EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON MONOAMINE LEVELS IN RAT BRAIN LIMBIC STRUCTURES DURING SATIATION

S. K. Sudakov, T. Kadar,
M. Varszegi, and G. Telegdy

UDC 612.391.014-46:615.357.3:577.175.734]-08:612.826.018

KEY WORDS: cholecystokinin; monoamines; limbic structures.

Research workers have recently paid great attention to the study of neuropeptides in the organization of various behavioral acts. Particular interest in this respect is being shown in the intestinal hormone cholecystokinin, which most workers regard as the hormone of satiation [3, 5], since peripheral and central administration of cholecystokinin to hungry animals reduces or completely suppresses their food consumption [1, 2]. During natural satiation the blood level of cholecystokinin of duodenal origin rises considerably, and this may be one of the factors inhibiting food behavior.

Cholecystokinin fragments, notably cholecystokinin octapeptide (CCK-8) have been found not only in the gastrointestinal tract, but also in different parts of the CNS [4]. This suggests that CCK-8 may play the role of neurotransmitter in the course of depression of food-motivated excitation. Meanwhile CCK-8 may play a regulatory role in these processes, and in that case the satiating effect is realized through other brain mediator systems. This last hypothesis is supported by the results of an investigation [7] which showed changes in brain monoamine levels after central injection of CCK-8.

Accordingly the aim of this investigation was to determine changes in monoamine levels in limbic structures of the rat brain during natural satiation and during satiation of animals receiving CCK-8.

EXPERIMENTAL METHOD

Experiments were carried out on 131 CFY male rats weighing 180-200 g. After food deprivation the rats were given CCK-8 intraperitoneally. The animals were offered food 10 min later. The rats were killed 30 min after presentation of the food. The quantity of food eaten was the criterion of the level of food motivation. Food consumption was analyzed individually for each animal. After appropriate morphological and histochemical treatment, the concentrations of dopamine, noradrenalin, and serotonin in sections of the hypothalamus, mesencephalon, amygdala, hippocampus, and corpus striatum were determined by the method in [6].

Food deprivation for 24 and 48 h was used, with two different doses of CCK-8: 5 and 10 μg/g body weight. Each group consisted of 24 rats.

Deprived animals receiving an intraperitoneal injection of isotonic NaCl solution (24 rats) and animals allowed food ad lib. (11 rats) were used as controls.

EXPERIMENTAL RESULTS

Intraperitoneal injection of CCK-8 considerably reduced the food consumption of the hungry rats. For instance, the mean quantity of food eaten by the control group was 2.4 times greater than in the group of rats receiving CCK-8 in a dose of 5 μg/kg and 2.5 times greater than in the group of animals receiving CCK-8 in a dose of 10 μg/kg (Table 1).

To discover individual differences in the action of CCK-8 and changes in the monoamine concentrations in limbic structures of the brain the animals as a whole were divided into three groups depending on the quantity of food eaten: 1) rats with a high level of food motiva-

Laboratory of Physiology of Motivations, P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. Department of Pathophysiology, Szeged Medical University, Hungary. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 290-293, March, 1984. Original article submitted April 8, 1983.

TABLE 1

Experimental conditions	Satiation after deprivation for 24 h			Satiation after deprivation for 48 h		
	mean quantity of food eaten, g	number of animals with low level of food motivation	number of animals with high level of food motivation	mean quantity of food eaten, g	number of animals with low level of food motivation	number of animals with high level of food motivation
Injection of 0.9% NaCl solution	2,22±0,36	1 (8,3%)	9 (75%)	1,86±0,26	2 (17%)	10 (83%)
Injection of CCK-8						
5 μg/kg	0,92±0,34	10 (42%)	6 (25%)	0,85±0,27	10 (42%)	7 (29%)
10 μg/kg	0,71±0,25	12 (52%)	6 (26%)	0,65±0,18	9 (37,5%	6 (25%)

TABLE 2. Monoamine Concentrations in Limbic Structures of Brain of Rats Allowed Food ad lib. (in μg/g)

