# POSTPONED EFFECT OF CHOLECYSTOKININ FRAGMENTS 30-33 (CCK-4), AND 31-33 (CCK-3), ON ALBINO RATS BEHAVIOR

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## SUMMARY

It is generally accepted that regulatory olygopeptides cause short-term physiological effects. However we demonstrate that CCK-4, and its shorter fragment CCK-3 evoke significant long-term changes in albino rat behavior. The anxiogenic action of CCK-4 (400 mg/kg) and CCK-3 (10 mg/kg) was measured by cross-maze and dark-light test. The tendency of rats to developing of depression-like states was evaluated by the Porsolt-test. In this study both CCK-4 and CCK-3 induced significant behaiour effects from 40$^{th}$ min to 12$^{th}$ day after the intraperitoneal administration. It was found also that there were significant changes in the level of bioamines and their derivatives not only during the first hours after the administration but also on the 5$^{th}$ and 12$^{th}$ day. The half-life of these peptides is less than 15 min, so it's possible the induction of some chain-reactions similar to memory consolidation process.

**KEY WORDS:** regulatory peptides; cholecystokinin; behavior

## INTRODUCTION

The half-life of the most of regulatory peptides (RPs) in plasma and tissues is relatively short. Therefore it is considered, that RPs evoke physiological effects during restricted time, which is less than several hours. However peptides possessing regulatory effect can induce numerous secondary processes such as the influence on other neuropeptides, neuromediator or hormone release (1). As a result a long-term physiological effects may arise and form a cascade-like processes. They can differ from initial effects of RPs or have the same direction if the secondary activities are similar the initial ones (2).

In the present study we have investigated such secondary effects induced by cholecystokinin (CCK) fragments CCK-4 and CCK-3. CCK-4 is a well-known powerful anxiogenic neuropeptide. It


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induces a wide spectrum of fear, anxiety and depression-like changes of behavior after the intraperitoneal administration in albino rats (3, 4, 5). The plasma half-life of CCK-4 in albino rats is about 13 min and is even smaller in crude synaptosomal fraction of brain - 3 min (6, 7). Such instability was probably the reason for the lack of interest of investigators to long-term effects of CCK-4. Below we present the results of observations during 20 days after a single administration of CCK-4 and CCK-3.

## MATERIALS AND METHODS

Studies were performed using male Wistar rats weighing 150-250 g. Two peptides were used in experiments: CCK-4 (**TRP-Met-Asp-Phe-$NH_2$**, ICN Biomedical Inc.) and CCK-3 (**Met-Asp-Phe-$NH_2$**) synthesized in the Institute of Molecular Genetics (Moscow, Russia). The peptides were administered intraperitoneally at a dose of 10 μg/kg (CCK-3) and 400 μg/kg (CCK-4) of body weight (0.2 ml). The control group received the same volume of physiological solution. Behavioral tests were performed starting from 40 min to 20 days after peptide administration. The functional state of rats was assessed by the following tests: the anxiety and fear components of rat behavior were estimated using a elevated cross-maze (5, 8) and "dark-light" test; the tendency of rats to developing of depression-like states was evaluated by the forced swimming method of Porsolt; movement and orientational-investigative activity was assessed using an automatic open-field test. The each group of rats was tested once only at a designated time after CCK administration.

The biochemical analysis of catecholamines and their metabolites (DA- dopamine; DOPAC – 3,4-dihydroxyphenylacetic acid; HVA – homovanillic acid; 5-HT – 5-hydroxytryptamine; 5-HIAA – 5-hydroxyindoleacetic acid) in striatum was done at 40 min and on the $5^{th}$ day after the peptide administration by the method of high-pressure liquid chromatography with electrochemical detection. Data were analyzed statistically using the non-parametric Wilcoxon-Mann-Whitney test.

## RESULTS

*The effects on behavior*

Recently we demonstrated that anxiety and fear are induced in albino rats in 40 min after a single parenteral administration of CCK-4 at a dose of 20-400 mg/kg of body weight (5). Simultaneously the development of the depression-like state was shown by forced swimming method of Porsolt.

In this study CCK-3 also induced significant anxiogenic effect at $40^{th}$ min after the intraperitoneal administration. Test in the cross-maze demonstrated decreased numbers of investigations and excursions into the open arms. The typical example of CCK-3 short-term effects is presented on Fig.1. The minimal effective dose of CCK-3, 10 μg/kg, was even smaller than that of

CCK-4. The analysis also demonstrated some dependence of CCK-3 and CCK-4 effectiveness on the initial level of rat anxiety: low anxious rats were more sensitive to these peptides.

