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Changes in Brain Monoamine Levels of Rats During Cholecystokinin Octapeptide-Induced Suppression of Feeding

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KÁDÁR, T., M. VÁRSZEGI, S. K. SUDAKOV, B. PENKE AND G. TELEGDY. *Changes in brain monoamine levels of rats during cholecystokinin octapeptide-induced suppression of feeding.* PHARMACOL BIOCHEM BEHAV **21**(3) 339–344, 1984.—Cholecystokinin octapeptide (CCK-8) in doses of 5 or 10 μg/kg was injected intraperitoneally to 24 hr food-deprived rats before a 30 min feeding period, and the dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) contents of the hypothalamus, mesencephalon, amygdala, hippocampus and striatum were measured thereafter. The experimental procedure (deprivation + food intake) alone could induce changes in the brain monoamine contents of saline-treated animals as compared to the nondeprived control group. The most striking effect was observed in the hypothalamus, in which the contents of all three monoamines decreased. In the deprived control group there was a significant positive correlation calculated by linear regression analysis between the amount of food eaten and the DA contents of the amygdala. Injection of CCK-8 before food intake testing decreased the DA contents of the hypothalamus. In the CCK-8-treated animals the correlation between food intake and amygdaloid DA contents disappeared. The CCK-8 treatment specifically gave rise to a significant positive correlation between the amount of food eaten and the NE content of the hypothalamus; such a relation could not be observed in the saline-treated group. The hypothalamic NE contents altered in parallel with the effectiveness of both doses of CCK-8 in inhibiting food intake. The results indicate the importance of the hypothalamic NE system in the food intake-suppressing effect of CCK-8.

Cholecystokinin octapeptide Suppression of feeding Brain monoamines Hypothalamic norepinephrine

IT is ten years since the first papers appeared on the food intake-depressing effect of cholecystokinin (CCK), a gastrointestinal peptide hormone [3, 7, 8]. In the past decade, one of the major ambitions of CCK research has been to clarify the mechanism of the effect, and to investigate the possible therapeutic efficacy of CCK in the treatment of obesity. The earlier review by Mueller and Hsiao [19] on the satiety effect of CCK and the more recent review by Morley [17] on CCK research clearly demonstrated the advances in this field.

In rats, CCK seems to exert a food intake-suppressing effect mainly after peripheral administration, as it had no effect on food intake when injected intracerebroventricularly [5, 13, 18], although it did specifically depress a food-related operant behavior [14], and inhibited the acquisition and facilitated the extinction of conditioned feeding behavior [6] of rats after central injection. The latter effects have been also demonstrated in a fear-motivated behavioral task [6,28], and thus the mechanism is presumably not specific for food.

In our view of the satiety effect of CCK, the peptide, at least in rats, produces food intake inhibition by exciting afferent vagal fibers, and the central input of afferent vagal mediation could be the medial hypothalamus [24]. Total abdominal or only gastric vagotomy abolishes the effect of peripherally administered CCK [13, 18, 25], but negative results have also been obtained [2]. McCaleb and Myers [16] demonstrated that peripheral CCK injection inhibited the feeding response produced by intrahypothalamic norepinephrine injection in sated rats, and that intrahypothalamic peptide treatment suppressed food intake by succeeding norepinephrine microinjection into the same brain loci. In addition, caerulein, a CCK analog, injected into the ventromedial hypothalamus, also depressed the food intake of rats [26], indicating that hypothalamic CCK receptors could be implicated in the regulation of feeding.

The present study was aimed at establishing what changes occur in the monoamine contents of certain areas of rat brain during food intake inhibition produced by intraperitoneally administered cholecystokinin octapeptide sulfate ester (CCK-8).

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TABLE 1

FOOD INTAKE OF RATS IN A 30 MIN SESSION AFTER 24 HR DEPRIVATION

Groups	Food intake (g) Mean ± SEM	Number of animals
A. Deprived control	2.22 ± 0.36	12
CCK-8 5 μg/kg IP	0.89 ± 0.19*	23
CCK-8 10 μg/kg IP	0.72 ± 0.20*	22
B. CCK-8 5 μg/kg IP		
well-responder	0.09 ± 0.05	10
non-responder	2.37 ± 0.26†	6
CCK-8 10 μg/kg IP		
well-responder	0.08 ± 0.03	12
non-responder	2.11 ± 0.35†	6

*Statistically significant versus deprived control, $p<0.001$ (Student's t-test).

