Korean J. of Pharmacology
Vol. 30, No. 1, pp. 39-48, 1994

Immunohistochemical and Biochemical Studies on Dopamine Content in Rat Brain During Cholecystokinin-Induced Suppression of Feeding

Joo-Ho Chung*, Yang-Che Kim, Sung-Vin Yim, Seung-Joon Park, Kye-Chang Ko and Jee-Chang Jung

Department of Pharmacology, College of Medicine, Kyung Hee University, Seoul 130-710, Korea

ABSRTACT

The present study was aimed at establishing what changes occur in the dopamine levels and pattern of TH-immunoreactive neurons of certain areas of rat brain during food intake suppression produced by intraperitoneally administration of CCK-8. CCK-8 in dose of 10 μg/kg was injected intraperitoneally to 48 h food-deprived rats. In the fasted group, the contents of dopamine were decreased in the frontal, striatum, hypothalamus and amygdala as compared to those of the fed control group. The administration of CCK-8 showed significant decrease on the dopamine levels of the hypothalamus, in comparison to those of the sated and starved group. During deprived condition, the density and number of TH-immunoreactive neurons in the paraventricular nucleus, arcuate nucleus, median eminence and substantia nigra were lower than those of the fed control group. After administration of CCK-8, the pattern and distribution of TH-positive neouons in the hypothalamic areas and substantia nigra were increased when compared to those of the starved group. It is concluded that the results domonstrate the partial involvement of hypothalamic dopamine-containing neurons in the feeding inhibition of CCK-8. Furthermore, the results indicate that TH-immunoreactive neurons play on important role in the hypothalamus and substantia nigra for eating behavior

Key Words: Dopamine, Tyosine hydroxylase, TH positive neuron, TH-immunoreactive neuron, CCK-8

INTRODUCTION

Multiple brain neurotransmitters are now believed to play a role in the control of food intake and patterns of meal-taking behavior. In this process, the medial and lateral portions of the hypothalamus, working in conjunction with forebrain and hindbrain sites and with peripheral autonomic endocrine systems, have a critical responsibility in balancing signals for hunger and satiety (Baile *et al.*, 1986; Leibowitz, 1986; Morley, 1987). Via its rich and biologically active neurotransmitter substances, the hypothalamus monitors and integrates the complex sensory and metabolic input concerning the nutritional status of the organism and transduces this information into appropriate quantitative and qualitative adjustments in food intake (Minano *et al.*, 1989; Tsai *et al.*, 1984).

These neurotransmitters include the monoamines, namely, norepinephrine, dopamine, epinephrine, and serotonin; the amino acid, γ-aminobutyric acid (GABA); and a variety of

*To whom correspondence should be addressed.

neuropeptides, such as cholecystokinin (CCK), pancreatic polypeptides, hormone-releasing factors (Leibowitz, 1986).

The role of dopamine in feeding behavior is controversial. The dopaminergic agonist, bromergocryptine, will enhance feeding at low doses while inhibiting it at higher doses, when the animal develop stereotypic behavior (Essatara *et al.*, 1984). Further evidence for the role of dopamine in feeding comes from the studies of Levin *et al.*, (1986) which showed that feeding a high calorie, high fat diet produced a diffuse acceleration of brain dopamine turnover. Chronic obesity, on the other hand, is associated with a decreased dopamine turnover in the hypothalamus.

CCK was originally described as a classical gastrointestinal peptide hormone that stimulates gall bladder contraction and pancreatic enzyme secretion (Noran and McHugh, 1982; Schick *et al.*, 1989; Shillabeer and Davison, 1987). CCK-containing neurons are also widely distributed throughout the brain, with the exception of the cerebellum, which is apparently devoid of CCK (Schick *et al.*, 1990). It has been reported that CCK may be involved in control of various physiological events including hypothermia, analgesia, sedation, anxiety and satiety (Singh *et al.*, 1991). Accumulating evidence has suggested that CCK concentrations vary with the fed or fasted condition of animal and that administration of CCK peptides decreased food intake (Kadar *et al.*, 1984). The importance of the hypothalamus in this function is reflected by neurochemical changes specifically in this structure that occur in relation to eating (Schick *et al.*, 1989).

Inasmuch as brain peptides and monoamines are known to coexist in a single neuron, it has been proposed that these substances may interact closely in their control of appetite for food (Debonnel and de Montigny, 1988; Rybarczyk *et al.*, 1990). It has been discovered that the neuropeptide CCK is co-localized with dopamine (Fukamauchi *et al.*, 1992; Hurd *et al.*, 1992; Oeth and Lewis, 1992; Seroogy *et al.*, 1989; Seroogy *et al.*, 1988).

However, the immunohistochemical and biochemical study on dopamine during fasting or CCK-8-induced feeding suppression has not been clearly documented.

