Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

The effects of CCK-8S on spatial memory and long-term potentiation at CA1 during induction of stress in rats

Malihe Sadeghi [1], Parham Reisi [1]*, Maryam Radahmadi [1]

[1] Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: May 28, 2017
Accepted: Sep 28, 2017

Keywords:
CA1
Cholecystokinin sulfated - octapeptide
Hippocampus
Long-term potentiation
Memory
Stress

ABSTRACT

Objective(s): Cholecystokinin (CCK) has been proposed as a mediator in stress. However, it is still not fully documented what are its effects. We aimed to evaluate the effects of systemic administration of CCK exactly before induction of stress on spatial memory and synaptic plasticity at CA1 in rats.

Materials and Methods: Male Wistar rats were divided into 4 groups: the control, the control-CCK, the stress and the stress-CCK. Restraint stress was induced 6 hr per day, for 24 days. Cholecystokinin sulfated octapeptide (CCK-8S) was injected (1.6 µg/kg, IP) before each session of stress induction. Spatial memory was evaluated by Morris water maze test. Long-term potentiation (LTP) in Schaffer collateral-CA1 synapses was assessed (by 100 Hz tetanization) in order to investigate synaptic plasticity.

Results: Stress impaired spatial memory significantly (P<0.01). CCK in the control rats improved memory (P<0.05), and prevented the impairments in the stress group. With respect to the control group, both fEPSP amplitude and slope were significantly (P<0.05) decreased in the stress group. However, there were no differences between responses of the control–CCK and Stress–CCK groups compared to the control group.

Conclusion: The present results suggest that high levels of CCK-8S during induction of stress can modulate the destructive effects of stress on hippocampal synaptic plasticity and memory. Therefore, the mediatory effects of CCK in stress are likely as compensatory responses.

► *Please cite this article as:*
Sadeghi M, Reisi P, Radahmadi M. The effects of CCK-8S on spatial memory and long-term potentiation at CA1 during induction of stress in rats. Iran J Basic Med Sci 2017; 20:1368-1376. doi: 10.22038/IJBMS.2017.9619

Introduction

Stress is defined as any challenge to the homeostatic balance that requires an adaptive reaction of the organism (1). It is well documented that both acute and chronic stress has profound effects on brain-body interactions and considerably result in cognitive deficits (2). One of the main physiological processes in response to stress is the activation of hypothalamic-pituitary-adrenal (HPA) axis (3) which regulates the cognition, memory and stress related behaviors (4).

Hippocampus plays a crucial role in the explicit memory function and spatial memory formation (5). It is involved in the regulation of HPA axis (6) and neuroendocrine regulation of stress hormones (7). Some previous data suggest that the hippocampus has an inhibitory effect on HPA activity (8). However, this brain structure is shown to be especially susceptible to stress. The responses of the hippocampus could be affected by the amount of stress-related hormones (4), and long-term exposure to the stress stimuli such as chronic restraint stress, reduces hippocampal volume and causes cognitive impairments (9). Long-term potentiation (LTP), a long lasting augmentation of excitatory synaptic transmission after a brief and high-frequency electrical stimulation (10), is also affected by stress. Increased glucocorticoid release in stress has destructive effects on LTP in the hippocampus (11). Depending upon the brain region, LTP is differently affected by stress exposure. For example, chronic stress has been shown to nearly abolish LTP induction in hippocampal CA1 area, whereas it exerts no effect on dentate gyrus LTP (12).

Cholecystokinin (CCK) was first found as a gastrointestinal hormone (13) and was later identified as one of the most widely distributed neuropeptides within the brain, where it acts as a neurotransmitter (14). CCK is greatly expressed and distributed in the hippocampus, which is involved in multiple physiological processes associated with memory functions (15). There are two types of G protein-coupled cholecystokinin receptors: CCK-A (mainly distributed in peripheral tissues and some brain regions) and CCK-B (the predominant form in the CNS) (16). Cholecystokinin sulfated octapeptide (CCK-8S) is one of the most abundant molecular isoforms of cholecystokinin in the brain (17). CCK results in marked neuromodulatory effects on the

*Corresponding author: Parham Reisi. Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: +98-31-37929033; Fax: +98-31-36688597; email: parhamzh@gmail.com

release of neurotransmitters such as gamma-amino-butyric acid (GABA) (18) and glutamate (16). It significantly mediates the endocannabinoid (central neuromodulatory lipid) system activities (19), the expression of neurotrophic factors specially brain-derived neurotrophic factor (BDNF) (20), neuronal apoptosis and neurogenesis (21). Increasing evidences show the ameliorative influences of CCK on hippocampal synaptic plasticity (22). Preclinical studies document that acute intraperitoneal (IP) injection of CCK-8S before each test in Morris water maze task, improved spatial memory in rats (23). As previously reported, CCK exerts an excitatory effect on perforant path-dentate gyrus (DG) granule cells, CA3-CA3 and Schaffer collateral-CA1 synapses (16). In stress, the CCK level fluctuations within the hypothalamus and hippocampus indicate the possible role of this neuropeptide in the regulation of HPA activity, glucocorticoid concentrations, learning and memory processing (24, 25). Nevertheless, the precise effects of cholecystokinin on memory formation at the time of stress exposure remains poorly understood. In this study, we examined the effect of CCK treatment just before induction of restraint stress using Morris water maze test and field potential recording at Schaffer collateral synapses in hippocampal area CA1.

Materials and Methods

The experiments were carried out on male Wistar rats (250-280 g) that were housed under standard conditions of temperature (22 ± 2 °C) and light (12h light-dark cycle). Food and water were available *ad libitum*, except during the stress situations. The Ethic Committee for Animal Experiments at Isfahan University of Medical Sciences approved the study, and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996.

The animals were randomly divided into four groups (n=10): the control, the control-CCK, the stress and the stress-CCK.