Monoamine	Hypothalamus	Mesencephalon	Amygdala	Hippocampus	Corpus striatum
Dopamine	0,73±0,04	0,397±0,06	0,65±0,005	0,18±0,04	4,81±0,22
Noradrenalin	2,15±0,08	0,69±0,05	0,36±0,05	0,29±0,05	
Serotonin	1,85±0,07	0,955±0,11	1,225±0,1	0,77±0,07	1,09±0,08

tion (food consumption over 0.88 g in 30 min), 2) rats with a low level of food motivation (food consumption under 0.38 g), 3) rats with an average level of food motivation (food consumption from 0.39 to 0.87 g). Within the context of the present investigation animals of group 1 and 2 are of particular interest.

Analysis of the animals' behavior after injection of different doses of CCK-8 following food deprivation for different periods and subsequent feeding gave the following results. An increase in the period of deprivation caused a decrease in food consumption by the control animals on average by 17%. The mean decrease in food consumption after administration of CCK-8 preceded by deprivation for 48 h was 30% less than when preceded by deprivation for 24 h. An increase in the dose of CCK-8 to 10 μg/kg, after deprivation for 48 h, led to even stronger depression of motivation in a smaller percentage of rats, whereas an increase in the dose of CCK-8 after deprivation for 24 h led to a smaller weakening of food motivation in a larger number of animals. The percentage of rats resistant to the action of CCK-8 was independent of its dose and of the duration of food deprivation (Table 1).

Changes in monoamine levels in each structure in experimental rats compared with those in the structures of intact animals with free access to food are given in Figs. 1 and 2.

Natural satiation of the animals was found to cause a characteristic change in dopamine, noradrenalin, and serotinin levels (Table 2). Changes in monoamine levels depended on the duration of food deprivation. Satiation after deprivation for 24 h led to a significant fall in monoamine levels in the hypothalamus, a decrease in noradrenalin and dopamine concentrations and an increase in the serotonin concentration in the amygdala, and an increase in dopamine and serotonin concentrations and a fall in the noradrenalin level in the hippocampus (Fig. 1a). Satiation after deprivation for 48 h did not cause such considerable changes in monoamine levels in limbic structures. These changes were expressed mainly as a decrease in the concentration of all monoamines in all structures tested (Fig. 2a).

Natural satiation of the animals after starvation for 24 h caused changes in monoamine levels in the limbic structures of the brain similar to those observed in animals whose hunger motivation was suppressed by injection of CCK-8 (Fig. 1b, c). Changes in monoamine levels in brain limbic structures of rats whose food consumption was not reduced after injection of CCK-8 also were similar to those accompanying natural satiation (Fig. 1d), but an increase in the dose of CCK-8 to 10 μg/kg reversed the reaction of dopamine and serotonin in the mesencephalon and of noradrenalin the amygdala, and changes in dopamine in the hippocampus were considerably reduced (Fig. 1e).

The response of the monoaminergic system of brain limbic structures to satiation induced by injection of CCK-8 after deprivation of food for 48 h differed qualitatively from that after deprivation for 24 h and from the response to natural satiation. In particular, the noradrenalin level was raised in all structures studied (Fig. 2c).

