Annales Médico-Psychologiques 175 (2017) 889–893

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Report

The significate of IGF-1 and IGF-1R in reducing PTSD cognitive function symptoms

L'importance de l'IGF-1 et de l'IGF-1R dans la réduction des symptômes de la fonction cognitive du troubles de stress post-traumatique

Yueqi Zhang [a], Peijun Hao [b], Xiuyu Yuan [b], Guiqing Zhang [b], Yuanjun Dong [b,*]

[a] *Psychological Department, the Fifth Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China*
[b] *Psychological Rehabilitation Department, the First Affiliated Hospital of the Medical College, Shihezi University, 832000 Shihezi, Xinjiang, China*

ARTICLE INFO

Article history:
Received 20 October 2015
Accepted 1st March 2016
Available online 25 July 2017

Keywords:
Animal experimentation
Cognitive function
Hippocampus
Insulin
Posttraumatic syndrome

ABSTRACT

Objective. – To study the expressions of insulin-like growth factor 1 (IGF-1) and IGF-1 receptor in the hippocampal CA1 region of posttraumatic stress disorder (PTSD) rats.
Methods. – Forty healthy adult male Wistar rats were adopted. The internationally established single prolonged stress (SPS) method was used to set up the PTSD rat model and the immunohistochemical (IHC) method was applied to detect the expressions of IGF-1 and its receptor in the hippocampal CA1 region of PTSD rats.
Results. – After SPS stimulus, the expression of IGF-1 protein in the rat hippocampal CA1 region increased with the development of PTSD and reached the maximum on the 14th day, which is statistically different from that of the control group ($P < 0.05$); While the expression of IGF-1 receptor protein showed no significant difference on the 1st day and 7th day before and after stress ($P > 0.05$), but slightly decreased on the 14th day and 28th day than before ($P < 0.05$).
Conclusion. – After stress, the expression of IGF-1 receptor didn't grow in pace with that of IGF-1 accordingly, but slightly lowered instead. This indicates that IGF-1 receptor may affect the positive role of IGF-1 to some degree and meanwhile involve the pathophysiological process of cognitive changes of PTSD.

Mots clés :
Expérimentation animale
Fonction cognitive
Hippocampe
Insuline
Syndrome post-traumatique

RÉSUMÉ

Objectif. – Étudier l'expression du facteur de croissance 1 de l'insuline (IGF-1) et du récepteur d'IGF-1 dans la région CA1 de l'hippocampe des rats soumis au syndrome de stress post-traumatique (SSPT).
Méthodes. – Sur quarante rats Wistar adultes adultes sains, la méthode de stress prolongé unique (SPS) établie à l'échelle internationale a été utilisée pour mettre en place le modèle PTSD et la méthode immunohistochimique (IHC) a été appliquée pour détecter les expressions de l'IGF-1 et de son récepteur dans la région CA1 de l'hippocampe des rats PTSD.
Résultat. – Après stimulation SPS, l'expression de la protéine IGF-1 dans la région CA1 de l'hippocampe du rat a augmenté avec le développement du SSPT et a atteint le maximum le 14^{e} jour, ce qui est statistiquement différent de celui du groupe témoin ($p < 0{,}05$). Alors que l'expression de la protéine du récepteur d'IGF-1 n'a montré aucune différence significative le 1er jour et le 7^{e} jour avant et après le stress ($p > 0{,}05$), elle a légèrement diminué les 14^{e} et 28^{e} jours précédents ($p < 0{,}05$).
Conclusion. – Après le stress, l'expression du récepteur de l'IGF-1 n'a pas augmenté par rapport à l'IGF-1 en conséquence, mais a été légèrement diminuée. Cela indique que le récepteur de l'IGF-1 peut affecter le rôle positif de l'IGF-1 dans une certaine mesure et implique le processus pathophysiologique des changements cognitifs du SSPT.