To induce chronic stress, the rats were individually restrained for 6 hr/daily in plexiglas cylinders (20×7 cm) which had holes for air exchange from 8:00 to 14:00 (26) for 24 days.

The rats in the CCK treated groups received CCK-8S (1.6 µg/kg, IP, dissolved in saline; Sigma-Aldrich) (23, 27, 28) before daily placing in restraint chambers. Animals in the control and stress groups received the same volume of saline.

Five days before beginning the induction of stress and the treatments, all rats were habituated to the water and apparatus. The apparatus was consisted of circular tank (180 cm in diameter) that filled with water (22±2 ◦C) made opaque and was surrounded by a variety of extra-maze cues. The tank was divided into four quadrants, and four start positions were located at the interactions of the quadrants. Data were recorded using custom software (*Radiab1*).

In the spatial acquisition phase that performed during 4 days before the stress induction and the treatments, the rats learned to find a submerged platform using extra-maze cues. A transparent Lucite platform (10 cm) was submerged 2 cm underneath the water in North-east quadrant of the tank, where it remained for all spatial trials. Each rat participated in 16 trials, which were organized into daily block of four trials (1 trial/start position within a block) for 4 consecutive days. For each trial, the rat was given a maximum time of 60 sec to locate the platform, after which the rat remained there for 30 sec; if the rat did not locate the platform within 60 sec, it was guided to it by the experimenter. The next trial started immediately after removal of rat from the platform. Escape latencies (s) was recorded.

In the retention phase, 24 hr after the last session of stress induction and treatments a 60 sec probe trial was conducted to examine how well the rats had learned the exact location of the platform. During this trial, the platform was removed from the tank. The swim time was measured inside a circular area (70 cm diameter) around the center of last place of the platform. To test possible deficits in sensorimotor processes, rats were tested in the water maze with a visible platform after probe trial. Four cued trials were conducted, with start locations randomized and the platform located in a different quadrant during each trial. Rats were allowed to swim for up to 60 sec to locate the platform. The criterion for impaired non-mnemonic aspects was inability to locate the platform within 60 sec, more than two times of four cued trials (29, 30). All rats located the platform within 60 sec.

After behavioral test, rats were anesthetized with urethane (1.8 g/kg, IP) and their heads were fixed in a stereotaxic frame. Body temperature kept at 36.5±0.5 °C with a heating pad. The skull was exposed and two small holes were drilled at the positions of the stimulating and recording electrodes. The exposed cortex was kept moist by the application of paraffin oil. A concentric bipolar stimulating electrode (stainless steel, 0.125 mm diameter, Advent, UK) was placed in the right Schaffer collateral pathway (AP=-4.2 mm; ML=3.8 mm; DV=-2.7–-3.8 mm), and a stainless steel recording electrode was lowered from the left side of skull into the right CA1 area until the maximal response was observed (AP=-3.4 mm; ML=1.5 mm; DV=-4.4–-5.1 mm; at an angle of 52.5 degrees) (31). In order to minimize trauma to brain tissue, the electrodes were lowered very slowly (2 mm/min). Implantation of electrodes in the correct position was determined by physiological and stereotaxic indicators.

Extracellular evoked responses were obtained from the CA1 pyramidal cell population following stimulation of the Schaffer collateral pathway.

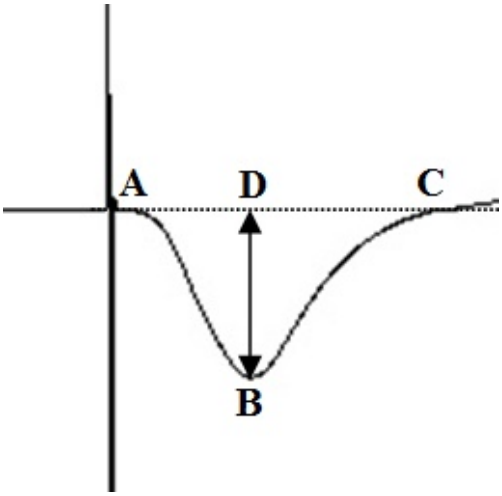

Figure 1. Schematic diagram of field excitatory post-synaptic potential (fEPSP). The fEPSP amplitude parameters analyzed as: difference between B and D, and fEPSP slope analyzed as: AB slope

Extracellular field potentials were amplified (×1000) and filtered (1 Hz to 3 KHz band pass). Signals were passed through an analogue to digital interface (Electro module D3111, Science Beam Institute, Tehran, Iran) to a computer and data were analyzed offline using eProbe software (Science Beam Institute, Tehran, Iran). As shown in Figure 1, the field excitatory post-synaptic potential (fEPSP) amplitude was measured as the difference in voltage between the negative peak of the fEPSP wave and the baseline (between B and D) and the fEPSP slope was measured as the slope between the baseline and the peak of the negative wave (between A and B). In order to evaluate synaptic potency, stimulus–response or input/output (I/O) functions were acquired by systematic variation of the stimulus current (50–1000 μA) before induction of LTP. After that, paired pulse facilitation was measured by delivering five consecutive evoked responses of paired pulses at 50ms inter-stimulus intervals at a frequency of 0.1 Hz (10 s interval). The fEPSP amplitude ratio (second fEPSP amplitude/first fEPSP amplitude) and the fEPSP slope ratio (second fEPSP slope/first fEPSP slope) were measured.

To evaluate long-term changes in the synaptic response of CA1 neurons, fEPSPs were evoked in the CA1 region using 0.1 Hz, 30 min prior to LTP induction and 90 min after that. LTP was induced using high-frequency stimuli protocols of 100 Hz (4 bursts of 50 stimuli, 0.15 ms stimulus duration, 10 s interburst interval). All potentials employed as baseline and high frequency stimuli were evoked at a stimulus intensity which produced 50% of the maximal response of the fEPSP (32).