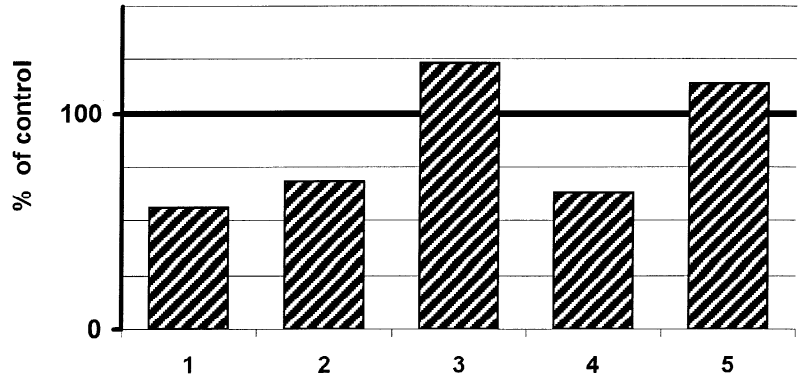


**Fig. 1** The example of short-term action of CCK-3 on 40th min after the parenteral administration. Horizontal axis: measure of behavior of rats in the cross-maze (% to control): 1 – the number of excursions and investigations of the open arms; 2 – the duration in the open arms; 3 – the duration of immobility; 4 – the ratio of number of excursions into the open arms to the total number of excursions into all arms; 5 – the risk behavior.

Continuing the rat behavior investigation we have found an unexpected effects. Complex picture of behavioral changes was observed on the 1st day after the CCK-3 and CCK-4 administration. There was a controversive combination of the anxiety and some signs of increased common motor activity in cross maze. However the development of depression-like behavior was also observed: the time of active swimming decreased and time of immobilization increased (Fig.2A and 2B).

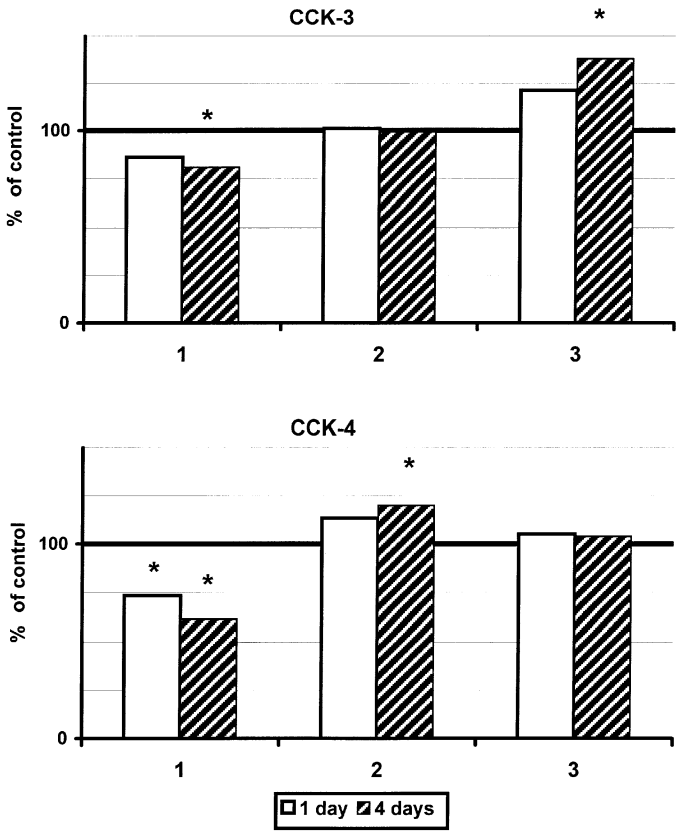


**Fig. 2** The behavior of rats in the forced swimming test on the 1st and 4th days after the parenteral administration of CCK-3 (A) and CCK-4 (B). Horizontal axis: 1 – the duration of active swimming; 2 - the duration of passive swimming; 3 – the duration of immobility (% to control).
* - $p < 0.05$.

Further on the 5th day the behavior of rats became less controversive and very similar in the most parameters to the behavior observed on the 40th minute after the CCK-3 and CCK-4 administration. (Fig. 3A and 3B). In cross-maze test we observed a significantly decreased number of investigations and excursions into the open arms, decrease in the time spent in the open arms and the reduced number of passages between arms, decreased risk behavior and increased duration of freezing.