* Corresponding author.
E-mail address: ktpdwawj@163.com (Y. Dong).

http://dx.doi.org/10.1016/j.amp.2016.03.023
0003-4487/

1. Introduction

As public emergencies like natural disasters, major traffic accidents, violent and terrorist activities increase constantly, the incidence of disorders related with psychological stress is rising year by year. And posttraumatic stress disorder (PTSD) is a kind of stress disorder characterized by the most severe clinical symptoms, worst prognosis and strongest possibility of brain damage. Currently, there is still no consensus regarding PTSD on the mechanism of changes in cognition, emotion and memory and other aspects. Some people hold that the formation of PTSD is influenced by dysfunction of emotional system [2,12,18,22], while some deem that the reason patients appearing symptoms like flashbacks, nightmares, or forcing to recall traumatic situations is simply because of their weakening cognitive control function [13,14,23]. To date, nevertheless, most studies tend to hold that some specific areas of brain, such as over-activation or inhibition of hippocampus and amygdala, result in neuroendocrine disorder and abnormal gene expression, but the mechanism involved mediating stress response remains unclear.

With further research, people began to link insulin with cognition. Most scholars deem that brain insulin has a protective effect on cognitive function [3,7]. Widely distributed in the brain, IGF-1 is overlapped with insulin on molecular structure, receptor and function. They have a high homology and both of them play an important role in cell proliferation, apoptosis and tumorigenesis. IGF-1 is involved in regulating hippocampal neurogenesis and formation of abnormal neural network as well as affecting the excitability of hippocampal neurons [16]. Nakajima et al. [17], in studying the effect of chronic stress on the perception of rat, discovered that chronic stress leads to a high expression of IGF-1 in the brain cortex and liver of rats, suggesting that IGF-1 is involved in the body anti-stress mechanism. Nowadays, the role of IGF-1 as an effective factor in neurogenesis processes associated with central nervous system (CNS) and peripheral nervous system (PNS) has been well known better than before and mechanisms of this factor about how it plays a role in this regard becomes more and more hot [1,4].

Therefore, we hypothesized that IGF-1 and its receptor may be involved in the incidence, development and prognosis of PTSD [21] and thereby the increase or decrease of their expression may be one of the mechanisms leading to the cognitive, emotional and memory changes in PTSD. Thus in this study, to explore the possible pathological mechanism of PTSD, animal model was adopted to study the expression of IGF-1 and its receptor in the hippocampal CA1 region of PTSD rats as well as their relationship with cognitive, emotional and memory changes.

2. Methods and materials

2.1. Experimental animal

The protocol for this study was approved by the institutional animal care and use committee of Shihezi University and all animal treatments were carried out at Shihezi University. All experiments were performed in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Forty 6-week-old male Wistar rats (weighing 180g ± 25 g) provided by the Experimental Animal Center of Xinjiang Medical University were used. All animals revealed no abnormality through general physical examination. They were fed in animal room under a regulated temperature of 20 to 23°C and a humidity of 50% to 60% and had free access to food and water. The feeding box was paved with soft sawdust inside to reduce foot irritation. Before the experiment, rats were provided 12/12 hour controlled light strictly, stroked daily for 3 minutes and helped to adapt to environment for one week. During the experiment, unnecessary suffering was minimized for experimental animals.

2.2. Methods

2.2.1. Experiment grouping

All rats were randomly divided into five groups, namely: post-stress 1d group (the 1st day, 8 rats), post-stress 7d group (the 7th day, 8 rats), post-stress 14d group (the 14th day, 8 rats), post-stress 28d group (the 28th day, 8 rats) and normal control group (normal, 8 rats).

2.2.2. The establishment of PTSD rats model

Based on the reference, the internationally mature SPS method [6,11] was employed to set up the animal model, which is specifically as below. The rats were first wrapped completely from head to tail and then bound with tape for 2 hours so that its body cannot move, but 3–5 holes were reserved near the nose to facilitate breath. After a break of two minutes, the rats were forced to swim for 20 minutes in a transparent rectangular tank made of food-grade plastics (400mm × 200mm × 350 mm, water depth 300 mm, water temperature 22 ± 2°C). Then, following a 15-minute break, the rats were placed in a box with an appropriate amount of 99.5% ether until they showed such symptoms as shortness of breath, staggering and loss of consciousness. Afterwards, they were removed to a well-ventilated cage (not the one for feeding) until their consciousness was recovered before being put back to the original feeding cage. And this method was the criterion for the animal affected with PTSD in this study.

