Brain Cholecystokinin and Nutritional Status in Rats and Mice

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ABSTRACT Under certain conditions, exogenously administered cholecystokinin (CCK) or its COOH-terminal octapeptide can terminate feeding and cause behavioral satiety in animals. Furthermore, high concentrations of CCK are normally found in the brains of vertebrate species. It has thus been hypothesized that brain CCK plays a role in the control of appetite. To explore this possibility, a COOH-terminal radioimmunoassay was used to measure concentrations of CCK in the cerebral cortex, hypothalamus, and brain stem of rats and mice after a variety of nutritional manipulations. CCK, mainly in the form of its COOH-terminal octapeptide, was found to appear in rat brain shortly before birth and to increase rapidly in cortex and brain stem throughout the first 5 wk of life. Severe early undernutrition had no effect on the normal pattern of CCK development in rat brain. Adult rats deprived of food for up to 72 h and rats made hyperphagic with highly palatable diets showed no alterations in brain CCK concentrations or distribution of molecular forms of CCK as determined by Sephadex gel filtration of brain extracts. Normal CCK concentrations were also found in the brains of four strains of genetically obese rodents and in the brains of six animals made hyperphagic and obese by surgical or chemical lesioning of the ventromedial hypothalamus. It is concluded that despite extreme variations in the nutritional status of rats and mice, CCK concentrations in major structures of the brain are maintained with remarkable constancy.

INTRODUCTION

Over the past few years, reports from several laboratories have suggested a new role for the gastrointestinal hormone cholecystokinin (CCK),[1] in addition to its known function as a stimulator of the gall bladder and exocrine pancreas. That this hormone may serve as a satiety signal was suggested by a series of experiments which showed that injections of either CCK-33 or CCK-8 could induce not only cessation of eating but the full sequence of "behavioral satiety" in rats (1–4). In addition, it has been shown that CCK injected directly into the cerebral ventricular system can diminish frequency of bar pressing for a meal in rats (5). These observations, combined with the intriguing findings of large concentrations of CCK within the central nervous system (6–9) and a recent demonstration of greatly diminished concentrations of CCK in the brains of a strain of genetically obese rodent (10) have increased interest in the potential function of CCK as a satiety signal within the central nervous system and in the periphery.

If brain CCK is somehow involved in the neuroregulation of appetite, it is likely that alterations in nutrition and feeding behavior might be associated with changes in the levels or anatomic distribution of this peptide within the central nervous system. Significant alterations in the feeding behavior and body composition of rodents are known to result from dietary manipulations, brain lesioning, and genetic mutations. In this study, an analysis of the concentration of brain CCK-like immunoreactivity of rats and mice in these conditions is presented to determine whether associated perturbations in concentration, molecular form, or regional anatomic distribution of brain CCK can be demonstrated.

METHODS

Development of radioimmunoassay. The antiserum employed in this study was produced, in a rabbit, to the desulfated COOH-terminal octapeptide of CCK (kindly supplied by Dr. M. Ondetti, Squibb Institute for Medical Research, Princeton, N. J.) coupled to bovine serum albumin with glutaraldehyde. The conjugate was injected in Freund's adjuvant at 3- or 4-wk intervals.

After five immunizations, the antiserum was found to be suitable for radioimmunoassay at a final dilution of 1:35,000. The desulfated COOH-terminal octapeptide, radioiodinated with ^{125}I (New England Nuclear, Boston, Mass.), served as

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[1] *Abbreviations used in this paper*: CCK, cholecystokinin; CCK-8, COOH-terminal octapeptide of CCK; CCK-33, triacontatriapeptide cholecystokinin; VMH, ventromedial hypothalamus.

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tracer in the assay. Iodination was achieved using minor modifications of the chloramine-T method (11). To 20 μl of 0.5 M PO_4 buffer, pH 7.5, were added in rapid sequence, 1.0 mCi ^{125}I, 500 ng CCK-8, and 25 μg chloramine-T. The total reaction volume was 35 μl. Less than 10 s after addition of chloramine-T, the reaction was terminated by adding 50 μg sodium metabisulfite to the mixture. Separation of the ^{125}I-CCK-8 from damaged components and from unreacted $^{125}I^-$ was effected by passing the remainder of the reaction mixture over a Bio-Gel P-2 column (Bio-Rad Laboratories, Richmond, Calif.).

The radioimmunoassay was performed in 0.02 M Veronal buffer pH 8.6 with 0.2% bovine serum albumin. Synthetic (sulfated) CCK-8 was used as standard. After a 2-d incubation at 4°C, 1 cm^3 of cold assay buffer containing 0.1 cm^3 human plasma was added to each tube. Immediately thereafter, bound and free ^{125}I-CCK-8 were separated by the addition of 20 mg charcoal (Norit A suspension) to each tube, followed by centrifugation at 4°C for 15 min to separate the unreacted ^{125}I-CCK-8 from antibody-bound radioactivity, damaged components, and free $^{125}I^-$.