In order to investigate the neuropharmacological linkage between biochemical and immunohistochemical changes on the dopamine in the rat brain, the dopamine contents of the frontal cortex, striatum, hypothalamus, amygdala, and hippocampus are measured by high performance liquid chromatography with electrochemical detection (HPLC-ECD) (Dizdar *et al.*, 1991), and the distribution and morphologic changes of tyrosine hydroxylase (TH)-immunoreactive neurons in the hypothalamus and substantia nigra are studied by immunohistochemical method and image analysis (Anthony and King, 1986; Hsu *et al.*, 1981).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, 250~350 g, were used throughout the experiment. Animals were housed in groups of six on a 12:12 hours light: dark cycle, with food and water provided *ad libitum*. With the exception of the fed control group, food was withdrawn from the animals 48 h before the start of the experiment.

On the day of the experiment, the starved animals were assigned to receive an intraperitoneal injection of either CCK dissolved in saline (10 μg/kg), or normal saline. For the food intake testing, each deprived rat was given to access to a preweighted amount of food (approximately 30g) for 30 min. Rats of the sated group were also injected with normal saline at the appropriate time. After the 30 min feeding test, animals were removed from the cages and sacrified (Kadar *et al.*, 1984).

Chemical reagents

The chemicals used and their souces were as follows: CCK-8, norepinephrine hydrochloride, 3-hydroxytyramine hydrochloride (dopamine hydrochloride), 3, 4-dihydroxybenzylamine hydrobromide (DHBA), nomochloroacetic acid, disodium ethylenediamine tetraacetic acid (disodium EDTA), 1-octanesulfonic acid, sodium acetate, silver nitrate, sucrose, 3, 3'-diaminobenzidine tetrahydrochloride (DAB), ascorbic acid, cetylpyridinium chloride, sodium tungstate from Sigma; anti-tyrosine hydroxylase from Eugene Tech;

paraformaldehyde from EMS; sodium hydroxide from Wako; xylene from Yakuri; perchloric acid from Hayashi; methanol from Mallinckrodt. All other chemicals were reagent grade best commercially available. Water was deionized and glass-distilled.

Determination of dopamine

All animals were sacrificed, and their brains were quickly removed and dissected into the following areas; frontal cortex, striatum, hypothalamus, amygdala and hippocampus. The dissected areas were deep-frozen on dry ice and stored at −70℃ until assayed. Individual brain regions were homogenized in 1.0 ml of 0.17 M perchloric acid, containing 200 ng of DHBA as an internal standard, for 30 sec with the use of a ultrasonic homogenizer at 300 watt. The homogenates were centrifuged at 12,000 g for 15 min at 4℃. The supernatants were seperated and filterd through a 0.2 μm sylinge filter (Nalgene). The resulting filtrates were either immediately analyzed or stored at −70℃ for later assay.

The dopamine concentrations were determined by HPLC-ECD. The HPLC systems were performed with a pump (Waters 510), an injector (Waters U6K), an integrator (Waters 745B), and an electrochemical detector (Waters 460). A glassy carbon working electrode was set at +0.75 V vs a Ag/AgCl reference electrode. Seperation was accomplished using a Nova-Pak C_{18} column (3.9×150 mm, Waters) preceded by a guard column (Waters). The mobile phase was Monochloroacetic acid buffer (0.15 M, pH 4.0) containing 20 mg/l EDTA·2Na, 144 mg/l sodium octanesufonate, and 17% methanol, which was pumped at a flow rate of 0.8 ml/min.

Statistical analysis of the results was perfomed using Kruskal-Wallis test and Mann-Whitney test. A p-value of less than 0.05 was accepted as a level of statistical significance.

Immunohistochemistry

The animals were terminally anesthetized with ether and perfused throught the heart with 0.012 M phosphate buffered saline (PBS,pH 7.4) for 9 min at a flow rate of 100 ml/min.

The brain was removed immediately and placed in a tube containing 4% paraformaldehyde in phosphate buffer for overnight at 4℃. For cryoprotection, the fixative was decanted and replaced with cold graded sucrose solutions of 12, 16, and 18% in 0.12 M PB. Frozen sections were cut at 40 μm thickness with a Cryocut (American Optical).

Immunohistochemical procedures were performed as described previously (Anthony and King, 1986; Hsu *et al.*, 1981). Briefly, antigens were stained on free-floating sections using the avidin-biotin-peroxidase complex (ABC) method. Sections were incubated at 4℃ in primary antibody solution (1 : 4000 dilution of anti-tyrosine hydroxylase) for 48～72 h. Then the tissues were incubated in secondary antibody solution (1 : 200 dilution of anti-rabbit IgG) for 1 h at room temperature. The sections were incubated for 1 h in ABC solution. The sections were incubated for 1 h in ABC solution. The primary antibody was visualized using ABC Kit (Vector Labs). The sections were developed for peroxidase reactivity with freshly prepared Ni-DAB solution. For increasing the sensitivity of immunoperoxidase staining, silver intensification was perfomed.

The atlas of Paxinos and Watson (1986) was used for the analysis of TH-immunoreactive neurons of the brain.