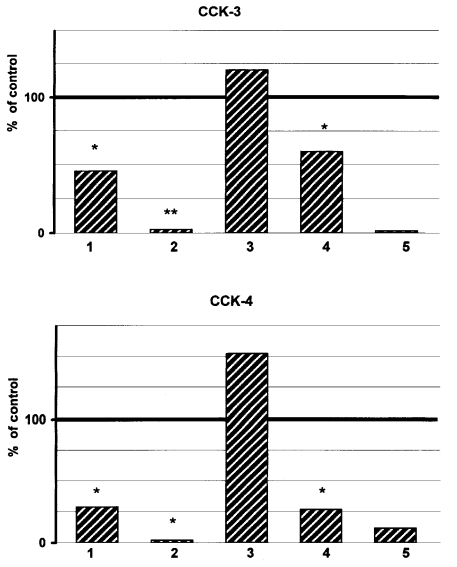


**Fig. 3** Measure of behavior of rats in the cross-maze on the 5th day after the parenteral administration of CCK-3 (A) and CCK-4 (B). 1 – the number of excursions and investigations of the open arms; 2 – the duration in the open arms; 3 – the duration of immobility; 4 – the ratio of number of excursions into the open arms to the total number of excursions into all arms; 5 – the risk behavior.
* - $p < 0.05$; ** - $p < 0.01$.

The investigation of behavior in the "dark-light" test demonstrated the decrease of latent time of the first exit from light compartment, and two-fold decrease of the time spent in the light compartment. At the same time there were no significant changes of behavior in automatic open-field test.

The level of depression-like behavior was high on the $5^{th}$ day (Fig.2A and 2B) after the CCK-3 and CCK-4 administration. There was a significant decrease of active swimming; the increase of immobilization time was observed for CCK-3 and the increase of passive swimming time for CCK-4.

Therefore we observed a close similarity of the most elements of rat behavior on the $40^{th}$ minute and the $5^{th}$ day after the peptide administration.

Much more modest changes of rats behavior were observed on the $12^{th}$ day after the CCK-3 and CCK-4 administration (Fig.4A and 4B). They demonstrated the same tendency as on the $5^{th}$ day, but the level of significance was lower. On the $20^{th}$ day there was no difference in behavior for experimental and control groups of rats.

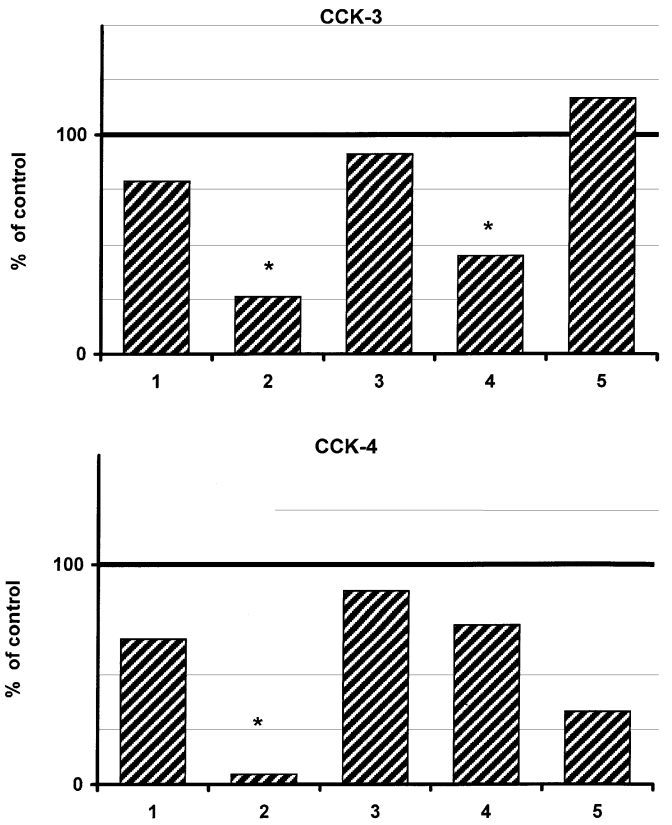


**Fig. 4** Measure of behavior of rats in the cross-maze on 12th day after the parenteral administration of CCK-3 (A) and CCK-4 (B). 1 – the number of excursions and investigations of the open arms; 2 – the duration in the open arms; 3 – the duration of immobility; 4 – the ratio of number of excursions into the open arms to the total number of excursions into all arms; 5 – the risk behavior.
* - $p < 0.05$.

*The effects on the level of bioamines and their metabolites in striatum.*

Significant decrease of DOPAK content and DOPAK/DA ratio in striatum was observed on the 40th min after the CCK-4 administration. There was also the tendency for HVA content and HVA/DA ratio decrease. The increase of 5-HT was low but statistically significant (Fig. 5A). On the $5^{th}$ day after the CCK-4 administration the changes in the level of DA and its metabolites had the same direction and were more pronounced. But the levels of 5-HT and HIAA were significantly decreased (Fig. 5A).

