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Original Article

Role of Chloroquine and Cocaine Injection on Synaptophysin Protein Level in PTSD Model of Male Wistar Rat

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Abstract

Introduction: Drug abuse could induce molecular changes in synapses, leading to mood-related disorders. In addition, some patients suffering from mood disease use drug to get comfort. In some behavioral disorders, autophagy inhibitor drugs are used.

Materials and Methods: In the current study, the effect of chloroquine (CQ, an autophagy inhibitor drug) in a rat model of Post-Traumatic Stress Disorder (PTSD), together with the role of cocaine abuse was examined. Rats were injected with the CQ and/or cocaine alone or following single-prolonged-stress exposure and were confirmed as PTSD, using elevated-plus maze (EPM) test and then protein level of synaptophysin (a synaptic vesicle glycoprotein) was investigated by western blotting tecnique. It should be noted that cocaine was administered intracerebroventricularly (i.c.v, 20µg/rat) and CQ was administered intraperitoneally (50 mg/kg, IP).

Results: Obtained data revealed that PTSD and chronic administration of cocaine (i.c.v) in PTSD animals could increase the level of Synaphtophysin. CQ injection in them decreased Synaptophysin. So cocaine increase Synaphtophysin while CQ decrease it in PTSD animals.

Conclusion: The current data suggests altering neural plasticity by Synaptophysin protein level changes in brain on PTSD rats.

Keywords: Cocaine, Chloroquine, PTSD, Synapthophysion

1. Introduction

Post-traumatic stress disorder (PTSD) often happens in individuals who experience a series of traumatic and stressful event in their life [1]. Some people have more risk factors to develop PTSD; hence, there are other variables apart from the traumatic event itself that influence those who get PTSD, such as cellular and molecular differences among people [2, 3]. The brain of PTSD patient responses to the stressors more than normal [4, 5]. Changes in brain cells lifetime are responsible for different diseases. Neural cell death has different pathways (autophagy, for instance) [6]. In the autophagy process, autophagosomes-containing materials and organelles attach to lysosomes which undergo degradation [7]. In normal condition, autophagy always accrue but as the level of autophagy changed, it may lead to diseases [8-11].

Chloroquine (CQ) was used to treat malaria at first [12, 13]. CQ increases lysosomal pH [14] and induces vacuolization in the cell [15]. It has food and drug administration, approved for treating tumors by autophagy inhibition [16].

In this study, the effect of autophagy inhibition by CQ in PTSD rat model was evaluated. For this purpose, single-

prolonged-stress (SPS) which is one of the approved models for PTSD inducing was used [17, 18]. It has been suggested that PTSD is related to cellular death in some brain regions including prefrontal cortex. [19-21]. One of the cellular death induced by stress is autophagy, which may change neural plasticity [22-25]. Microtubule-associated proteins 1A/1B light chain 3B (LC3) is a central protein in the autophagy system. There are different proteins involved in synaptic plasticity, one of which is synaptophysin, also known as the major synaptic vesicle protein p38, which is a synaptic vesicle glycoprotein participates in synaptic transmission and neural plasticity[26].

Medications that help PTSD sufferers are not always helpful, so some of the patients use opium to reduce their symptoms. Cocaine abuse in PTSD sufferers is prevalent. Exposure to cocaine- a psycho-stimulant- induces autophagy and inflammation [27, 28]. In addition, cocaine increases the anxiety-like behavior and changes molecular pathways in animals [29].

The main aim of the current study was to evaluate the effect of exposure of SPS on rats – as an animal model of PTSD- on synaptophysin level. In addition, the effect of CQ as an autophagy inhibitor and cocaine were investigated in SPS-induced synaptophysin level changes.

2. Materials and Methods

2.1. Animals

Adult (8 weeks old) male Wistar rats were obtained from Pasteur Institute, Tehran, Iran and kept in the lab under a controlled temperature (22±2°C) and 12/12-h light-dark cycle. All animals had free access to fresh water and food. All experiments were in accordance with the guidelines for the Care and Use of Laboratory Animals of the Islamic Azad University of Damghan ethics committee.

2.2. Stereotaxic Surgery

Each rat (weighting 220-250 gr) was anesthetized by i.p injection of ketamine and xylazine (5:2, IP injection). Then, the rat was placed into stereotactic apparatus (Stoelting Co, USA) and was subjected to the stereotaxic surgery for lateral ventricular canalization. After fixing the rats' head into the stereotaxic apparatus, the head was shaved and was incised and the soft tissue was removed. A guide cannula was placed into the lateral ventricle according to Paxinos atlas [30] (Bregma-Lambda: 9mm; anterior-posterior: 0.5 mm, mediolateral: 5.1 mm, dorsoventral: 4). After drilling the skull and making a hole in the skull, a 22 gauge stainless steel guide cannula was placed into the lateral ventricle and was fixed by dental cement. One week after surgery, one µl of cocaine was injected through a 27-gauge injection needle that connected using a polyethylene tube to a Hamilton syringe. Drug microinjection into the ventricle was performed for 1 min.

