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Effects of Caerulein and Cholecystokinin-Octapeptide on Acetylcholine and Choline Contents in the Brains of Intact and Vagotomized Mice

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MIYATE, H. *Effects of caerulein and cholecystokinin-octapeptide on acetylcholine and choline contents in the brains of intact and vagotomized mice.* PHARMACOL BIOCHEM BEHAV **35**(1) 143–149, 1990.—The effects of caerulein (CLN; 0.5, 5, and 50 μg/kg, IP) and cholecystokinin-octapeptide (CCK-8; 5, 50, and 400 μg/kg, IP) on the acetylcholine and choline contents in the discrete brain regions were examined, 30, 60, and 120 min after injection into intact and vagotomized mice. In all of the discrete brain regions of the intact mice, CLN and CCK-8 was found to have a complex effect on the acetylcholine and choline contents depending on the brain region, dosage and treatment time. On the other hand, the effect of CLN was abolished completely in the vagotomized mice. Thus, the present study indicates that peripherally administered CLN and CCK-8 have an effect on the central cholinergic system, mainly mediated via the vagus.

Caerulein (CLN) Cholecystokinin-octapeptide Acetylcholine Choline Discrete brain Vagotomy Mouse

CHOLECYSTOKININ octapeptide (CCK-8) is a hormone found in the gastrointestinal and central nervous tissues of several species (12, 26, 41, 44). This peptide and its analogue caerulein (CLN), originally extracted from the skin of the frog (1), have various behavioral and physiological actions, e.g., sedation (23,49), analgesia (47,49), antinociception (21), satiety (2,36), palpebral ptosis (47), antistereotypy (50), and hypothermia (51) in a variety of species. In addition, it has been shown that the behavioral profile of CLN also resembles that of antipsychotic drugs (33,45). Furthermore, clinical information has shown that CLN is effective against various symptoms of chronic schizophrenic patients for whom neuroleptic therapy has been ineffective (34), and tardive dyskinesia (39,40). However, the mechanism of these clinical effects is still unclear.

Recently, it has been reported that depending on the dosage (30), CLN and CCK-8 had both a stimulative and depressive effect on the release of acetylcholine (ACh) from the cerebral cortex in the urethane-anesthetized rat, and that the release of CCK-8 from rat cortical synaptosome increased with ACh (24). This information suggests that CLN and CCK-8 may exert the effect not only on the central monoaminergic system that was reported earlier (15), but also on the central cholinergic system. However, the interaction between the central cholinergic system and these peptides is still unclear. In addition, the question concerning the central effect of these peptides administered peripherally arises as to whether the site of the action of these peptides is peripheral or central, due to conflicting reports (7, 16, 20, 32, 35, 42, 46).

In the present study, the effects of peripherally administered CLN and CCK-8 on the ACh and choline (Ch) content in the discrete brain regions of the intact and vagotomized mice were examined.

METHOD

Animals

Male ddY mice, weighing 28–32 g, were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan). The mice were housed in a room with a controlled temperature of 22 ± 1°C, and a humidity of 55 ± 5%, and a 12-hour light cycle from 7 a.m. to 7 p.m. The mice were given free access to both water and laboratory chow.

Experimental Procedure

The effects of CLN and CCK-8 on ACh and Ch content. The ACh and Ch content were measured by a method established in our department, using a high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (37). Briefly, after administration of CLN (0.5, 5, and 50 μg/kg, IP), CCK-8 (5, 50, and 400 μg/kg, IP) or saline, the mice (n = 6–9) were sacrificed by