Brain extraction. All animals were fed standard Purina laboratory rat chow (Ralston Purina Co., St. Louis, Mo.) unless otherwise specified. The animals were maintained on alternating 12-h light-dark cycles and were sacrificed in the afternoon by rapid decapitation. The brains were immediately dissected and each anatomic area to be analyzed was weighed and then boiled in a 10-fold volume of distilled water for 3 min. During boiling, the tissues were ground with glass rods. The time taken from decapitation to boiling the tissue was minimized (<4 min) in an attempt to limit proteolysis. Brain extracts were stored frozen at −20°C until assayed. Assays were carried out at multiple dilutions of each sample to optimize precision and ensure that the unknowns and CCK-8 standard exhibited parallel binding inhibition curves.

Gel filtration patterns. Brain extracts from a number of animals under each experimental condition were further analyzed by Sephadex gel filtration using Sephadex G-50 fine columns (column dimensions were 1.5 × 60 cm).

500-μl samples were applied to the columns and eluted with buffer identical to that used in the assay. 1.0-cm^3 fractions were collected. The flow rate was 10 cm^3/h, and all gel filtration procedures were done at 4°C. Marker molecules (blue dextran 2000, porcine CCK-33 [V. Mutt, Karolinska Institute, Stockholm, Sweden], CCK-8, $^{125}I^-$) were used to identify the peaks of immunoreactivity. To rule out noncovalent aggregation as a source of higher molecular weight immunoreactivity, the integrity of immunoreactive peaks eluting earlier than CCK-33 was tested by incubating G-50 void volume material in 8 M urea and rerunning it on Sephadex columns in the presence of 4 M urea.

Dietary manipulations. Experiments were carried out to test the effects of over- and undernutrition on the concentration of brain CCK in rats. After each experiment, animals were sacrificed and their brains dissected and extracted as described above.

To study the effect of undernutrition on the concentration of brain CCK, groups of adult, male Sprague-Dawley rats were fasted for 72 h. Control rats had ad lib. access to standard laboratory chow. Both the control and food-deprived groups were housed in individual hanging cages and were given ad lib. access to water. Animals were weighed before sacrifice to verify food deprivation and weight loss. After the 72-h fast, the rats had lost 10–15% of their body weights. Brain weights in the fasted group were identical to those of controls.

The effect of chronic undernutrition in early life was examined by placing newborn rats in artificially enlarged litters of 22 pups each. Animals reared in this manner suffer significant early malnutrition and remain stunted as adults (12–14). At ages 2, 3, and 4.5 wk, large-litter animals were sacrificed and their brains extracted as described above. Control rats reared in normal size litters of 10 pups each were also sacrificed at ages 2, 3, and 4.5 wk. In addition, rats from normal sized litters were sacrificed at various times in early life to determine when CCK reaches its maximum concentration in the developing rat brain.

To determine the effects of chronic overfeeding on brain CCK, Osborne Mendel rats were fed a diet consisting of 55% fat for 5 mo. This diet is highly palatable to these rats, as was evidenced by hyperphagia and marked weight gain relative to chow-fed controls. At the end of the 5-mo period the obese, high fat-fed animals weighed 808±20 g, compared with a mean weight of 592±15 in the control group ($P < 0.01$).

Similar diet-induced hyperphagia and obesity can be achieved by supplementing the standard chow diet with sucrose solutions (15). Osborne Mendel rats were made obese by feeding them a chow diet with ad lib. access to a 16% sucrose solution for 7 mo. At the end of this period, the sucrose-fed animals were markedly obese, weighing 924±51 g.

An additional group of Sprague-Dawley rats made hyperphagic by feeding the sucrose-supplemented diet for 2 wk was also studied.

Finally, a group of chow-fed Sprague-Dawley rats was sacrificed during the dark phase of their day-night cycle to determine whether their marked predilection for nocturnal eating is associated with changes in brain CCK levels.

Genetically obese rodents. Four strains of genetically obese, hyperphagic rodents were studied. Obese animals and their lean littermates were sacrificed and their brains dissected and extracted as described above. In each group, the obese and lean animals were sacrificed on the same day, in the fed state, and during the light phase of their 12-h light-dark cycles.

Groups of young (age 5 wk) and adult (age 1 yr) Zucker fatty rats (fafa), together with their lean littermates (Fa/?) were studied (kindly donated by Dr. P. R. Johnson and Dr. R. Hemmes, Department of Biology, Vassar College, Poughkeepsie, N. Y.). Adult obese animals had a mean brain weight of 1.88±0.003 g, whereas their lean littermates had a mean brain weight of 1.93±0.01 g ($P < 0.01$). In the younger group, the obesity had been evident on gross inspection for 2 wk; although the obese and lean animals do not usually differ significantly in total body weight at this time in life, there are marked differences in body composition, the fatties having a smaller lean body mass and a greater amount of total body fat than their lean littermates (16). These differences in body composition are easily recognizable in the intact 5-wk-old animals; the fatties appear shorter and rounder than their lean littermates. The 5-wk-old fatties had a mean brain weight of 1.45±0.02 g, whereas the lean littermates had a mean brain weight of 1.66±0.03 g ($P < 0.01$).