Implantation of electrodes in the correct location was determined by histological verification. For histolo - gical verifications at the end of each experiment, rats were perfused transcardially with a 10% formalin solution and the brain removed and fixed in 10% formalin for at least 3 days. Subsequently, transverse sections through the brain were cut using a freezing microtome to locate electrode tracts. The sections were examined under a microscope and compared to the rat brain atlas (Figure 2) (31).

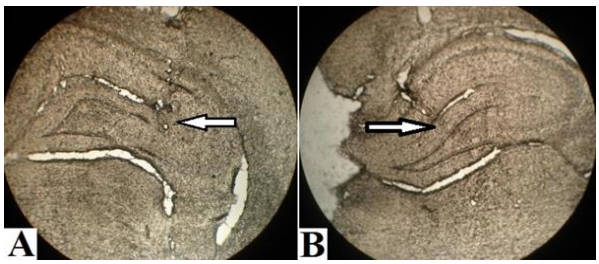

Figure 2. Histological representation of A) stimulating electrode location in Schaffer collateral B) recording electrode location in CA1. The arrow indicates the stained places

Adrenal weights were evaluated to assess the HPA activity (33). Hence, at the end of the electrophysio - logical study, rats were dissected and adrenal glands were removed and weighed.

Data were analyzed using the SPSS version 21 for windows (IBM Corporation). The data were analyzed statistically by repeated measures analyze of variance (ANOVA) followed by Tukey *Post hoc* for between subjects' differences and within effects, across the blocks of MWM and the data from LTP induction and maintenance; and the data from paired pulse facilitation, probe trial of MWM and adrenal weight were analyzed by one-way ANOVA followed by Tukey. The significant level was set at $P<0.05$. Results are expressed as mean±SEM.

Results

All rats showed a reduction in escape latencies (BLOCK effect, $F(3, 108) = 60.59$, $P<0.001$; Figure 3) across the blocks of trials, indicating spatial acquisition. The pattern of reduction in escape latencies to locate the platform across the blocks was the same between the groups.

For the results of probe trial as measured by the mean time spent inside a circular area (70 cm diameter) around the center of the plat form, the between group

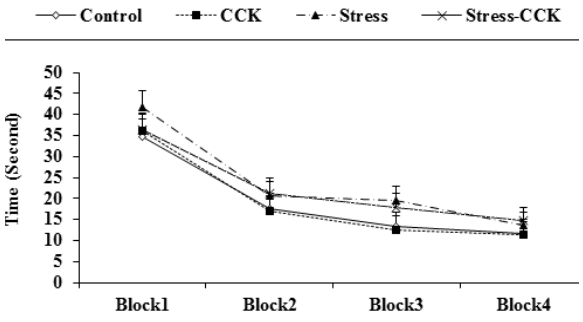

Figure 3. The escape latencies at different blocks of Morris water maze, before stress induction and treatments. Each point represents the block, mean±SEM of 4 swims. Lower numbers indicate better performance (n=10)

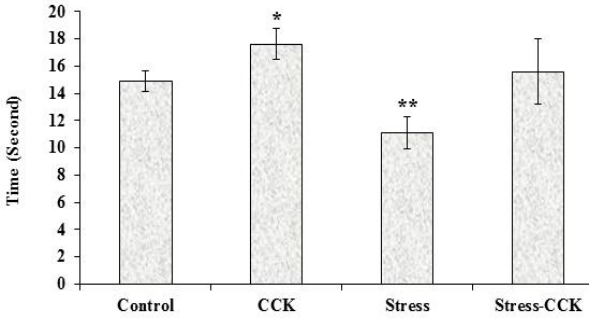

Figure 4. Effects of systemic administration of cholecystokinin sulfated octapeptide (CCK-8S) exactly before induction of stress on performance during the probe trial of Morris water maze as measured by the mean time spent inside a circular area (70 cm diameter) around the center of the platform, 1 day after the last session of stress induction and treatment. * P<0.05 and ** P<0.01 with respect to the control group (n = 10)

comparison indicated that CCK-8S significantly increased this time in CO-CCK group with respect to the CO group (17.6±1.14 s and 14.9±0.78 s, respectively; P<0.05; Figure 4). Stress decreased the time significantly in ST group (11.1±1.15 s; P<0.01). However, CCK-8S prevented this reduction in stressed rats as there was no difference between the CO and the ST-CCK groups (15.6±2.42 s; Figure 4).

As it is shown in Figure 5, the effects of high systemic levels of CCK-8S on LTP induction and maintenance in CA1 area of the stressed and intact rats were determined. A repeated measures ANOVA revealed that fEPSP amplitude (Time effect, F (8, 256) = 30.86, P<0.001; Figure 5A) and fEPSP slope (Time effect, F (8, 256) = 17.9, P<0.001; Figure 5B) reduced in all the groups over the time.