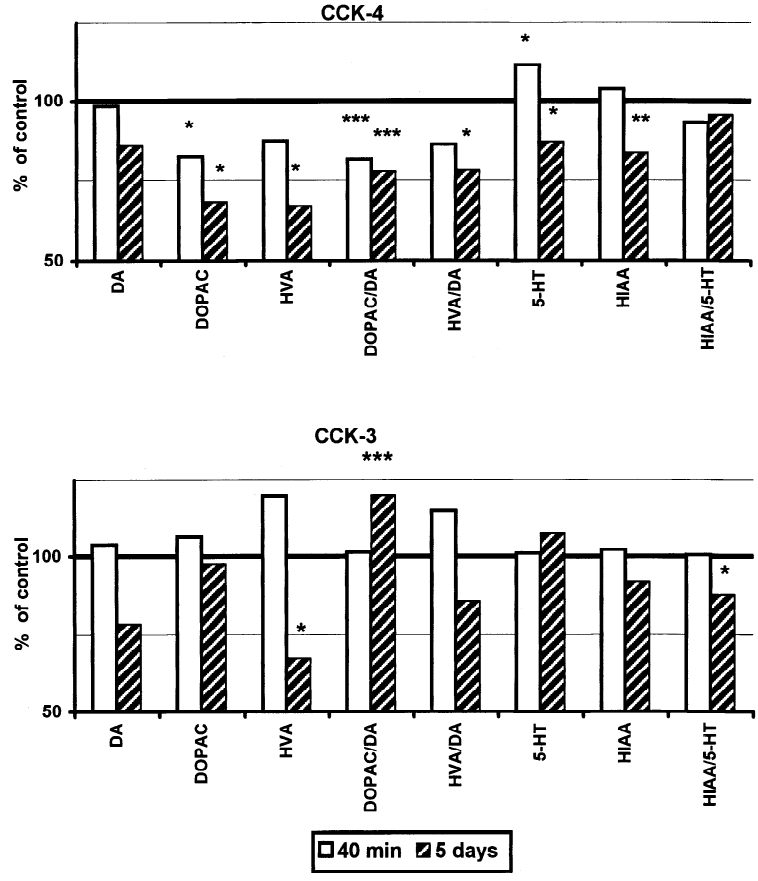


**Fig. 5** The biochemical changes in striatum on the 40th min and on the 5th day after the parenteral administration of CCK-4 (A) and CCK-3 (B) ( % to control).
* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.005$.

In contrast, on the 40$^{th}$ min after the CCK-3 administration there weren't significant changes in content of bioamines and theirs derivatives (Fig. 5B). However on the 5$^{th}$ day the level of HVA significantly decreased and DOPAK/DA ratio increased. The decline of DA content was not statistically significant while HIAA/5-HT ratio significantly decreased (Fig. 5B).

Therefore there were significant changes in the level of bioamines and their derivatives, but in contrast to behavior changes they were pronounced only on the 5$^{th}$ day after the CCK-3 and CCK-4 administration.

## DISCUSSION

There are numerous data on the physiological activity of CCK-4 and its interaction with B-receptors in the brain. But bioactivity of the shorter fragment **Met-Asp-Phe-$NH_2$** (CCK-3) is not so far sufficiently investigated in vivo. Only some data on CCK-3 effects in tissue preparations were published (9,10). We have found a close similarity between the direct behavioral effects of CCK-3 and CCK-4. Since the CCK-3 effective doses are less than for CCK-4 for the induction of anxiety and depression-like behavior, we suppose that tripeptide sequence **Met-Asp-Phe-$NH_2$** is sufficient for the activation of B-receptors. However we have also noticed some small peculiarities of direct rapid behavioral effects of CCK-3. Further investigations are necessary to reveal an exact difference between mechanisms of CCK-3 and CCK-4 rapid action. So far there is no much data on postponed effects of regulatory peptides on experimental animals. There are many examples of long-term effects of sterouid hormones and iodothyronines, but not regulatory olygopeptides. The observations of the changes in 5-HT content and in monoaminoxydase activity on the 3d-5th day after the administration of enkephalin analog (**Tyr-D-Ala-Gly-Phe-$NH^2$**) and study the ability of vasopressin and short vasopressin analogues to facilitate consolidation and retrieval of memory processes were published (11, 12, 13, 14).

So, the very most intriguing is the phenomenon of the postponed (on the $5^{th}$ - $12^{th}$ day) effects of CCK-3 and CCK-4 on behavior. It is impossible to explain it by the direct action on B-receptors since a half-life time of these peptides in body fluids is only 3-13 min (6, 7). The reason of the phenomenon may be certain chain of reactions or the induction of a memory-like process. However any hypothesis about memory involvement in behavioral experiments usually implies the recollection by rats of a distinct initial behavior. It is important to stress that each group of rats in our experiments passed behavioral testing only once at the designated time after the CCK administration. So since they could not have any preliminary experience of the test procedure the postponed effects cannot be characterized as a trivial recollection. It is possible that the chain of biochemical and physiological reactions caused by CCK-4 and CCK-3 leads to switching off systems associated with memory processes. The assumption of memory-like nature of the phenomenon does not contradict with the data on postponed bioamines changes.

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