2.2.3. Preparation of Brain Specimens

Rats from the 1st day, 7th day, 14th day, 28th day and normal groups were taken for heart perfusion to draw their brains.

First, 1.5 mL 0.1% chloral hydrate anesthesia was injected into the rats and then their abdominal cavity was cut open to expose their heart. The perfusion needle was inserted from cardiac apex into the left ventricle and to the aorta end. Then, the right atrial appendage was cut open and 250 mL ice saline (4°C) was infused rapidly until incision effluent from the right atrial appendage becomes crystal clear and liver pale. Then, 250 mL 4% precooled paraformaldehyde solution (4°C) was infused. The total infusion time was controlled around 1.5 hours. Brains were surgically removed from the rats and rapidly frozen in liquid nitrogen and stored at −80°C until use.

2.2.4. Detection of the Expressions of IGF-1 and IGF-1 Receptor in Hippocampal CA1 Region by Immunohistochemsy

Some samples of corresponding brain regions were taken out and placed in the 4% paraformaldehyde solution (4°C) for 24 hours. After dehydration, transparency, wax dipping, paraffin embedding and wax block precooling, the brain tissue was subject to serial coronal sectioning with a thickness of 3 μm. All brain slices were selected at regular intervals. Six hippocampal slices were taken from about the same position of each rat. These brain slices were dried and saved for the subsequent immunohistochemistry.

After dewaxing, de-xylene, removal of endogenous peroxidase activity and antigen retrieval successively, the selected paraffin sections were dripped primary antibody (Rabbit Anti-Rat IGF-1 and Rabbit Anti-Rat IGF-1 Receptor) respectively. The blank control group (only adding phosphate buffer) and antibody specificity control group (adding primary antibody or secondary antibody (Goat Anti-Rabbit IgG) only) were established. All sections were conducted immunohistochemical staining simultaneously. After added with primary antibody, sections were placed into a wet box overnight under 4 °C and in the next day incubated at 37 °C for 30 minutes after being rewarmed at 37 °C, washed with PBS for

3 times (5 minutes per time) and added with biotin-labeled secondary antibody drop by drop successively. Then, these sections were conducted DAB coloration, hematoxylin redyeing, dehydration and transparency successively. Finally, they were mounted with neutral resin and detected with microscopy. Four sections were taken from each rat to observe the hippocampus by 400-times optical microscope, wherein 10 vision fields (× 400) were taken continuously to capture images. By the image analysis software ImagePro Plus 6.0, the integrated optical density (IOD) and area of positive expression positions in each vision field were measured to obtain the average optical density (mean density = IOD/area). Finally, the arithmetic mean of average optical density values of 10 vision fields was obtained.

2.2.5. Expressions of IGF-1 and IGF-1 Receptor in Hippocampal CA1 Region measurement by real-time florescent quantitative PCR

The other samples of corresponding brain regions were reduced to powder by comminuting the frozen tissue in liquid nitrogen with a pestle. Total RNA was extracted from the brain tissues using trizol reagent according to the manufacturer's protocol (Ambion, US). Briefly, 1 mg tissue powder were homogenized in 0.01 mL trizol reagent at room temperature. A first strand cDNA synthesis kit (Sangon Biotech Co., Ltd, Shanghai, China) was used for cDNA synthesis according to the manufacturer's instructions. Gene sequences for primer design were obtained by Sangon Biotech Co., Ltd. (Shanghai, China). The primers were dissolved in 100 μL of distilled water after centrifugation at a speed of 10,000 rpm for 20 seconds. The solution was centrifuged again at a speed of 5,000 rpm for 10 seconds and stored as stock at −20 °C with the final concentration of 100 μM. The working concentration was 5 μM stored at −4 °C. The primer sequences are listed in Table 1. The PCR conditions for IGF-1, IGF-1 Receptor and internal gene β-actin were the same as for the two-step method by ABI Prism 7500 sequence detection system (Applied Biosystems, US): the reaction volume was denatured at 95 °C for 2 minutes first, followed by 40 cycles of denaturing at 95 °C for 20 seconds, and annealing at 60 °C for 30 seconds and elongating at 68 °C for 1 minute.