†Statistically significant versus well-responder subgroup, $p<0.001$. (Student's t-test).

METHOD

Male rats of the CFY strain (Sprague-Dawley) weighing 180–200 g were used throughout the experiments. The animals were maintained on a 12 hr light-dark cycle (lights on at 6 a.m.) and were given access to food and tap water ad lib. With the exception of the non-deprived control group, food was withdrawn from the animals 24 hr before the start of the experiments. All behavioral experiments were carried out in the early afternoon.

For the measurement of food intake, deprived animals were placed individually in testing cages, which were the same as their home cages, except that the wood chip litter was absent. After 10 minutes' accomodation to the testing cages, saline or 5 μg/kg or 10 μg/kg CCK-8 (dissolved in saline) was injected intraperitoneally (IP) into the animals in a volume of 0.20 ml/100 g body weight. After a second 10 min interval each animal was given to access to a preweighed amount of food (approximately 40 g) for 30 min. Following the feeding period the amount of food eaten was recorded. Rats of the non-deprived control group were also injected with saline at the appropriate time and were placed in testing cages for 50 min altogether, but no food was available for them.

After the 30 min food intake testing, animals were removed from the cages and decapitated. Brains were removed and dissected into the following areas: hypothalamus, mesencephalon (between pons and mamillary body), amygdala (dissected area contains amygdaloid complex together with the surrounding pyriform cortical tissue), hippocampus (total), striatum (caudate-putamen complex). The dissected areas were deep-frozen as soon as possible, and the dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) contents of the brain areas were determined on the following day by the fluorometric method of Jacobowitz *et al.* [12], as modified by Szabó *et al.* [27].

For statistical analyses one-way analysis of variance and t-test was used. Linear regression analysis too was carried out for the transmitter contents in relation to the amount of food ingested in order to determine the corresponding correlation coefficients, and in some cases was followed by analysis of covariance. A p-value of less than 0.05 was accepted as a level of statistical significance.

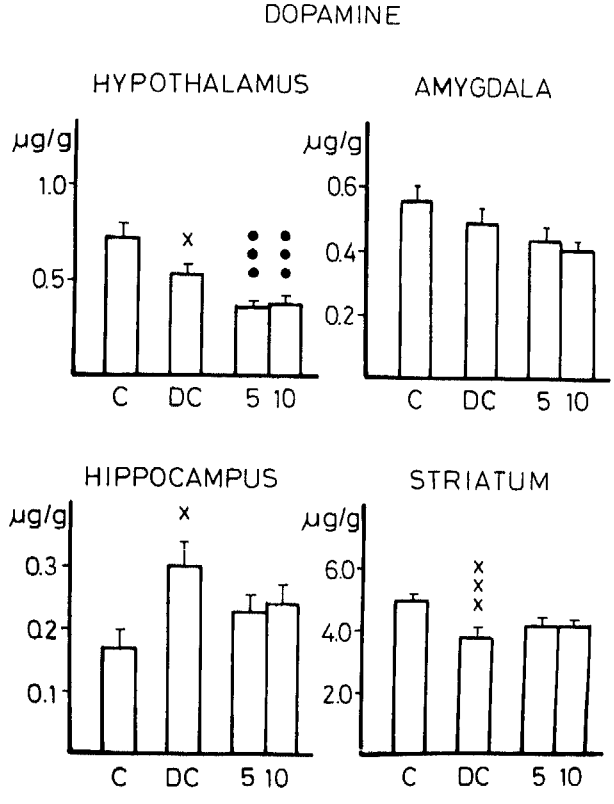

FIG. 1. Effects of intraperitoneal CCK-8 injection on the dopamine levels of denoted rat brain areas, when 24 hr deprivation was followed by 30 min feeding test. C: non-deprived control group (food was not available in testing cages); DC: deprived control group; 5, 10: deprived animals following 5 or 10 μg/kg CCK-8 IP. Asterisks: significant changes versus non-deprived controls; dots: significant changes versus deprived controls. ×: $p<0.05$; ×××,●●●: $p<0.001$ (t-test). For the number of animals see Table 1A. Further details in text.

