Original Article

Effects of repeated treatment with cholecystokinin sulfated octapeptide on passive avoidance memory under chronic restraint stress in male rats

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Abstract

Background: Cholecystokinin (CCK), a peptide hormone found in the gut is the most abundant peptide neurotransmitter in the brain as well, and its effects on learning, memory, and anxiety have been shown. However, it is not clear whether this substance acts as a mediator for anxiety and stress induction or inhibits them. Hence, the purpose of this study was to evaluate the effects of CCK on memory function under stress conditions.

Materials and Methods: Male Wistar rats were divided into four groups: The control, the control-CCK, the stress, and stress-CCK. To induce stress, the rats were placed within adjustable restraint chambers for 6 h daily, for 24 days. CCK-8S (cholecystokinin sulfated octapeptide was injected before induction of stress (1.6 μg/kg, intraperitoneal) for 24 days. Passive avoidance learning test was used for evaluation of learning and memory. Rats received foot electrical shock before stress induction and CCK injection and step through latencies were evaluated 1-day after the last session of stress and treatments.

Results: Stress impaired memory significantly ($P < 0.05$). Although CCK *per se* decreased memory ($P < 0.05$), it prevented the memory impairments in the stress group as there was no significant difference between the control and stress-CCK groups.

Conclusion: Stress has a profound effect on cognition and CCK probably acts as a mediator for its action. Our results showed that a high concentration of CCK during stress may be helpful in alleviating the effects of stress on the brain.

Key Words: Cholecystokinin, memory, rat, restraint stress

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INTRODUCTION

Stress is an internal response applied to internal or external stimuli from the environment, which changes the organism's homeostasis.[1] It is well known that stress activates the release of stress-related hormones like corticosteroids from the adrenal cortex.[2] The hypothalamus-pituitary-adrenal (HPA) axis has been shown to regulate the cognitive processes, memory, and stress related behaviors in stress conditions.[3] Moreover, hippocampus is known as one of the most important areas involved in memory processing in the brain.[4] Previous studies have demonstrated direct and indirect anatomical connections between the HPA axis and hippocampus. Some previous studies have

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demonstrated neurogenesis inhibition, neuronal death in the hippocampus, and memory deficit in passive avoidance test following the chronic stress.[5,6]

On the other hand, cholecystokinin (CCK) is a gastrointestinal hormone and one of the most abundant neuropeptides within the central nervous system (CNS).[7,8] The CCK is shown to be able to pass across the blood-brain barrier.[9] A couple of receptors named CCK-A and CCK-B are known in CNS suggesting that CCK can play widespread central roles in mammalians.[10,11] The CCK is extensively expressed in the hippocampus, where it crucially impacts various physiological processes associated with memory function.[12] The CCK sulfated octapeptide (CCK-8S) is one of the most abundant molecular isoforms of CCK in the CNS[13] which remarkably has modulatory effects on the release of neurotransmitters such as gamma-amino-butyric-acid (GABA) and serotonin.[14,15] It significantly mediates the endocannabinoid (central neuromodulatory lipids) system activities,[16] as well as the expression of neurotrophic factors like brain-derived neurotrophic factor (BDNF).[8] In the stress conditions, the changes of CCK levels in hypothalamus and hippocampus probably indicate the regulatory role of this neuropeptide on HPA function, glucocorticoid levels, learning, and memory processing.[17,18]

With regard to the role of CCK in cognitive processes and the changes of CCK levels in the brain, the aim of the present study was to investigate the effects of a repeated CCK-8S treatment (1.6 μg/kg, 24 days) associated with chronic restraint stress (6 h/day for 24 days) on memory function by passive avoidance test in rats.

MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats provided from Pastor Institute, Tehran, Iran, and weighing 250–300 g at the beginning of the experiment. Rats were housed in groups of 5/cage and maintained under light control (12 h light/dark; lights on 07:00–19:00). Room temperature was qualified (22°C ± 2°C). Food and water were available *ad libitum*, except during the stress situations. All experiments were executed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996 and the Ethic Committee for Animal Experiments in the Isfahan University accepted the study plan.

The animals were randomly assigned into four groups (10 in each):

- Control group (CO): Receiving saline for 24 days without stress
- Control-CCK (CO-CCK): Receiving CCK-8S for 24 days without stress
- Stress (ST): Receiving saline and stress for 24 days
- Stress-CCK (ST-CCK): Receiving stress and CCK-8S for 24 days.

In this study, the groups receiving CCK were injected by CCK-8S (1.6 μg/kg, intraperitoneal, dissolved in saline; Sigma-Aldrich),[19,20] before stress induction, and the CO and ST groups received an equivalent volume of saline as placebo.

Stress paradigms

To induce chronic stress model, rats were placed and tightly fitted in plexiglas cylindrical restrainers for 6 h/day from 8:00 to 14:00 (the time known as their active period)[6] for 24 days according to a confirmed protocol. During the restraint, animals had no access to food and water.

