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Original Article

Acute effect of cholecystokinin on short-term synaptic plasticity in the rat hippocampus

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Abstract

Cholecystokinin (CCK), a peptide hormone found in the gut, is the most abundant peptide neurotransmitters in the brain, and its acute effects on the brain activity have been shown. In this study we aimed to evaluate the acute effects of CCK on short-term synaptic plasticity in the dentate gyrus (DG) of the rat hippocampus. Via stereotaxic surgery, the stimulating and the recording electrodes were placed in the perforant pathway and dentate gyrus, respectively and 30 min after intraperitoneal (i.p.) injection of CCK octapeptide sulfated (CCK-8S, 1.6 μg/kg), evoked responses were recorded after delivering of paired-pulse stimulations at 10 to 500 ms inter-stimulus intervals. With respect to the control group that received saline instead of CCK, in baseline responses, slope of field excitatory postsynaptic potential (fEPSP) 5 min and 10 min after injection of CCK-8S ($p<0.05$) and population spikes (PS)-amplitudes 5 min after injection of CCK-8S ($p<0.05$) were significantly increased. In paired pulse responses, PS amplitudes were increased in the CCK group, but these enhancements only were significant at inter-stimulus interval 40 ms ($p<0.05$). However fEPSP slopes were decreased at inter-stimulus intervals 70 ms ($p<0.05$), 120 ms ($p<0.01$), 150 ms ($p<0.001$) and 300 ms ($p<0.001$). The results showed that CCK-8S has a transient excitatory effects on baseline responses, but it inhibits paired pulse indices in acute. Therefore, in a short period of time, effect of CCK on the function of synapses is time dependent, and it has stimulatory or inhibitory effects at different time periods.

Keywords: Cholecystokinin; Paired pulse facilitation; Paired pulse depression; Hippocampus; Dentate gyrus; Rat

INTRODUCTION

Cholecystokinin (CCK), a known digestive system hormone, is the most abundant peptide neurotransmitter in the brain and has important roles in various physiological functions of the brain, including memory (1). CCK has different molecular forms; however, cholecystokinin sulfated octapeptide (CCK-8S) is the most common form in the central nervous system and probably is the only form that has physiological effects as a neurotransmitter (2). CCK and its receptors have been found in the necessary areas for memory with high concentrations, including the hippocampus (3). CCK has two types of receptors; CCKA and CCKB (4). Both receptors are present in the hippocampus with a high concentration and have same affinity to CCK-8S (3). In addition to being a neurotransmitter in the brain, CCK has modulatory effects on other neurotransmitters, such as GABA, glutamate, dopamine and acetylcholine (2,5-7). Previous studies have shown that intraperitoneal (i.p.) application of a single dose of CCK-8S, leads to acute effects on the brain activity after 30 min, including changes in the levels of neurotransmitters in different brain regions such as the hippocampus (2,5). Previous studies have shown that acute i.p. injection of CCK-8S, 10 min before each test in Morris water maze, improved learning in rats. These effects are likely resulted from the temporary chemical changes, such as changes in the release or uptake of neurotransmitters (8).

Synaptic plasticity is one of the functional aspects of hippocampus that is involved in learning and memory (9,10). At the synapse

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level, the phenomenon of paired-pulse facilitation (PPF) and paired-pulse depression (PPD) have been identified as short-term forms of synaptic plasticity that generally used as a model for assessing hippocampal glutamatergic presynaptic neurons and GABAergic inhibitory recurrent neurons (9). Therefore, the aim of this study was to evaluate the acute effects of CCK on short-term synaptic plasticity in the rat hippocampus.

MATERIALS AND METHODS

Animals and the treatment

Male Wistar rats (280 ± 20 g) were housed four per cage and maintained on a 12 h light–dark cycle in an air-conditioned constant temperature (23 ± 1 ºC) room, with food and water made available *ad libitum*. 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 1996. The animals were divided into two groups: controls (n=6) and CCKs (n=6). The CCK group received CCK-8S (1.6 μg/kg in saline, I.P.; Sigma-Aldrich) (8) and the control group received an equivalent volume of saline as placebo, during the study.