RESULTS

CCK-8 in doses of 5 or 10 μg/kg IP reduced the 30 min food intake of the animals by 60 and 68%, respectively, after 24 hr deprivation, $F(2,54)=9.45$, $p<0.01$. The reductions in food intake were highly significant as compared to the food intake of the deprived control group (Table 1A.).

In our preliminary experiments, when the food intake-suppressing potency of our CCK-8 preparation was evaluated, we observed that a proportion of CCK-8-treated animals failed to respond to CCK-8 treatment, and their food intake remained at about the control level. In the present series of experiments, from the large number of CCK-8-treated rats two subgroups were selected according to the amount of food eaten in the behavioral test. The subgroups were designated well-responder and non-responder, and their respective data represent animals with food intakes less (well-responder) or more (non-responder) than the mean food intake of all the 5 or 10 μg/kg CCK-8-treated rats minus or plus twice the standard error of the mean (Table 1B). There is a highly significant difference between the well-

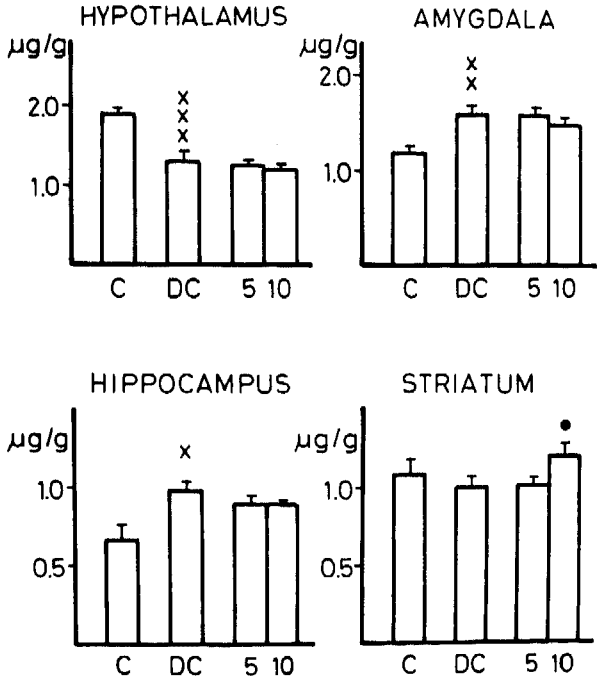

FIG. 2. Effects of intraperitoneal CCK-8 injection on the 5-hydroxytryptamine levels of denoted rat brain areas, when 24 hr deprivation was followed by 30 min feeding. For abbreviations see Fig. 1. ×,●: $p<0.05$; ××: $p<0.01$; ×××: $p<0.001$ (t-test). Further details in text.

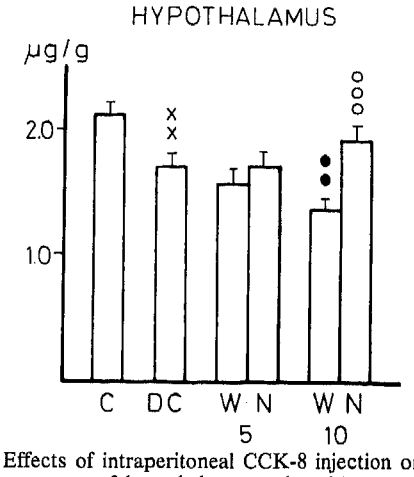

FIG. 3. Effects of intraperitoneal CCK-8 injection on the norepinephrine contents of hypothalamus, when 24 hr deprivation was followed by 30 min feeding. W: well-responder; N: non-responder subgroups within the 5 or 10 μg/kg CCK-8-treated groups. Open circles: significant changes versus well-responder subgroup. For abbreviations see Fig. 1. For the number of animals see Table 1A and 1B. Further details in text.