The analysis was conducted by the integrated software to determine Ct value by negative control and baseline and to determine the validness of Ct value according to the dissolution curve. The triplicate reactions were performed in parallel for each sample, and the average Ct value was calculated. The negative control reaction was also performed for each round of PCR. The ratio of IGF-1(R)/β-actin (T/S) was calculated by the formula of $2^{-\Delta Ct}$, and $\Delta Ct = Ct_{IGF\neg 1(R)} - Ct_{\beta\text{-actin}}$, relative T/S ratio $= 2^{-\Delta\Delta Ct} = 2^{-\left(\Delta Ct_{IGF-1\mathbf{af};(R)} - \Delta Ct_{\beta\text{-actin}}\right)}$

2.2.6. Statistical Method

The statistical software SPSS 19.0 was used for data analysis. Data were expressed as mean ± standard deviation (SD). The mean values of animals in each group were detected with the least significant difference method (LSD) and correlation between groups by Spearman. All statistical tests were two-tailed ones, the significance level was set at 0.05 and various tests were free of special calibration.

Table 1
PCR primers list.

Name	Primer sequence	PCR product length (bp)
IGF-1	forward: 5′- CAGGCAGGTATGCTAGGAGC -3′ reverse: 5′- TCAAGGTATTTCCCAGTGCC -3′	224
IGF-1R	forward: 5′- GTCCTTCGGGATGGTCTA-3′ reverse: 5′- AACTTGTTGGCATTGAGGT-3′	369
β-actin	forward: 5′- TCAGGTCATCACTATCGGCAAT -3′ reverse: 5′- AAAGAAAGGGTGTAAAACGCA -3′	432

3. Results

3.1. Effect evaluation of PTSD Rat Model

In the response rate test (Fig. 1a), the PTSD group scored significantly higher than the control group ($P < 0.01$), indicating an increased awareness. In the open field test (Fig. 1b), the PTSD group had fewer behaviors like shuttling and standing than those of the

