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Original Article

Acute application of cholecystokinin and its effect on long-term potentiation induction at CA1 area of hippocampal formation in rat

Farzane Dehghani[1,2], Parham Reisi[1,2,3]*

1. Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
3. Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Abstract

Introduction: It has been demonstrated that cholecystokinin sulfated octapeptide (CCK-8s) can affect synaptic transmission in the hippocampus. Because one of the major experimental models to understand the events happening in synaptic plasticity is To Study the long-term potentiation (LTP), we decided to investigate the effect of concomitant administration of CCK-8s and tetanic stimulation of Schaffer collateral path-CA1 synapses on LTP induction and maintenance.

Materials and Methods: Experimental groups were control, CCK-5min and CCK-30min. CCK-8s was injected 5 or 30 min (1.6 µg/kg; i.p.) prior to induction of LTP. The stimulating and the recording electrodes were placed in the Schaffer collateral pathway and hippocampal CA1, respectively. LTP was induced by 100 Hz tetanization and field excitatory postsynaptic potentials (fEPSP) slope, area and amplitude were measured and compared during 30 minutes Interval before, and 90 minutes Interval after LTP induction in each group.

Results: The results showed that maintenance of the induced LTP was significantly improved in the CCK-30min group comparing to the control group. This improvement was particularly visible in the fEPSP slope ($p<0.001$) and the fEPSP area ($p<0.001$). Seventy minutes after the LTP induction, fEPSP was similar in both the CCK-5min and the CCK-30min groups and there was Also a significant difference between the treated groups comparing to the control group ($p<0.05$).

Conclusion: These results indicated that LTP induction and maintenance is carried out effectively, at higher levels of CCK in the brain. The data suggest that CCK-8s has pronounced effects on synaptic plasticity in the hippocampus and the consequent cognitive functions.

Keywords:
Cholecystokinin sulfated octapeptide;
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Hippocampus;
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***Correspondence to:**
P. Reisi

Tel: +98 313 7929133
Fax: +98 313 6688597

Email:
p_reisi@med.mui.ac.ir

Introduction

Cholecystokinin (CCK) , in Addition to its hormonal function in gastrointestinal system, is one of the most abundant neuropeptide in the brain (Whissell et al., 2015). CCK interacts with 2 types of receptors: CCK-A and CCK-B receptors. CCK-A is a peripheral receptor, a few of which are present in the brain, whereas CCK-B is considered to be the most common form in the brain (Deng et al., 2010). CCK

and its receptors are present in high concentration in the hippocampus including CA1 area (Kohler and Chan-Palay, 1988; Rezayat et al., 2005), and its postulated role in learning and memory, has been demonstrated (Sadeghi et al., 2015). Studies have shown that both systemic administration (Baptista et al., 2007; Dolatabadi and Reisi, 2014) and central injection of CCK (Altar and Boyar, 1989; Blandizzi et al., 1995) affected different brain functions. In the central nervous system (CNS), there are several molecular forms of CCK, however, cholecystokinin octapeptide sulfated (CCK-8S) is the most prominent form in the brain and is probably the only form that acts as a neurotransmitter (Miller et al., 1997).

Studies have shown contradictory effects of the CCK in CA1 area of hippocampus , some of them have the excitatory effects of CCK (Miller et al., 1997), while others have been observed to exert inhibitory effects (MacVicar et al., 1987). It has been shown that CCK-8s via increasing glutamate release induces a marked excitatory response in the CA1 (Bohme et al., 1988). Other studies have shown that CCK has a dual effect on GABA release, an initial transient increase followed by a persistent reduction (Deng and Lei, 2006).

Therefore, considering the role of hippocampus in the cognitive process and the effects of CCK on glutamatergic and GABAergic neurons in CA1, we aimed to investigate the effect of concomitant administration of CCK-8s and tetanic stimulation of Schaffer collateral path-CA1 synapses on long-term potentiation (LTP), a major experimental model for studying the synaptic plasticity, in the hippocampus.

Materials and methods

Experiments were performed on 18 male Wistar rats weighing 180 ± 20 g. The animals were housed four per cages in a 12 h light/dark cycle with food and water available ad libitum, under an air-conditioned constant temperature (23 ± 1 ºC). The experimental procedures used in the present study were approved by the Institution for Animal Care and Use of the Isfahan University of Medical Sciences which are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Experiments carried out in 3 groups of animal (n=6 in each group), the control, the CCK 5min and CCK 30min. The rats in the CCK groups received acute administration of 1.6 µg/kg CCK-8S in saline 5 or 30 minutes prior to LTP induction (I.P.; Sigma-Aldrich) (Voits et al., 2001) and the control group received an equivalent volume of saline.

