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Action of peripherally administered cholecystokinin on monoaminergic and GABAergic neurons in the rat brain.

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Abstract

In an acute study, cholecystokinin octapeptide sulfate (CCK) in doses of 1, 10 or 100 micrograms/kg body weight was injected intraperitoneally into rats just prior to the dark cycle. Rats were sacrificed two hours following the CCK injection. Norepinephrine levels were elevated in the dorsal amygdala of rats injected with 10 micrograms of CCK as well as in the septum of rats injected with 1 and 10 micrograms of CCK. The dopamine level in the septum of rats injected with 1 microgram of CCK as well as the gamma-aminobutyric acid (GABA) level in the lateral hypothalamus of rats injected with 10 micrograms of CCK were also elevated. In a chronic study, CCK (1 microgram/kg body weight/h) was subcutaneously infused into rats with Alzet osmotic minipump for seven consecutive days. The daily food consumption did not change during the 7 days of CCK infusion. The dopamine turnover in the striatum accelerated and the GABA level increased. On the contrary, dopamine metabolism in the substantia nigra and locus coeruleus decreased. Furthermore, the serotonin level in the substantia nigra decreased. Norepinephrine levels decreased in the nucleus paraventricularis, the locus coeruleus and the substantia nigra. The results suggest that peripherally administered CCK may act on the monoaminergic neurons and GABAergic neurons in the brain.

KEYWORDS: cholecystokinin, nigro-striatum, dopamine, serotonin, ?-aminobutyric acid

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Action of Peripherally Administered Cholecystokinin on Monoaminergic and GABAergic Neurons in the Rat Brain

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In an acute study, cholecystokinin octapeptide sulfate (CCK) in doses of 1, 10 or 100 μg/kg body weight was injected intraperitoneally into rats just prior to the dark cycle. Rats were sacrificed two hours following the CCK injection. Norepinephrine levels were elevated in the dorsal amygdala of rats injected with 10 μg of CCK as well as in the septum of rats injected with 1 and 10 μg of CCK. The dopamine level in the septum of rats injected with 1 μg of CCK as well as the γ-aminobutyric acid (GABA) level in the lateral hypothalamus of rats injected with 10 μg of CCK were also elevated. In a chronic study, CCK (1 μg/kg body weight/h) was subcutaneously infused into rats with Alzet osmotic minipump for seven consecutive days. The daily food consumption did not change during the 7 days of CCK infusion. The dopamine turnover in the striatum accelerated and the GABA level increased. On the contrary, dopamine metabolism in the substantia nigra and locus coeruleus decreased. Furthermore, the serotonin level in the substantia nigra decreased. Norepinephrine levels decreased in the nucleus paraventricularis, the locus coeruleus and the substantia nigra. The results suggest that peripherally administered CCK may act on the monoaminergic neurons and GABAergic neurons in the brain.

Key words: **cholecystokinin, nigro-striatum, dopamine, serotonin, γ-aminobutyric acid**

The administration of cholecystokinin octapeptide sulfate (CCK) has been shown to produce satiety, analgesia and central depression (1). In addition, CCK and dopamine (DA) have been shown to coexist in many neurons in the substantia nigra and ventral tegmental area (2). The functional interaction between CCK and DA in these brain regions may be of interest in view of the hypothesized over-activity of the dopaminergic system in schizophrenia (3). Locomotor hyperactivity induced by methamphetamine was suppressed by intracerebroventricular injection of CCK (4).

The blood CCK probably does not cross the blood-brain barrier to act on CNS sites (5). However, it has been suggested that the vagus may act as a means of transmitting

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signals from peripheral receptors to the central sites of action (6). Peripheral or central administration of CCK alters dopamine metabolism in some brain regions (7-10). Accordingly, the effects of CCK on animal behavior may in part be due to its action on the function of the mesolimbic dopamine system. The injection of either norepinephrine (NE) or serotonin (5-HT) into an animal's brain alters feeding desires (11, 12). In addition, the inhibitory transmitter, γ-aminobutyric acid (GABA), has been found to regulate some dopamine systems (13).

The aim of the present study was to examine the effects of peripherally administered CCK on monoamine metabolism and GABA levels in the brain nuclei.

Methods

Male wistar rats were individually housed in stainless-steel wire bottomed cages. The room temperature and relative humidity were kept at approximately 24°C and 55%, respectively, and a 12-h light-dark cycle was maintained (lights on from 7:00 to 19:00). Each rat weighed about 300 g at the beginning of the study.

