Possible Involvement of the Central Nervous System in Long-Term Effect of Caerulein on Amphetamine-Induced Hyperactivity in Rats

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Abstract—The present experiments were performed in order to ascertain whether the long-term antagonistic effect of caerulein on amphetamine-induced hyperactivity is produced in the central nervous system. In the vagotomized rats, animals treated with a combination of caerulein and haloperidol and exposed to amphetamine on the 1st day became less sensitive to amphetamine for 24 hr to 1 week when their ambulatory activities were measured in an open field apparatus. The same results were also observed in the experiments where caerulein was applied directly into the lateral ventricle. However, the animals given caerulein in the nucleus accumbens showed no reduced susceptibility to amphetamine. Thus, the present results suggest that caerulein may indirectly modulate some dopaminergic activity in the central nervous system. One possible mechanism involved in the antagonistic effect of caerulein on amphetamine-induced hyperactivity is changes in activities of endocrine systems.

Caerulein (CLN), chemically related to cholecystokinin octapeptide (CCK-8), when peripherally administered to mice, showed a number of pharmacological effects, i.e., sedation, analgesia, catalepsy, palpebral ptosis, inhibition of rearing, prolongation of hexobarbital sleeping time and anticonvulsant effect (1-4).

Recently, we demonstrated that CLN has a long-lasting antagonistic effect on amphetamine (AMP) hyperactivity in rats when it is given together with haloperidol (HLP) (5, 6). There are several reports indicating that behavioral changes induced by peripherally administered CLN or CCK-8, i.e., suppression of food intake and decrease in exploratory behavior, are mediated via the vagal nerve (7, 8). Since the durations of these effects are generally short-lasting, the mechanism underlying our observed long-term effect of CLN may differ from that involving vagal nerve activities.

The present study was conducted to ascertain whether the long-term antagonistic

effect of CLN has a CNS origin or depends on vagal nerve activities.

Materials and Methods

Animals: Male Wistar rats (Shizuoka Laboratory Animal Center), weighing 250-300 g at the beginning of the experiments, were maintained on ad libitum food and water on an 8 a.m.-8 p.m. lighting schedule during the experiments.

Vagotomy: Bilateral subdiaphragmatic vagotomy was performed by making a 3-cm midline incision just below the sternum. The stomach was pulled gently toward the posterior, and the lobes of the liver were held aside to expose the esophagus. Under a microscope, the two subdiaphragmatic branches of the vagus were separated from the esophagus and severed. The esophagus was then carefully examined with the microscope, and all smaller trunks of the vagal nerve were also severed. The animals were allowed at least 1 week to recover from the operation before the experiment began.

Cannulae implantation for intracerebro-ventricular or intra-nucleus accumbens injection: The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and positioned in a stereotaxic apparatus. After exposure of the skull, 23-gauge stainless steel cannulae were either implanted bilaterally in the nucleus accumbens or the same size cannula was implanted unilaterally in the right lateral ventricle by employing an optical brain tracer (9). Coordinates for the nucleus accumbens were A 9.4, L 1.4, H -0.6, and for the lateral ventricle, A 6.4, L 1.4, H 1.8, according to the atlas of König and Klippel (10). The cannulae were fixed onto the skull with acrylic dental cement. Animals were allowed 1 week to recover before testing.

CLN was injected into the nucleus accumbens bilaterally in a volume of 0.2 μ l over a period of 10 sec or into the lateral ventricle in a volume of 5 μ l over a period of 30 sec through a 30-gauge needle passed into each cannula. The injection needle was kept in position for 30 sec before being withdrawn. The tip of the needle was extruded into the brain 1.5 mm beyond the tip of the cannula.

Experimental procedures: CLN effects were evaluated in three types of experimental models: 1) peripheral injections to vagotomized rats, 2) intracerebroventricular injections and 3) intra-nucleus accumbens injections. Each study consisted of 24 or 30 animals, with five or six animals per group. On the 1st day, animals were treated with saline, HLP (0.1 mg/kg), CLN (40 μ g/kg), or a combination of both drugs, and 60 min later, all groups received AMP (2 mg/kg). At 30 min after AMP administration, each animal was placed in the center of the floor in an open field apparatus (11), and its ambulatory activity was measured for 3 min. On the 2nd, 8th and 15th day, each animal was injected with AMP (2 mg/kg) alone, and 30 min later, the activity was measured in the same manner as on the 1st day.

Drugs: The drugs used were: caerulein (ceruletide diethylamine, synthesized in our laboratory), dl-amphetamine sulfate (Zedrin, Takeda), and haloperidol (Shionogi). All drugs were dissolved in physiological saline and administered subcutaneously, unless

otherwise stated.

Statistical analysis: The experimental results were initially subjected to one-way analysis of variance. Further statistical comparisons of the means were based on Dunnett's *t*-test.

Results

We reported that only rats given CLN plus HLP on the 1st day became less sensitive to AMP for about 2 weeks (5, 6).

The vagotomized animals showed results similar to those of the intact animals. On the 1st day, treatment with HLP or the combination of CLN and HLP significantly reduced AMP-induced hyperactivity in comparison to the saline-treated control group ($F=48.14$, df 3/20, $P<0.01$) (Fig. 1). On the 2nd day, only animals given CLN plus HLP showed reduced susceptibilities to AMP, and the activities of the remaining two drug-treated groups were similar to that of the saline-control group ($F=14.47$, df 3/20, $P<0.01$) (Fig. 1). This reduction lasted for about 1 week ($F=4.25$, df 3/20, $P<0.05$) and disappeared on Day 15 ($F=0.97$, df 3/20, $P>0.1$).