Behavioral apparatus and method

Learning and memory function were assessed by a step-through passive avoidance test[21] which is a hippocampus-dependent task.[6] Since the hippocampus-dependent learning and memory tasks have been shown to be sensitive to stress that induced HPA activity,[22] the passive avoidance apparatus was chosen and divided into two parts (light and dark). Training was performed 1-day prior to the start of restraint stress and treatment in which rats were individually placed in the light compartment for 30 s. After that, the door between two compartments was raised. When the rat entered the dark compartment, the door was closed, and a single foot electric shock (50 Hz, 0.2 mA, 3 s) was delivered through the grid floor by a stimulator.[6] The initial latency of entrance into the dark compartment was recorded. During the probe trial for evaluation of memory, 1-day after the last session of stress and treatment rats were located in the apparatus again with no shock.[23] The delay time before entering to the dark compartment was recorded as latency (up to a maximum of 600 s). When an animal avoided from entering the dark compartment within 600 s, the trial was ended. The passive avoidance defined the ability of the animal to recall the delivered foot shock.

Measurement of adrenal weight

Adrenal weights were evaluated for assessing the HPA activity.[24] Hence, animals were anesthetized at the end of the experiments and were decapitated at 12:00–13:00 on day 25. Then, the adrenal glands were dissected.

Data analysis

All data are reported as mean ± standard error of the mean. Data were analyzed using one-way

ANOVA followed by Tukey's *post-hoc* test for multiple comparisons. $P < 0.05$ was considered as significant.

RESULTS

Entrance latency to the dark compartment

In the acquisition trial, the mean initial latencies were same in all groups [Figure 1], whereas step through latency showed that stress significantly impaired memory with respect to the control group ($P < 0.05$). The CCK administration significantly decreased memory ($P < 0.05$). In stress-CCK group, latency had no significant enhancement compared to CO group.

The retention latency had significant ($P < 0.05$) enhancement in ST-CCK group when compared to ST group. Therefore, the CCK administration prevented the memory impairments in the ST group [Figure 2].

Adrenal glands weight

The adrenal glands weight was significantly ($P < 0.001$) increased in the ST group compared to the CO group. CCK had no significant effects on the adrenal weight in CO-CCK group. However, it decreased the adrenal weight in the ST-CCK group compared to ST group ($P < 0.001$) [Figure 3].

DISCUSSION

Our results indicated that the chronic restraint stress-induced memory deficit in ST group [Figure 2]. In line with our results, other studies have demonstrated the deleterious effects of chronic restraint stress on memory function.[6,25] Stress can impair hippocampal-dependent memory and passive avoidance task. However, cumulative data demonstrated that the destructive effects of stress on memory probably depend on the reduction of expression, synthesis and levels of BDNF within the hippocampus.[5,6]

According to our results, long-term administration of CCK-8S had beneficial effects on the memory improvement in stressed rats [Figure 2]. The chronic local treatment by CCK-8S has been shown to promote hippocampal dendritic growth and neuronal network interconnections like stimulatory synapses. Therefore, it leads to protect the hippocampus against atrophy and damage.[26] Furthermore, the CCK develops the neuroprotection through enhancing the expression and function of BDNF.[27] With regard to the prominent role of the hippocampus in inhibition of HPA axis,[28] it could be suggested that the CCK may indirectly modify the HPA hyperactivity following a protective effect on hippocampus in stress conditions. Hence, that HPA inhibition of CCK leads to an enhanced memory function in stressed rats. In addition, it was

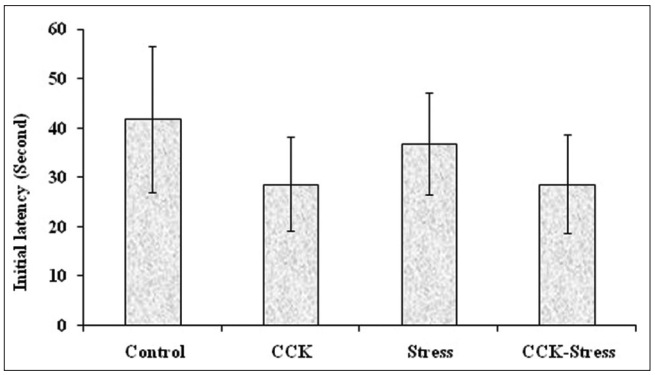

Figure 1: Initial latency in passive avoidance test before induction of electrical shock and treatments ($n = 10$). Data are expressed as mean ± standard error of the mean