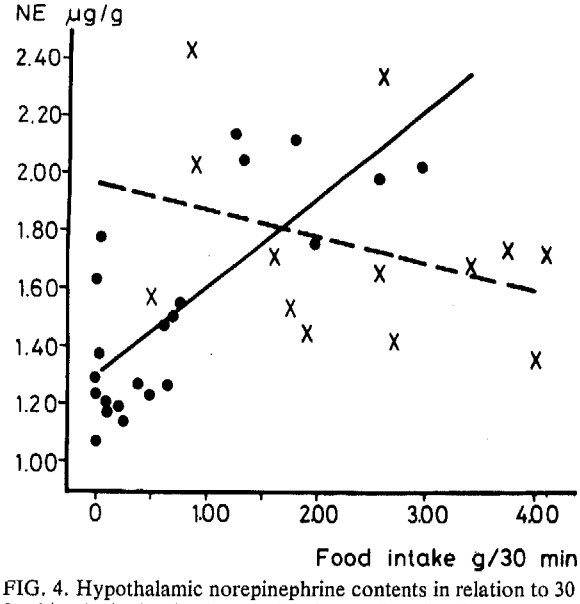

FIG. 4. Hypothalamic norepinephrine contents in relation to 30 min food intake in deprived control and 10 μg/kg CCK-8-treated groups. Individual data and corresponding regression lines are shown. Asterisks and dotted line: deprived control group; dots and continuous line: CCK-8-treated group. For the equations of regression lines and for correlation coefficients see Table 2.

responder and non-responder subgroups, and the animals in the non-responder groups ingested as much food as those in the saline-treated deprived groups.

Transmitter levels in the deprived control group were changed as compared to the non-deprived control group in the brain areas dissected immediately after food intake testing (with the exception of the mesencephalon, in which no changes could be observed). As compared to the non-deprived control group (n=12, t-test) the DA levels were significantly decreased in the hypothalamus and striatum, and significantly increased in the hippocampus (Fig. 1). There was no change in the DA level of the amygdala. The 5-HT levels of the hypothalamus were decreased as compared to the non-deprived control group, while those of the amygdala and hippocampus were increased and no difference could be observed in the striatum (Fig. 2). The NE content was decreased only in the hypothalamus in the deprived control group (Fig. 3).

When all the CCK-8-treated animals' data were compared to the deprived control group, both doses of CCK-8 decreased the DA content of the hypothalamus (for the deprived groups, $F(2,54)=3.88$, $p<0.05$, Fig. 1). No other changes were observed in the DA contents after CCK-8 treatment. CCK-8 injection had no significant effect on the 5-HT levels of the brain areas, with the exception of the striatum, where it was increased by 10 mg/kg CCK-8, $F(2,54)=3.53$, $p<0.05$, Fig. 2. The NE contents of the brain structures were not changed by either dose of CCK-8, when the data of all of the 5 or 10 μg/kg CCK-8-treated animals were compared with those of the deprived control group, and no changes of transmitter contents were observed in the mesencephalon after CCK-8 treatments (data not shown).

The well-responder and non-responder subgroups of CCK-8-treated groups did not differ significantly from each other as regards the respective DA or 5-HT contents of the brain areas (data not shown). Figure 3 shows that after treatment with 10 μg/kg CCK-8 the NE contents of the hypothalamus were significantly, $F(4,41)=5.83$, $p<0.01$, lowered in the well-responder group as compared to the deprived control group, and significantly enhanced in the non-responder group as compared to the well-responder group

TABLE 2

LINEAR REGRESSION ANALYSIS OF HYPOTHALAMIC NE LEVELS IN RELATION TO AMOUNT OF FOOD EATEN IN DEPRIVED CONTROL AND CCK-8-TREATED GROUPS (FOR FURTHER DETAILS SEE TEXT)

Groups	Y'=a+bX	r	*df*	Significance
Deprived control	Y'=1.955−0.089X	−0.319	10	N.S.
CCK-8 5 μg/kg	Y'=1.361+0.168X	0.460	20	$p<0.05$
CCK-8 10 μg/kg	Y'=1.297+0.311X	0.773	20	$p<0.01$

Y'=a+bX: equation of regression line.
r: Correlation coefficient.
df: Degree of freedom.

