Effect of Injection of CCK-8 into the Nucleus Caudatus on the Behavior of Rats

Yasuo TAKEDA, Yoshiko KAMIYA*, Kenji HONDA, Yukio TAKANO and Hiro-o KAMIYA**
Department of Pharmacology, School of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-01, Japan
*Department of Research Center, Fukuoka Dental College, Fukuoka 814-01, Japan
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Abstract—This report deals with the effect of cholecystokinin octapeptide (CCK-8) on the regulation of the behavior stimulated by dopaminergic drugs. Bilateral injection of CCK-8 (1 μg per side) into the nucleus caudatus significantly reduced the locomotor hyperactivity induced by methamphetamine. Stereotyped sniffing and yawning occurred after intrastratal administration of apomorphine (20 μg per side). Injections of CCK-8 into the nucleus caudatus completely inhibited the sniffing, but did not affect the yawning induced by apomorphine. It also had no effect on the basal dopamine (DA) level or the methamphetamine-induced DA level in the striatum. These results suggest that the injection of CCK-8 into the nucleus caudatus selectively inhibited the function of the dopaminergic system in the striatum, and blocked post-synaptic DA receptors.

Cholecystokinin (CCK), which was first recognized as a gut hormone by Ivy and Oldberg (1928) (1), has been found in the central nervous system (2, 3). In the nucleus caudatus, CCK-octapeptide (CCK-8)-like immunoreactivity has been found in the terminals and fibers of neurons originating from the claustrum and the piriform cortex (4). Recently, it was found to modulate the release of dopamine (DA) from slices of the nucleus caudatus (5–7). Furthermore, the release of CCK-like immunoreactivity from the nucleus caudatus was shown to be regulated by the activity of dopaminergic D-1 and D-2 receptors (8). Biochemical studies have demonstrated that an intraventricular injection of CCK-8 affects the DA content and DA turnover in the striatum (5, 9, 10). These findings suggest that CCK-8 may modulate the activity of dopaminergic neurons in the nucleus caudatus. However, the exact physiological actions of CCK-8 and related peptides in this region are unknown.

In the present study, we injected CCK-8 into the nucleus caudatus of rats and examined its effects on the behavior induced by stimulation of the dopaminergic system.

Materials and Methods

Animals: Male Wistar rats (200±20 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan). They were housed in a room at 25°C with a 12 hr light-dark cycle (light on 7:00 a.m.) and given free access to commercial food (CE-2, Clea Ltd., Japan) and tap water.

Surgery: All animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and then placed in a stereotaxic apparatus. Guide cannulae (20 gauge stainless steel) were inserted bilaterally into the nucleus caudatus (A, 8.0; L, 2.3; V, −1.0) according to the atlas of the rat forebrain by König and Klippel. The cannulae were fixed to the skull with dental cement. Animals were allowed to recover from surgery for at least 1 week before experiments. At the end of experiments, the sites of injections were determined histologically.
Behavioral studies: All experiments on behavior were carried out between 9:00 and 18:00. Locomotor activity was measured by the open-field method. The open-field apparatus of 60 cm diameter and 53 cm height, divided into 19 divisions was used (11). For observation of stereotyped behavior, the animals were placed in a square wire-mesh cage. Stereotypy, sniffing behavior, was observed for 60 min after the injection of saline or drugs and recorded according to the scale of Creese and Iversen (1975) (12): 0) totally inert, asleep or no movement; 1) very weak sniffing, brief in duration (less than 60 sec in 5 min); 2) occasional sniffing without locomotor hyperactivity; 3) occasional sniffing with locomotor hyperactivity; 4) sniffing and locomotor hyperactivity fairly frequent, but not constant (for more than 3 min in 5 min); 5) constant and intense sniffing with locomotor hyperactivity. Yawns in 5 min period after the injection of drugs were counted as total numbers of mouth openings.

Statistical significances were examined by the two-tailed Student's t-test.

Determination of catecholamines: DA and norepinephrine (NE) were determined by the trihydroxyindole method with some modifications for DA (13). Standard curves for DA and NE were linear between 10 and 100 pmol of DA and 0.5 and 5 pmol of NE per 1 ml, respectively, when standards of these catecholamines were injected into the analytical system for HPLC (Toyo soda Manufacturing, Model HLC-825CA, Tokyo, Japan). The average recovery in this procedure was 73% for DA and 90% for NE.