Five groups of ob/ob mice (all mice used in this study were obtained from Jackson Laboratories, Bar Harbor, Maine), together with their lean (C 57/BL 6J +/?) littermates were studied. The groups ranged in age from 5 wk to 9 mo. With the exception of the 5-wk-old group, the ob/ob animals were much heavier than their lean littermates (Table III). In each group for which total brain weight data is available, the brains of ob/ob mice weighed significantly less ($P < 0.01$) than their lean littermates; at age 11 wk, 380.9±6.4 mg for the obese, 430.3±4.3 mg for the lean; at age 15 wk, 391.2±4.2 mg for the obese, 433.7±3.0 mg for the lean; at 6 mo, 378.8±2.0 mg for the obese, 422.1±3.0 mg for the lean; at age 9 mo, 389.0±10.0 mg for the obese, 437.2±10.2 mg for the lean. Additionally, portions of brains of ob/ob mice and their lean littermates at ages 15 wk and 6 mo were boiled in 0.1 N HCl for 3 min as described previously (8, 10) to determine whether the mode of

extraction had any effect on our ability to detect differences between the obese and lean animals.

Two separate groups of obese yellow mice (C57BL/6J A^y/a), together with their normal weight C57BL/6J a/a littermates were analyzed for brain CCK content. Animals were studied at ages 4 and 8 wk. There was no difference in body weights between yellow and black littermates at age 4 wk. However, at 8 wk, the yellow mice had a significantly greater body weight than their lean littermates ($P < 0.01$). These data are essentially identical with an earlier report on the development of obese yellow mice (17). There were no differences in brain weights between yellow mice and lean controls at either age. An additional group of three yellow mice, aged 20 wk, was studied although lean littermates were not available for this group.

Finally, six obese C57BL/6J db/db mice and their lean littermates C57BL/6J +/? were studied at age 15 wk.

Brain-lesioned animals. Chemical or physical lesions to the ventromedial hypothalamus (VMH) result in hyperphagia and obesity (18, 19). Accordingly, rats made obese by surgical lesioning of the VMH and mice lesioned with gold-thioglucose were studied to determine whether alterations in brain CCK accompanied these hyperphagic states.

Three female Sprague-Dawley rats with knife-cut lesions to the ventromedial hypothalamus were kindly provided by Dr. A. Sclafani (Brooklyn College, Brooklyn, N. Y.), along with sham-operated controls. The animals had been lesioned 6 wk before sacrifice, were hyperphagic, and had gained significantly more weight than the controls (preoperative weights: VMH, 266±19 g; controls, 270±23; 6-wk postoperative weights: VMH, 431±6 g; controls, 292±7, $P < 0.01$). The total brain weights of the lesioned animals were the same as those of controls (1.81 g for both groups).

6-wk-old mice of the C57BL/6J strain were injected intraperitoneally with gold-thioglucose in 0.5 mg/g doses. Body weights were monitored in the treated and control groups. 4 mo after injection of the gold-thioglucose, obese animals in the treated group achieved a mean body weight of 47.3±4.2 g, whereas the controls weighed 29.6±9 g ($P < 0.01$). The brains of the gold-thioglucose-injected group weighed slightly less than those of the sham-injected controls: 414±4 mg for the gold-thioglucose animals and 430±7 mg for the controls. At this time they were sacrificed and their brain CCK concentrations were determined.

RESULTS

Radioimmunoassay. When the products of the CCK-8 radioiodination reaction were filtered through a Bio-Gel P-2 column, the elution pattern of radioactivity appeared as shown in Fig. 1. The specific activity of the ^{125}I-CCK-8 produced in this reaction, calculated from measuring the radioactivity in column eluates, is ≅800 μCi/μg. Generally, 95% of the radioactivity present in the radiolabeled octapeptide in peak II was capable of binding to charcoal. After a 2-d incubation under radioimmunoassay conditions, 92–94% of the radioactivity present in control tubes (incubated under identical conditions but without antibody), remained charcoal-bindable.

Characteristics of the antiserum employed in this study are shown in Fig. 2. The antibody reacted with

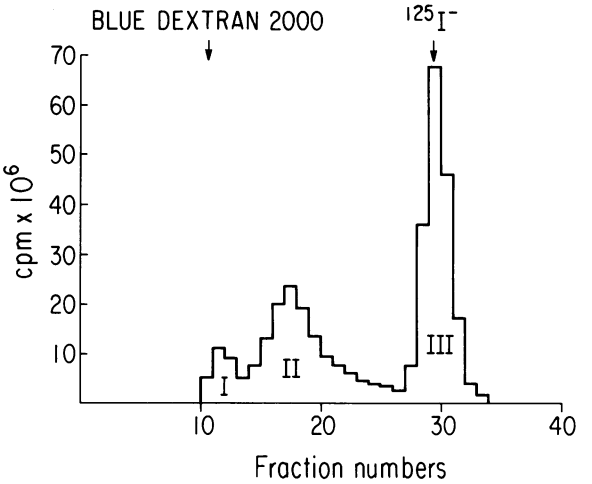

FIGURE 1 Iodination of desulfated CCK-8: purification of products of iodination reaction on a Bio-Gel P-2 column. Peak I consists of column void volume material that binds poorly to charcoal and to anti-CCK-8 antibodies. Peak II consists of ^{125}I-CCK-8 which was used as a tracer for radioimmunoassay. This material was 95% charcoal-bindable. Peak III consists of unreacted ^{125}I$^-$.