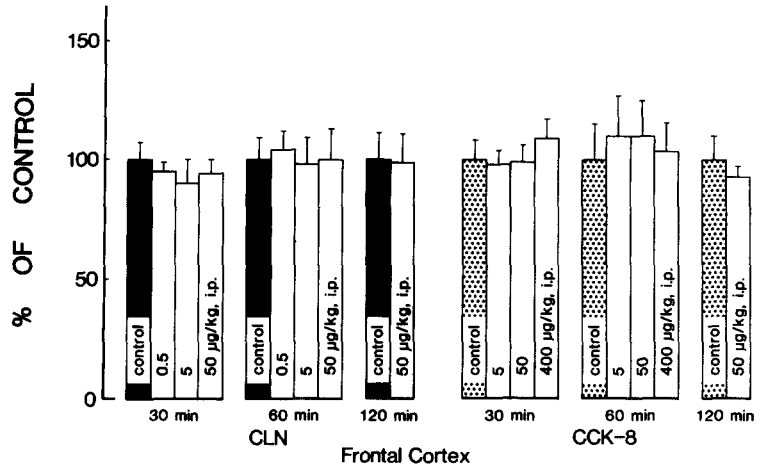

FIG. 1. Effects of CLN and CCK-8 on the ACh content in the frontal cortex of mice. Vertical bars indicate ±S.D.

microwave irradiation (1 kW at 2450 MHz for 8 sec, Model RE-3000, Sharp Co., Osaka, Japan), 30, 60, and 120 min after the injection, and their brains removed. Only the head of the mouse was irradiated with microwave using a handmade applicator. Although the mice died within 2 sec of the onset of the irradiation, 8 sec were used for it made separation of the brain easier. The frontal cortex, striatum, hypothalamus, hippocampus and nucleus accumbens were isolated on an ice-chilled glass plate, and immediately frozen on dry ice, then weighed and stored at −80°C until extraction. Frozen tissues were homogenized with an ultrasonic cell disruptor (60 W, 50% pulsed power for 10 sec, Model 200, Branson, Danbury, CT) in 200 μl of ice-cooled 0.1 M perchloric acid solution containing 0.1 mM EDTA and 100 μM ethylhomocholine (EHC, as an internal standard) for the striatum, 160 μl for the frontal cortex, hypothalamus, and hippocampus, and 120 μl for the nucleus accumbens. Homogenates were centrifuged at 12,000 × g for 15 min at 4°C, and the supernatants were filtered through a 0.45 μm filter (Type HV, Nihon Millipore, Yonezawa, Japan) and stored at −80°C until assay. Twenty μl of the filtrates were injected onto the HPLC system. ACh and Ch were separated on an AC-GEL column (EICOM, Kyoto, Japan) using the mobile phase of a phosphate buffer (pH 8.0), containing 300 mg/l octanesulfonic acid sodium salt (Aldrich, Milwaukee, WI), 65 mg/l tetramethylammonium chloride (Wako Chemical, Osaka, Japan), and 6 mg/l EDTA. An electrochemical detector (VMD-501, Yanagimoto, Kyoto, Japan) with platinum electrode (EICOM) was also used.

Bilateral cervical vagotomy. Bilateral cervical vagotomy was carried out under urethane anesthesia (2.0 g/kg, IP). A cervical midline incision was made, and the vagal nerve trunks of each side were separated from its surrounding tissues and sympathic nerve near the common carotid artery and the nerves were cut. It is well-known that the general condition worsens rapidly after bilateral cervical vagotomy. So the general condition of mice after vagotomy was observed. Although the mice were panting after the operation, they lived for at least 4 hours in a preliminary experiment. In sham-operated mice, the vagal nerves of each side were kept intact after separation. Immediately following the operation, CLN (50 μg/kg, IP) or saline was administered, and 30 min later, they were sacrificed by the same manner as described above. The animals were not awake at the time of sacrifice. (The urethane anesthesia affected for about 3 hours.)

TABLE 1

BASAL CONTENTS OF Ch AND ACh IN THE DISCRETE BRAIN REGIONS OF MICE

	Choline	Acetylcholine
Frontal cortex	25.2 ± 4.4 (6)	26.9 ± 1.9 (6)
Striatum	31.4 ± 3.6 (6)	76.5 ± 5.5 (6)
Hypothalamus	31.6 ± 3.6 (7)	32.5 ± 0.8 (7)
Hippocampus	23.4 ± 2.3 (7)	25.5 ± 1.4 (7)
Nucleus accumbens	49.6 ± 4.4 (6)	78.1 ± 5.8 (6)

Mean ± S.D. nmoles/g of tissue weight. The number of animals is in the parentheses.