The rats were decapitated 20 min after the administration of drugs. The brain was rapidly removed, and thin sections (500 μm in thickness) were cut with a McIlwain tissue slicer. Then the nucleus caudatus was punched out from the dorso-lateral part of the striatum (from A: 8.0 to A: 7.5) (14). Tissues were soaked in 1.1 ml of ice-cold 0.1 N perchloric acid containing 0.11 mM ascorbic acid and 5 μM pargyline, rapidly frozen, and kept at −70°C until assayed for catecholamines. For this, the frozen tissues were thawed and homogenized by sonication (Kontes, model 881440). Then 10 μl of the homogenate was removed for protein determination (15), and the remainder was assayed for DA and NE.

Electrophysiological experiments: To rule out the possibility that CCK-8 was causing some non-specific effects on the methamphetamine-induced locomotor hyperactivity, a series of in vitro electrophysiological experiments was done. The rat striatal slice (1 mm) was placed into a small chamber superfused (1 ml/min) with 95% O2−5% CO2 oxygenated Krebs-Ringer-bicarbonate solution. The composition of the solution was as follows (mM): NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 21; NaH2PO4, 1.2; and glucose, 10, pH 7.4. The striatum was stimulated continuously with rectangular pulses of 10–15 V, 0.25 Hz, 5–10 msec delay, and 0.05 msec duration.

Drugs: The drugs used in this study were CCK-8 (Peptide Research, Osaka, Japan), methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Tokyo, Japan) and apomorphine hydrochloride (Sigma, St. Louis, MO, U.S.A.). CCK-8 and methamphetamine were dissolved in physiological saline, and apomorphine was dissolved in distilled water. All drugs in vivo experiments were directly injected bilaterally in a total volume of 2 μl into each nucleus caudatus.

Results

The effect of CCK-8 on methamphetamine-induced hyperlocomotion is shown in Fig. 1. The maximum response was detected 15 to 30 min after bilateral injection of methamphetamine (10 or 25 μg per side) with or without CCK-8 (1 μg per side) into the nucleus caudatus. As shown in Fig. 1, bilateral injections of 1 μg of CCK-8 into the nucleus caudatus decreased the dose-dependent hyperlocomotion induced by bilateral injection of methamphetamine (10 μg and 25 μg, respectively), into the nucleus caudatus. However, spontaneous locomotor activity was not affected within 2 hr after the injection of CCK-8 into the nucleus caudatus (data not shown).

Bilateral injection of 20 μg of apomorphine per side into the nucleus caudatus caused a significant increase in sniffing. This apomorphine-induced sniffing was com-
pletely blocked by the bilateral injection of CCK-8 into the nucleus caudatus (1 μg per side) (Fig. 2A). Bilateral injection of the same dose of apomorphine also induced yawning with marked protrusion of the tongue, but this effect of apomorphine was not affected.

Fig. 1. Time course of the effect of CCK-8 bilaterally injected into the nucleus caudatus (1 μg x 2) on the locomotor hyperactivity induced by methamphetamine (25 μg x 2) in rats. Values are mean activities ± standard errors for the numbers of rats shown in parentheses. *P<0.05 vs. methamphetamine (MP) alone.

Fig. 2. Time course of the effect of CCK-8 injected bilaterally into the nucleus caudatus (1 μg x 2) on (A) sniffing and (B) yawning behaviors induced by apomorphine. *P<0.05, **P<0.01 vs. saline control; *P<0.05, **P<0.01 vs. apomorphine alone.
by CCK-8 (Fig. 2B). CCK-8 given alone did not induce both sniffing and yawning behaviors at 1 µg per side into the nucleus caudatus. No sniffing or yawning was observed on the bilateral injection of apomorphine (20 µg per side) into the nucleus accumbens.

In order to test whether or not CCK-8 at 1 µg into the nucleus caudatus induced depolarization block, a series of the nerve recordings was performed. Electrophysiological parameters such as the amplitude, time of onset, rate of rise, and duration of the action potential due to stimulus were essentially unchanged in the absence or presence of 0.5 mM CCK-8, equivalent to in vivo administration of CCK-8 (1 µg/2 µl), in the striatum. Lidocaine (4 mM) caused a complete blockade within 2.5 min after the treatment. These results indicate that CCK-8 does not exert a non-specific blockade of dopaminergic neurons in the striatum at the concentration used in our in vivo experiments.