Image analysis

Image analysis as applied to immunohistochemistry was designated to extract labeled profiles from background and measured them. Image analyzer (Nireco) analyzes images obtained from a television camera (Ikegami) attached to an optical microscope (Olympus). In this experiment, the measurements of TH-immunoreactive cell bodies were number and area per unique field (8,500 μm^2).

Statistical analysis of the results was perfomed using one-way analysis of variance and Student's t-test. A p-value of less than 0.05 was accepted as a level of statistical significance.

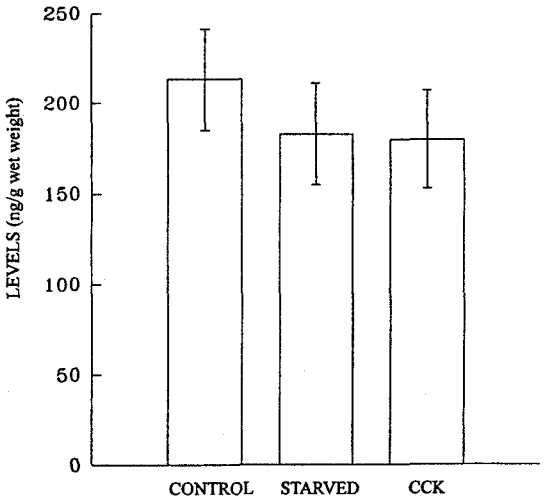

Fig. 1. Effect of CCK-8 on the dopamine levels of the frontal cortex.
CONTROL: non-deprivation, 6 cases
STARVED: 48 h deprivation, 6 cases
CCK: CCK-8 injection after 48 h deprivation (10 μg/kg, i.p.), 6 cases

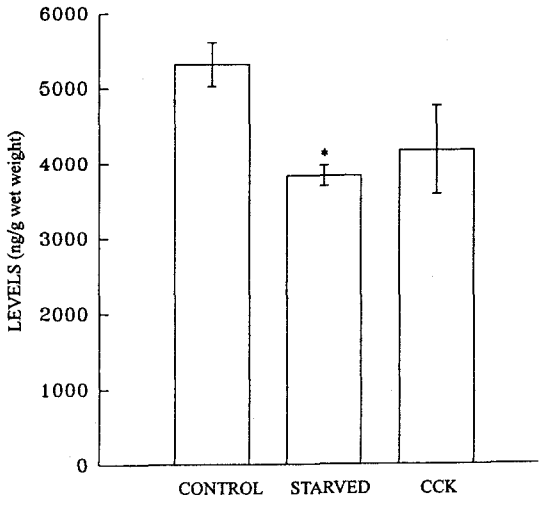

Fig. 2. Effect of CCK-8 on the dopamine levels of the striatum.
CONTROL: non-deprivation, 6 cases
STARVED: 48 h deprivation, 6 cases
CCK: CCK-8 injection after 48 h deprivation (10 μg/kg, i.p.), 6 cases
*$p<0.05$, compared to control group, obtained with Kruskal-Wallis and Mann-whitney tests.

Table 1. Effedt of CCK-8 on the 30 min food intake testing after 48 hr deprivation

Groups	Food intake(g) Mean±standard error	Number of amimals
Deprived control	2.33±0.27	8
CCK-8(10μg/kg, i.p.)	0.70±0.12*	8

Data are means ±standard error of the mean.
*Statistically significant versus deprived control, $p<0.05$ (Student's t-test)

RESULTS

Effect of CCK-8 on food intake

Under the 48 h starved condition, the food intake during 30 min testing period was measured as 2.33±0.27 g. After administration of CCK-8 in the fasted rats, the food intake during 30 min testing was measured as 0.7±0.12 g (Table 1).

CCK-8 reduced the 30 min food intake of the rats by 70%, after 48 h deprivation. The reduction in food intake was significant as compared to that of the deprived animals.

Dffect of CCK-8 on content of dopamine in different brain regions

The administration of CCK-8 in the 48 h deprived rats showed slight decrease on the dopamine contents of the frontal cortex, in comparison to those of the fed contol group and fasted group (Fig. 1)

It is showed that by the administration of CCK-8 the content of dopamine in the striatum was decreased as compared to the sated control group. And in the 48 h deprived group, the dopamine level of the striatum was significantly decreased, when compared to the fed control group (Fig. 2).

It is evident that the fasted group and CCK-8-administered group produced significant decrease of the dopamine levels in the hypothalamus when

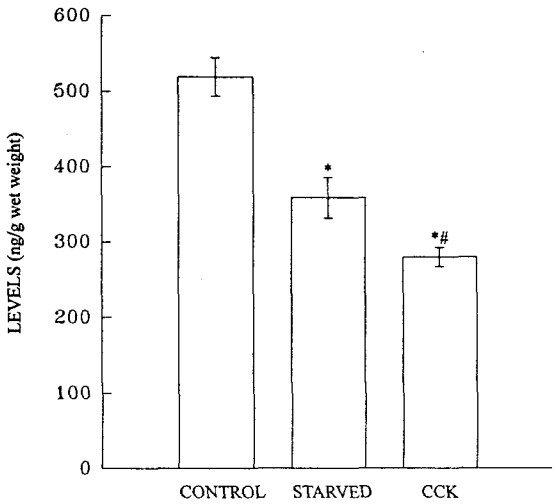

Fig. 3. Effect of CCK-8 on the dopamine levels of the hypothalamus.
CONTROL: non-deprivation, 6 cases
STARVED: 48 h deprivation, 6 cases
CCK: CCK-8 injection after 48 h deprivation (10 μg/kg, i.p.), 6 cases
*$p<0.05$, compared to control group, obtained with Kruskal-Wallis and Mann-Whitney tests.
#$p<0.05$, compared to starved group, obtained with Kruskal-Wallis and Mann-Whitney tests.