Surgical procedure

The animals were anesthetized with urethane (1.6 g/kg, i.p.) and their heads were fixed in a stereotaxic head-holder. A heating pad was used to maintain body temperature at 36.5 ± 0.5 °C. 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 perforant pathway (AP = −7.8 mm; ML = 3.5 mm; DV = 3.2-3.5 mm), and a stainless steel recording electrode was lowered into the DG (AP = −3.8 mm; ML = 2 mm; DV = 3.2-3.5 mm) until the maximal response was observed (11). In order to minimize trauma to brain tissue, the electrodes were lowered very slowly (0.2 mm/min). Implantation of electrodes in the correct position was determined by physiological and stereotaxic indicators. Extracellular evoked responses were obtained from the dentate granule cell population following stimulation of the perforant pathway. Extracellular field potentials were amplified (×1000) and filtered (1 Hz to 3 KHz band pass). Signals were passed through an analogue to digital interface (Data Acquisition ScienceBeam-D3111) to a computer, and data were analyzed using Potentalize software (ScienceBeam).

Input/output functions

Stimulus–response or input/output (I/O) functions were acquired by systematic variation of the stimulus current (100–1000 μA) in order to evaluate synaptic potency before induction of paired pulse. Stimulus pulses were delivered at 0.1 Hz and five responses at each current level were averaged. As shown in Fig. 1, the population spike (PS) amplitude was measured using following equation:

PS amplitude = [(VB-VC)+(VD-VC)]/2

where, VB is the voltage of the peak of the first positive wave, VC is the peak of the first negative deflection, and VD is the voltage of the peak of the second positive wave.

The field excitatory postsynaptic potential (fEPSP) slope was measured as the slope between the baseline and the peak of the first positive wave (AB slope) (Fig. 1).

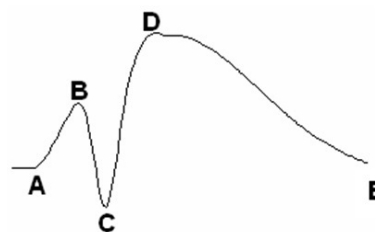

Fig. 1. Schematic diagram of population spike and field excitatory postsynaptic potential analysis. The population spike parameters analyzed as: [the difference in voltage between the peak of the first positive wave and the peak of the first negative deflection (VB−VC) + the difference in voltage between the peak of the second positive wave and the peak of the first negative deflection (VD−VC)]/2 or [(VB-VC) + (VD-VC)]/2, and the field excitatory postsynaptic potential slope was measured as the slope between the baseline and the peak of the first positive wave (AB slope).

Electrophysiological recordings

Stimulation intensity was adjusted to evoke about 40% of the maximal response of the PS and fEPSP. PS and fEPSPs were evoked in the dentate gyrus (DG) region using 0.1 Hz stimulation. Baseline recordings were taken at least 30 min prior to each experiment and after ensuring a steady state of baseline response, the rats received the treatment and after that, baseline was recorded again for 30 min and then, paired pulse facilitation/depression was measured by delivering five consecutive evoked responses of paired pulses at 10, 20, 30, 40, 50, 60, 70, 100, 120, 150, 300 and 500 ms inter-stimulus intervals to the perforant pathway at a frequency of 0.1 Hz (10 s interval). The paired pulse index (PPI) defined as PS amplitude ratio [(second PS amplitude minus first PS amplitude)/first PS amplitude at percent; (PS2-PS1)/PS1%]and the fEPSP slope ratio [(second fEPSP slope minus first fEPSP slope)/first fEPSP slope at percent; (fEPSP2-fEPSP1)/fEPSP1%] were measured at different inter-stimulus intervals.

For histological verifications at the end of each experiment, rats were perfused transcardially with a 10% formalin solution, and the brain was 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 the electrodes tracts. The section were examined under a microscope and compared to the rat brain atlas (11).

Statistical analysis

Results are given as mean ± S.E.M. Data were analyzed statistically using unpaired t-test. Probabilities less than 0.05 were considered significantly different.