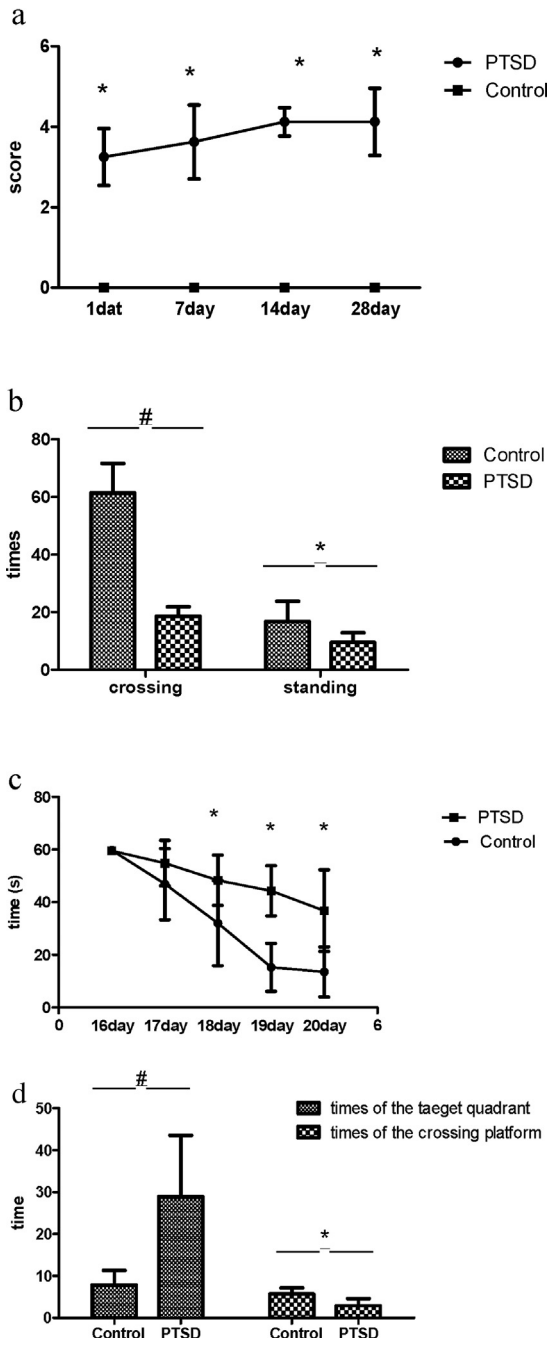

Fig. 1. Effect evaluation on PTSD model. a: the response rate scores. The response rate to the capture of the PTSD group was higher than that of the control group ($^{*}P < 0.05$); b: the open field-test scores. The PTSD group showed significant difference in the crossing, standing and grooming behaviors compared with the normal control group ($^{*}P < 0.05$, $\#P < 0.01$); c and d: the water maze test scores. The learning impairments induced by the combined stress in rats. Escape latency is to find a hidden platform during 5 consecutive days training. The PTSD group spent more time to find the hidden platform than the control group. ($^{*}P < 0.05$). There were differences in the times of the target quadrant and the crossing platform between the PTSD group and the control group ($^{*}P < 0.05$, $\#P < 0.01$).