Rats were anesthetized with urethane (1.8 g/kg, i.p.) and their heads 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 drilled as 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, 0.125mmdiameter, Advent, UK) was placed in the Schaffer collateral pathway (AP=−4.2 mm; ML=3.8 mm; 2.3–2.7 mm ventral to dura), and a stainless steel recording electrode lowered into in the CA1 stratum radiatum (AP=−3.4 mm; ML=2.5 mm; 1.8–2.2 mm ventral to dura) (Paxinos and Watson, 2004). In order to minimize trauma to brain tissue, the electrodes were lowered very slowly (2 mm/min). The Correct location of the electrodes was determined according to the Physiological and stereotaxic standards.

Extracellularly- evoked responses were obtained following stimulation of the Schaffer collateral pathway. Extracellular field potentials were amplified (×1000) and filtered (1Hz to 3 KHz band pass). Signals were passed through an analogue to digital interface (ScienceBeam-D3111, Iran) to a computer and data analyzed using custom software (eProbe). 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), the fEPSP slope was measured as the slope between the baseline and the peak of the negative wave (between A and B) and fEPSP area was considered as the area under the curve (between A, B and C). In order to evaluate the synaptic potency, stimulus-response or input/output (I/O) functions were acquired by systematic variation of the stimulus current (50-1000µA) before induction of LTP. fEPSPs evoked in the CA1 region using 0.1Hz stimulation. Baseline recordings were taken 30 min prior to LTP induction and 90 min after that, in order to determine any changes in the synaptic response of CA1 neurons. LTP was induced using high-frequency stimuli protocols of 100 Hz (4bursts of 50 stimuli, 0.15 ms

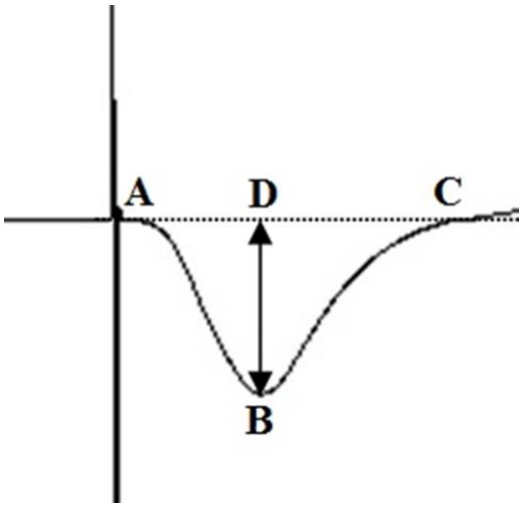


Fig.1. Schematic diagram of fEPSP. The fEPSP amplitude parameters analyzed as: difference between B and D, fEPSP slope analyzed as: AB slope and fEPSP area analyzed as: the area under the curve between A, B and C.

stimulus duration, 10 s inter burst interval). All potentials employed as the baseline and high frequency stimuli was evoked at an intensity, which produced 50% of the maximal fEPSP.

Data were statistically analyzed using two-way analysis of variance (two-way ANOVA) and one-way ANOVA followed by Tukey's test. The significant level was set at $p<0.05$. Results are expressed as means±S.E.M.

Results

The activity of postsynaptic AMPA and NMDA receptors is commonly presented by waveform of Field excitatory postsynaptic potential (fEPSP). Electrical stimulation at the Schaffer collaterals commissure evoked potential of fEPSP and the recording is done extracellularly in the CA1 apical dendritic layer. In our study, fEPSP components including area, slope and amplitude were used as criteria for the evaluation of synaptic plasticity (Liu et al., 2008).

Fig. 2, illustrates the effects of CCK8s on LTP induction and maintenance in CA1 area of the rats. A comparison among all subjects revealed that fEPSP slope (Time*CCK effect, $F(16,136) = 3.5$, $p<0.001$; Fig. 2B) and fEPSP area (Time*CCK effect, $F(16,136) = 5.99$, $p<0.001$; Fig. 2C) were reduced in control rats which reflects the weakness in the maintenance of induced LTP over time. However, CCK-8s, especially when injected 30 minute before induction of LTP demonstrated the opposite effect.