Drugs

The following drugs were employed: CLN (Shionogi Pharmaceutical Company, Osaka, Japan), CCK-8 (Peptide Laboratory, Osaka, Japan), and urethane (Kanto Chemical, Tokyo, Japan). They were dissolved in a sterile saline solution, and then injected intraperitoneally.

Statistics

The results are expressed as percentage of controls. The mean values were presented with their standard deviation. The statistical comparisons of the data were analyzed by the Dunnett's test (2-sided) to determine the acute effects of CLN and CCK-8, and the Student's *t*-test (2-sided) to determine the effects of CLN in vagotomized mice.

RESULTS

Basal Contents of ACh and Ch

The basal contents of ACh and Ch in discrete brain regions are shown in Table 1.

ACh Content in the Frontal Cortex, Striatum, and Other Parts

CLN and CCK-8 had no effect on the ACh content of the frontal cortex (Fig. 1). As shown in Fig. 2, the ACh content in the striatum increased significantly 30 min after administration of 50

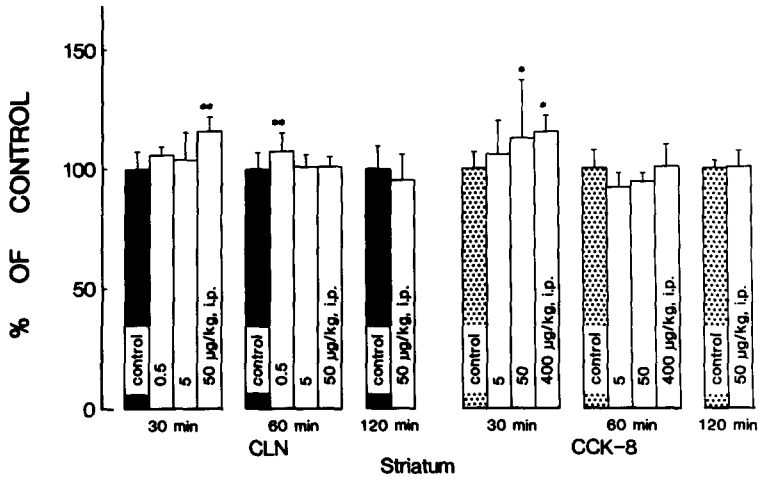

FIG. 2. Effects of CLN and CCK-8 on the ACh content in the striatum of mice. Vertical bars indicate ±S.D. *$p<0.05$; **$p<0.01$ vs. control (Dunnett's test, N=5–7).

μg/kg (+15.9%) CLN and 60 min after injection of 0.5 μg/kg (+7.5%) CLN. The ACh content in the striatum increased significantly 30 min after administration of CCK-8 at 50 μg/kg (+12.7%) and 400 μg/kg (+15.5%). In the hypothalamus and nucleus accumbens, CLN had no effect on the ACh content, and the ACh content decreased significantly in the hippocampus only 30 min after administration of 5 μg/kg CLN (see Table 2). The effect of CCK-8 on the ACh content in the hypothalamus (data not shown) and nucleus accumbens was similar to that of the striatum.

TABLE 2

EFFECT OF CAERULEIN AND CCK-8 ON THE ACh CONTENT IN THE DISCRETE BRAIN REGIONS OF MICE

	Dose (μg/kg)	Treatment Time (min)	% of Control Caerulein	CCK-8
Hippocampus	0.5	30	100.0 ± 7.4	—
		60	102.8 ± 5.0	—
	5	30	91.4 ± 7.4†	94.5 ± 3.9
		60	98.9 ± 6.4	101.3 ± 3.8
	50	30	96.5 ± 4.3	97.3 ± 3.1
		60	96.4 ± 8.9	100.4 ± 3.8
		120	96.6 ± 4.7	103.8 ± 10.6
	400	30	—	98.0 ± 3.5
		60	—	99.6 ± 4.3
Nucleus accumbens	0.5	30	97.6 ± 5.2	—
		60	100.1 ± 6.3	—
	5	30	98.1 ± 10.6	107.3 ± 9.4
		60	96.4 ± 3.0	116.3 ± 5.6†
	50	30	105.0 ± 8.7	104.6 ± 8.6
		60	101.0 ± 11.9	93.3 ± 2.0
		120	—	—
	400	30	—	107.2 ± 1.7
		60	—	92.1 ± 6.4*

Mean ± S.D. *$p<0.05$, †$p<0.01$ vs. saline control (Dunnett's test, 2 sided). Number of animals are 6 to 9.

