, iNOS plays an important role in stress induced damage. iNOS is expressed in various regions of the hippocampus and dentate gyrus, and the iNOS activity in hippocampus may also affect its function (Amrouni *et al.* 2010). This study found that high temperature and high humidity stress can up-regulate the expression of iNOS in hippocampus, causing hippocampal damage, resulting in weakened negative feedback regulation from hippocampuson HPA.

Previous studies have found that TRPV1 was an important thermoreceptor channel widely expressed in nervous tissues. TRPV1 is an important non-selective cation channel on the cell membrane (Hurtado-Zavala *et al.* 2017). It is also a ligand-gated non-selective cation channel in the body that can be activated by high temperature. At normal physiological pH, the temperature threshold at which TRPV1 being activated is approximately higher than 43°C. However, the temperature threshold can be lowered to normal body temperature during tissue acidosis, such as ischemia and inflammation (Caterina *et al.* 2001). TRPV1 is expressed in the hypothalamus, cerebellum, cerebral cortex, striatum, hippocampus, etc. It can be activated by a variety of noxious stimuli. In addition, TRPV1 participates in cytokines, neurotransmitter release, and neurodegeneration, which may be a stress protein in the central nervous system (Anstötz *et al.* 2018; Ho *et al.* 2012). However, the role of TRPV1 as a thermo sensory channel protein in high temperature and high humidity stress of hippocampus has not been reported. The results of this study showed that high temperature and high humidity stress significantly increased the expression level of TRPV1 mRNA, but making no effect on the expression of TRPV1 protein. Further study is needed to clarify the specific mechanism. It suggested

that TRPV1 may not be involved in high temperature and high humidity stress damage.

The results of this study indicated that high temperature and high humidity stress may up-regulate the expression of iNOS in hippocampus, decrease the activity of GC receptor, lead to the decrease of effective GR-GC binding, inhibit the negative feedback regulation of hippocampus on HPA axis. Mifepristone inhibited the local expression of iNOS in hippocampus by specifically blocking GR, and improved the negative feedback regulation of rat hippocampus on HPA axis, under high temperature and high humidity stress. However, in addition to the GC receptor blocking effects, mifepristone is also an antagonist of progesterone, which is commonly applied in anti-early pregnancy, contraception, etc. (Singer *et al.* 2018; Jin *et al.* 2015). Thus, its clinical applications have been limited by its effects in reproductive endocrine, and it is necessary to develop specific GC receptor blockers to prevent HPA axis function disorder caused by high temperature and high humidity stress.

CONCLUSIONS

High temperature and high humidity stress may up-regulate the local expression of iNOS in hippocampus and decrease the activity of glucocorticoids (GC) receptor, then the effective binding of GR-GC would be decreased and the negative feedback regulation of hippocampus on HPA axis would be inhibited. The glucocorticoid receptor antagonist can improve the negative feedback regulation of hippocampus on HPA axis in rat.

CONFLICT OF INTEREST

There is no conflict of interest.

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