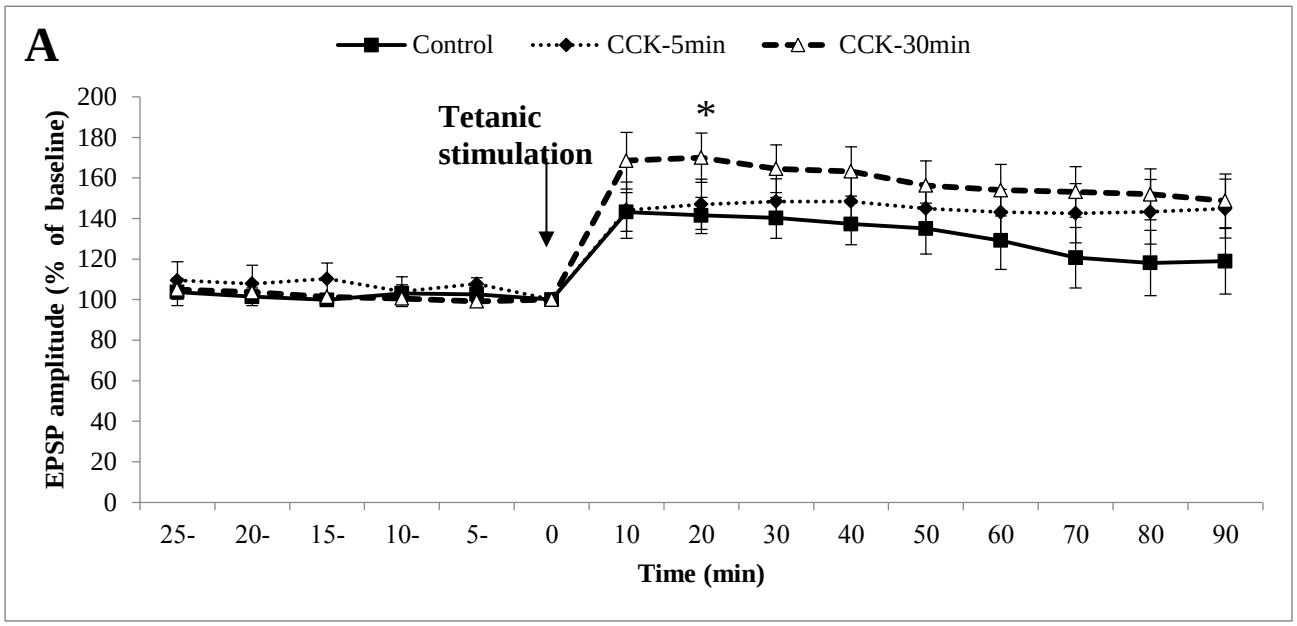
The fEPSP area after tetanization was significantly increased in the CCK-30min group with respect to the control group (161.27±11.97% and 128.39±10.37%, respectively; $p < 0.05$; Fig. 2C). However, fEPSP amplitude and fEPSP slope showed no significant difference between the groups (Fig. 2A, B). Over time, fEPSP indices of the CCK-5min and the CCK-30min groups showed similarity, especially after 90 minutes of LTP induction (Fig. 2).

Discussion

Our results showed that CCK-8s has significant effects on LTP induction and maintenance in CA1 area of hippocampus. When CCK-8s was injected 30 minutes before induction of LTP, it improved both induction of LTP and its maintenance. However, when it was administered 5 minutes before induction, had no effects on induced LTP, but improved its maintenance.

Studies have demonstrated that CCK has excitatory effects on hippocampus (Miller et al., 1997). CCK facilitates presynaptic glutamate release (Deng et al., 2010) and markedly increases the sensitivity of NMDA receptors to glutamate in hippocampus (Gronier and Debonnel, 1996). Therefore, there is a possible constructive interaction between CCK and synaptic plasticity in hippocampus.

According to our results, the duration of long-term potentiation and its maintenance could be linked to the levels of CCK in the brain. However, when CCK was injected at 5 or 30 minutes before the high frequency stimulation, of response patterns was different between the groups. It is important to note