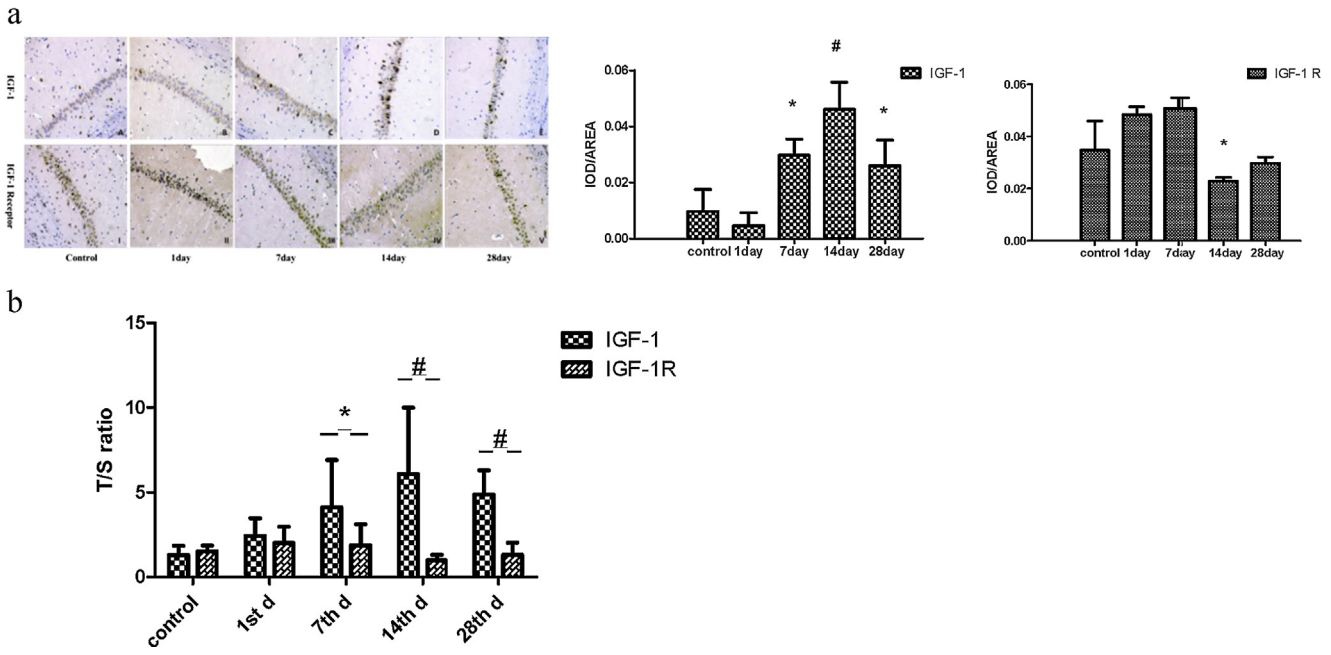

Fig. 2. Expressions of IGF-1 and IGF-1 Receptor in Hippocampus. a: the immunohistochemistry results. The IHC staining of the IGF-1 and IGF-1 receptor expression in the hippocampal CA1 region of the PTSD group and the normal control group. The differences of the integrated optical density (IOD) were statistically significant between the control group and PTSD group ($^{*}P < 0.05$, $\#P < 0.01$). The IGF-1 receptor protein expression levels of mean optical density in the hippocampal CA1 region on the 1st d before and after stress and the 7th d after stress didn't show significant difference ($P > 0.05$), which, however, decreased slightly on the 14th d and 28th d than before ($^{*}P < 0.05$); b: the qRT-PCR results. The effects of SPS stress on the expression of IGF-1 mRNA and IGF-1R mRNA of rats in hippocampal. Compared with control group, the expression of IGF-1 mRNA increased with significant association ($P < 0.05$). However, IGF-1R mRNA increased, but there was no significant association along with time passing by ($P > 0.05$).

control group ($P < 0.05$), indicating a decreased desire to explore. In the water maze test (Fig. 1c and 1d), within five days of learning, the latency of rats in the control group significantly reduced, whereas that of PTSD rats decreased not so notably ($P < 0.05$), revealing that the learning ability of PTSD rats lowered. In the sixth day of the water maze test, compared with rats in the control group, PTSD rats were slower in discovering the platform for the first time, and had fewer times of crossing the platform, having difference in statistics ($P < 0.05$).

3.2. Expressions of IGF-1 and IGF-1 Receptor in Hippocampus

The expressions of IGF-1 and IGF-1 receptor in the hippocampus of the post-stress 1st day, 7th day, 14th day and 28th day groups as well as the normal control group were measured. It can be seen from Fig. 2a that IGF-1 was mainly distributed within cytoplasm. With time passing by after stress, the mean optical density in hippocampal CA1 region increased with varying degrees than those of the control group ($P < 0.05$). IGF-1R protein was also mainly distributed in the cytoplasm. However, as time became longer after stress, the IGF-1R expression levels of mean optical density in the hippocampal CA1 region on the 1st day before and after stress and the and 7th day after stress didn't show significant difference ($P > 0.05$), which, however, decreased slightly on the 14th day and 28th day than before ($P < 0.05$). It can be seen from Fig. 2b that the expressions of IGF-1 mRNA and IGF-1R mRNA in the hippocampus variation tendency as same as the immunohistochemistry results shows. As shown in Table 2, analysis of Spearman rank correlation between the expression of IGF-1 protein and that of IGF-1 receptor protein of the post-stress 1st day, 7th day, 14th day and 28th day groups and the normal control group was conducted. The analysis results indicate that the two types of protein have no correlation (r = −0.400, $P > 0.05$).

Table 2
Correlation between Expressions of IGF-1and IGF-1 Receptor.

			IGF1	IGF1R
Spearman's rho	IGF1 $n = 5$	Correlation coefficient	1.000	−0.400
		Sig. (2-tailed)		0.505
	IGF1R $n = 5$	Correlation coefficient	−0.400	1.000
		Sig. (2-tailed)	0.505	

Analysis of Spearman rank correlation between the expression of IGF-1 protein and that of IGF-1 receptor protein of the post-stress 1st d, 7th d, 14th d and 28th d groups and the normal control group was conducted. The analysis results indicate that the two types of protein have no correlation ($r = -0.400$, $P > 0.05$).

4. Discussion

Studies have demonstrated most of the PTSD patients suffer social and behavioral abnormalities owing to the hippocampal atrophy in these people [5]. Numerous theories have been published on the causes of atrophy; yet, there are not certain evidences on the intervention of apoptosis in hippocampal atrophy [24].

The incidence of PTSD is directly related to mental trauma. Nowadays, because of ethical issues with the molecular studies in human models, different animal models including predator stress, single prolong stress (SPS), etc. have been suggested for studying the causes of hippocampal atrophy in PTSD. And as we all know that PTSD is regarded as a unique diagnosis according to the disease classification given by Diagnostic and Statistical Manual (Fifth Edition, DSM-5), which is deemed as the most authoritative diagnostic system. PTSD has the following four core symptoms:

- pathological recurrence of traumatic events;
- continued avoidance of trauma-related events and selective amnesia of traumatic experiences;
- heightened state of arousal;
- feeling or emotional numbness [21].