2.3. PTSD Induction

Single-Prolonged Stress (SPS) was used to induce PTSD [31, 32]. The protocol of SPS induction was as follows:

a) Restrain: Rats were restrained in a chamber (7 cm diameter, 21 cm length) for two hours

b) Swim stress: Rats were put in an acrylic cylinder (20 cm diameter), filled with water (24 °C)

c) Anesthesia: With 15 min rest after swim stress, animals were exposed to isoflurane until deep anesthesia. SPS was repeated for 7 following days.

2.4. Western Blotting

Hippocampus , an important part of brain involved in PTSD was extracted carefully and tissue segments were rapidly collected at 1 hour after the PTSD confirmation and were kept in liquid nitrogen immediately for 24 hours; then, they were transported to -80˚C refrigerator, until the beginning of the Western blotting procedure. Protein concentration was

determined by spectrophotometer (Picodrop, UK). Sixty µg of total protein of each sample were loaded and separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes.

Electroblotted proteins were put onto PVDF membranes after blocking with skim milk, probed with synaptophysin and LC3 antibodies overnight at 4 °C and then incubated with relevant secondary antibodies. Following extensive washing, immunoreactivity was visualized using the enhanced chemiluminescence method. Also antibody was used against Beta-Actin as housekeeping protein to normalize all of the treatments. The bands were analyzed by densitometric quantification using ImageJ software and normalized to the appropriate loading controls.

2.5. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using prism software (ver 5). Following Tukey Post hoc., data were expressed as means ± S.E.M and the p-value lower than 0.05 was considered as significant.

3. Results

Effect of CQ Injection in LC3-II/I Protein Level

In this study, LC3-II/I protein, a marker of autophagosomes, verifying the role of CQ in autophagy inhibition were evaluated in CQ injected group compared to control group. As Fig.1 shows, the protein level of LC3-II/I decreased about 4 times in CQ injected rats compared with the control group.

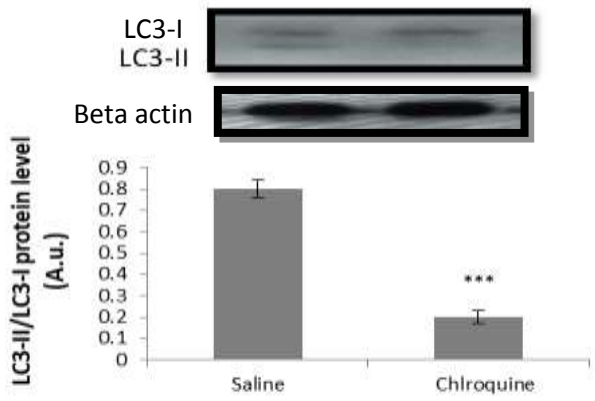

Figure 1. Shows the result of western blot technique for the evaluation of LC3-II/LC3-I protein level between control and CQ groups to confirm CQ effects on autophagy inhibition. $^{***}P < 0.001$. N=5

Effect of PTSD, CQ, and Cocaine on Synaptophysin Protein Level

In the current research, the effect of PTSD on the synaptophysin protein level and the role of autophagy in it were investigated. As is shown in the current study, PTSD increased synaptophysin in the rat while CQ (as an autophagy inhibitor) decreased it. In addition, administration of cocaine in PTSD animals caused more increase in synaptophysin protein level. The effect of PTSD, cocaine, CQ, and co-administration of these with PTSD is shown in Figure 2. The level of synaptophysin

increased 1.4 times in PTSD rats compared to the control (saline) group. Moreover, the level of synaptophysin increased 1.6 times in cocaine-injected rats compared to the PTSD group ($p<0.001$). A decrease in synaptophysin level between control group and CQ group ($p<0.05$) was observed. Both administration of CQ and cocaine had a less synaptophysin protein level when injected at the same time to PTSD rats ($p<0.001$) compared to the PTSD group, while not being statically significant.