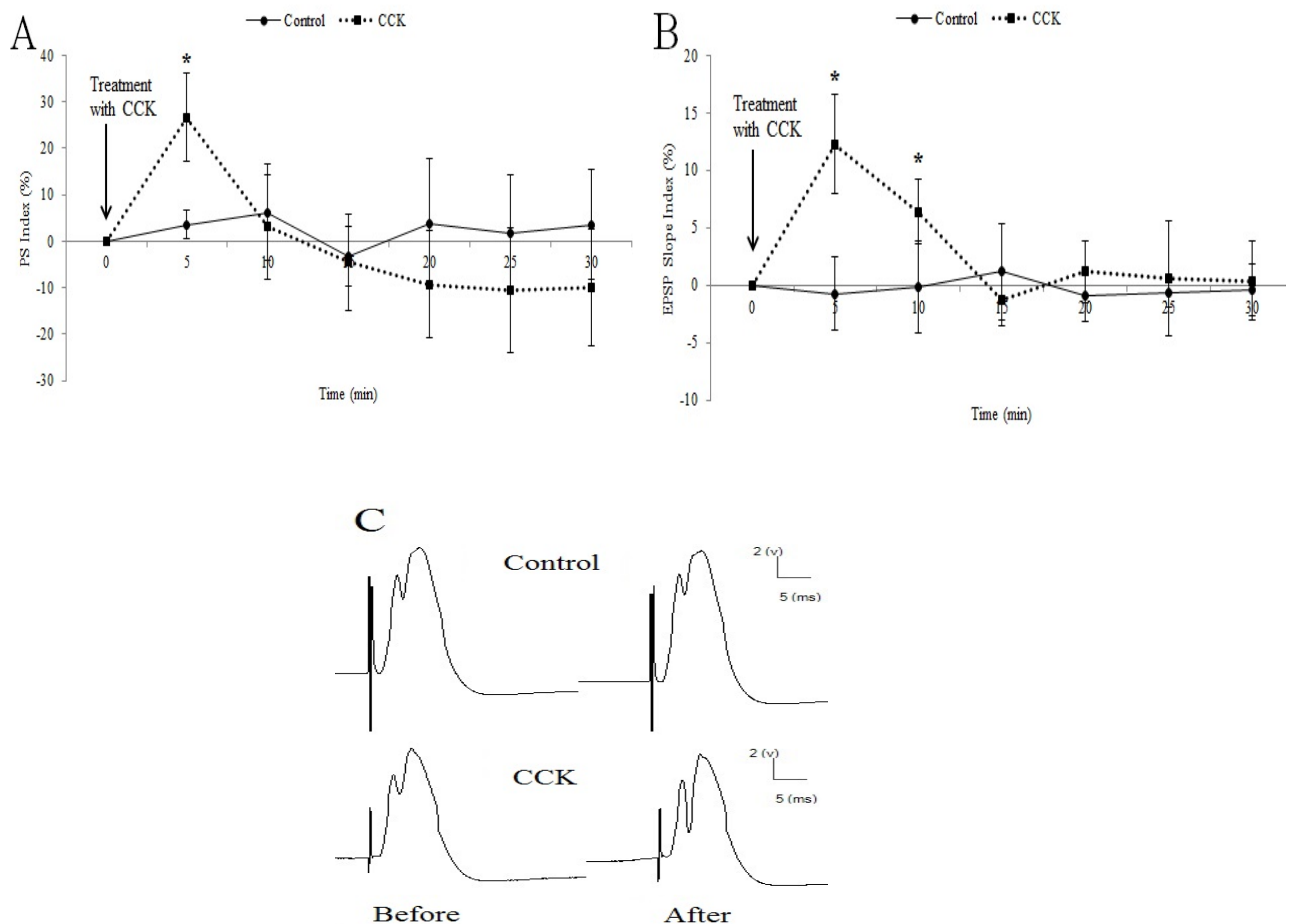

Fig. 2. The effect of cholecystokinin sulfated octapeptide on field excitatory postsynaptic potential in dentate gyrus of the hippocampus at A: the population spike amplitude, and B: field excitatory postsynaptic potential slope. C: single traces recorded from responses before and 5 min after the treatment. (*$p<0.05$ with respect to field excitatory postsynaptic potential before the treatment in cholecystokinin group. n=6 for each experimental group).