A
Control
CCK-5min
CCK-30min
Tetanic stimulation
*
EPSP amplitude (% of baseline)
200
180
160
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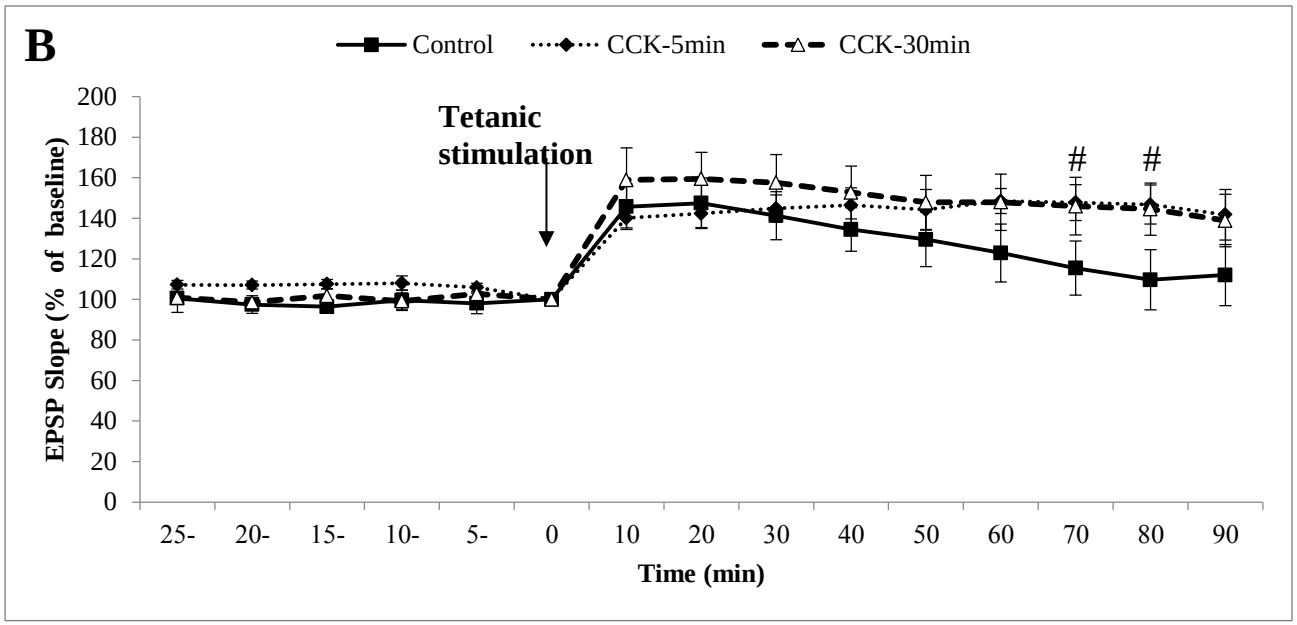
25- 20- 15- 10- 5- 0 10 20 30 40 50 60 70 80 90
Time (min)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CCK-5min
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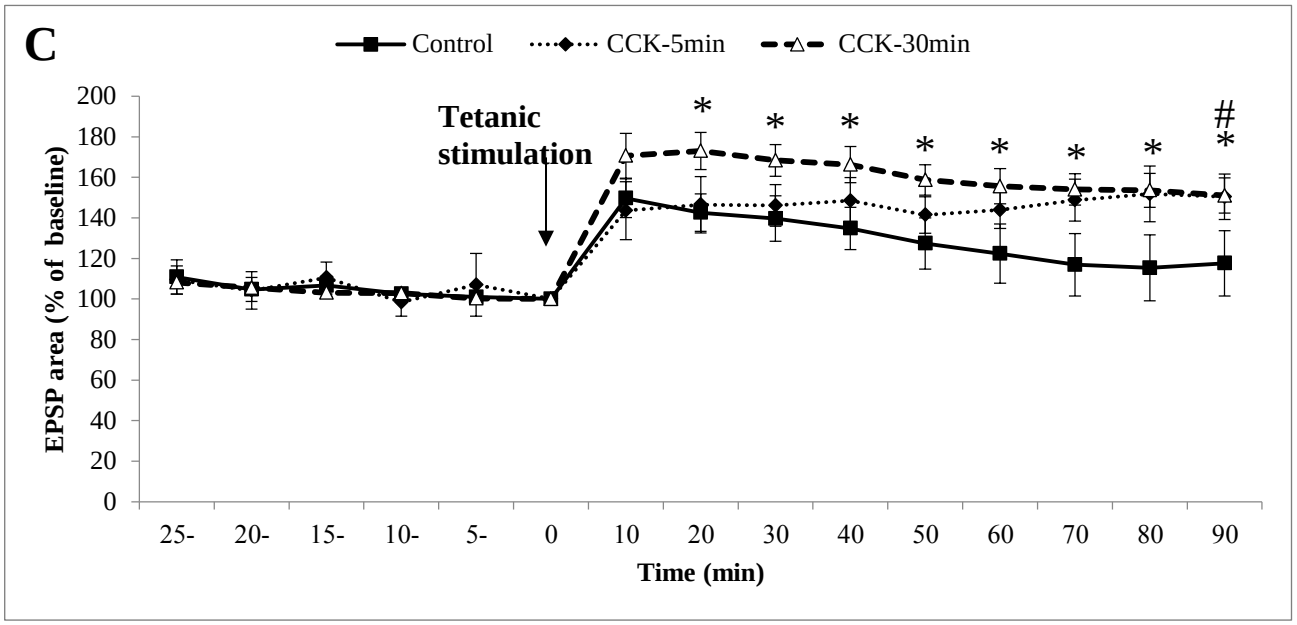
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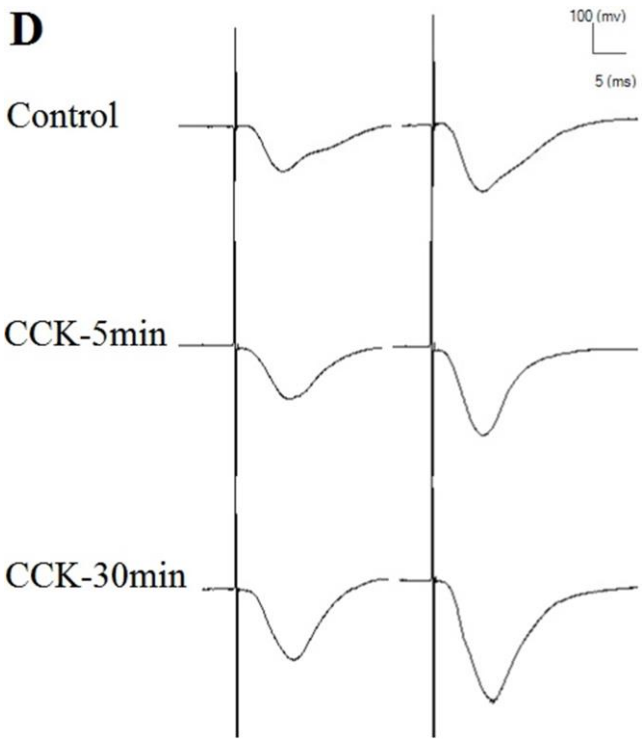