Also, only animals treated with intracerebroventricular CLN (100 ng or 1000 ng) and subcutaneous HLP exhibited the reduction of AMP effects on the 2nd day (Fig. 2). On the 8th day, animals given a dose of 1000 ng of CLN were still less sensitive to AMP. On the 15th day, the reduced susceptibility to AMP disappeared ($F=0.65$, df 5/24, $P>0.1$) (Fig. 2).

Unlike the results described above, no reduction in AMP hyperactivity was observed on the 2nd day when CLN (10 ng or 100 ng) was introduced into the nucleus accumbens ($F=0.51$, df 5/24, $P>0.1$) (Fig. 3).

Discussion

With regard to the sites of action of CLN or CCK-8, Crawley et al. (7) and Smith et al. (8) showed that a primary site may exist in the periphery. On the other hand, Anika et al. (12) proposed that satiety effects of these peptides were not mediated via the vagal nerve, as suppression of food-intake was observed even after vagotomy. Also, Eigyo et al. (13) reported that vagal transection did

not affect CLN-induced locomotor changes in rats. These findings suggest that CLN may act on both peripheral and central sites when

administered peripherally.

In addition, there is no clear answer on the question of whether systemically adminis-

Fig. 1. Time course of the antagonistic effect of caerulein with haloperidol on amphetamine-induced hyperactivity in vagotomized animals. On Day 1, each group received the following treatments at 60 min prior to amphetamine (2 mg/kg, s.c.): group A, saline; group B, haloperidol (0.1 mg/kg, s.c.); group C, caerulein (40 µg/kg, s.c.); group D, caerulein and haloperidol (doses the same as for B and C). At 30 min after amphetamine administration, the open field test was conducted for 3 min. On Day 2, 8 and 15, each group was treated with amphetamine alone. Results are expressed as the mean ± S.E. of 6 animals. Statistical comparisons between group A and the other groups were based on Dunnett's *t*-test (* $P < 0.05$, ** $P < 0.01$).

Fig. 2. Time course of the antagonistic effect of intra-cerebroventricular (I.C.V.) injected caerulein with haloperidol on amphetamine-induced hyperactivity. On Day 1, each group received the following treatments at 60 min prior to amphetamine (2 mg/kg, s.c.): group A, saline (I.C.V.) and saline (s.c.); group B, saline (I.C.V.) and haloperidol (0.1 mg/kg, s.c.); group C, caerulein (100 ng, I.C.V.) and saline; group D, caerulein (100 ng, I.C.V.) and haloperidol; group E, caerulein (1000 ng, I.C.V.) and saline; group F, caerulein (1000 ng, I.C.V.) and haloperidol. The results are expressed as the mean ± S.E. of 5 animals. Abbreviations, see Fig. 1.

Fig. 3. Absence of the long-lasting antagonistic effect of intra-nucleus accumbens (I.N.A.)-injected caerulein with haloperidol on amphetamine-induced hyperactivity. On Day 1, each group received the following treatments at 60 min prior to amphetamine (2 mg/kg, s.c.): group A, saline (I.N.A.) and saline (s.c.); group B, saline (I.N.A.) and haloperidol (0.1 mg/kg, s.c.); group C, caerulein (10 ng, I.N.A.) and saline; group D, caerulein (10 ng, I.N.A.) and haloperidol; group E, caerulein (100 ng, I.N.A.) and saline; group F, caerulein (100 ng, I.N.A.) and haloperidol. Results are expressed as the mean $\pm$ S.E. of 5 animals. Abbreviations, see Fig. 1.

tered CLN can penetrate the blood-brain barrier. Although it was reported that no clear amount of CCK-8 passed the blood-brain barrier (14), Zetler (1) speculated that some pharmacological effects of CLN could be produced if only a small amount of the peptide entered into the brain.

In the present experiments, the long-term effect of CLN persisted after subdiaphragmatic vagotomy. Consequently, peripherally administered CLN does not seem to act in the abdomen through gastric vagal fibers to produce the antagonistic effect of CLN on AMP hyperactivity.

Intracerebroventricular injections of CLN were done to ascertain whether the CLN effect is relevant to the central nervous system. As the result was similar to those after peripheral injections of CLN to the intact and vagotomized rats, it is likely that the long-term effect is elicited by a direct or indirect central action of the peptide.

Enhanced locomotor activity induced by AMP is assumed to result from released dopamine from dopaminergic nerve terminals in the central nervous system (15–18). In addition, 6-hydroxydopamine lesions restricted to the terminal regions of the mesolimbic dopamine system attenuate the

increase in locomotor activity after low dose of AMP, suggesting that the nucleus accumbens is an important structure for locomotor-stimulant drugs (16, 19).

However, the finding that the applications of CLN into the nucleus accumbens produced no effect negates a direct action of CLN on dopaminergic synapses in the nucleus accumbens.

Since a systemic administration of CLN causes a variety of changes in the endocrine system, for example, release of ACTH, β -endorphin, substance P, or prolactin (20–23), the observed effect of CLN may result from some indirect changes in these systems. Further detailed analysis is in progress to clarify the mode of CLN actions in endocrine systems.

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