CCK-8, human gastrin I,[2] and porcine CCK-33 equally on a molar basis. The sensitivity of the assay was 2 pg CCK-8/ml. All brain extracts displayed complete parallelism with the CCK-8 standard. Somatostatin, arginine vasopressin, methionine enkephalin, adrenocorticotropin, gonadotropin-releasing hormone, and thyrotropin-releasing hormone all failed to inhibit the binding of ^{125}I-CCK-8 to the antibody (Fig. 2).

The intraassay coefficient of variation was 9% at 25 pg/ml ($n = 10$). The interassay coefficient of variation was 13% at 25 pg/ml ($n = 8$). When aqueous brain extracts were stored at −20°C the interassay coefficient of variation was generally <15% over a 6-mo period. However, acid extracts (0.1 N HCl) of brain failed to retain full immunoreactivity on storage in the frozen state, with losses of up to 50% after 2 mo at −20°C. Greater stability of acid extracts could be achieved by diluting the extracts in assay standard buffer before freezing.

When exogenous CCK-8 was added to brain tissue just before extraction the recovery of immunoreactive CCK-8 after boiling of the tissue in water for 3 min was essentially 100%.

Regional distribution of CCK immunoreactivity in rat brain. CCK immunoreactivity, expressed as CCK-8 mass equivalent, present in extracts of major anatomic regions of brains of adult Sprague-Dawley rats maintained on standard laboratory chow is

[2] Unsulfated heptadecapeptide gastrin.

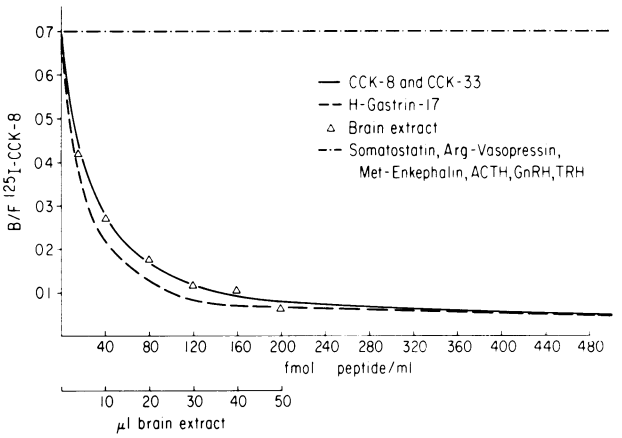

FIGURE 2 Binding of ^{125}I-CCK-8 to antibody 10. CCK-8, porcine CCK-33, and boiled water extracts of rat brain exhibited parallel inhibition curves. Other peptides shown in the figure occur in brain and fail to inhibit binding of ^{125}I-CCK-8 to the antibody. Human gastrin I reacted equipotently with CCK-8 on a molar basis. Antibody 10 was used at a dilution of 1:30,000; the assay was incubated at 4°C for 48 h.

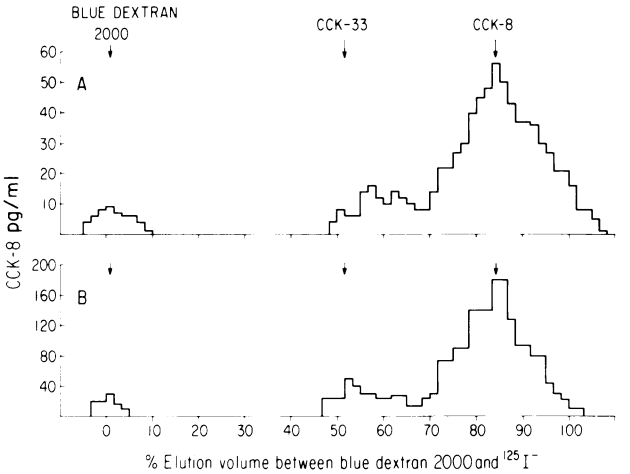

FIGURE 3 Sephadex G-50 fine gel filtration patterns of CCK immunoreactivity in boiled water extracts of rat cerebral cortex. (A) Profile of the CCK immunoreactivity of an extract of the cortex of a mature Sprague-Dawley rat maintained on a standard laboratory diet. Most of the immunoreactivity has the elution pattern of CCK-8, with minor peaks in the column void volume and in the region of CCK-33. Essentially identical patterns are seen in boiled water extracts of cerebral cortex of animals subjected to 72 h of food deprivation (B). Columns were precalibrated with blue dextran 2000, porcine CCK-33, CCK-8, and ^{125}I$^-$.

shown in Table I. These data are essentially in agreement with other published reports on the anatomic distribution of CCK in human and porcine brain (6, 20).

