The C-terminal tetrapeptide of cholecystokinin decreases hunger in rats

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Summary. Intraperitoneal injection of the C-terminal tetrapeptide amide of cholecystokinin (CCK) suppressed feeding behaviour and food intake in rats deprived of food for 12 h in a dose-dependent manner. The CCK-4 possesses a hunger suppressive property similar to the full molecule of CCK.

It is well documented that cholecystokinin (CCK) and the COOH-terminal octapeptide (CCK-8) suppress food intake and reduce behaviour associated with hunger in rats[1-3]. Both CCK and CCK-8 are present in brain tissue, which suggests a physiological role of these peptides in neuro-regulation[4-5]. Indeed, it was found that the cerebral cortex of genetically obese mice contained a much smaller amount of CCK-8 than that of nonobese littermates[6]. Recently it was found that the C-terminal tetrapeptide of CCK (CCK-4) is also present in the brain and peripheral nerves[7-8]. The probable functional significance of CCK-4 in the brain is suggested by the findings of the present experiments, that CCK-4 suppressed different variables of feeding behavior and food intake in fasted rats.

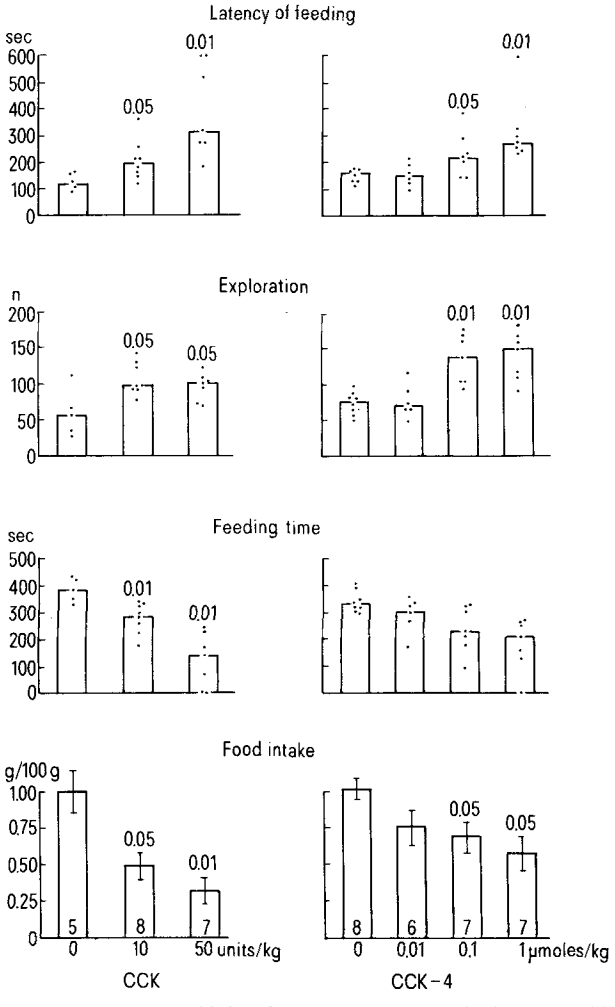
Methods. 48 male Wistar rats of 180-200 g b.wt were used. The rats were subjected to 12 h food deprivation before the behavioral test, but water was available ad libitum. The peptides were administered i.p. in 0.5 ml physiological saline 10 min prior to the test. The same volume of saline was given to the controls. The CCK (45% pure natural CCK from procine duodenum, Kabi Diagnostika, Stockholm) was injected in doses of 10 or 50 Ivy dog units per kg, and the C-terminal tetrapeptide (L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide HCl, Sigma) in 0.01, 0.1, and 1.0 µmoles/kg.

During the behavioral test the rat was placed in an experimental box measuring 30×30×30 cm. Only the front wall was transparent, and through this the interior of the box was lit. The floor was divided into 4 squares to score locomotion, and in the middle solid food pellets were available in a small dish. During the 10 min observation period the following variables of feeding behavior were measured: 1. Latency of feeding; the time in sec from the placement of animals into the experimental box till the initiation of feeding, 2. feeding time in sec., 3. food intake in g per 100 g b.wt. Since exploratory activity of fasted rats is approximately a mirror-image of consuming activity in a novel environment[9] and reflects the general motor activity of drug treated animals as well, we scored all crossings and rearings and the duration of rearings after every sec in the course of observation period. The exploratory activity was expressed by the sum of these scores.

Results. 10 min after the i.p. injections of CCK and CCK-4 the fasted rats were put into the experimental box and the following changes in behavior and food intake were observed (fig. 1). Both doses of CCK delayed the latency of feeding (picking up a piece of food and chewing), decreased feeding time, increased exploratory activity, and decreased the amount of food consumed. The CCK-4 also delayed the latency of feeding, increased exploration, and decreased feeding time and food intake (right side of figure). The effect of CCK-4 proved to be dose-dependent and the smallest effective dose was 0.1 µmoles/kg.

Discussion. The findings of this experiment show that the C-terminal tetrapeptide amide of CCK suppresses hunger in fasted rats. CCK-4 has been shown to be present in both the brain and peripheral nerves and probably has a role as a neurotransmitter[7-8]. We suggest that CCK-4 in the brain might be involved in the central nervous control of feeding behaviour and food intake.