So, behavioral variation in rats on SPS is considered as a symptom of this disorder. The PTSD animal model reveals strong

similarity with PTSD patients behaviorally and physiologically, expressed by such phenomena as increased negative feedback inhibition of hypothalamic-pituitary-adrenal axis system (HPA axis), strengthened acoustic startle response (ASR), continued preservation of conditioned fear response and spatial memory impairment. We have established PTSD animal model through SPS method. As shown in Fig. 1a, b and c, stressed rats showed an increased awareness, a lowered desire to explore and learning and memory loss, which mimics the clinical symptoms of PTSD patients relatively well. Our experiment results are similar to those in previous studies.

Prevention of cell-programmed death may be caused by various molecular mechanisms like neurotrophins, growth factors synthesis and expression adjustment of apoptotic regulators [10]. The expression of IGF-1 is high in the developing brain tissue, decreases after adulthood and increases when adult brain is suffered from internal and external environmental stimulates or injury [9,15,20]. This research result has been verified again in the researches of Rusch et al. [19] and ours. In this study, the expressions of IGF-1 and IGF-1 receptor in rat hippocampal CA1 region were detected through the immunohistochemistry method and meanwhile the mean optical density (IOD/area) was measured with the software Imagepro Plus 6.0. As shown in Fig. 2a, b and Table 2, after SPS stress the IGF-1 expression in the hippocampal CA1 region of the 1st day, 7th day, 14th day and 28th day groups is higher than that of normal group and peaked in the 14 day group. These results are similar to those in previous research results. Afterwards, we studied the expression of IGF-1 receptor further, finding that the expression level of IGF-1 receptor on the 1st day and 7th day before and after stress showed no significant difference, but decreased slightly on the 14th day and 28th day than before. Then, we conducted Spearman correlation analysis on the expressions of IGF-1 and its receptor, which revealed that the expression of two proteins has no correlation. Recent studies demonstrated that IGF-1 possesses insulin-like biological activity, which plays an important role in promoting anabolism of brain as well as formation of synapse and brain myelination. All we know is that a declined level of insulin-like growth factor may promote the development of cognitive dysfunction [8]. Rusch's et al. paper [19] tends to make readers believe that the increase of IGF-1 has an effect on the improvement of patients' cognitive function. However, our research discovered that the expression of IGF-1 receptor didn't increase simultaneously with that of IGF-1 and they are not related, although IGF-1 has protective effects on stress. Thus, it is concluded that IGF-1 receptor may limit the positive role of IGF-1 to a certain extent and may be involved in the pathophysiological process of PTSD in cognitive function changes. In fact, it is still unknown and requires further researches to verify.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Anacker C, Cattaneo A, Luoni A, Musaelyan K, Zunszain PA, Milanesi E, et al. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. Neuropsychopharmacology 2013;38:872–83.

[2] Dębiec J, Bush DEA, LeDoux JE. Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats – a possible mechanism for the persistence of traumatic memories in PTSD. Depress Anxiety 2011;28:186–93.

[3] Desideri G, Kwik-Uribe C, Grassi D, Necozione S, Ghiadoni L, Mastroiacovo D, et al. Benefits in cognitive function, blood pressure and insulin resistance through cocoa flavanol consumption in elderly subjects with mild cognitive impairment novelty and significance. Hypertension 2012;60:794–801.

[4] Dietrich MO, Muller A, Bolos M, Carro E, Perry ML, Portela LV. Western style diet impairs entrance of blood-borne insulin-like growth factor-1 into the brain. Neuromolecular Med 2007;9:324–30.

[5] Fink G. Stress controversies: posttraumatic stress disorder, hippocampal volume, gastroduodenal ulceration. J Neuroendocrinol 2011;23:107–17.