RESULTS

In baseline responses as illustrated in Fig. 2, PS amplitude was significantly increased 5 min after injection of CCK-8S ($p<0.05$; Figs. 2A, 2C), but after that it backed to the initial level, so that there were no significant differences with respect to before injection during 10-30 min. In the control group injection of saline had no effects on PS amplitude (Figs. 2A, 2C).

Also, slope of fEPSP was increased 5 min and 10 min after injection of CCK-8S ($p<0.05$; Figs. 2B, 2C), however it backed to the initial level in following. Same to PS amplitude, fEPSP slope was unchanged in the control group after injection of saline (Figs. 2B, 2C).

As illustrated in Fig. 3, the effects of CCK-8S on paired pulse indices in DG were determined. Statistical analysis revealed that paired pulse indices were increased, as measured by the population spike (PS) ratio, at inter-stimulus intervals 40-150 ms in the CCK group compared to the control group (Fig. 3A). However, these increments only were significant at inter-stimulus interval 40 ms ($p<0.05$). CCK reduced paired pulse facilitation, as measured by the fEPSP slope ratio, at inter-stimulus intervals 60-500 ms in the CCK group compared to the control group (Fig. 3B). These reduction were significant at inter-stimulus intervals 70 ms ($p<0.05$), 120 ms ($p<0.01$), 150 ms ($p<0.001$) and 300 ms ($p<0.001$). (Fig. 3B).