TABLE 3

LINEAR REGRESSION ANALYSIS OF DA LEVELS IN THE HYPOTHALAMUS AND AMYGDALA IN RELATION TO AMOUNT OF FOOD EATEN IN DEPRIVED CONTROL AND CCK-8-TREATED GROUPS (FOR FURTHER DETAILS SEE TEXT)

Groups	Y'=a+bX	r	*df*	Significance
Hypothalamus				
Deprived control	Y'=0.688−0.087X	−0.387	10	N.S.
CCK-8 5 μg/kg	Y'=0.436−0.072X	−0.394	20	N.S.
CCK-8 10 μg/kg	Y'=0.381+0.015X	0.143	20	N.S.
Amygdala				
Deprived control	Y'=0.132+0.184X	0.651	10	$p<0.05$
CCK-8 5 μg/kg	Y'=0.403+0.024X	0.115	20	N.S.
CCK-8 10 μg/kg	Y'=0.456−0.062X	−0.339	19	N.S.

For abbreviations see Table 2.

(*t*-test). The hypothalamic NE contents did not change significantly, when the data of all CCK-8-treated animals were compared to those of the saline-treated deprived animals (means: deprived control: 1.75±0.10, 5 mg/kg CCK-8: 1.53±0.08, 10 mg/kg CCK-8: 1.52±0.08; $F(2,53)=1.77$; N.S.).

The individual hypothalamic NE contents were also plotted as a function of the corresponding food intake values for the deprived control and CCK-8-treated groups. The results obtained after linear regression analysis are shown in Table 2. In the control group no significant correlation could be demonstrated between the two variables. After CCK-8 treatments the correlation coefficients were enhanced dose-dependently and showed significant positive correlations. Figure 4 shows the individual points for the deprived control and 10 mg/kg CCK-8-treated groups, together with the corresponding regression lines obtained. Analysis of covariance was also carried out for the three populations of samples, and clarified that the variances in the data populations were significantly different, $F(4,50)=3.784$, $p<0.01$, and the variances of the regression coefficients calculated were also significantly different, $F(2,50)=7.492$, $p<0.01$.

Linear regression analysis demonstrated no significant correlation for the hypothalamic DA contents and the amount of food eaten in the deprived control and CCK-8-treated groups (Table 3); indicating that the hypothalamic DA contents decreased equivocally in all the CCK-8-treated animals, and the relation observed for the NE contents is not the result of application failures.

Linear regression analysis also revealed that there is a positive correlation between the DA contents of the amygdala and the amount of food ingested in the deprived control group (Table 3). This correlation disappeared after CCK-8 treatments.

DISCUSSION

Several metabolic, hormonal and neural factors may influence the control of nutrition of animals under normal conditions. The catecholamine neuron systems have been implicated by several authors in the regulation of feeding behavior. Destruction of the nigrostriatal DA pathway by 6-hydroxydopamine has been shown to result in aphagia and adipsia as well as impaired sensory-motor functions [29]. In contrast, selective chemical lesions of the ascending noradrenergic fibers in the ventral part of the central tegmental tract cause overeating and weight gain [1]. The intrahypothalamic application of NE can elicit feeding [9,20] by activating alpha-adrenergic receptors [4]. In the hypothalamus, which has a crucial role in control over feeding (for a review see [11]), changes have been observed in the *in vivo* release of NE in rats during food ingestion [15, 23, 31], and there is a significant positive correlation between the *in vitro* uptake of NE in the hypothalamic tissue and the

amount of food eaten, if the last meal terminated within 15 min before decapitation of the animals [30]. The above results clearly demonstrate a vital role of NE in the mechanisms underlying the hypothalamic control of feeding.