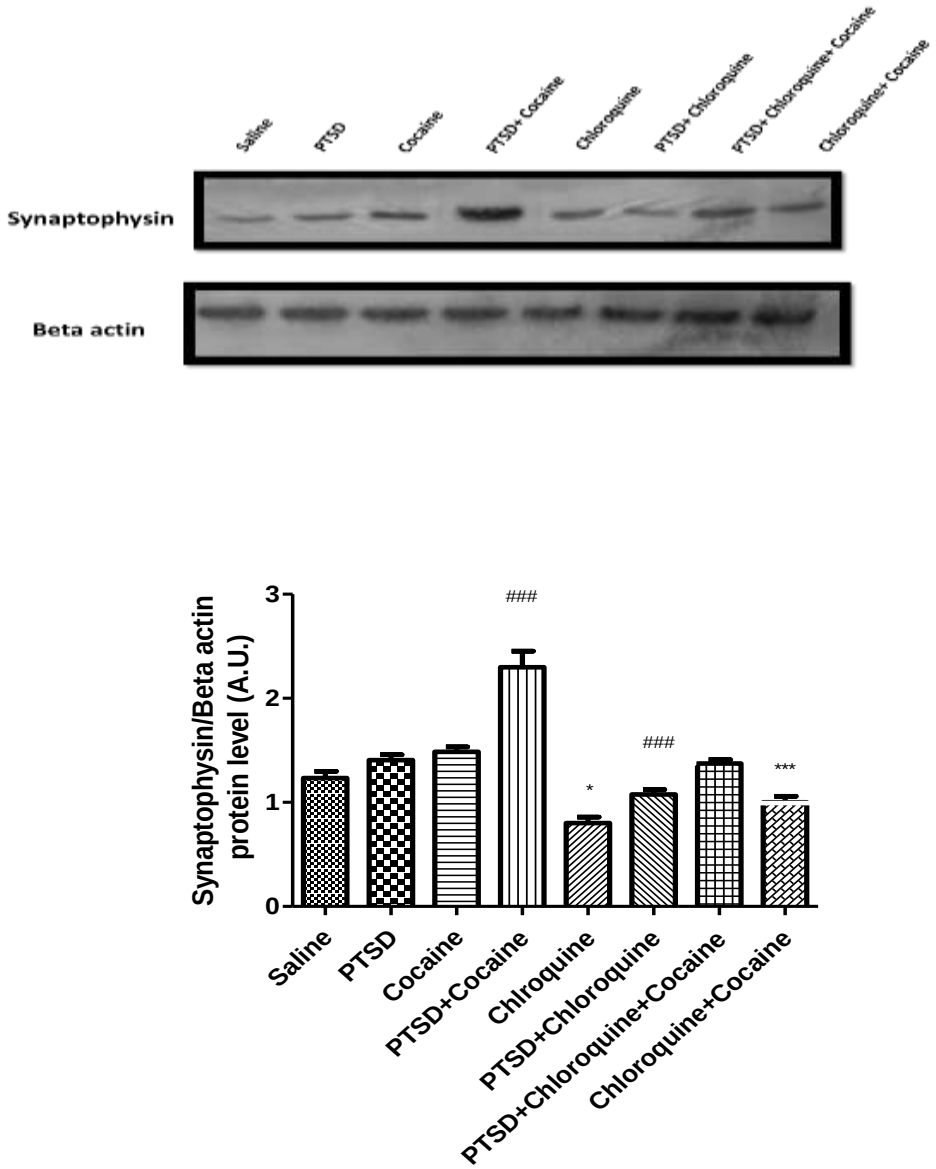

Figure 2. Shows the result of western blot technique for the evaluation of Synaptophysin protein level among groups. The density of Synaptophysin/ β -actin was determined by Image J software. $^{***}P < 0.001$ and $^{*}P < 0.05$ vs. control group and $^{###}P < 0.001$ vs. PTSD group. (One-way ANOVA followed by Tukey's multiple comparisons test). N=5

4. Discussion

PTSD model used in this experiment was acquired from previous work [33]. To understand the mechanisms underlying SPS-induced PTSD and finding the role of autophagy in this process, the role of CQ on synaptophysin protein level in PTSD animals was examined. It has previously

been shown that autophagy plays a major role in anxiety-like behaviors [25, 34, 35]. Then, the effect of the administration of an autophagy inhibitor drug (CQ) was examined as for understanding the role of autophagy in PTSD-induced neural plasticity changes [36]. There is a clinical study indicating that antimalarial drug such as CQ could enhance the mental illnesses such as anxiety among veterans who used these drugs [37]. Correspondingly, one study revealed that CQ might induce some psychiatric disorders such as anxiety [38]. The real activity of synaptophysin is not clear: its interaction with other important synaptic vesicle. Recent research has manifested that elimination of synaptophysin in mice induces behavioral changes including increased exploratory behavior, impaired object novelty recognition, and reduced spatial learning. The present study revealed that synaptophysin increased in PTSD rats which received CQ and cocaine together, which may suggest the possible role of pharmacological usage of these two drug combined for patients suffering from PTSD. It has been previously reported that using endocannabinoids could increase synaptophysin as a potential treatment for PTSD [39]. Furthermore, it is reported that stress induces the synaptophysin level in mice [40] and using cocaine would increase it; hence, it could increase neural plasticity [41]. CQ as an autophagy inhibitor has proved to decrease synaptophysin in previous works which is in parallel with the findings of the current study [42].

5. Conclusion

The present study suggest adverse effect of cocaine for patients suffering from PTSD, while cannabinoids and other drugs have appeared to be as a therapeutic target for its treatment. In addition, it suggests drugs inhibiting autophagy like CQ as a suppressor of synaptophysin and neural plasticity induced by devastating memory which cusses PTSD.

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Conflict of interest

The authors declare no conflict of interest.

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