We investigated the effect of CCK peptides on the feeding behavior of fasted rats in a novel environment, which is a stressful situation and brings into operation brain structures involved in the behavioral stress response. In this experimental circumstance feeding behavior motivated by hunger is in conflict with exploratory activity evoked by external stimuli. The increment in exploration was considered as a shift from consuming activity to exploratory activity since



Effects of cholecystokinin (CCK) and its C-terminal tetrapeptide amide (CCK-4) on feeding behavior (latency and duration of feeding), on exploratory activity, and on food intake of 12 h fasted male rats tested in a novel environment. Groups contained 5-8 animals. Individual values and medians are shown for the upper 3 variables, means ± SEM show the food intake. The statistically significant differences are marked by 5 and 1% probability levels above the columns. The Mann-Whitney test and t-test were used respectively in comparison with the control values.

in satiated animals the CCK peptides do not increase exploration but even possess central depressant effects[10]. Thus the increased exploration in the presence of decreased consuming activity seems to be an additional measure of hunger suppression.

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PRO EXPERIMENTIS

Development of an enzyme linked immunosorbent assay (ELISA) for measurement of vitellogenin concentrations in cockroaches

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Summary. A competitive enzyme linked immunosorbent assay was developed for the quantification of a large lipoprotein, namely the yolk protein vitellogenin, in the haemolymph of cockroaches *(Nauphoeta cinerea).* This assay was found to be specific, reproducible and it has a high sensitivity (approximately 10 ng).

Vitellogenins are female specific proteins which in the cockroach *Nauphoeta cinerea* are synthesized in the fat body and incorporated into the oocytes under the control of juvenile hormone[2-4]. For our studies on the production and incorporation of vitellogenin in different physiological stages and under various experimental conditions we needed a sensitive and specific assay for the measurement of vitellogenin concentrations. An enzyme linked immunosorbent assay (ELISA) seemed to meet our demands[5,6]. Enzyme immunoassays depend on the assumption that either an antigen or an antibody can be linked to an enzyme whilst retaining both immunological and enzymic activity in the resultant conjugate. The technique described here involves the following steps: adsorbtion of antivitellogenin to polystyrene tubes, incubation of known quantities of standard vitellogenin or unknown samples together with enzyme-labelled vitellogenin (competition step), incubation with enzyme substrate and measurement of resulting colour change.

Material and methods. Nauphoeta cinerea is an ovoviviparous cockroach. Under the rearing conditions used (26 °C, 60% relative humidity) its oocyte maturation cycle lasts 12 days and the pregnancy 35–40 days.

For developing an ELISA, purified vitellogenin and a vitellogenin-specific antibody had to be prepared. For purification of vitellogenin, which is a large lipoprotein, haemolymph was collected from reproducing females, added on a DEAE column and eluted with a gradient of NaCl 0.15–0.6 M at pH 6.5 (for details see Buschor and Lanzrein[7]). Antibodies were raised in rabbits against egg case homogenates[2] and the antiserum was run over a sepharose affinity column containing male haemolymph in order to remove cross reacting antibodies. IgG fractions were obtained after precipitation with 45% $(NH_4)_2SO_4$ by chromatography on a protein A Sepharose column. The competitive ELISA established here was set up according to methods described[5,6]. Vitellogenin was conjugated to alkaline phosphatase; first the enzyme was activated using the cross linking agent glutaraldehyde[8,9], and then the activated enzyme was reacted with DEAE purified vitellogenin.

Assay procedure. 1 ml coating buffer (0.05 M carbonate/bicarbonate buffer, pH 9.6) containing 180 ng IgG from an antivitellogenin serum was incubated in polystyrene tubes (3 ml volume) at 4 °C during 16 h whilst shaking. After 3 washes with phosphate buffered saline (PBS-Tween) the tubes were filled with 3 ml of PBS-Tween containing 0.5% bovine serum albumine and incubated in a shaking water bath for 8 h at 4 °C in order to saturate the polystyrene tube walls. Thereafter, the tubes were washed with PBS-Tween followed by the competition step. The vitellogenin standard solution and the diluted haemolymph samples respectively were incubated together with the vitellogenin-alkalinephosphatase conjugate (1:7000) in 1 ml PBS Tween during 15 h at 4 °C whilst shaking. This was followed by 3 washes with PBS-Tween. For quantification, 1 mg enzyme substrate (p-nitrophenylphosphate) in 1 ml diethanolamine buffer (pH 9.8) was incubated at 20 °C during 40 min. The reaction was stopped by the addition of 100 µl of 3 M NaOH. Photometric measurement was made using a PYE UNICAM spectrophotometer by reading the absorption at 405 nm.

Results and discussion. A typical standard curve obtained by using DEAE purified vitellogenin without and with addition of male haemolymph as control is shown in figure 1 and demonstrates a linear slope between concentrations of

Calculation of haemolymph vitellogenin (VG) concentrations from figure 2

	Dilution factor	VG (mg/ml)	$\bar{X} \pm SE$
Day-11 females	1.6×10^5	35.2	
	3.2×10^5	35.2	
	6.4×10^5	36.8	
	1.3×10^6	35.8	
			35.9 ± 0.33
Day-3 females	2.6×10^4	5.5	
	5.1×10^4	4.8	
	1.0×10^5	5.9	
	2.0×10^5	6.5	
			5.7 ± 0.36
Day-37 females	3.2×10^3	0.74	
	6.4×10^3	0.87	
	1.3×10^4	0.83	
	2.6×10^4	0.74	
			0.80 ± 0.03
Day-7 males	2×10^2	< 0.01	