The results presented here also indicate that deprivation followed by feeding could induce changes in the monoamine contents of the brain structures studied. The most pronounced effects were observed in the hypothalamus, where the contents of all three monoamines were decreased after the 30 min feeding period in deprived rats. In addition, the DA and 5-HT levels were increased in the hippocampus, as were the 5-HT levels in the amygdala, while the DA levels were decreased in the striatum, and there was a significant positive correlation between the amount of food ingested and the DA content of the amygdala in the deprived control group. Lesion and stimulation studies revealed that parts of the limbic system could have a modulatory role in the execution of feeding behavior (for a review see [10]), probably by changing its emotional and/or sensory components, and the alterations presented here in the transmitter levels of limbic areas might reflect changes in neuronal activity specific for feeding.

The action of CCK-8 on the hypothalamic noradrenergic pathways (presumably transmitted by vagal fibers from the periphery) seems to be relevant for its satiety-inducing effect, as peripheral CCK-8 injection antagonized NE-evoked food intake, and elevated *in vivo* NE release in hypothalamic loci, where intraduodenal food exerted the same effect [21,22]. In our experiments CCK-8 injected intraperitoneally caused the most striking changes in the DA and NE contents of the hypothalamus and the DA contents of the amygdala.

The DA contents of hypothalamus were equivocally decreased after CCK-8 as compared to the deprived control group, and no relation to the amount of food ingested could be demonstrated, thus this effect is not unambiguously connected with the food intake-suppressing effect of the peptide. The deterioration of positive correlation shown in the control group between food intake and amygdaloid DA contents is obviously resulted by the peptide treatment, and might reflect the altered motivational state of CCK-8-treated animals.

The most important finding seems to be the relation of the hypothalamic NE content to the amount of food eaten, shown after CCK-8 treatment. The effect was dose-dependent and could not be observed in the control animals, and seems to be specific for the ability of CCK-8 to suppress feeding.

The mechanism by which CCK-8 interconnects the hypothalamic NE contents to the amount of food eaten is not clear. It is possible that the sensitivity of the peripheral (and probably the central) CCK receptors are simply different in the population of rats studied, or, as there was no correlation between the hypothalamic DA content and the food intake following CCK-8 treatment, the animals in the higher motivational state can specifically override the food intake-suppressing effect of CCK-8, and the consequent changes in noradrenergic transmission, and thus the relation observed between the hypothalamic NE contents and the amount of food ingested are overall resultants of much more complex synaptic events.

In conclusion, the present study confirms the involvement of the hypothalamic noradrenergic system in the "satiety" effect of CCK, but additional investigations must be designed to acquire an insight into the biochemical background of the central neuronal changes underlying the mechanism by which the peptide exerts its food intake-suppressing effect.