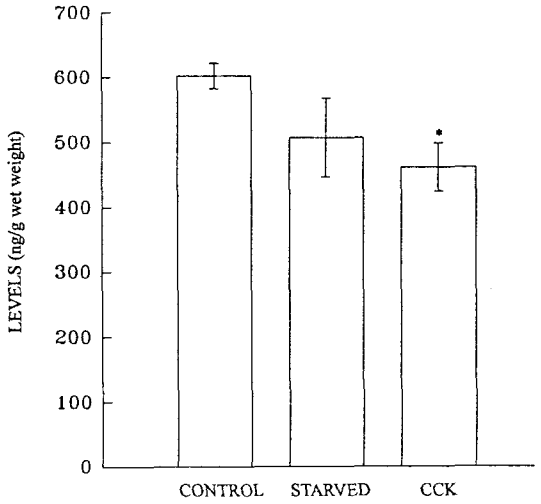

Fig. 4. Effect of CCK-8 on the dopamine levels of the amygdal.
CONTRAL: non-deprivation, 6 cases
STARVED: 48 h deprivation, 6 cases
CCK: CCK-8 injection after 48 h deprivation (10μg/kg, i.p.), 6 cases
*$p<0.05$, compared to control group, obtained with Kruskal-Wallis and Mann-Whitney tests.

compared to that of the fed control group (Fig. 3).

The administration of CCK-8 showed significant decrease on the dopamine content of the amygdala, as compared to the fed control group. In the 48 h starved group, the level of dopamine was decreased in the amygdala when compared to the sated control data, but no significance was shown between two groups (Fig. 4).

In the 48 h deprived group, the content of dopamine in the hippocampus was increased as compared to the fed control level. It is also evident that CCK-8 injection produced only slight decrease on the dopamine levels of the hippocampus when compared to those of the sated control group (Fig. 5).

Immunohistochemistry and image analysis on tyrosine hydroxylase in hypothalamus and substantia nigra

Paraventricular nucleus: In the fed control group, TH-immunoreactive nerve fibers were gen-

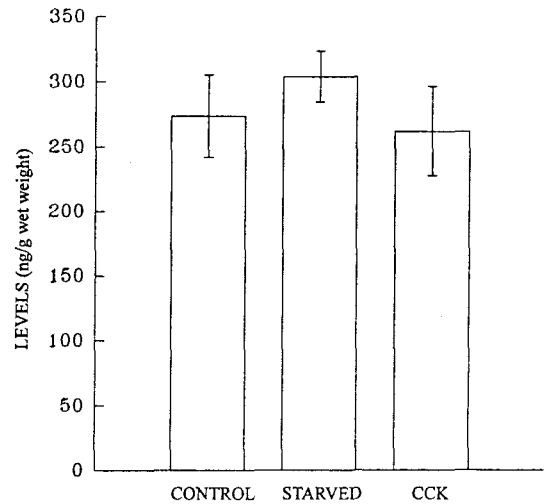

Fig. 5. Effect of CCK-8 on the dopamine levels of the hippocampus.
CONTROL: non-deprivation, 6 cases
STARVED: 48 h deprivation, 6 cases
CCK: CCK-8 injection after 48 h deprivation (10μg/kg,i.p.), 6 cases

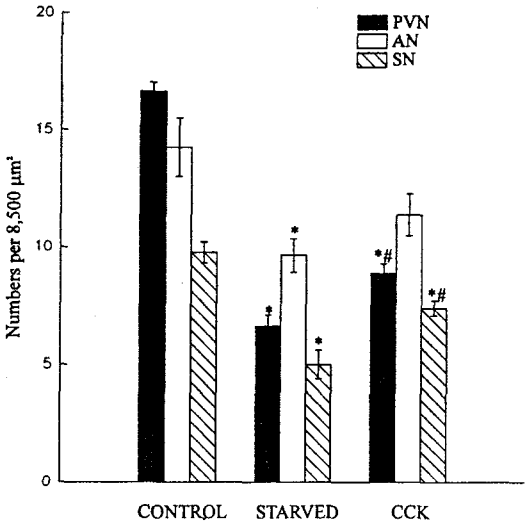

Fig. 6. Number of tyrosine hydroxylase immunoreactive neurons.
CONTROL: non-deprivation, 8 cases
STARVED: 48 h deprivation, 8 cases
CCK: CCK-8 injection after 48 h deprivation (10μg/kg,i.p.), 8 cases
PVN: Paraventricular nucleus
AN: Arcuate nucleus
SN: Substantia nigra
Data are mean+/−S.E. of the numbers per 8, 500μm².
*$p<0.05$, compared to control group, obtained with one-way analysis of variance and Student's *t*-test.
#$p<0.05$, compared to starved group, obtained with one-way analysis of variance and Student's *t*-test.