CCK-8 has no effect on the ACh content in the hippocampus as in the frontal cortex.

Ch Content in the Frontal Cortex

Depending on the dosage, CLN and CCK-8 exerted a biphasic effect on the Ch content of the frontal cortex. As shown in Fig. 3, at 30 min after administration of 5 μg/kg CLN, a significant increase in the Ch content was noted in the frontal cortex (+32.1%). With a dosage of 50 μg/kg CLN, an increase of +63.1% occurred after 30 min followed by a smaller increase at 60 and 120 min. Whereas with 0.5 μg/kg CLN, a significant decrease of the Ch content (−24.3%) was noted 60 min after injection. On the other hand, with CCK-8 at dosages of 5, 50, and 400 μg/kg, a significant increase in the Ch content of the frontal cortex was noted 30 min after administration (+33.7%, +60.6%, and +44.4%). Conversely, with 5 μg/kg CCK-8, a significant decrease in the Ch content (−25.0%) was noted 60 min after injection.

Ch Content in the Other Parts

Table 3 shows that CLN and CCK-8 exerted the same biphasic effect on the Ch content in the striatum as they did in the frontal cortex, and the increase caused by 50 μg/kg CCK-8 continued for at least 120 min.

In the hypothalamus and hippocampus, a significant increase in the Ch content, depending on the dosage of CLN, was noted 30 min after administration (see Table 4). With 50 μg/kg CLN, the Ch content in the nucleus accumbens increased significantly. The effect of CCK-8 on the Ch content in the hypothalamus, hippocampus, and nucleus accumbens was similar to that of the frontal cortex and striatum.

Effect of CLN on the ACh and Ch Content in Vagotomized Mice

In the sham-operated mice, 30 min after injection of 50 μg/kg CLN, the ACh content increased in the frontal cortex and striatum by 10.1% and 13.3%, respectively (Fig. 4), whereas the effects of CLN disappeared entirely in the vagotomized mice. Similarly, increases in the Ch content, 30 min after administration with 50 μg/kg CLN, of the frontal cortex (24.9%) and striatum (25.2%) were completely inhibited by vagotomy (see Fig. 5).

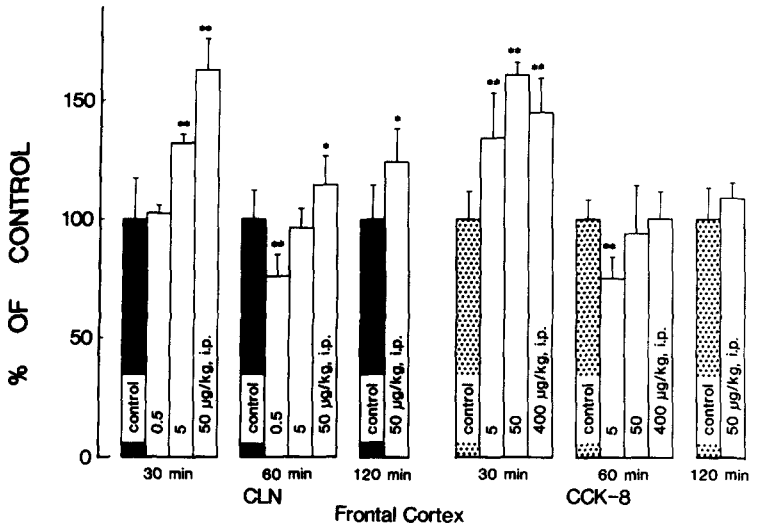

FIG. 3. Effects of CLN and CCK-8 on the Ch content in the frontal cortex of mice. Vertical bars indicate ±S.D. *$p<0.05$; **$p<0.01$ vs. control (Dunnett's test, N=5–7).