Sephadex G-50 gel filtration patterns. Fig. 3 shows a representative Sephadex G-50 fine gel filtration pattern of CCK immunoreactivity found in boiled water extracts of cerebral cortex taken from Sprague-Dawley rats maintained on standard laboratory chow. Consistent with several published reports on the molecular heterogenity of brain CCK (6–8, 20, 21), most of the immunoreactivity (at least 80%) has an elution volume identical with that of CCK-8. Minor peaks were found in the column void volume, in the CCK-33 region, and between the CCK-33 and CCK-8 markers. Hypothalamus and brain stem extracts showed similar patterns on Sephadex G-50 gel filtration. When a portion of the void volume material was pooled and rechromatographed under identical conditions on the same G-50 column, most of the immunoreactivity reappeared in the void volume. However, after incubation of the void volume material in 8 M urea and rechromatography in 4 M urea, nearly all of the immunoreactivity appeared to co-elute with the CCK-8 markers (Fig. 4) suggesting noncovalent aggregation of CCK-8, or binding of CCK-8 to cellular constituents, as the source of most of the void volume immunoreactivity on G-50 gel filtration.

TABLE I
Regional Distribution of CCK Immunoreactivity in the Brains of Mature Sprague-Dawley Rats

Brain region	CCK concentration*
Cortex	550
Olfactory bulb	185
Hypothalamus	179
Hippocampus	103
Brainstem	45
Spinal cord	3
Cerebellum	<0.5
Optic nerve	<0.5
Pineal gland	<0.5

* Expressed as CCK-8 nanogram equivalent per gram tissue, wet weight.

Dietary manipulation studies. As shown in Table II no change was found in the concentration of CCK-like immunoreactivity in cerebral cortex, hypothalamus, or brainstem in rats which experienced 72 h of total food deprivation. Sephadex gel filtration of brain extracts of fasted animals showed no differences in the molecular size distribution of CCK immunoreactivity from patterns seen in fed animals (Fig. 3).

Very little CCK is present in extracts of brains taken from rats at the time of birth. The CCK immunoreactivity appears in the brain stem and cortex at approximately the same time in early development and rapidly increases throughout the brain during the 1st mo of life (Fig. 5A). When the data is expressed as percent

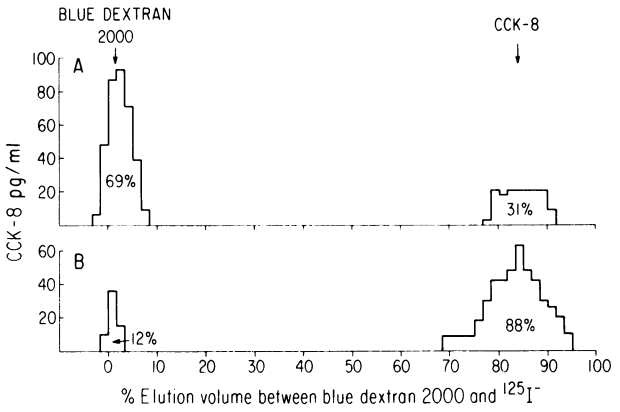

FIGURE 4 Sephadex G-50 fine gel filtration. The void volume material obtained from Sephadex G-50 fine gel filtration of rat cortex was pooled. Half of the material was boiled for 3 min and rerun on the same column using 0.02 M Veronal buffer pH 8.6 with 0.2% bovine serum albumin as elution medium (A). The other half was incubated at 4°C for 8 h in 8 M urea and rerun on a Sephadex G-50 fine column using 4 M urea as elution medium (B). Percentage of the recovered immunoreactivity is indicated for each peak.

adult level, the rates of increase of CCK concentration in the brain stem and cortex are the same (Fig. 5B). Sephadex gel filtration patterns of extracts taken from various parts of the brain in early life were all identical to the adult pattern.

While the levels of CCK in the brains of rats close to the time of birth were extremely low, boiled water extracts of duodenum in these same animals contained levels of immunoreactivity comparable to those found in adult animals.[3]

Rats undernourished from birth showed no differences in brain CCK concentrations at 2, 3, and 4.5 wk of age, from those found in rats reared in normal sized litters (Table III).

No differences in the whole brain concentrations of CCK were seen in animals made obese by diets high in fat or supplemented with sucrose (Table II). Sephadex gel filtration patterns of brain extracts from these obese animals showed no differences in the molecular distribution of CCK immunoreactivity from that found in chow-fed controls.

The brains of animals sacrificed during the dark phase of their day-night cycle had concentrations of CCK immunoreactivity which were identical to those found in animals sacrificed during the light phase (Table II).

Genetically obese rodents. As shown in Table II, the concentrations of CCK in the brains of Zucker fatty

[3] It should be appreciated that immunoreactivity detected with COOH-terminal antibody in duodenal extracts is not exclusively CCK, as the antibody can detect gastrin on an equimolar basis.