Acute CCK (Peptide Institute Inc., Osaka Japan) treatment was undertaken just before the dark phase by intraperitoneally injecting CCK into the rats in doses of 1, 10 or 100 μg/kg body weight, during which time food and water were available *ad libitum*. The control animals were given saline following the same procedures. Food and water consumption was measured for two hours after the CCK injection. The rats were sacrificed two hours after CCK administration by the near-freezing method (14). Their brains were rapidly removed and frozen on dry ice, then stored at −70°C until dissection.

Chronic CCK treatment was performed using Alzet osmotic minipump (Alza Corp., Palo Alto, CA USA; Model 2001) which delivered 1 μg per kg body weight of saline or CCK per h for 7 days. Under ether anesthesia, pump was surgically implanted in the subcutaneous tissue. Surgery was carried out between hours 8 and 9 of the 12-h light period. Body weight, food consumption and water intake of each animal were measured daily. In addition, the locomotor activity was measured with ANIMEX (LKB, Farad Electronics, Sweden) for 7 days following the Alzet osmotic minipump implantation. Rats were sacrificed at time 2 of the dark phase. Their brains were rapidly removed and stored as described in the acute experiment. Red bulbs were used to provide dim illumination in the dark phase experiment.

Stored brains were sliced into coronal sections of one millimeter thickness in a cryostat kept at −20°C. Sliced tissues were dissected with stainless-steel needles (#11-17), according to the atlas of Pellegrino *et al.* (15), into the following sections: ventromedial hypothalamus (VMH), lateral hypothalamus (LH), dorsal amygdala, ventral amygdala, nucleus paraventricularis (N. paravent.), striatum, nucleus accumbens (N. acc.), substantia nigra (S. nigra), nucleus raphe dorsalis (raphe D.) and locus coeruleus. The dissected regions were kept frozen in an Eppendorf-type tube, containing 100 μl of 50 mM tris buffer (pH 7.5), at −70°C until assayed. All manipulations of samples were carried out while they were frozen. The brain tissue in the Eppendorf-type tube was dissolved and homogenized with a piston rod which was form-fitted to the inside of the Eppendorf-type tube. The homogenate was centrifuged twice at 12,500×g, each time for 10 min at 4°C. Monoamines and their metabolites were separated by chromatography on a reverse phase HPLC column (Lichrosorb RP-18, 5 μm, 4 mm×250 mm, Cica-Merck) and were measured electrochemically using an amperometric detector with an Ag/AgCl electrode (Model E-308, Irica Instrument Inc., Kyoto, Japan). Aliquots of 10 μl of supernatant were injected into the HPLC-ED system. The mobile phase was composed of 13% acetonitrile, 87% 0.1 M phosphate buffer (pH 3.4), 0.1 mM EDTA 2-sodium salt and 0.078% sodium octanesulfonate. The detector potential was set at +0.80 V against the Ag/AgCl reference electrode. The GABA level in the brain nuclei was estimated using the method of Okada *et al.* (16). The protein content of the homogenate was determined by the procedure of Lowry *et al.* (17).

Data are shown as the mean ±SD. Results were statistically analyzed using Student's t-test, one way analysis of variance, and Duncan's multiple

range test by the Muscot series program (Y. D. K Co., Ltd.) on a computer.

Results

Food consumption decreased in rats injected with 10 and 100 μg/kg body weight of CCK (acute treatment) when compared with the control group (Table 1). Effects of CCK on the levels of NE, DA, DOPAC (3, 4-dihydroxyphenylacetic acid), HVA (homovanillic acid) and 5-HT are shown in Table 2. NE levels in both the dorsal amygdala of rats injected with 10 μg of CCK, and the septum of rats injected with 1 and 10 μg of CCK were significantly higher than the control group levels. HVA levels in the S. nigra of rats injected with 1, 10 and 100 μg of CCK decreased, whereas DOPAC levels remained unchanged. The DA level increased in the septum of rats injected with 1 μg of CCK,

Table 1 Effects of intraperitoneally injected cholecystokinin (CCK) on food and water consumption of rats[a]

Dose of CCK (μg/kg body weight)	2-h Food intake (g)	2-h Water intake (g)
0 (Saline)	3.60±1.72	7.58±1.16
1	2.67±0.78	6.30±1.48
10	1.90±0.77*	6.34±3.63
100	1.62±1.12*	5.86±1.18*

a: Results are expressed as the mean±SD of 6 rats. *: Significantly different from the saline group (Student's *t*-test, $p < 0.05$).