[6] George SA, Rodriguez-Santiago M, Riley J, Abelson JL, Floresco SB, Liberzom I. Alterations in cognitive flexibility in a rat model of posttraumatic stress disorder. Behav Brain Res 2015;286:256–64.

[7] Guo LL, Tian GQ, Insulin. Insulin resistance in diabetes with cognitive dysfunction. Chin J Rehabil Theory Pract 2012;18:433–5.

[8] Holly JMP, Perks CM. Insulin-like growth factor physiology: what we have learned from human studies. Endocrinol Metab Clin North Am 2012;41:249–63.

[9] Kim YK, Na KS, Hwang JA, Yoon HK, Lee HJ, Hahn SW, et al. High insulin-like growth factor-1 in patients with bipolar I disorder: a trait marker. J Affect Disord 2013;151:738–43.

[10] Lee EG, Son H. Adult hippocampal neurogenesis and related neurotrophic factors. BMB Rep 2009;42:239–44.

[11] Liberzon I, Krstov M, Young EA. Stress-restress: effects on ACTH and fast feedback. Psychoneuroendocrinology 1997;22:443–53.

[12] Louchart-de la Chapelle S, Nkam I, Houy E, Belmont A, Ménard JF, Roussignol AC, et al. A concordance study of three electrophysiological measures in schizophrenia. Am J Psychiatry 2005;162:466–74.

[13] MacDonald AW, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science 2000;288:1835–8.

[14] Milham MP, Banich MT, Barad V. Competition for priority in processing increases prefrontal cortex's involvement in top-down control: an event-related fMRI study of the stroop task. Cogn Brain Res 2003;17:212–22.

[15] Muller AP, Fernandez AM, Haas C, Zimmer E, Portela LV, Torres-Aleman I. Reduced brain insulin-like growth factor I function during aging. Mol Cell Neurosci 2012;49:9–12.

[16] Muller AP, Gnoatto J, Moreira JD, Zimmer ER, Haas CB, Lulhier F, et al. Exercise increases insulin signaling in the hippocampus: physiological effects and pharmacological impact of intracerebroventricular insulin administration in mice. Hippocampus 2011;21:1082–92.

[17] Nakajima S, Ohsawa I, Ohta S, Ohno M, Mikami T. Regular voluntary exercise cures stress-induced impairment of cognitive function and cell proliferation accompanied by increases in cerebral IGF-1 and GST activity in mice. Behav Brain Res 2010;211:178–84.

[18] Rabinak CA, MacNamara A, Kennedy AE, Angstad M, Stein MB, Liberzon I, et al. Focal and aberrant prefrontal engagement during emotion regulation in veterans with posttraumatic stress disorder. Depress Anxiety 2014;31:851–61.

[19] Rusch HL, Guardado P, Baxter T, Mysliwiec V, Gill JM. Improved sleep quality is associated with reductions in depression and PTSD arousal symptoms and increases in IGF-1 concentrations. J Clin Sleep Med 2015;11:615–23.

[20] Schober ME, Block B, Beachy JC, Statler KD, Giza CC, Lane RH. Early and sustained increase in the expression of hippocampal IGF-1, but not EPO, in a developmental rodent model of traumatic brain injury. J Neurotrauma 2010;27:2011–20.

[21] Van Rooij SJ, Geuze E, Kennis M, Rademaker AR, Vink M. Neural correlates of inhibition and contextual cue processing related to treatment response in PTSD. Neuropsychopharmacology 2015;40:667–75.

[22] Williamson JB, Heilman KM, Porges E, Lamb D, Porges S. A possible mechanism for PTSD symptoms in patients with traumatic brain injury: central autonomic network disruption. Front Neuroeng 2013;6:13.

[23] Zalta AK, Gillihan SJ, Fisher AJ, Mintz J, McLean CP, Yehuda R, et al. Change in negative cognitions associated with PTSD predicts symptom reduction in prolonged exposure. J Consult Clin Psychol 2014;82:171.

[24] Zhang L, Zhou R, Li X, Ursano RJ, Li H. Stress-induced change of mitochondria membrane potential regulated by genomic and non-genomic GR signaling: a possible mechanism for hippocampus atrophy in PTSD. Med Hypotheses 2006;66:1205–8.