TABLE II

CCK Immunoreactivity in the Brains of Several Strains of Rats Under a Variety of Experimental Conditions

Strain	Experimental condition	Age	Body weight	n	CCK level*			
					Cortex	Brain stem	Hypothalamus	Whole brain
		wk	*g*					
Sprague-Dawley	Chow-fed	36	604±24	7	553±14	45±5	173±8	—
	Chow-fed	104	850±40‡	3	526±12 NS	47±10 NS	159±10 NS	—
	72 h fasted	36	556±30	9	546±20 NS	51±6 NS	166±9 NS	—
	Chow-fed; sacrificed during dark cycle	36	618±18	6	542±21 NS	51±3 NS	179±10 NS	—
	Fed a sucrose-supplemented chow diet (2 wk)	36	631±21	3	563±30 NS	64±10 NS	170±21 NS	—
	VMH lesioned	20	443±15‡	3	532±23 NS	38±4 NS	154±16 NS	—
	Sham VMH lesioned	20	305±10	3	514±3	41±1	186±17	—
Osborne Mendel	Chow-fed	36	592±15	3	—	—	—	269±12
	High fat fed (5 mo)	36	808±20‡	3	—	—	—	237±44 NS
	Fed a sucrose-supplemented chow diet (7 mo)	44	924±51‡	6	—	—	—	245±20 NS
Zucker	Obese (fafa)	6	152±19	6	404±19 NS	65±2 NS	189±12 NS	—
	Lean littermate (Fa/?)	6	153±23	6	349±9	60±6	175±9	—
	Obese (fafa)	54	625±39‡	3	416±7 NS	39±2 NS	158±12 NS	—
	Lean littermate (Fa/?)	54	414±38	3	435±7	61±37	151±9	—

* Mean CCK level expressed as nanogram equivalents CCK-8 per gram tissue, wet weight.
NS indicates no significant difference from control values.
‡ Indicates significant difference ($P < 0.001$) between obese and lean groups.

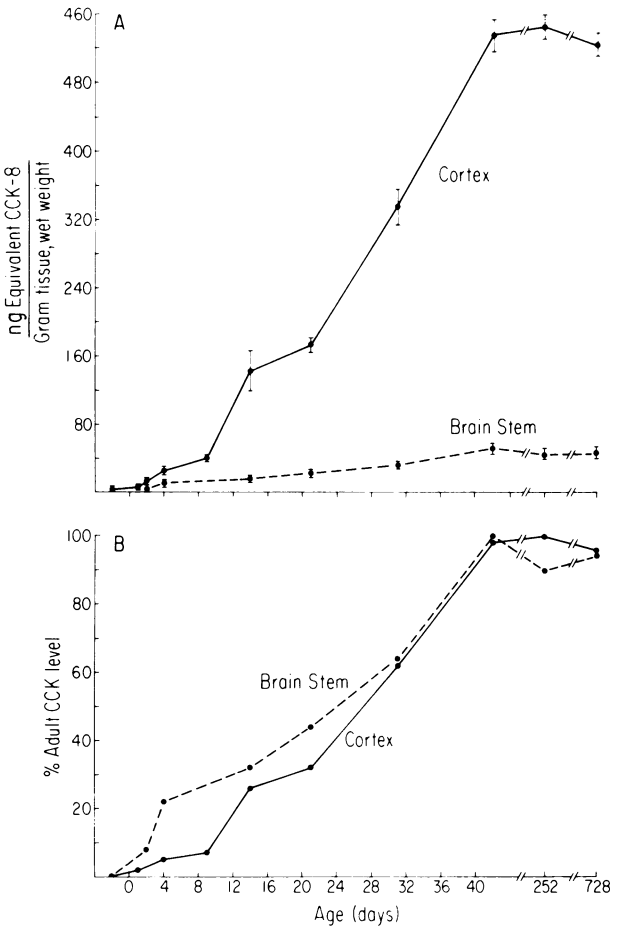

FIGURE 5 CCK immunoreactivity in cerebral cortex and brain stem of developing rats. The CCK immunoreactivity first appears in cortex and brain stem around the time of birth and increases in both regions of the brain throughout the first 6 wk of life (A). When expressed as percent adult level, concentration of CCK in cortex and brain stem appear to increase at similar rates (B).

rats did not differ from those found in their lean littermates. Sephadex gel filtration patterns of immunoreactive CCK in the boiled water extracts of obese Zucker rat brains and in extracts of lean Zucker rat brains were identical with the usual patterns found in Sprague-Dawley rats (Fig. 6).

Similar results were obtained from the three strains of genetically obese mice: the CCK concentrations found in the brains of the obese animals did not differ from those found in the brains of their lean littermate controls (Table IV). Boiled 0.1 N HCl extracts of mouse brains generally contained somewhat less immunoreactive CCK than boiled water extracts. However, the CCK content of boiled acid extracts of ob/ob mouse brains did not differ from that of their lean littermates (Table IV).

Brain-lesioned animals. As shown in Tables II and III no differences were detected in the concentrations of brain CCK in major dissected brain regions of rats or mice made hyperphagic and obese by chemical or surgical lesions to the VMH.