DISCUSSION

The present experiment had primarily two purposes: 1) whether or not peripherally administered CLN and CCK-8 had an effect on ACh and Ch content in the discrete brain of mice, and if they did, 2) whether the site of the action of CLN was peripheral or central.

CLN and CCK-8 had complex effects on the ACh release of the rat cortex (30) and also on the monoamine contents of the rat brain (15). These results and the present result suggest that CLN and CCK-8 effected not just monoaminergic neurons, but cholinergic neurons as well. Moreover, CLN and CCK-8 may be also associated with the central gamma-aminobutyric acidergic (GABA-ergic) system (48). Thus, these results suggest that these peptides have an effect on central nervous system, widely and complexly. It was reported that there were interactions among cholinergic, dopaminergic and GABAergic neurons (3,11). However, the problem of whether these nervous systems are independently affected by CLN and CCK-8 or not, is still unclear.

TABLE 3

EFFECT OF CAERULEIN AND CCK-8 ON THE CHOLINE CONTENTS IN THE STRIATUM OF MICE

Dose (μg/kg)	Treatment Time (min)	% of Control Caerulein	% of Control CCK-8
0.5	30	100.3 ± 5.7	—
	60	90.3 ± 9.5*	—
5	30	113.4 ± 3.8*	119.9 ± 10.9*
	60	92.7 ± 6.8	94.5 ± 9.9
50	30	142.0 ± 5.7†	131.0 ± 18.7†
	60	108.3 ± 9.5	98.4 ± 5.2
	120	106.8 ± 16.4	108.7 ± 4.6*
400	30	—	124.0 ± 5.9†
	60	—	102.9 ± 9.4

Mean ± S.D. *$p<0.05$, †$p<0.01$ vs. saline control (Dunnett's test, 2 sided). Number of animals are 6 to 9.

Our results also demonstrated that vagotomy clearly abolished the effects of CLN on the ACh and Ch contents. It is not clear as of yet where the primary action site of the peripherally administered CLN is. It has been reported earlier that the primary action site of CLN was probably peripheral, because the presence of CCK binding sites is a characteristic of the vagus nerve and that other peripheral nerves do not have CCK-receptors (32,46), also because abdominal vagotomy blocks several effects of CLN (7, 27, 42). Thus, the present result suggests that the effect on central ACh and Ch of peripherally administered CLN, is mediated via the peripheral CCK receptors and the vagus. This suggestion supports the earlier study that stated peripherally administered CLN reduces dopamine release from the striatum mediated via the vagal afferent system (16). However, there are some reports that the action site of CLN may be centrally located, because vagotomy does not prevent some of the behavioral and neurochemical effects of systemically administered CLN (8, 14, 20, 35). Furthermore, small amounts of peptide may cross the blood-brain barriers (22), and since CCK-8 has an effect on dopamine release from slices of cat caudate nucleus (31), it may be speculated that peripherally administered CLN exerts effects on the central nervous system mainly by the vagus, and partly by the brain. This speculation supports the earlier studies that stated vagotomy to not abolish, but reduce the satiety and increase of ACh release from cerebral cortex in rat, induced by peripheral administered CLN (29,43). The suggestion, reported earlier, that peripherally administered CCK has two components to its excitatory action in the substantia nigra (19), also supports this speculation.

The effects of CLN and CCK-8 on the Ch content of the frontal cortex and striatum showed biphasic patterns. The Ch content decreased at CLN 0.5 μg/kg, while at 5 and 50 μg/kg it increased. The Ch content decreased at CCK-8 5 μg/kg, and increased at 50 and 400 μg/kg. On the other hand, earlier results of ACh release in urethane-anesthetized rat showed the same biphasic pattern (26). Low dosage of CLN (up to 5 μg/kg, IP) increased the release of ACh from the frontal cortex 30 min after its injection, and this increase slowly diminished after 60 min. At a high dosages of CLN (20 μg/kg, IP), the release of ACh decreased in the frontal cortex 30 min after administration and this decrease lasted for at least 60 min (30). The earlier results of ACh release and results of the present study suggested that Ch increased as a result of a