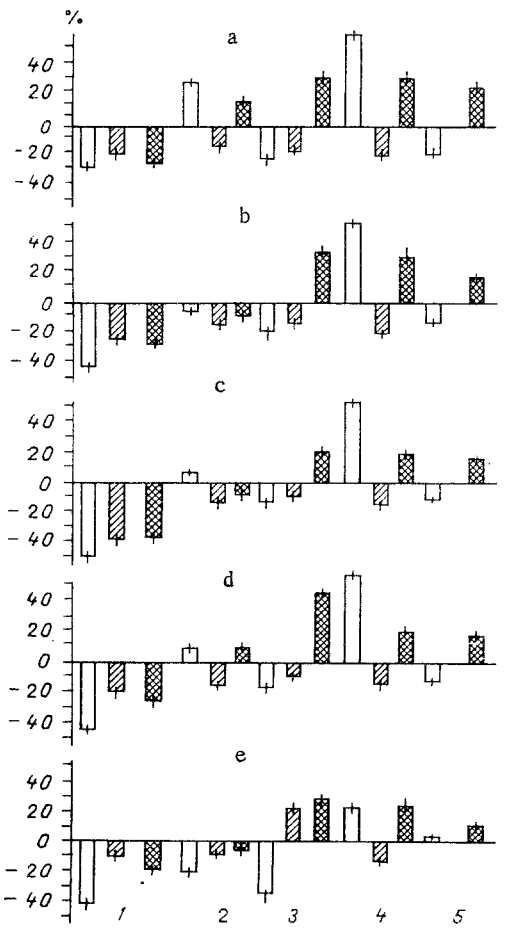

Fig. 1

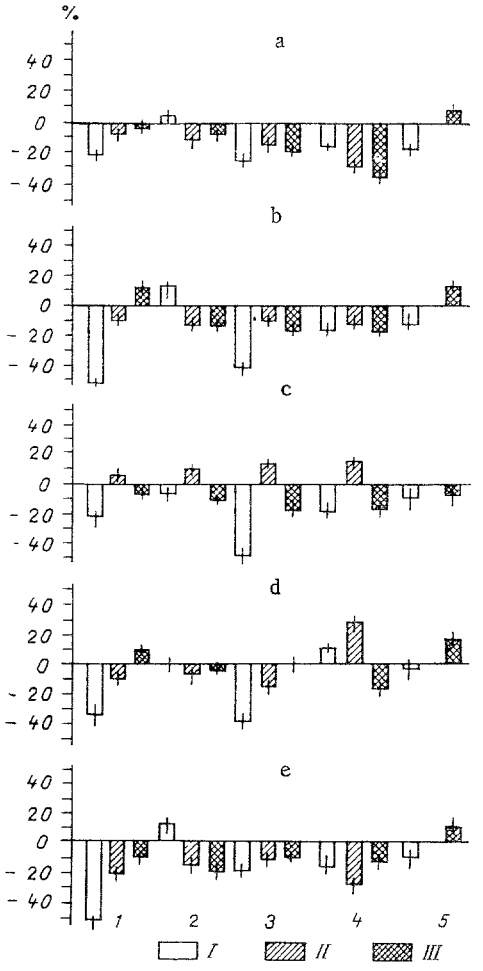

Fig. 2

Fig. 1. Changes in monoamine levels in limbic structures of rat brain during satiation after food deprivation for 24 h. Ordinate, changes in monoamine levels in deprived rats (in % of their levels in animals allowed food ad lib.). a) Control animals, b, c) rats with a low level of food motivation after injection of 5 and 10 μg/kg CCK-8 respectively, d, e) rats with a high level of food motivation after injection of 5 and 10 μg/kg CCK-8 respectively. 1) Hypothalamus, 2) mesencephalon, 3) amygdala, 4) hippocampus, 5) corpus striatum. Empty columns — dopamine, obliquely shaded — noradrenalin, cross-hatched — serotonin.

Fig. 2. Changes in monoamine concentrations in limbic structures of rat brain during satiation after food deprivation for 48 h. Legend as to Fig. 1.

When injected intraperitoneally, CCK-8 thus had a satiating effect, and the higher the level of food motivation, the stronger this effect. The inhibitory action of CCK-8 on food motivation evoked in rats by deprivation for 24 h is probably mediated through the monoamine system of the hypothalamus, amygdala, and hippocampus. However, the results of the present investigation suggest the presence of a different mechanism of the satiating action of CCK-8 under conditions of marked food motivation after long periods of starvation.