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REFERENCES

1. Ahlskog, J. E. and B. G. Hoebel. Overeating and obesity from damage to a noradrenergic system in the brain. *Science* **182:** 166–169, 1973.
2. Anika, S. M., T. R. Houpt and K. A. Houpt. Satiety elicited by cholecystokinin in intact and vagotomized rats. *Physiol Behav* **19:** 761–766, 1977.
3. Antin, J., J. Gibbs, J. Holt, R. C. Young and G. P. Smith. Cholecystokinin elicits the complete behavioral sequence of satiety in rats. *J Comp Physiol Psychol* **89:** 784–790, 1975.
4. Booth, D. A. Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J Pharmacol Exp Ther* **160:** 336–348, 1968.
5. Della-Fera, M. A. and C. A. Baile. Cholecystokinin octapeptide: Continuous picomole injections into the cerebral ventricles of sheep suppress feeding. *Science* **206:** 471–473, 1979.
6. Fekete, M., A. Szabó, M. Balázs, B. Penke and G. Telegdy. Effects of intraventricular administration of cholecystokinin octapeptide on active avoidance and conditioned feeding behaviour of rats. *Acta Physiol Acad Sci Hung* **58:** 39–45, 1981.
7. Gibbs, J., R. C. Young and G. P. Smith. Cholecystokinin decreases food intake in rats. *J Comp Physiol Psychol* **84:** 488–495, 1973.
8. Gibbs, J., R. C. Young and G. P. Smith. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* **245:** 323–325, 1973.
9. Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* **132:** 301–302, 1960.
10. Grossman, S. P. Neurophysiologic aspects: extrahypothalamic factors in the regulation of food intake. In: *Hunger and Satiety in Health and Disease, Advances in Psychosomatic Medicine*, vol 7, edited by F. Reichsman. Basel: Karger, 1972, pp. 49–72.
11. Grossman, S. P. Role of hypothalamus in the regulation of food and water intake. *Psychol Rev* **82:** 200–224, 1975.
12. Jacobowitz, D. M. and J. S. Richardson. Method for rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. *Pharmacol Biochem Behav* **8:** 515–519, 1978.
13. Lorenz, D. N. and S. A. Goldman. Vagal mediation of the cholecystokinin satiety effect in rats. *Physiol Behav* **29:** 599–604, 1982.
14. Maddison, S. Intraperitoneal and intracranial cholecystokinin depress operant responding for food. *Physiol Behav* **19:** 819–824, 1977.
15. Martin, G. E. and R. D. Myers. Evoked release of (^{14}C)-norepinephrine from rat hypothalamus during feeding. *Am J Physiol* **229:** 1547–1555, 1975.
16. McCaleb, M. L. and R. D. Myers. Cholecystokinin acts on the hypothalamic noradrenergic system involved in feeding. *Peptides* **1:** 47–49, 1980.
17. Morley, J. E. The ascent of cholecystokinin (CCK)—from gut to brain. *Life Sci* **30:** 479–493, 1982.
18. Morley, J. E., A. S. Levine, J. Kneip and M. Grace. The effect of vagotomy on the satiety effects of neuropeptides and naloxone. *Life Sci* **30:** 1943–1947, 1982.

19. Mueller, K. and S. Hsiao. Current status of cholecystokinin as a short-term satiety hormone. *Neurosci Biobehav Rev* **2**: 79–87, 1978.
20. Myers, R. D. Chemical mechanisms in the hypothalamus mediating eating and drinking in the monkey. *Ann NY Acad Sci* **157**: 918–933, 1969.
21. Myers, R. D. and M. L. McCaleb. Feeding: satiety signal from intestine triggers brain's noradrenergic mechanism. *Science* **209**: 1035–1037, 1980.
22. Myers, R. D. and M. L. McCaleb. Peripheral and intrahypothalamic cholecystokinin act on the noradrenergic "feeding circuit" in the rat's diencephalon. *Neuroscience* **6**: 645–655, 1981.
23. Myers, R. D., M. L. McCaleb and K. A. Hughes. Is the noradrenergic "feeding circuit" in the hypothalamus really an olfactory system? *Pharmacol Biochem Behav* **10**: 923–927, 1979.
24. Ricardo, J. A. and E. T. Koh. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* **153**: 1–26, 1978.
25. Smith, G. P., C. Jerome, B. J. Cushin, R. Eterno and K. J. Simansky. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* **213**: 1036–1037, 1981.
26. Stern, J. J., C. A. Cudillo and J. Kruper. Ventromedial hypothalamus and short-term feeding suppression by caerulein in male rats. *J Comp Physiol Psychol* **90**: 484–490, 1976.
27. Szabó, G., G. L. Kovács and G. Telegdy. A modified screening method for rapid simultaneous determination of dopamine, noradrenaline, and serotonin in the same brain region. *Acta Physiol Hung* **61**: 51–57, 1983.
28. Telegdy, G., M. Fekete and T. Kádár. Cholecystokinin in the brain. *Intern Med* **2**: 2–5, 1982.
29. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. *Acta Physiol Scand (Suppl)* **367**: 95–122, 1971.
30. Van der Gugten, J. and J. L. Slangen. Norepinephrine uptake by hypothalamic tissue from the rat related to feeding. *Pharmacol Biochem Behav* **3**: 855–860, 1975.
31. Van der Gugten, J. V. and J. L. Slangen. Release of endogenous catecholamines from rat hypothalamus *in vivo* related to feeding and other behaviors. *Pharmacol Biochem Behav* **7**: 211–219, 1977.