Table 2 Effects of acutely administered cholecystokinin (CCK) on norepinephrine (NE), dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and serotonin (5-HT) levels in the rat brain nuclei[a]

	NE	DA	DOPAC	HVA	5-HT
Substantia nigra					
Saline	5.5± 1.4	15.5±4.1	14.3±4.3	5.4±1.8	19.2±4.8
CCK 1 μg	6.9± 2.1	16.1±3.8	9.6±4.3	3.0±0.9*	19.6±6.9
CCK 10 μg	8.5± 3.5	16.3±1.5	11.9±5.1	3.5±0.9*	20.7±2.9
CCK 100 μg	5.2± 1.3	15.6±2.6	13.4±2.2	3.7±0.9*	18.7±1.7
Septum					
Saline	11.5± 3.4	5.8±1.9	10.1±4.7	4.2±2.6	7.9±1.5
CCK 1 μg	18.9± 2.6**	11.9±5.3*	13.8±5.3	4.3±0.9	9.9±1.9
CCK 10 μg	19.4± 3.3**	9.8±2.7	10.8±3.0	3.5±1.1	9.5±2.1
CCK 100 μg	13.5± 3.0	6.7±1.7	12.0±3.5	4.8±2.0	8.5±0.9
Locus coeruleus					
Saline	20.0± 6.6	2.1±0.9	4.7±2.6	2.3±0.8	6.6±1.2
CCK 1 μg	21.5±11.3	2.6±1.5	7.0±4.8	1.1±0.5	9.6±1.3**
CCK 10 μg	19.7± 2.4	2.0±0.9	6.8±2.7	0.9±0.3	7.6±2.2
CCK 100 μg	12.0± 7.8	1.3±0.3	5.2±2.4	1.2±0.5	6.7±1.0
Ventral amygdala					
Saline	7.8± 1.7	2.1±0.5	3.1±1.2	1.7±0.8	6.3±0.5
CCK 1 μg	7.1± 0.9	1.8±0.6	3.2±1.1	0.9±0.4	8.6±1.8
CCK 10 μg	8.4± 1.0	1.1±0.2*	2.1±0.4	0.8±0.2	7.8±1.8
CCK 100 μg	9.5± 2.0	1.6±0.4	2.4±0.7	1.0±0.5	6.9±1.6
Dorsal amygdala					
Saline	6.7± 1.5	5.4±2.7	11.3±5.7	5.6±2.7	7.9±1.8
CCK 1 μg	10.3± 4.6	4.3±1.6	6.6±0.7	1.7±0.6	10.6±2.1
CCK 10 μg	9.7± 1.4*	4.5±1.4	7.5±2.5	2.0±0.6	10.5±1.6
CCK 100 μg	8.4± 1.0	3.9±1.5	9.1±4.3	2.7±0.6	9.2±1.9

a: Results are expressed as the mean±SD of 6 rats in units of pmoles/mg protein. *, **: Significantly different from saline group (Duncan's multiple range test: * $p < 0.05$; ** $p < 0.01$).

but decreased in the ventral amygdala of rats injected with 10 μg. The 5-HT level in the locus coeruleus of rats injected with 1 μg of CCK was elevated slightly but significantly. However, levels of monoamines and their metabolites in the VMH, LH, N. paravent., striatum, N. acc., and raphe D. were not significantly different from the control values (data not shown). The GABA level was measured in the S. nigra, N. acc., striatum, VMH, LH, ventral amygdala and dorsal amygdala. As indicated in Table 3, there was an elevation of the GABA level in the LH of rats injected with 10 μg of CCK, whereas the amino acid level in both the S. nigra and ventral amygdala of rats injected with 1 μg of CCK, significantly decreased from the control levels. The GABA level did not differ from the control value in the N. acc. and VMH (data not shown).

The total daily food and water consumption of rats subcutaneously infused with CCK (chronic treatment) was not significantly different from that of the saline-infused control

Table 3 Effects of acutely administered cholecystokinin (CCK) on γ-aminobutyric acid (GABA) in rat brain nuclei[a]

Dose of CCK (μg/kg body weight)	S. nigra	Striatum	LH	V. amygdala	D. amygdala
0 (Saline)	110.6±13.1	54.0±2.6	76.6±6.9	36.2±4.2	28.9±2.8
1	78.0±19.0*	57.7±3.5	83.8±7.2	30.6±2.6*	33.1±2.6
10	88.6±23.0	57.6±6.3	92.2±7.5*	35.5±3.8	33.0±6.1
100	99.6±10.0	53.6±3.1	83.3±7.3	36.8±3.4	30.9±3.1

a: Results are expressed as the mean±SD of 6 rats in units of nmoles/mg protein. *: Significantly different from the saline group (Duncan's multiple range test, $p < 0.05$). Abbreviations: S. nigra, substantia nigra; LH, lateral hypothalamus; V. amygdala, ventral amygdala; D. amygdala, dorsal amygdala.