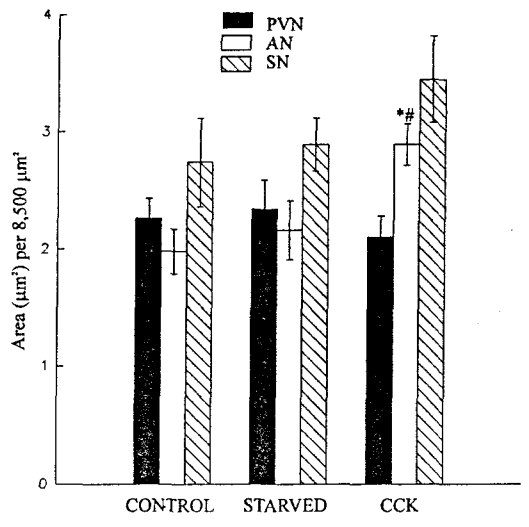

Fig. 7. Area of tyrosine hydroxylase immunoreactive neurons.
CONTROL: non-deprivation, 8 cases
STARVED: 48 h deprivation, 8 cases
CCK: CCK-8 injection after 48 h deprivation (10 μg/kg, i.p.), 8 cases
PVN: Paraventricular nucleus
AN: Arcuate nucleus
SN: Substantia nigra
Data are mean+/−S.E. of the area (μm²) per 8, 500 μm².
*$p<0.05$, compared to control group, obtained with one-way analysis of variance and student's *t*-test.
#$p<0.05$, compared to starved group, obtained with one-way analysis of variance and Student's *t*-test.

erally fine and transverse. The TH-positive cell bodies in this region showed pyramidal or oval pattern (photomicrograph not shown). In the fasted group, the number of TH-immunoreactive cell bodies were reduced, when compared to those of the fed control group. And the density and number of TH-positive neurons of the CCK-8 administered group were increased as compared to the starved group (Fig. 6).

Arcuate nucleus: In the sated control group, TH-positive fibers located near the third ventricle were more dense than those of lateral area. Biopolar or multipolar neurons were observed in this region (photomicrograph not shown). In the starved group, the number of TH-immunoreactive cell bodies were reduced, when compared to the sated control group (Fig. 6). The administration of CCK-8 produced increase on the number and area of TH-positive cell bodies when compared to those of the deprived group (Fig. 6, 7).

Median eminence: In the fed control group, TH-immunoreactive fibers were highly distributed in all regions of the median eminence. Under the 48 h deprived condition, the density of TH-positive neuron was lower than that of the sated control group. After administration of CCK-8, the im-