DISCUSSION

If the large quantities of CCK that reside within the brain function as a link in the chain of neurochemical events leading to the perception of hunger or satiety, one might expect to detect changes in brain CCK levels in association with short-term or chronic alterations in nutritional status and feeding behavior. Our data demonstrate that extremes in nutritional status and feeding behavior have no apparent effect on the concentration of immunoreactive CCK in major anatomic regions of the brains of rats and mice. Animals made hungry by 72 h of total food deprivation showed no differences in the levels, molecular forms, or regional distribution of brain CCK from those found in control animals. Unaltered concentrations and rates of appearance of brain CCK were also found in stunted animals which had been chronically undernourished from birth by placing them in artificially enlarged litters. In addition, analysis of genetic, diet-induced, and brain-lesioned models of hyperphagia and obesity showed no associated alterations in brain CCK concentrations.

Our data showing that ob/ob mice have normal concentrations of brain CCK are in contrast to a recent report describing greatly diminished levels of CCK immunoreactivity in the cerebral cortex of this obese strain (10). In studying these animals, both laboratories employed COOH-terminal assay systems which presumably have the same specificity. The concentration, regional anatomic distribution, and molecular heterogeneity of immunoreactive CCK which we have observed in the brains of rodents are similar to results published in several studies of brain CCK in other mammalian species. In addition, a major fraction of the CCK immunoreactivity present in brain homogenates is detectable in a synaptosomal fraction using our assay system,[4] in agreement with the published report of Pinget et al. (22). Straus and Yalow used 0.1 N HCl in their extraction procedure, whereas we used boiling distilled water for our nutritional experiments. However, when we assayed boiled 0.1 N HCl extracts of the brains of two groups of adult ob/ob mice, we again found no differences between obese animals and their lean littermates. Both laboratories obtained the ob/ob mice from Jackson Laboratories. Straus and Yalow report no differences in brain weights between ob/ob mice and their lean littermates. In contrast, we find that at every age studied, the ob/ob mice had significantly lower brain weights than their lean littermates,

[4] Goldman, S., and B. Schneider. Unpublished data.

TABLE III

CCK Immunoreactivity in the Brains of Large and Normal Litter Rats

Age	Litter size	n	Body wt	Brain wt	CCK level*		
					Cortex	Stem	Hypothalamus
wk			*g*	*g*			
2	Large	4	16.2±0.3‡	0.91±0.02‡	159±12§	—	—
	Normal	4	23.0±0.3	1.01±0.02	141±23	—	—
3	Large	4	26.3±1.9‡	1.29±0.04‡	186±9§	23±2§	121±10§
	Normal	4	39.9±1.4	1.43±0.01	172±7	22±3	138±6
4½	Large	4	59.5±5.2‡	1.42±0.04‡	356±5§	36±1§	154±9§
	Normal	4	95.4±1.7	1.61±0.02	335±9	32±1	148±15

* Expressed as CCK-8 nanogram equivalents per gram tissue, wet weight.

‡ $P < 0.01$ using Student's t test.

§ Not significant using Student's t test.

essentially in agreement with the results reported by other investigators (23–25). Whether the conflicting data on CCK levels found in the brains of ob/ob mice are a result of differences in the animals used or are technical in origin is not known.

The fact that we have been unable to demonstrate changes in brain CCK concentrations in association with alterations in nutritional status cannot be taken as proof that brain CCK does not function as a satiety factor. Methods of analysis such as the one employed in this study consist of regional brain dissection combined with radioimmunoassay and permit very reproducible quantitative determinations of the amount of peptide per gram of tissue. Such a system of analysis depends on the existence or induction of significant changes in levels of peptides in an entire region of brain, although more refined dissection techniques might show local physiologic perturbations.

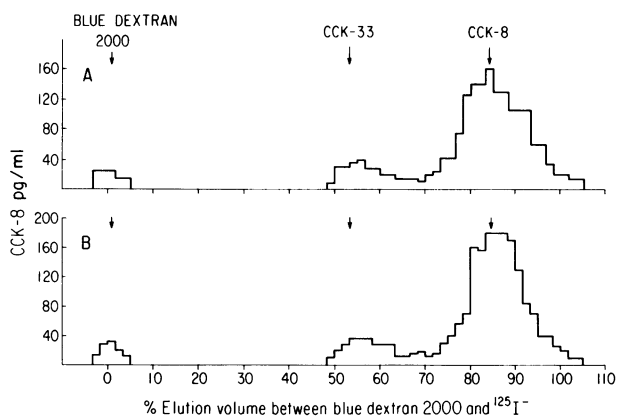

FIGURE 6 Sephadex G-50 fine gel filtration. Patterns of immunoreactivity of boiled water extracts taken from Zucker fatty (A) and Zucker lean (B) rats are essentially the same.

Specific immunohistochemical staining, on the other hand, provides only semiquantitative information, but with greater refinement of localization. Both techniques using the same antibody (26) gave similar results on a regional anatomic basis in rat brain, although intense immunochemical reactivity in specific neuronal bundles is revealed via the staining techniques. It should be appreciated that neither method allows for estimation of synthetic or degradative rates of central nervous system peptides unless combined with other manipulations. Moreover, it is conceivable that the tissue content of CCK remains unchanged under altered nutritional conditions, as a result of parallel changes in the synthetic and degradative rates of the peptide. If such changes in peptide turnover times involve only a small fraction of the total regional CCK content, then the methodology employed in this study would probably not detect what might be a physiologically labile "pool" of the peptide.