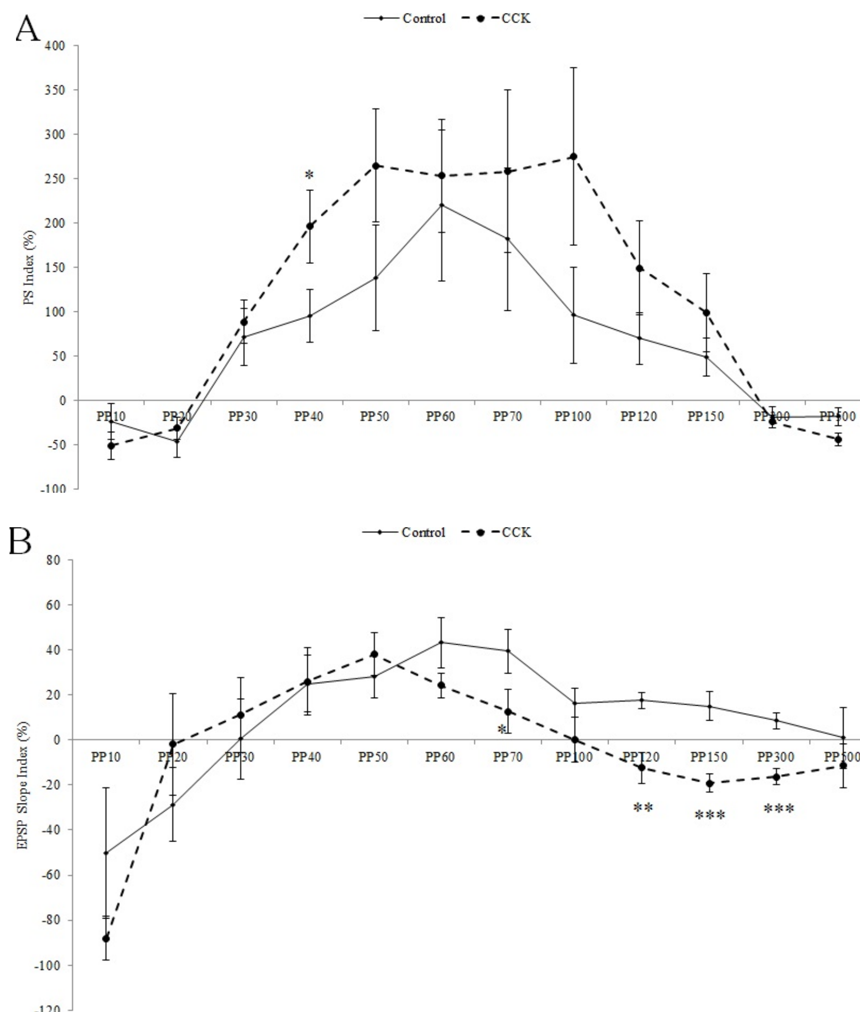

Fig. 3. The effect of cholecystokinin sulfated octapeptide on paired-pulse facilitation and depression in dentate gyrus of the hippocampus at A: the population spike amplitude ratio, (percentage of mean [(PS2-PS1)/PS1] ± SEM), and B: field excitatory postsynaptic potential slope ratio (percentage of mean [(fEPSP2- fEPSP1)/fEPSP1] ± SEM) (*$p<0.05$ with respect to the control group. n=6 for each experimental group). PS; Population spike. fEPSP; Field excitatory postsynaptic potential.

DISCUSSION

At perforant path-DG granule cell synapses, the fEPSP slope is a criterion of synaptic strength. When the intensity of the stimulation reaches the threshold for action potential of granule cell, fEPSP is accompanied by a PS and the amplitude of the PS reflects the number and synchrony of discharging granule cells (12). Our results showed that treatment with a single dose of CCK-8S (1.6 µg/kg) produced a significant increase in fEPSP slope and PS amplitude after a short time of treatment.

The maximal increases were observed at 5 min post-injection and returned to the baseline by 10-15 min. The changes are indicative of an increase in excitability; because for a given synaptic current, a larger voltage displacement is achieved and a larger number of granule cells were brought to firing threshold following CCK-8S injection.

CCK specified in the systemic application can rapidly cross the blood-brain barrier and appears in different brain regions, including the hippocampus (5). CCK receptors are present in the hippocampus and these receptors are expressed on neurons (13). In this study, it was determined immediately after i.p. injection of CCK, excitatory state increases at the perforant path-DG granule cell synapses that could result from decreased inhibition or increased excitation situations.

These results are consistent with previous studies that showed CCK-8S can increase the release of glutamate in different regions of the hippocampus including DG. This increase is probably via B-type receptors of CCK and inhibition of 4-AP-sensitive K_channel (13). It has been demonstrated that CCK increases the excitabilities of DG granule cells (14) and other regions of the hippocampus (15,16) and modulates GABAergic transmission in the hippocampus (17,18).

In a short period of time, CCK known to cause a transient increase in GABA release that followed by a steady decline (13). When both GABAergic and glutamatergic systems are functioning, the transient increase in GABA release can't decrease excitability of granule cells significantly, because increased levels of glutamate abate action of GABA.

As evidenced by paired pulse tests (Fig. 3), 30 min after the treatment, CCK-8S at short inter-stimulus intervals had no effects on paired pulse inhibition, but at longer intervals (paired pulse facilitation) it produced a significant decrease in fEPSP slope and a non-significant increase in PS amplitude in most inter-stimulus intervals.

One mechanism whereby CCK-8S may produce a smaller voltage displacement to the second stimulus pulse (decreased fEPSP slope), but an increased likelihood of cell discharge (increase PS), is through the induction of a relative state of depolarization at the dendrites. Overall, the results showed, at the inter-stimulus intervals that there is a significant decrease in the fEPSP slope; PS was unchanged, indicating an increase in tonic inhibition.

These changes can be caused by an increased release of GABA or glutamate loss. Consistent with these results, a study that measured contents of amino acids in the different brain regions 30 min after injection of CCK, have shown CCK reduced glutamate and increases GABA in the hippocampus (5).

In view of the fact that CCK receptors are widely exist in the hippocampus and DG (19), therefore direct effects of CCK that is not mediated by other neurotransmitters, can't be ignored.

It has been demonstrated that CCK-8S alters the membrane electrophysiological characteristics and synaptic activity of hippocampal neurons. However, a better understanding of the cellular mechanisms of these functions requires further studies (20).

CONCLUSION

These results indicate acute effects of CCK on synaptic plasticity in the hippocampus. These effects are probably due to chemical changes that can be caused by direct action of CCK or modulation of other neurotransmitters such as glutamate and GABA. Notably, in a short period of time, an effect of CCK on the function of synapses is time dependent, and it

has stimulatory or inhibitory effects at different time periods.

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