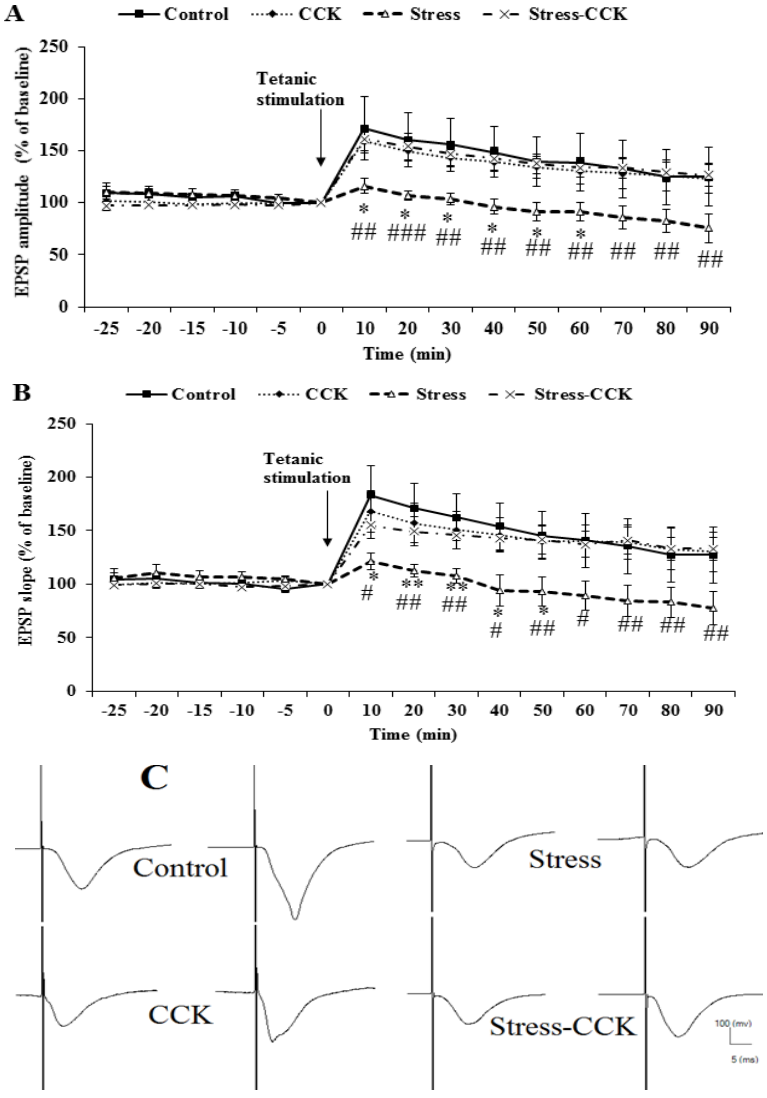

Figure 5. Effects of systemic administration of cholecystokinin sulfated octapeptide (CCK-8S) exactly before induction of stress on long-term potentiation (LTP) induction and maintenance in CA1 area of the hippocampus using tetanic stimulation at A: the field excitatory postsynaptic potential (fEPSP) amplitude and B: fEPSP slope. Data are plotted as the average percentage change from baseline responses. Values are % mean±SEM. * P<0.05 and ** P<0.01 with respect to the control group. # P<0.05, ## P<0.01 and ### P<0.001 with respect to the stress-CCK group (n=10). C: Single traces recorded before and after induction of LTP in CA1 area of the hippocampus

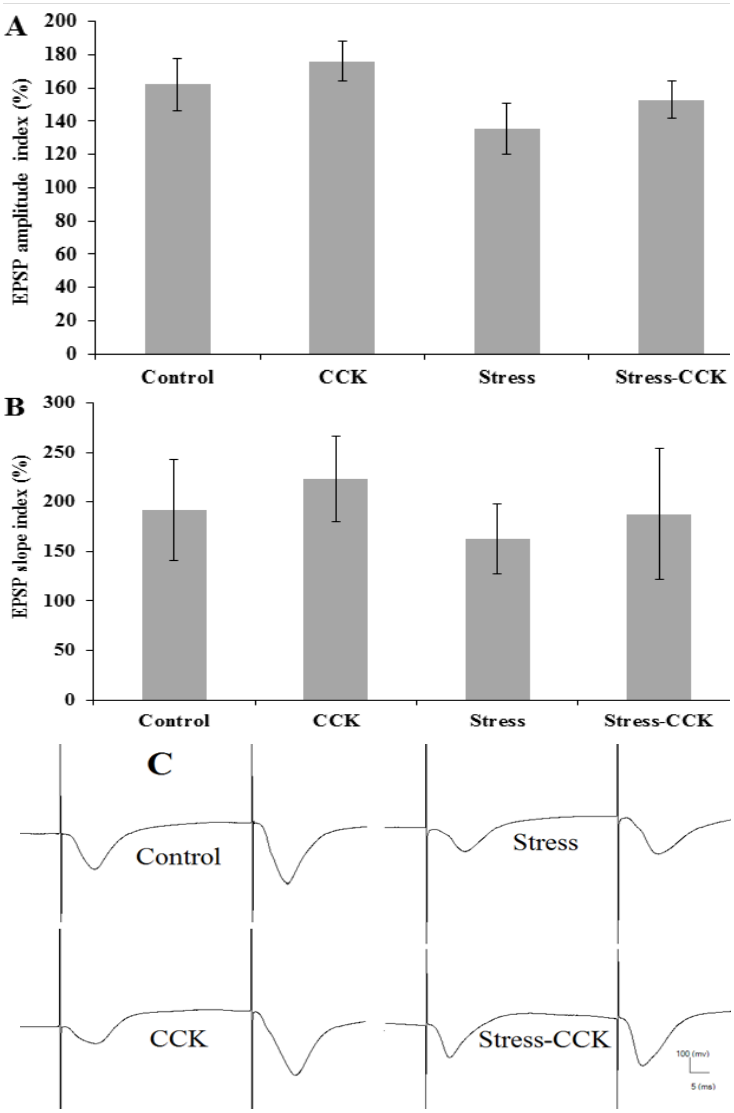

Figure 6. Effects of systemic administration of cholecystokinin sulfated octapeptide (CCK-8S) exactly before induction of stress on paired-pulse facilitation at inter-stimulus interval 50 ms in CA1 area of the hippocampus. A: the fEPSP amplitude ratio, (percentage of mean Amp2 / Amp1±SEM), and B: fEPSP slope ratio (percentage of mean fEPSP2 / fEPSP1 ± SEM). C: single traces recorded at inter-stimulus interval 50 ms were shown in (n= 10)

In stress group, stress decreased the fEPSP amplitude (94.37±13.72) and fEPSP slope (96.46± 14.43%) significantly with respect to the control group after tetanization (amplitude: 144±15.34% and slope: 149.76±16.47%, respectively; $P<0.05$; Figure 1 A, B). Although CCK-8S had no effects on fEPSP in CCK group, but in the stress-CCK group, it increased fEPSP amplitude (136.97±14.46% and 140.58±14.46%, respectively) and fEPSP slope (145.04±15.52% and 142.08±15.52%, respectively), as there was no significant difference between the control and the stress-CCK groups (Figure 5). CCK-8S and stress had no significant effects on paired-pulse facilitation at inter-stimulus interval 50 ms in CA1 area of the hippocampus (Figure 6).