It should also be observed that we have consistently expressed our data as weight of peptide per wet weight of brain tissue. Although no changes in the concentration of peptide, expressed in this manner, were observed between experimental and control animals in any altered nutritional situation, in circumstances in which experimental animals had lower brain weights than controls, the total brain content of CCK would be correspondingly reduced. This would be true of undernourished large-litter rats, Zucker fatty rats, and ob/ob mice. In these animals, the total brain content of CCK would be roughly 90% of that of controls. Whether concentration of brain CCK is more physiologically relevant than total brain content of CCK remains to be determined. In addition, the problem of expressing data of this sort is rendered even more complex by the fact that brain size reduction may not be caused by parallel diminution of all brain constituents. Indeed,

TABLE IV

CCK Immunoreactivity in the Brains of Obese and Lean Mice

Strain	Experimental condition	Age	Body weight	n	CCK level*			
					Cortex	Brain stem	Hypothalamus	Whole brain
		wk	*g*					
ob/ob; +/?	Obese (ob/ob)	5	24.5±2.6	3	301±25 NS	117±18 NS	—	—
	Lean littermate (+/?)	5	19.7±2.0	3	330±27	99±8	—	—
	Obese (ob/ob)	11	53.1±1.6§	10	325±15 NS	90±7 NS	161±27 NS	—
	Lean littermate (+/?)	11	30.9±1.5	10	346±18	92±5	185±8	—
	Obese (ob/ob)	15	53.9±1.3§	5	335±23 NS (241±21)¶	98±10 NS	183±16 NS	—
	Lean littermate (+/?)	15	31.5±1.1	6	325±17 (225±21)¶	83±5	172±11	—
	Obese (ob/ob)	24	67.5±1.0§	3	—	—	—	255±17 (160±11)‖
	Lean littermate (+/?)	24	34.3±2.3	3	—	—	—	240±9 (146±9)‖
	Obese (ob/ob)	36	74.5±6.3§	2	315±18 NS	88±7 NS	156±12 NS	—
	Lean littermate (+/?)	36	34.4±3.9	2	300±16	83±6	168±10	—
A^y/a, a/a	Obese (A^y)	4	14.1±0.9	4	385±45 NS	99±8 NS	—	—
	Lean littermate (a/a)	4	11.6±1.1	4	330±27	126±10	—	—
	Obese (A^y)	8	27.9±1.2§	4	354±26 NS	107±8 NS	—	—
	Lean littermate (a/a)	8	20.8±1.6	4	326±18	111±6	—	—
	Obese (A^y)	20	41.7±3.5	3	390±31‡	112±5‡	—	—
db/db; +/?	Obese (db/db)	15	52.0±0.2§	6	327±25 NS	107±9 NS	—	—
	Lean littermate (+/?)	15	24.2±0.9	6	337±7	99±5	—	—
C57BL/6J	Gold thioglucose injected	24	47.3±4.2§	3	308±22 NS	94±7 NS	160±15 NS	—
	Sham-injected gold thioglucose control	24	29.6±0.9	3	300±10	86±5	181±13	—

* Mean CCK level expressed as nanogram equivalents CCK-8 per gram tissue, wet weight.
NS indicates no significant difference between obese and lean groups.
‡ Indicates no significant difference between this group and 8-wk-old lean controls.
§ Indicates significant difference ($P < 0.01$ Student's t test) between obese and lean groups.
‖ CCK levels of contralateral brain half extracted in 0.1 N HCl.
¶ CCK levels of contralateral cortical hemisphere extracted in 0.1 N HCl.

several observations in rats undernourished from birth have shown preferential loss of glia and myelin, whereas neuronal cell number did not diminish. Neuronal cell size and morphology may also be altered in these animals (13–15, 27). A recent report on ob/ob mouse brains demonstrated brain weight, cerebroside content, and DNA content to be 90, 48, and 74%, respectively, of that found in the lean littermates (24). Therefore, until more is learned about the physiology of brain CCK, the mode of expression of levels of immunoreactivity (whole brain content, content per gram wet weight of brain, content per microgram of DNA, etc.) must be approached with some caution.

In summary, our data show that extreme alterations in nutritional status, accompanied in a variety of ways, do not change cerebral cortical, hypothalamic, or brain stem concentrations of CCK in rats and mice. The only significant changes we have thus far observed in brain CCK concentrations accompany the early postnatal development of the rat, and these increases occur during a period of rapid brain growth and closely parallel those reported for many brain constituents, including the neurotransmitters dopamine and norepinephrine (28, 29). Certainly, our study does not rule out the possibility that CCK may serve as a neuroregulator of appetite in some part of the brain, most likely the hypothalamus. More refined anatomic and neurophysiologic studies are required to prove such a function. Until these are available, the role of central nervous system CCK-like peptides remains uncertain.

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