Fig.2. The effect of CKK-8s on LTP induction and maintenance in CA1 area of the hippocampus using tetanic stimulation at A: the field excitatory postsynaptic potential (fEPSP) amplitude, B: fEPSP slope and C: fEPSP area. Data are plotted as the average percentage change from baseline responses. Values are % mean±S.E.M. #p < 0.05 significant differences between the control and the CCK-5min groups and *p < 0.05 significant differences between the control and the CCK-30 min groups (n=6-7). D: Single traces recorded before and 60 min after induction of LTP in CA1 of the hippocampus.

that the responses showed some similarity after 60 to 70 minutes in both groups. Considering that CCK was administered systemically and that it takes time for CCK to cross the blood-brain barrier and to exert its effects on hippocampus, it is therefore natural to expect different response patterns between the groups. Although it was previously believed that CCK cannot cross the blood-brain barrier (Acosta, 1998), but our results, consistent with previous studies (Tirassa and Costa, 2007; Dolatabadi and Reisi, 2014) lends credence to the notion that CCK-8s can pass the blood brain barrier.

In addition, our findings show that high frequency stimulation of Schaffer collateral path-CA1 synapsis at the time that the levels of CCK is elevated in the brain, can be more effective in induction of LTP (Figure 2, Group CCK-30min). Based on the responses of the CCK-5min group, and considering the delay for CCK to cross the blood-brain barrier, if CCK levels become elevated in the brain after LTP induction, it would have preventive effects; it will increase plasticity and helps to maintain it. One possible mechanism for these events could be the changes observed in release of both stimulatory and inhibitory neurotransmitters. It has been demonstrated that CCK induces release of GABA after 3-5 minutes of application which subsequently will be followed by predominant release of glutamate (Deng and Lei, 2006; Deng et al., 2010). It is speculated that in the group were CCK was injected 5minprior to induction, synchronicity of LTP induction with the maximum release of GABA, between 3rd and -5 minutes of CCK injection, plays a role in reducing the effect of CCK on synaptic plasticity. However, in the CCK-30min group, high frequency stimulation was probably concomitant with over expression of glutamate.

Conclusion

In conclusion, our results indicated that acute injection of CCK-8s in rats enhances the effectiveness of high frequency stimulation for induction of LTP and its maintenance. The data suggest that systemic administration of CCK-8s can affect synaptic plasticity in the hippocampus and the resultant cognitive functions.

Acknowledgment

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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