The weight of the adrenal glands was increased significantly ($P<0.001$) in the stress group compared to the control group. CCK-8S significantly ($P<0.001$) decreased weight of adrenal gland in control-CCK group and there was a significant difference between the stress and the stress-CCK group (Figure 7).

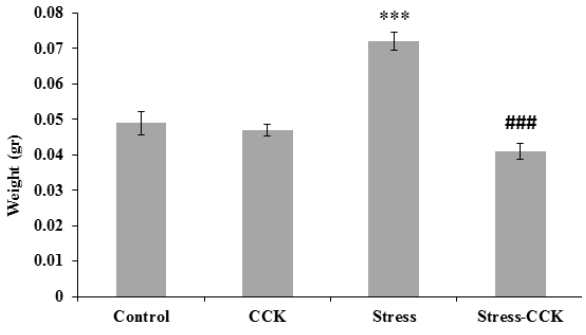

Figure 7. Effects of cholecystokinin octapeptide sulfated (CCK-8S) and stress on adrenal gland weight. Data are expressed as mean±SEM. (n = 10). *** $P< 0.001$ with respect to the control group, ### $P < 0.001$ with respect to the stress group

Discussion

In this study, we report that chronic stress impairs recall performances of the rats in Morris water maze task (Figure 4) as well as long-term plasticity in the hippocampal CA1 area (Figure 5). These results are consistent with previous data that demonstrated the detrimental effects of stress on hippocampal LTP (10) and spatial memory tasks (34). As previously shown, chronic restraint stress leads to increase in HPA activity and adrenal glucocorticoid production (35, 36). High glucocorticoid levels are shown to alter some of the brain characteristics and functions such as hippocampal dendritic structures (37), the expression of neurotrophic factors like BDNF and subsequent neurogenesis (38), action of neuromedu - latory systems including endocannabinoids (39), release of neurotransmitters like GABA (40) and Glutamate (41), and postsynaptic LTP induction at CA1 area of the hippocampus (42). Stress reduces the after-hyperpolarization amplitude, while it enhances the amplitude of Calcium influx, the activity of NMDA receptors and glutamatergic transmission in CA1 pyramidal neurons. All of these changes can affect the membrane potential and LTP induction (43). On the other hand, some evidence suggests that prolonged corticotropin-releasing hormone (CRH) augmenta - tion and its interaction with CRH receptor type-1 (located in dendrites of CA1 pyramidal cells) in the hippocampus underlies the impairment of LTP and cognition associated with chronic early life stress (44).

As a secondary observation, our results indicated that CCK-8S significantly increases the time spent around the center of the platform within the control-CCK group, indicating an enhanced retention of the prior platform location in the water maze performance (Figure. 4). The same result has been shown in aged rats following repeated IP injection of CCK-8S with a same dose (1.6 µg/kg) (23). CCK-B receptors probably play a key role in the performance of visual recognition tasks (45). Moreover, studies have documented impaired spatial memory performance of the rats lacking CCK-A receptors (46). However, this spatial memory improvement in intact rats was not supported by our electrophysiological data, because there was no significant difference in the fEPSP slope and amplitude between the control-CCK and control groups. Therefore, the exact mechanisms underlying the memory enhancement followed by long-term CCK treatment remains to be understood.

CCK-8S increased fEPSP amplitude and slope within the stress-CCK group compared to those of the stress group, as there was no significant difference between the control and stress-CCK groups (Figure. 5). Analogously, cholecystokinin reduced spatial memory impairment in the stress-CCK group, and there was no difference between the control and stress-CCK groups (Figure 4). In other words, CCK administration substantially improved special memory performance after a long period of merging with chronic restraint stress, and this result was supported by increased LTP induction and maintenance in CA1 area of the hippocampus. The enhancement of the LTP induction could be due to a reduction of K^+ conductance at CA1 pyramidal cells mediated by CCK-B receptors (47). CCK has been shown to induce the $[Ca^{2+}]$ signaling via an influx of extracellular calcium, leading to inhibition of NMDA receptors (48). Furthermore, a reduction of glutamate transmission in excitatory synapses via CCK-B receptors is reported (49). The decrease in GABA transmission via the activation of endocannabinoid system (50) as well as the enhancement of BDNF expression (2) are another mechanism probably mediated by CCK (19, 20).

Prolonged administration of CCK-8S significantly compensates the stress-dependent memory impairment and notably reduces HPA activity in chronic stress (Figure. 7) (27). It is well documented that both the CCK-A and CCK-B receptors contribute to the regulation of the HPA axis (51, 52), and repeated treatment with CCK decreases the expression of these receptors within the pituitary gland (53). Subsequently, the activation of the HPA axis may be suppressed by lower efficacy receptors. Furthermore, increasing evidence suggests that chronic stress results in a marked reduction of BDNF (a neurotrophic protein linked to various aspects of synaptic plasticity in CNS) expression mediated by glucocorticoids, leading to suppressed hippocampal neurogenesis and memory deficits (54). CCK was shown to augment the expression and function of BDNF (2). A repeated local administration of CCK-8S was reported to enhance neuronal network interconnections such as hippocampal excitatory synapses (49) and dendritic growth, leading to protect the hippocampus against atrophy (55). According to the inhibitory influence of the hippocampus on the HPA response to stress (8), it could be supposed that the activity of the HPA axis may be indirectly modified by CCK following a protective effect on hippocampus in stress conditions. As reported previously (56), expression of the cholecystokinin gene is increased

during periods of chronic stress. We suggest that such enhancement in CCK expression is probably to prevent destructive effects of HPA responses on hippocampal synaptic plasticity and spatial memory formation.