Table 4 Effects of chronically administered cholecystokinin (CCK) on norepinephrine (NE), dopamine (DA), 2, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and serotonin (5-HT) levels in rat brain nuclei[a]

	NE	DA	DOPAC	HVA	5-HT
Striatum					
Saline	—	340 ±43	189 ±21	63.2± 8.3	4.6±0.7
CCK	—	371 ±29	241 ±39*	69.3± 6.7	4.6±1.0
Nucleus accumbens					
Saline	—	159 ±16	133 ±32	40.0±12.2	12.6±3.2
CCK	—	161 ±15	129 ±17	33.9± 6.0	13.0±2.8
Substantia nigra					
Saline	9.4± 2.8	51.3±12.5	23.4± 4.4	6.6± 1.5	19.6±2.8
CCK	6.3± 1.2#	30.8± 4.8##	15.5± 2.3**	4.2± 0.9*	15.8±2.4*
Nucleus paraventricnlaris					
Saline	68.6± 8.1	5.2± 0.6	4.6± 0.3	1.0± 0.5	10.8±1.5
CCK	50.1±10.5**	5.0± 1.3	6.3± 1.0	1.0± 0.5	8.8±1.7
Locus coeruleus					
Saline	25.1± 8.1	2.8± 0.5	6.3± 1.4	1.9± 0.6	14.4±3.3
CCK	15.8± 3.9**	1.6± 0.4**	3.5± 0.7**	1.8± 0.6	12.5±1.2

a: CCK (1 μg/kg body weight/h) was infused subcutaneously into rats for seven consecutive days using Alzet osmotic minipump. Results are expressed as the mean±SD of 6 rats in units of pmoles/mg protein. *, **, #, ##: Significantly different from the saline group (Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, and Aspin-Welch test: # $p < 0.05$, ## $p < 0.01$).

rats at any point in time during the 7 day infusion period. As shown in Fig. 1, although the locomotor activity of the CCK infused rats was reduced at time point 1 of the dark period, but increased at time point 9 as compared with the saline infused rats (Student's *t*-test), the locomotor activity pattern itself was not altered (one-way ANOVA). Table 4 shows the effects of CCK on the levels of NE, DA, DOPAC, HVA and 5-HT. The NE level in rat brain regions decreased in the N. paravent., locus coeruleus and S. nigra. The levels of DA, DOPAC and HVA decreased in the S. nigra, and the DA and DOPAC levels decreased in the locus coeruleus. The DOPAC level increased in the striatum. The 5-HT level decreased in the S. nigra. However, the levels of the monoamines and their metabolites in the VMH, LH, dorsal amygdala, ventral amygdala and septum were not significantly different from the control values (data not shown). The GABA level in the striatum increased (71.1 ± 4.7 *vs* 61.5 ± 8.4 nmoles/mg protein, $p < 0.05$), but it