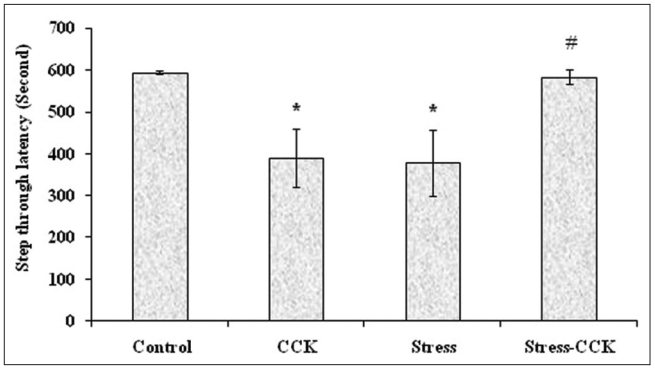

Figure 2: Effects of cholecystokinin and stress on step through latency in passive avoidance test, after induction of electrical shock in all groups ($n = 10$). Data are expressed as mean ± standard error of the mean $^{*}P < 0.05$ with respect to the control group and $^{\#}P < 0.05$ with respect to the stress group

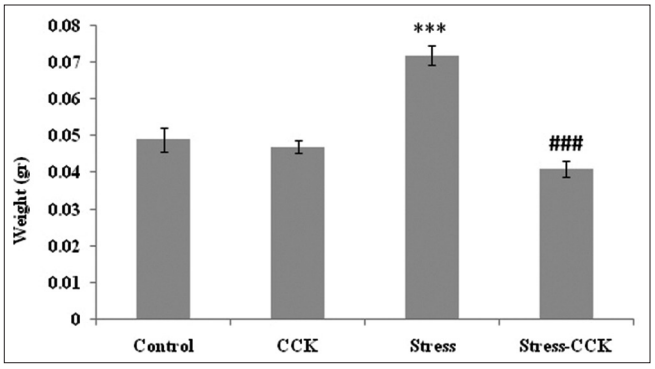

Figure 3: Effects of cholecystokinin octapeptide sulfated and stress on adrenal glands weight. Data are expressed as mean ± standard error of the mean ($n = 10$). $^{***}P < 0.001$ with respect to the control group, $^{\#\#\#}P < 0.001$ with respect to the stress group

reported that the CCK gene expression is increased in the hippocampus following chronic stress.[29] Increased CCK possibly plays a pivotal role in compensating the HPA activation impacts on memory function. On the other hand, it is documented that both the CCK-A and CCK-B receptors activate the HPA axis,[10,11] and chronic CCK administration lowers

the expression of these receptors in the pituitary.[30] Therefore, the CCK can also prevent HPA response to stress by diminishing the CCK-A and CCK-B activity. Moreover, contrarily to our results, some studies have indicated that systemic administration of CCK stimulates HPA activity.[11,31] Our previous study showed that learning and memory functions did not significantly improve after chronic CCK treatment.[32] A possible explanation for these contradictory results might be related to the duration and type of stress,[6] the differences in dose of CCK,[31] the duration and type of treatment protocol,[32] the administration before or after the shock[33] or other variables that have not yet been defined.

It seems that repeated CCK administration affects behavioral responses and probably modulates the activity of the HPA axis in stressful conditions. The CCK probably can be used as a treatment for memory improvement in stress experiments, although it needs more research. Hence, we considered these findings as a dilatory effect of long-term CCK administration in chronic stress.

Other present results indicated that prolonged CCK-8S administration had harmful effects on memory processing in the absence of stress [Figure 2]. Despite the stimulatory role of acute CCK treatments, chronic CCK injection showed nearly ineffective in HPA activation. Presumably, other mechanisms are involved in memory destruction here.

There are divergent views about the CCK effects on memory function; some studies referred to recognition enhancement,[34] whereas the others indicated memory destructive impressions.[20] It is recognized that CCK-8S can depolarize hippocampal inhibitory interneurons were called CCK-positive, which express CCK, GABA, and endocannabinoid (CB1) receptor.[35,36] CCK augments the activity of CB1 receptors by exciting CCK-positive interneurons.[37] Therefore, CCK inhibits the release of presynaptic neurotransmitters.[38] Furthermore, simultaneous activation of CCKergic and serotonergic systems is involved in anxiety formation.[39] The items mentioned above represent the eventual regulatory role of CCK in anxiety-related behaviors or memory dysfunctions. However, the more accurate mechanisms remain to be understood in line with this result.

On the basis of present results, adrenal glands weight had a significant enhancement only in ST group compared with CO group. As largely known, the glucocorticoid productions increase via HPA activation in chronic restraint stress.[40] In addition, the CCK-8S separately had no effect on adrenal glands weight, whereas adrenal weight in ST-CCK group had significant decreases compared to ST group [Figure 3]. Consistent with the results of the present investigation, previous data suggested that chronic stress acts through HPA axis and induces hypertrophy and hyperplasia in the adrenal fasciculate zone, and increases adrenal glands weight.[24] However, chronic CCK administration has been shown to decrease the volume of adrenocortical zones.[30] It seems that repeated injection of CCK significantly prevents the HPA response to stress.

CONCLUSION

Taken together, our findings demonstrated that chronic restraint stress impairs memory, whereas the repeated systemic administration of CCK-8S compensates this memory impairment possibly by exerting an inhibitory effect on HPA axis in stressed rats. Hence, we suggest that the CCK probably acts as a central modulatory component in stress conditions, and prolonged treatment by low-dose CCK may alleviate the memory damage in chronic stress.

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