TABLE 4

EFFECT OF CAERULEIN AND CCK-8 ON THE CHOLINE CONTENTS IN THE DISCRETE BRAIN REGIONS OF MICE

	Dose (μg/kg)	Treatment Time (min)	% of Control Caerulein	CCK-8
Hypothalamus	0.5	30	106.6 ± 7.3	—
		60	95.2 ± 9.4	—
	5	30	114.5 ± 6.0†	111.0 ± 9.1
		60	103.9 ± 8.5	95.1 ± 6.1
	50	30	129.4 ± 4.4†	119.0 ± 7.6†
		60	107.2 ± 7.2	96.6 ± 5.8
		120	106.2 ± 10.3	101.8 ± 7.8
	400	30	—	115.0 ± 10.3†
		60	—	101.5 ± 7.8
Hippocampus	0.5	30	96.3 ± 6.9	—
		60	91.1 ± 10.4	—
	5	30	122.5 ± 6.4†	121.6 ± 20.5*
		60	87.5 ± 10.7	85.0 ± 7.3
	50	30	151.4 ± 14.7†	133.3 ± 13.2†
		60	112.8 ± 9.5	93.0 ± 19.5
		120	109.9 ± 14.7	113.6 ± 9.5
	400	30	—	123.5 ± 17.5*
		60	—	99.0 ± 9.1
Nucleus accumbens	0.5	30	102.0 ± 2.4	—
		60	98.4 ± 5.6	—
	5	30	98.6 ± 7.1	101.5 ± 11.6
		60	102.9 ± 8.2	86.1 ± 7.1*
	50	30	112.1 ± 6.3†	100.8 ± 10.2
		60	107.9 ± 11.1	99.2 ± 12.6
		120	—	—
	400	30	—	100.0 ± 7.1
		60	—	104.5 ± 13.8

Mean ± S.D. *$p<0.05$, †$p<0.01$ vs. saline control (Dunnett's test, 2 sided). Number of animals are 6 to 9.

feedback inhibition by the high ACh concentration achieved by 5 μg/kg CLN 30 min after administration. Since earlier studies have shown that Ch levels in the brain increases following administration of oxotremorine, physostigmine, and muscarine (25,28), it has been suggested that ACh increase may enhance phosphatidylcholine hydrolysis via muscarinic receptor stimulation (13,25). On the other hand, our studies showed 50 μg/kg CLN to increase the Ch content, though earlier studies revealed 20 μg/kg CLN to decrease the release of ACh from the frontal cortex (30). Thus, at a high dosages of CLN, the increase of Ch level in mouse brain cannot be fully explained by a muscarinic receptor-mediated mechanism. This contradiction is probably caused by differences in experimental condition, e.g., anesthesia, dosage, and species used, or possibly by other mechanisms which controls the Ch level. Anesthesia, in particular, may be more important than the other factors to explain this contradiction, because many anesthetics alter the ACh release in many brain regions (4). Dosage may be also one of the factors, because the more CLN administered probably exert more permeation of CLN through blood-brain barrier. As to the relevance of Ch as a marker of ACh release, the clarification of this problem requires further investigations.

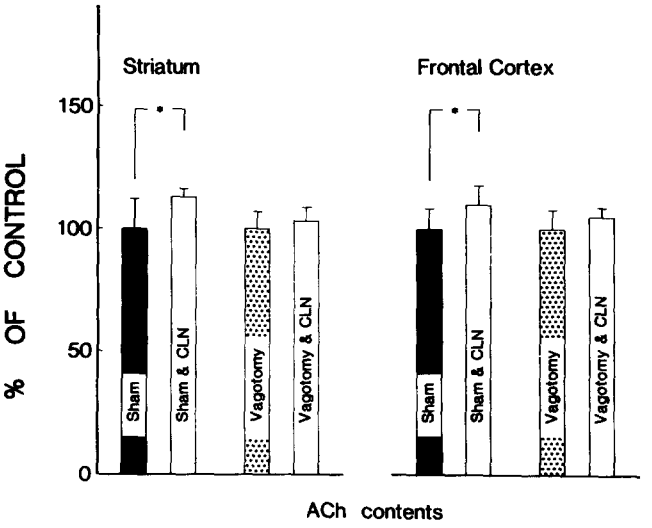

FIG. 4. Effect of caerulein (50 μg/kg, 30 min after IP injection) on the ACh content in the striatum and frontal cortex of sham-operated and vagotomized mice. Vertical bars indicate ±S.D. *$p<0.05$ vs. control (Student's t-test, N = 6–8).