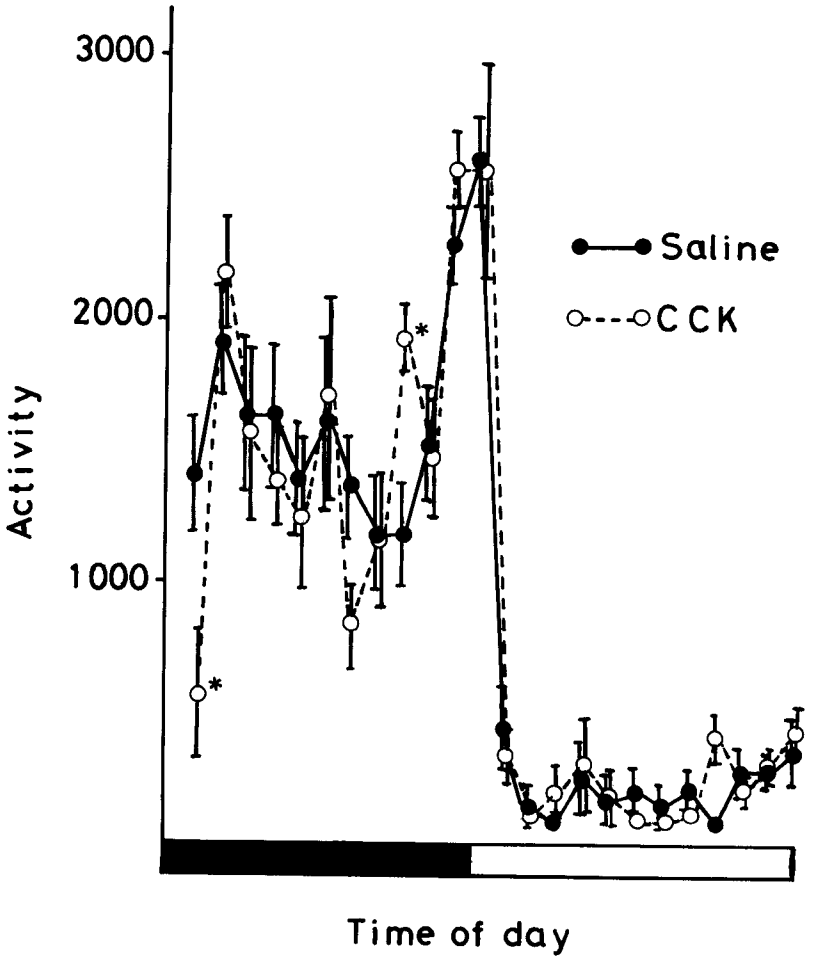

Fig. 1 Effects of chronically administered CCK on locomotor activity of rats. CCK (1 μg/kg body weight/h) was infused subcutaneously into rats for seven consecutive days using Alzet osmotic minipump. Points and corresponding vertical lines (not illustrated if smaller than the symbol) represent the mean ± SEM (locomotor counts/h/animal, N = 4). *: Significantly different from the saline group (Student's *t*-test, $p < 0.05$). ■, Dark phase; □, light phase.

did not change in any of the other brain regions examined.

Discussion

The results of this study provide evidence that the peripheral administration of CCK can alter monoamine metabolism and the GABA level in the central nervous system. The central effects of CCK following parenteral administration have rarely been investigated, although positive effects have been found (7, 8). Furthermore, it is important to know whether CCK administered intraperitoneally or subcutaneously is acting via a peripheral site or whether the peptide is penetrating the blood brain barrier and acting centrally. It seems unlikely that CCK may act directly on any central site following peripheral administration, since this peptide probably does not cross the blood-brain barrier (5). It has been hypothesized that the peripheral action might be mediated to the brain via visceral afferent fibers (18). Several studies have shown that the behavioral and satiety-inducing effects of peripherally administered CCK are abolished by vagotomy (6). Furthermore, the central relay nuclei of the vagus is the nucleus of the solitary tract. The action of peripherally administered CCK in the brain may be mediated through fibers from the nucleus of the solitary tract (19).

In this study, monoamine metabolism in some brain regions was altered by acute or chronic CCK administration. However, changes in the levels of monoamines and metabolites in the brain after CCK administration did not occur in a dose-dependent manner. This finding is in agreement with reports (10, 20, 21) that effects of systemically administered CCK are not always produced in a dose-dependent manner.

In our study, monoamine metabolism was not clearly altered in the VMH, LH, N. paravent. and amygdala, which play a role in the control of food intake. Kádár *et al.* (8) reported that CCK-8 injected intraperitoneally caused striking changes in the DA and NE contents of the hypothalamus, and in the DA contents of the amygdala. The difference in the effect of CCK on monoamine metabolism in certain brain regions may have been due to differences in the time of decapitation following the CCK injection, because CCK has been shown to participate in short-term satiety (22).

The interaction between CCK and monoamine neurotransmitters in the brain has been extensively studied since CCK was shown to coexist with dopamine in some mesolimbic neurons (2). Satiety, analgesia and central depression have been studied in animals following central administration of CCK (1), and the antipsychotic (3) and anti-dyskinetic (23) properities of CCK have been studied in schizophrenic patients. As would be expected from the fact that dopaminergic neurons receive striato-nigral inhibitory GABA projections (24), striato-nigral GABAergic mechanisms have been found to directly and indirectly control the activity of dopamine (25). Additionally, it has been shown that GABA and 5-HT coexist in the raphe dorsalis (26). Neurons containing 5-HT were shown to be distributed from B7 to both the striatum and S. nigra (27). Our study suggested that chronic CCK administration does not affect the pattern of locomotor activity. DA, 5-HT and GABA levels in S. nigra-striatum system were altered. These neurochemical changes may reflect the changes in a certain functional activity of this brain area.

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