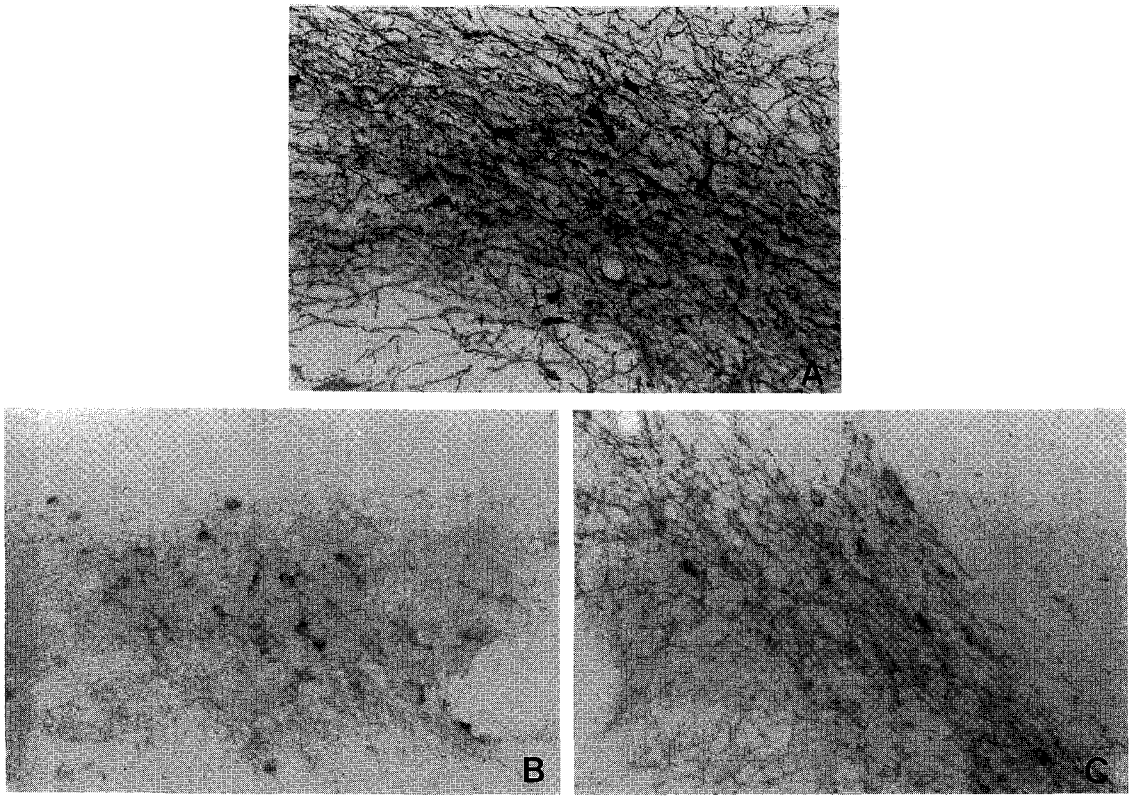

Fig. 8. Photomicrography of tyrosine hydroxylase immunoreactive nerve fibers in substantia nigra. X200.
A: CONTROL, B: STARVED, C: CCK

munoreactivity of TH-positive neuron was increased in this region when compared to that of the fasted group (photomicrograph not shown).

Substantia nigra: In the fasted group, the number of TH-immunoreactive cell body was lower than that of the sated control gruop, In the CCK-treated group, the number and area of TH-positive neuron was increased when compared to those of the starved group (Fig. 6, 7, 8).

DISCUSSION

Studies of brain monoamines and neuropeptides have provided extensive evidence in support of their role in the control of normal eating behavior (Baile *et al.*, 1986; Leibowitz, 1986). The specific neurotransmitters for which there is the most extensive evidence for a physiological function include the eating-stimulatory substances norepinephrine, opioid peptides, pancreatic polypeptides, growth hormone-releasing factor, and γ-aminobutyric acid; the eating-inhibitory substances dopamine, epinephrine, serotonin, cholecystokinin, neurotensin, calcitonin, glucagon, and corticortropin-releasing factor; and possible other gut-brain peptides (Leibowitz, 1986; Morley, 1987).

The potential physiological importance of lateral hypothalamic catecholaminergic system in the control of natural eating behavior has recently become clearer through a variety of biomedical and pharmacological studies. For example, food deprivation, the eating process, and diet infusion into the gut each produce predictable changes in catecholamine release and receptor activity within the perifornical hypothalamus (Tsai *et al.*, 1984). Further, with blockade of dopamine recep-

tors in this area via hypothalamic as well as peripheral administration of neuroleptics, food ingestion and ultimately body weight gain are enhanced (Leibowitz *et al.*, 1981). The dopamine antagonist, pimozide, suppressed the intake of sacchrine-glucose solution more than plane water, suggesting that dopaminergic neurons mediate the opsitive effects of sweet taste on consumption (wise, 1982).

In the starved conditions, it has been reported in the literatures that the monoamine levels in the rat brain reduced. Minano *et al.*, (1989) observed that the content of dopamine in the hypothalamus during fasted state was slightly decreased as compared to the sated group. When the rats were fasted, the administration of CCK reduced dopamine concentrations in the hypotha-lamus (Kadar *et al.*, 1984).

In the present study, CCK-treated rats showed decrease on dopamine levels of the hypothalamus, in comparison to those of the fed control group and fasted group. These results may confirm the involvement of the hypothalamic dopaminergic system in the "satiety" effect of CCK.

Subsequent studies have demonstrated that systemically adminstered CCK can inhibit food consumption in a variety of species, such as rats (Smith *et al.*, 1985), guinea pigs (Larsson and Rehfeld, 1979), pigs (Rehfeld, 1978), monkeys (Beinfelt *et al.*, 1983), and humans (Emson *et al.*, 1982). The effect of fast length on the response to CCK had been measured in few studies. In rats, 1 μg/kg CCK-8 decreased food intake by 70% of control after a 12 h fast but had no effect after a 48 h fast; 10 μg/kg was required to produce a simiar reduction in 48 h fasted rats (Billington *et al.*, 1983).

From this study, CCK-8 in dose of 10 μg/kg (i. p.) reduced the 30 min food intake of the animals by 70%, after 48 h deprivation. The reduction in food intake was significant as compared to the food intake of the deprived group. These results are consistent with the previous data on the food intake-depressing effect of CCK.

Immunohistochemistry is one of the most powerful techniques available for identifying and mapping the distribution of characterized neuronal systems in the brain (Anthony and King, 1986; Hsu *et al.*, 1981). TH is the rate-limiting enzyme of the first step in catecholamine biosynthesis. The enzyme can also convert phenylalanine into tyrosine and then into 3-4-dihydroxyphenylalanine by successive hydroxylation (Dizdar *et al.*, 1991; Raisman-Vozari *et al.*, 1991). CCK act through catecholaminergic mechanisms in the hypothalamus to influence feeding behaviour (Crawly and Kiss, 1985; Kadar *et al.*, 1984).