Previous data demonstrated that chronic restraint stress reduces the DSI (depolarization-induced supperssion of inhibition / a short-term plasticity at GABAergic synapses) in the hippocampus, which is possibly associated with a dysfunction in presynaptic CB1 (cannabinoid receptor1- notably distributed in stress-responsive brain regions such as the hippocampus) modulation of GABAergic transmission (57). Another study has indicated the ameliorative effects of the CB1-dependent endocannabinoid signaling on impaired hippocampal long-term poten - tiation following chronic restraint stress (58). Endo - cannabinoids organize a neurotransmitter system, which is activated by glucocorticoid rising during stress. They inhibit the excitation of the HPA axis regulating endocrine response to stress (59). CB1 receptors are expressed on CCK positive interneurons in the hippocampus (60). Therefore, the stress induced destruction of DSI is suggested to result from altered functional integrity of the CCK interneuron network (57). Cholecystokinin activates postsynaptic mGlu-R5-mediated retrograde endocannabinoid signaling via CCKA/B receptors (19). A recent data indicated that CB1 is also mostly expressed in pyramidal cells of CA1 and CA3, and the granular cells of the dentate gyrus (61). The above data suggest that CCK can possibly activate the endocannabinoid system by which may reduce the detrimental effects of chronic stress on synaptic plasticity and memory formation within the hippocampus through yet poorly known regulatory mechanisms (62).

Furthermore, stress also affects synaptic plasticity via altering glutamate transmission. It has been indicated that LTP may be induced due to the activation of glutamate receptors (63), and N-methyl-D-aspartate (NMDA) receptor is crucially needed for LTP induction in the CA1 area (64). Furthermore, chronic stress leads to a considerable increase in the expression of AMPA and NMDA receptor subunits, presumably due to an overproduction of glucocorticoids (65, 66). Enhanced expression of these receptor subunits may raise vulnerability to glutamate neurotoxicity (67). Preclinical studies suggest that cholecystokinin protects neuronal cells against glutamate neurotoxicity via CCK-B receptors. This neuronal protection is probably through inhibition of NO-formation triggered by NMDA receptors (68). On the other hand, cholecystokinin crucially modulates glutamatergic synaptic transmission mediated by CCK-B receptors (49).

In paired-pulse experiments, we observed no significant effect of CCK-8S and stress on paired-pulse facilitation at inter-stimulus interval 50 ms in hippocampal CA1 sub-region (Figure. 6). Contrarily to our result, other data demonstrated altered paired-pulse responses following stress at CA1 area. These contradictory results could be dependent on the type (69) and duration (70) of stress.

Conclusion

Taken together, our findings demonstrated that chronic immobilization stress attenuates the LTP induction and maintenance in CA1 area, with a parallel destruction of spatial memory in the water maze task. A repeated treatment with CCK-8S along with induction of restraint stress improved both the LTP deficit and spatial memory impairment toward normal, indicating reversibility of stress-related changes in the hippocampus. The present data represent a compensatory role of cholecystokinin in stress. Hence, we suggest that the high concentration of CCK during exposure to stressful stimuli may inhibit the response to stress.

Acknowledgment

The present study was financially supported by Isfahan University of Medical Sciences, Isfahan, Iran. Grant number was 393635. The results described in this paper were part of student thesis.

References

1. Santos TB, Cespedes IC, Viana MB. Chronic corticosterone administration facilitates aversive memory retrieval and increases GR/NOS immunoreactivity. Behav Brain Res 2014; 267:46-54.
2. Radahmadi M, Alaei H, Sharifi MR, Hosseini N. Effects of different timing of stress on corticosterone, BDNF and memory in male rats. Physiol Behav 2015; 139:459-467.
3. Levine S. Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 2005; 30:939-946.
4. Vazquez E, Barranco A, Gallo M, Rueda R. Chronic psychological stress impairs immune system as well as object and spatial memory in a water-avoidance stress (WAS) mouse model. Brain Behav Immun 2010; 24:63.
5. Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002; 3:453-462.
6. McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci 1999; 22:105-122.
7. Diamond DM, Bennett MC, Fleshner M, Rose GM. Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. Hippocampus 1992; 2:421-430.
8. Frodl T, O'Keane V. How does the brain deal with cumulative stress? a review with focus on developmental stress, HPA axis function and hippocampal structure in humans. Neurobiol Dis 2013; 52:24-37.
9. Zhang Y, Liu W, Zhou Y, Ma C, Li S, Cong B. Endoplasmic reticulum stress is involved in restraint stress-induced hippocampal apoptosis and cognitive impairments in rats. Physiol Behav 2014; 131:41-48.
10. Kumar A. Long-term potentiation at CA3-CA1 hippocampal synapses with special emphasis on aging, disease, and stress. Front Aging Neurosci 2011; 3:7.
11. Kamal A, Ramakers G, Altinbilek B, Kas M. Social isolation stress reduces hippocampal long-term