LITERATURE CITED

1. K. V. Anokhin, in: Systemic Mechanisms of Motivations [in Russian], Moscow (1982), pp. 219-222.
2. J. Gibbs, R. C. Young, and G. P. Smith, Fed. Proc., 31, 397 (1972).
3. J. Gibbs, P. C. Young, and G. P. Smith, J. Comp. Physiol. Psychol., 84, 488 (1973).
4. A. J. Loonen and W. Soudijn, J. Physiol. (Paris), 75, 831 (1979).
5. K. Mueller and S. Hsiao, Neurosci. Biobehav. Rev., 2, 79 (1978).

6. M. K. Shellenberger and J. H. Gordon, Anal. Biochem., 39, 356 (1971).
7. G. Telegdy, M. Fekete, M. Varszegi, et al., in: Third Congress of the Hungarian Pharmacological Society. Satellite Symposium. Aminergic and Peptidergic Receptors, Szeged (1979), pp. 169-185.

INDUCTION OF ELECTROGENIC PHOSPHATE TRANSPORT THROUGH THE MITOCHONDRIAL MEMBRANE BY THERMOSTABLE CYTOPLASMIC FACTOR IN HYPERTHYROIDISM

M. Kh. Gainutdinov, S. I. Mirmakhmudova, N. M. Mullagalieva, and Academician Ya. Kh. Turkulov*

UDC 612.352.1]-06:[612.391+612.766.1

KEY WORDS: hyperthyroidism; mitochondria; oxidative phosphorylation; electrogenic phosphate transport.

One of the main effects of thyroid hormones is stimulation of oxygen consumption in target cells [2]. The mechanism of stimulation of basal metabolism in hyperthyroidism is not yet known. It has been shown that mitochondria isolated from tissues of hyperthyroid rats perform oxidative phosphorylation in experiments *in vitro* with high P/O and ADP/O ratios and high respiratory control [6, 10]. In the intact cell the state of mitochondrial function is controlled by cytoplasmic regulators, whose action as a rule is to change activity of cation carriers and metabolites in the inner mitochondrial membrane [1, 5, 11].

It can be tentatively suggested that stimulation of oxygen consumption in hyperthyroidism is due to changes in activity of cytoplasmic regulators of mitochondrial metabolism. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 100-120 g. Mitochondria were isolated from the rats' liver by the method in [8]. The mitochondrial isolation medium consisted of 0.3 M sucrose, 5 mM Tris-HCl, 1 mM EDTA, pH 7.5. Oxidation of succinate by the mitochondrial suspension was measured polarographically. The kinetics of mitochondrial swelling was determined from the change in optical density at 540 nm on the LMF-69 photometer. Hyperthyroidism was induced by injection of thyroxine into the rats in a dose of 100 μg/100 g body weight daily for 5 days. The last injection of thyroxine was given 20 h before sacrifice. Cytoplasm was isolated from the liver and heart of satiated rats by centrifugation of the homogenate (1 g/ml of isolation medium containing 0.12 M KCl, 5 mM Tris-HCl, pH 7.65) for 20 min at 30,000g. Immediately after centrifugation in the cold the supernatant was heated to 97°C for 7 min, after which the denatured proteins and membrane fragments were centrifuged. The thermostable fraction of cytoplasm was kept in a refrigerator at −10°C. In the experiments with addition of thermostable cytoplasmic fraction to the mitochondrial suspension, 0.12 M KCl was added in the control experiment.

EXPERIMENTAL RESULTS

Preincubation of rat liver mitochondria for 2 min with thermostable cytoplasmic fraction of rat liver increased the rate of swelling of the mitochondria in iso-osmotic solution of potassium phosphate in the presence of valinomycin (Fig. 1). Under these conditions valinomycin is known to induce high permeability of the inner mitochondrial membrane for K^+ and swelling of the mitochondria is limited by the rate of transport of the anion through the mitochondrial membrane [5]. Consequently, in these experiments preincubation of the mito-

*Academy of Sciences of the Uzbek SSR.

Laboratory of Hormone Biochemistry, Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental-noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 293-295, March, 1984. Original article submitted March 21, 1983.

 0007-4888/84/9703-0268$08.50