In the rat, CCK and dopamine coexist in a subpopulation of neurons of the ventral tegmental area projecting to the nucleus accumbens (Wang *et al.*, 1992). CCK-8-like immunoreactivity is increased in the medial frontal cortex in response to the stimulation of pre-synaptic dopamine receptor, suggesting a control of CCK-8 release, at least in part, by the pre-synaptic dopamine receptor (Fukamauchi *et al.*, 1992). Recently, Kiyama *et al.*, (1991) demonstrate the co-expression of TH mRNA and CCK mRNA in the ventral mesencephalic dopaminergic neurons of the rat.

In this investigation, the density and number of TH-immunoreactive neurons in various hypothalamic areas during fasting condition were greatly lower than those of the those of the sated control group. However, the administration of CCK-8 produced increase on the number of TH-positive cell bodies when compared to those of the starved group. The pattern of TH-immunoreactive neurons in the substantia nigra was similar to the general pattern described above.

These results suggest that CCK-8 act to augment the dopamine-containing neurons in the rat hypothalamus. It also suggest that the increase of TH-positive neurons may partially compensate for food-intake suppression effect of CCK-8.

REFERENCES

Anthony ELP and King J: *Combined light and electron microscope immunocytochemical localization of scattered peptidergic neurons in the central nervous system. Am J Anat 175: 179-195, 1986*

Baile CA, McLaughlin CL and Della-Fera MA: *Role of cholecystokinin and opioid peptides in control of feeding. Physiol Rev 66: 172-234,1986*

Beinfelkt MC, Lewis ME, Eiden Le, Nilaver G and Pert CB: *The distribution of cholecystokinin and vasoactive intestinal peptide in rhesus monkey brain as determined*

by radioimmunoassay. Neuropeptides 3: 337-344, 1983

Billington CJ, Levine AS and Morey JE: *Are peptides truly satiety agents? A method of testing for neurohumoral satiety effects. Am J Physiol 245: R920-926, 1985*

Crawly JN and Kiss JZ: *Paraventricular nucleus lesions abolish the inhibition of feeding induced by systemic cholecystokinin. Peptides 6: 927-935,1985*

Debonnel G and de Nontigny C: *Increase neuronal responsiveness to cholecystokinin and dopamine induced by lesioning mesolimbic dopaminergic neurons: an electrophysiological study in the rat. Synapse 2: 537-545, 1988*

Dizdar N, Henriksson A and Kagedal B: *Determination of l-3, 4-dihydroxyphenylalanine in biological fluids and tissue. J Chromatogr 565: 1-26, 1991*

Emson PC, Rehfeld JF and Rossor MN: *Distribution of cholecystokinin-like peptides in the human brain. J Neurochem 38: 1177-1179, 1982*

Essatara MB, McClain VJ, Levine AS and Morley JE: *Zinc deficiency and anorexia in rats: the effect of central administration of norepinephrine, muschmol and bromergocryptine. Phyusiol Behav 32: 479, 1984*

Fukamauchi F, Yoshikawa T and Shibuya H: *Presynaptic dopaminergic control mechanism for CCK-8 like immunoreactivity in the rat medial frontal cortex. Neuropeptides 23: 55-59, 1992*

Hsu SM, Raine L and Fanger H: *Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577-580, 1981*

Hurd YL, Lindefors N, Brodin E, Brene S, Persson H, Ungerstedt U and Hoekfelt T: *Amphetamine regulation of mesolimbic dopamine/cholecystokinin neurotransmission. Brain Res 578: 317-326, 1992*

Kadar T, Varszwgi M, Sudakov SK, Penke B and Telegdy G: *Changes in brain monoamine levels of rats during cholecystokinin octapeptide-induced suppression of feeding. Pharmacol Biochem Behav 21: 339-344, 1984*

Kiyama H, McGowen EM and Emson PC: *Co-expression of cholecytokinin mRNA and tyrosine hydroxylase mRNA in populations of rat substantia nigra cells; a study using a combind radioactive and non-radioactive in situ hybridization procedure. Brain Res Mol Brain Res 9: 87-93, 1991*

Larsson LI and Rehfeld JF: *Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res 165: 201-218, 1979*

Leibowitz SF: *Brain monoamines and peptides: role in the control of eating behavior. Federation Proc 45: 1396-1403, 1986*

Leibowitz SF, Jhanwar-Uniyal M and Levin BE: *Turnover of catecholamines (CA) in discrete hypothalamic areas and the effects of chlorpromazine (CPZ). Soc Neurosci Abstr 7: 928, 1981*

Levin BE, Triscari J and Sullivan AC: *The effects of diet and chronic obesity on brain catecholamine turnover in the rat. Pharmacol Biochem Behav 24: 299, 1986*

Morley JE: *Neuropeptide regulation of appetite and weight. Endocr Rev 8: 256-287, 1897*

Minano FJ, Peinado JM and Myers RD: *Neurotensin perfused in hypothalamus of sated or fasted rat: HPLC analysis of release of DA, NE and 5-HT and their metabolites. Peptides 9: 1381-1387 1989*