potentiation: effect of animal strain and involvement of glucocorticoid receptors. Neuroscience 2014; 256:262-270.
12. Gerges NZ, Stringer JL, Alkadhi KA. Combination of hypothyroidism and stress abolishes early LTP in the CA1 but not dentate gyrus of hippocampus of adult rats. Brain Res 2001; 922:250-260.
13. Ivy A, Janeuek H. Assay of jorpes-mutt secretin and cholecystokinin. Acta Physiol Scand 1959; 45:220-230.
14. Wang H, Wong PT-H, Spiess J, Zhu YZ. Cholecystokinin-2 (CCK 2) receptor-mediated anxiety-like behaviors in rats. Neurosci Biobehav Rev 2005; 29:1361-1373.
15. Greenwood RS, Godar SE, Reaves TA, Hayward JN. Cholecystokinin in hippocampal pathways. J Comp Neurol 1981; 203:335-350.
16. Deng P-Y, Xiao Z, Jha A, Ramonet D, Matsui T, Leitges M, *et al.* Cholecystokinin facilitates glutamate release by increasing the number of readily releasable vesicles and releasing probability. J Neurosci 2010; 30:5136-5148.
17. Zanoveli JM, Netto CF, Guimarães FS, Zangrossi H. Systemic and intra-dorsal periaqueductal gray injections of cholecystokinin sulfated octapeptide (CCK-8S) induce a panic-like response in rats submitted to the elevated T-maze. Peptides 2004; 25:1935-1941.
18. Acosta GB. A possible interaction between CCKergic and GABAergic systems in the rat brain. Comp Biochem Physiol C Toxicol Pharmacol 2001; 128:11-17.
19. Mitchell VA, Jeong H-J, Drew GM, Vaughan CW. Cholecystokinin exerts an effect via the endocannabinoid system to inhibit GABAergic transmission in midbrain periaqueductal gray. Neuropsychopharmacology 2011; 36:1801-1810.
20. Bariohay B, Lebrun B, Moyse E, Jean A. Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. Endocrinology 2005; 146:5612-5620.
21. Reisi P, Ghaedamini AR, Golbidi M, Shabrang M, Arabpoor Z, Rashidi B. Effect of cholecystokinin on learning and memory, neuronal proliferation and apoptosis in the rat hippocampus. Adv Biomed Res 2015; 4:227.
22. Wen D, Zang G, Sun D, Yu F, Mei D, Ma C, *et al.* Cholecystokinin-octapeptide restored morphine-induced hippocampal long-term potentiation impairment in rats. Neurosci Lett 2014; 559:76-81.
23. Voits M, Hasenöhrl RU, Huston JP, Fink H. Repeated treatment with cholecystokinin octapeptide improves maze performance in aged Fischer 344 rats. Peptides 2001; 22:1325-1330.
24. Kim H, Whang W-W, Kim H-T, Pyun K-H, Cho S-Y, Hahm D-H, *et al.* Expression of neuropeptide Y and cholecystokinin in the rat brain by chronic mild stress. Brain Res 2003; 983:201-208.
25. Greisen MH, Bolwig TG, Wörtwein G. Cholecystokinin tetrapeptide effects on HPA axis function and elevated plus maze behaviour in maternally separated and handled rats. Behav Brain Res 2005; 161:204-212.
26. Azadbakht AA, Radahmadi M, Javanmard SH, Reisi P. The effects of doxepin on stress-induced learning, memory impairments, and TNF-á level in the rat hippocampus. Res Pharm Sci 2015; 10:460-465.
27. Sadeghi M, Radahmadi M, Reisi P. Effects of repeated treatment with cholecystokinin sulfated octapeptide on passive avoidance memory under chronic restraint stress in male rats. Adv Biomed Res 2015; 4:150.
28. Dolatabadi K, P Reisi L. Acute effect of cholecystokinin on short-term synaptic plasticity in the rat hippocampus. Res Pharm Sci 2014; 9:331-336.
29. Hamidi G, Arabpour Z, Shabrang M, Rashidi B, Alaei H, Sharifi MR, *et al.* Erythropoietin improves spatial learning and memory in streptozotocin model of dementia. Pathophysiology 2013; 20:153-158.
30. Eidelkhani N, Radahmadi M, Rafiee L, Gharzi M, Alaei H, Reisi P. Effects of doxepin on spatial memory, TNF-α and Bcl-2 family genes expression in rat hippocampus. Physiol Pharmacol 2015; 19:185-192.
31. Paxinos G, Watson, C. The Rat Brain in Stereotaxic Coordinates, 4th ed. Academic Press, San Diego; 2005.p. 61-68.
32. Dehghani F, Reisi P. Acute application of cholecystokinin and its effect on long-term potentiation induction at CA1 area of hippocampal formation in rat. Physiol Pharmacol 2015; 19:241-246.
33. Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab 2006; 291:965-973.
34. Ortiz JB, Mathewson CM, Hoffman AN, Hanavan PD, Terwilliger EF, Conrad CD. Hippocampal brain-derived neurotrophic factor mediates recovery from chronic stress-induced spatial reference memory deficits. Eur J Neurosci 2014; 40:3351-3362.
35. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. Endocr Rev 2000; 21:55-89.
36. Maghsoudi N, Ghasemi R, Ghaempanah Z, Ardekani AM, Nooshinfar E, Tahzibi A. Effect of Chronic Restraint Stress on HPA Axis Activity and Expression of BDNF and Trkb in the Hippocampus of Pregnant Rats: Possible Contribution in Depression during Pregnancy and Postpartum Period. Basic Clin Neurosci 2014;5:131-137.
37. McEwen BS. Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. Ann N Y Acad Sci 2010; 1204:38-59.
38. Numakawa T, Adachi N, Richards M, Chiba S, Kunugi H. Brain-derived neurotrophic factor and glucocorticoids: reciprocal influence on the central nervous system. Neuroscience 2013; 239:157-172.
39. Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, *et al.* Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 2005; 30:508-515.
40. Hu W, Zhang M, Czéh B, Flügge G, Zhang W. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacology 2010; 35:1693-1707.
41. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nature Rev Neurosci 2012; 13:22-37.
42. Korz V, Frey JU. Stress-related modulation of hippocampal long-term potentiation in rats: Involvement of adrenal steroid receptors. J Neurosci 2003; 23:7281-7287.