Incidentally, the mechanism of reduction in Ch in striatum induced by 0.5 μg/kg CLN cannot be explained at the present time, and this will be the subject of further study.

In this study, 50 μg/kg CLN was found to have an effect on the ACh content in the frontal cortex of sham-operated mice, but not in normal mice (see Figs. 1 and 4). In addition, 50 μg/kg CLN exerted the same effect on the ACh content in the frontal cortex, also, of urethane anesthetized mice (nonsham-operated mice) in the preliminary experiment (data not shown). Many anesthetics reduced ACh release from cerebral cortex in sheep and cat (4). Thus, the increase of ACh content exerted by 50 μg/kg CLN may be caused by alteration of ACh synthesis induced by CLN, though this problem cannot be resolved from the present study.

Both CLN and CCK-8 had similar complex effects on the central ACh and Ch contents, but CLN was clearly more potent than CCK-8. This result corresponded to the earlier results which have demonstrated that CLN was more potent than CCK-8 in their

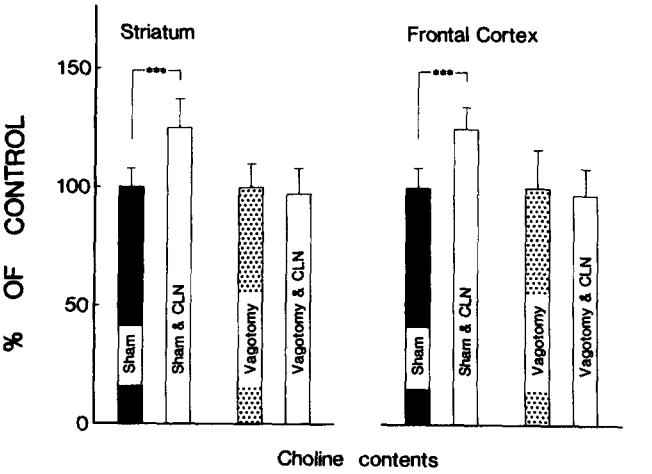

FIG. 5. Effect of caerulein (50 μg/kg, 30 min after IP injection) on the Ch content in the striatum and frontal cortex of sham-operated and vagotomized mice. Vertical bars indicate ±S.D. ***$p<0.001$ vs. control (Student's t-test, N = 6–8).

antinociceptive effects, sedation, stereotypy, and palpebral ptosis in rats or mice (21,49). It has also been reported that compared to CCK-8, CLN was more resistant to degradation by rat brain synaptosomes (10).

Clinical information has shown that peripheral administration of CLN is potent against tardive dyskinesia (39,40). However, the mechanism of CLN against tardive dyskinesia still remains unsolved. Earlier reports have observed that systemically administered Ch was potent against tardive dyskinesia, and increased the Ch level of brain (6,18). The present result also showed that CLN increased the Ch level of the brain, at dosages of over 5 μg/kg. Therefore, the increase of central Ch level induced by CLN may be part of the CLN's mechanism against tardive dyskinesia.

It is noteworthy that injection of 0.5 μg/kg CLN systemically exerts an effect on the ACh and Ch content of the frontal cortex and striatum, because 0.5 μg/kg is smaller than any other dosage used earlier in previous experiments, and is very close to the clinical dosage used against schizophrenia (38).

In conclusion, both CLN and CCK-8 were found to have complex effects on the ACh and Ch levels of the mouse brain regions, and that the vagus is probably a necessary factor in producing this effect in terms of peripherally administered CLN.

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