Moran TH and McHugh PR: *Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am J Physiol 242: R491-497, 1982*

Oeth KM and Lewis DA: *Cholecystokinin- and dopamine-containing mesencephalic neurons provide distinct projections to monkey prefrontal cortex. Neurosci Lett 145: 87-92, 1992*

Paxinos G and Watson C: *The rat brain in sterotaxic coordinates. 2nd ed. San Diego, Academic Press, 1986*

Raisman-Vozari R, Hirsch Em HJavoy-Agid F, Vassort C, Savasta M, Feuerstein C, Thibault J and Agid Y: *Quantitive autoradiography of tyrosine hydroxylase immunoreactivity in the rat brain. J Neurochem 57: 1212-1222, 1991*

Rehfeld JF: *Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system of man and hog. J Biol Chem 253: 4022-4030, 1978*

Rybarczyk MC, Orosco M, Rouch C, Jacquot C and Cohen Y: *Interaction of cholecystokinin octapeptide (CCK-8) acts to suppress feeding in rats: a mapping study. Neuropharmacology 29: 109-118, 1990*

Schick RR, Harty GJ, Yaksh TL and Go VL: *Release of hypothalamic cholecystokinin in cats: effects of nutrient and volume loading. Am J Physiol 256: R248-254, 1989*

Seroogy K, Schalling M, Brene S, Dagerlind A, Chai SY, Hoekfelt T, Persson H, Brownstein M, Huan R, Cixon J, Filer D, Schlessinger D and Goldstein M: *Cholecystokinin and tyrosine hydroxylase messenger RNAs in neurons of rat mesencephalon: peptide/monoamine coexistence studies using in situ hybridization combined with immunocytochemistry. Exp Brain Res 74: 149-162, 1989*

Seroogy K, Tsuruo Y, Hoekfelt T, Walsh J, Farenkrug J, Emson PC and Goldstein M: *Further analysis of presence of peptides in dopamine neurons. Exp Brain*

Res 72: 523-534, 1988

Shillabeer G and Davison JS: *Endogenous and exogenous cholecystokinin may reduced food intake by different mechanisms. Am J Physiol 253: R379-382, 1987*

Singh L, Field MJ, Hughes J, Menzies R, Oles RJ, Vass CA and Woodruff GN: *The behavioural properties of CI-988, a selective cholecystokinin B receptor antagonist. Br J Pharmacol 104: 239-245, 1991*

Smith GP, Jerome C and Norgren R: *Afferent axons in abdominal vagus mediate satiety effects of cholecystokinin in rats. Am J Physiol 249: R638-641, 1985*

Tsai SH, Passaro EJr and Lin MT: *Cholesystokinin acts through catecholaminergic mechanisms in the hypothalamus to influence ingestive behaviour in the rat. Neuropharmacology 23: 1351-1356, 1984*

Wang ZJ, Rao ZR and Shi JW: *Tyrosine hydroxylase-, neurotensin-, or cholecystokinin-containing neurons in the nucleus tractus solitarii send projection fibers to the nucleus accumbens in the rat. Brain Res 578: 347-350, 1992*

=국문초록=

Cholecystokinin에 의한 음식물 섭취 억제시 흰쥐 뇌내 Dopamine 함량에 대한 면역조직화학 및 생화학적 연구

경희대학교 의과대학 약리학교실

정주호 · 김양제 · 임성빈 · 박승준 · 고계창 · 정지창

포만중추 (satiety center)를 자극하여 음식섭취를 억제한다고 알려진 CCK-8을 흰쥐 복강에 투여하여, 흰쥐 뇌의 도파민 변화에 대한 CCK-8의 효과를 관찰하였다. 흰쥐 뇌의 부위별 도파민 함량은 HPLC-ECD방법으로 측정하였으며, 시상하부와 흑질에서의 TH-immunoreactive neuron은 면역조직화학법과 영상분석법을 시행하였다. 굶긴 쥐에서는 정상 쥐에 비하여, 도파민 함량이 전두피질, 해마, 시상하부 및 편도체에서 각각 감소하였다. CCK-8을 투여한 쥐는 정상 쥐와 굶긴 쥐에 비하여, 도파민 함량이 시상하부에서 의미있게 감소하였다. 또한 굶긴 쥐는 정상 쥐와 비교하여, TH-positive neuron의 분포와 수가 뇌실옆핵, 깔때기핵, 정중융기 및 흑질에서 현저히 감소하였다. CCK-8투여시, 시상하부와 흑질에서의 TH-immunoreactive neuron의 수는 굶긴 쥐에 비하여 증가하였다.

이상의 실험 결과로 보아 음식물 섭취를 억제하는 작용이 있는 CCK-8은 시상하부의 도파민 신경계와 일부분 관련되어 있으며, 또한 시상하부와 흑질에 존재하는 TH-positive neuron은 음식물 섭취 행위에 중요한 역할이 있음을 시사하고 있다.