43. Karst H, Joëls M. Brief RU 38486 treatment normalizes the effects of chronic stress on calcium currents in rat hippocampal CA1 neurons. Neuropsychopharmacology 2007; 32:1830-1839.
44. Ivy AS, Rex CS, Chen Y, Dubé C, Maras PM, Grigoriadis DE, *et al.* Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. J Neurosci 2010; 30:13005-13015.
45. Sebret A, Léna I, Crété D, Matsui T, Roques BP, Daugé V. Rat hippocampal neurons are critically involved in physiological improvement of memory processes induced by cholecystokinin-B receptor stimulation. J Neurosci 1999; 19:7230-7237.
46. Matsushita H, Akiyoshi J, Kai K, Ishii N, Kodama K, Tsutsumi T, *et al.* Spatial memory impairment in OLETF rats without cholecystokinin–a receptor. Neuropeptides 2003; 37:271-276.
47. Yang S, Wen D, Dong M, Li D, Sun D, Ma C, *et al.* Effects of cholecystokinin-8 on morphine-induced spatial reference memory impairment in mice. Behav Brain Res 2013; 256:346-353.
48. Wei X, Zhang Z, Zhao L, Si J. CCK-8S inhibited the NMDA-activated current of cultured hippocampal neuron under normal and ethanol exposure conditions. Neurosci Lett 2009; 449:34-37.
49. Wei X-f, Zhang Y-h, Zhang L-l, Xu S-j, Chen X-w, Wang C, *et al.* CCK-8S increases the firing frequency of CCK-positive neurons and facilitates excitatory synaptic transmission in primary rat hippocampal neurons. Neurosci Lett 2013; 549:34-39.
50. Reich CG, Karson MA, Karnup SV, Jones LM, Alger BE. Regulation of IPSP theta rhythm by muscarinic receptors and endocannabinoids in hippocampus. J Neurophysiol 2005; 94:4290-4299.
51. Abelson JL, Liberzon I. Dose response of adrenocorticotropin and cortisol to the CCK-B agonist pentagastrin. Neuropsychopharmacology 1999; 21:485-494.
52. Katsuura G, Ibii N, Matsushita A. Activation of CCK-A receptors induces elevation of plasma corticosterone in rats. Peptides 1992; 13:203-205.
53. Malendowicz LK, Spinazzi R, Majchrzak M, Nowak M, Nussdorfer GG, Ziolkowska A, *et al.* Effects of prolonged cholecystokinin administration on rat pituitary-adrenocortical axis: role of the CCK receptor subtypes 1 and 2. Int J Mol Med 2003; 12:903-909.
54. Suri D, Vaidya V. Glucocorticoid regulation of brain-derived neurotrophic factor: relevance to hippocampal structural and functional plasticity. Neuroscience 2013; 239:196-213.
55. Zhang L-l, Wei X-f, Zhang Y-h, Xu S-j, Chen X-w, Wang C, *et al.* CCK-8S increased the filopodia and spines density in cultured hippocampal neurons of APP/PS1 and wild-type mice. Neurosci Lett 2013; 542:47-52.
56. Lisowski P, Juszczak GR, Goscik J, Wieczorek M, Zwierzchowski L, Swiergiel AH. Effect of chronic mild stress on hippocampal transcriptome in mice selected for high and low stress-induced analgesia and displaying different emotional behaviors. Eur Neuropsychopharmacol 2011; 21:45-62.
57. Hu W, Zhang M, Czéh B, Zhang W, Flügge G. Chronic restraint stress impairs endocannabinoid mediated suppression of GABAergic signaling in the hippocampus of adult male rats. Brain Res bulletin 2011; 85:374-379.
58. Abush H, Akirav I. Cannabinoids ameliorate impairments induced by chronic stress to synaptic plasticity and short-term memory. Neuropsychopharmacology 2013; 38:1521-1534.
59. Gray JM, Vecchiarelli HA, Hill MN. Endocannabinoid Signaling and Synaptic Plasticity During Stress. Synaptic Stress and Pathogenesis of Neuropsychiatric Disorders: Springer; 2014.p.99-124.
60. Lee SH, Soltesz I. Requirement for CB1 but not GABAB receptors in the cholecystokinin mediated inhibition of GABA release from cholecystokinin expressing basket cells. J Physiol 2011; 589:891-902.
61. Zhu F, Wang X-q, Chen Y-n, Yang N, Lang S-y, Zuo P-p, *et al.* Changes and overlapping distribution in the expression of CB1/OX1-GPCRs in rat hippocampus by kainic acid-induced status epilepticus. Brain Res 2015; 1597:14-27.
62. Segev A, Rubin AS, Abush H, Richter-Levin G, Akirav I. Cannabinoid receptor activation prevents the effects of chronic mild stress on emotional learning and LTP in a rat model of depression. Neuropsychopharmacology 2014; 39:919-933.
63. Grover LM. Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. J Neurophysiol 1998; 79:1167-1182.
64. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004; 44:5-21.
65. Reagan LP, McEwen BS. Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. J Chem Neuroanat 1997; 13:149-167.
66. Sterlemann V, Rammes G, Wolf M, Liebl C, Ganea K, Müller MB, *et al.* Chronic social stress during adolescence induces cognitive impairment in aged mice. Hippocampus 2010; 20:540-549.
67. Nacher J, Pham K, Gil-Fernandez V, McEwen B. Chronic restraint stress and chronic corticosterone treatment modulate differentially the expression of molecules related to structural plasticity in the adult rat piriform cortex. Neuroscience 2004; 126:503-509.
68. Tamura Y, Sato Y, Akaike A, Shiomi H. Mechanisms of cholecystokinin-induced protection of cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. Brain Res 1992; 592:317-325.
69. Gao Y, Han H, Xu R, Cao J, Luo J, Xu L. Effects of prolonged exposure to context following contextual fear conditioning on synaptic properties in rat hippocampal slices. Neurosci Res 2008; 61:385-389.
70. Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. Proc Natl Acad Sci U S A 2005; 102:19204-19207.