The DA levels in the nucleus caudatus of rats were determined 20 min after administration of drugs, when the animals showed maximal changes in behavior induced by methamphetamine (Fig. 1). As shown in Fig. 3, bilateral injection of methamphetamine (25 µg into each nucleus caudatus) significantly increased the DA level by about 30% in both the centrolateral and posterolateral regions of the nucleus caudatus over those in saline-treated rats. This increase in the DA level was correlated with the marked increase in score of locomotor activity. However, intrastriatal injection of CCK-8 (1 µg per side) did not alter the DA content of the nucleus caudatus, and it also had no influence on the increase in DA level induced by intrastriatal injection of methamphetamine (23–26% increase by methamphetamine with CCK-8). Changes in the NE levels in these regions of the nucleus caudatus were parallel with those in the DA level, but the increases of NE induced by methamphetamine were slightly lower than those of DA (increase of DA, 30.7%; increase of NE, 26.3%). The levels of these catecholamines in the A 9 area, cell body regions of the nigrostriatal dopaminergic pathway, were not changed by these treatments.

Discussion

The present study showed that 1) the bilateral injection of CCK-8 into the nucleus caudatus significantly reduced the locomotor hyperactivity induced by methamphetamine and 2) an injection of CCK-8 into the nucleus caudatus completely inhibited the sniffing, but not the yawning induced by intrastriatal administration of apomorphine.

At the start of this study we assumed that yawning induced by a lower dose of apomorphine was due to activation of the presynaptic DA auto-receptors (16–19) and that the sniffing was produced by the post-DA receptors (12, 20, 21). Yamada and Furukawa showed that an intraperitoneal injection of apomorphine at doses ranging from 0.5 to 1 mg/kg produced both sniffing and yawning in rats (19).

In the pilot experiments, we observed that 1) bilateral injection of apomorphine (from 5 to 40 µg per side) into the nucleus caudatus did not affect locomotor activity; 2) sniffing,
one of the stereotypies, was observed by the bilateral injection of apomorphine into the nucleus caudatus in a dose-dependent manner, but yawning was not observed by the bilateral injection of the doses of apomorphine except 20 \( \mu g \) per side. In the present study, we injected 20 \( \mu g \) of apomorphine into each nucleus caudatus so that it might evoke both sniffing and yawning in rats.

Recently, it has been demonstrated that an intraventricular injection of CCK-8 reduced the number of \(^3\)H-spiperone binding sites, which are thought to be DA D-2 receptors in rat striatum (22) and that CCK-8 also interfered with \(^3\)H-spiperone binding to D-2 receptors in the striatal membranes in vitro (23).

The present results suggest that injection of CCK-8 into the nucleus caudatus selectively antagonizes the post-synaptic function of dopaminergic neurons (Figs. 1 and 2A) and that these inhibitory effects of CCK-8 on dopaminergic function may be caused by reduction of DA D-2 receptor sites. However, Van Ree et al. (1983) (24) reported that CCK-8-related peptides (10 ng) do not affect the stereotyped sniffing response elicited by injection of apomorphine into the nucleus caudatus (10 \( \mu g \)). The reason for this difference from our results may be due to a difference in the dose of CCK or time after administration of the peptides: they observed the behavior one hour after the injection of the peptides, but as shown in Fig. 1, we found that the effect of CCK-8 disappeared within 60 min.

There are many reports concerning the effects of CCK-8 on dopaminergic responses, but the results have been conflicting. Researchers have employed a wide range of CCK-8 dosages in their experiments: 9 \( \sim \) 27.5 pg (9), 10 ng (24) and \( \sim \) 1.25 \( \mu g \) (25, 26) into each nucleus caudatus or nucleus accumbens. We chose a relatively high dose of CCK, 1 \( \mu g \) per nucleus caudatus.

The present results with catecholamine assays showed that methamphetamine increased significantly DA levels in the nucleus caudatus. This increase was similar to the results of Kuczynski, who found that DA in the striatum increased by an intraperitoneal injection of amphetamine to rats (27).

Intraventricular administration of CCK-8 has been demonstrated to increase DA turnover in rat striatum, but to have opposite effects in certain areas of the nucleus caudatus and nucleus accumbens of rats (9). In this study, bilateral injection of CCK-8 into the nucleus caudatus had no effect on the basal DA level and methamphetamine-induced DA level in the striatum.

The diffusion of 2 \( \mu l \) of microinjected drugs such as methamphetamine, apomorphine and CCK-8 to the neighboring structures has to be considered, (e.g., into the nucleus accumbens). We previously demonstrated some difference in the effects of CCK-8 on the locomotor hyperactivity induced by methamphetamine administered into the nucleus caudatus and the nucleus accumbens, respectively (28). Therefore, we presumed that the present results reflected effects of these drugs mostly in the nucleus caudatus.

Meyer et al. reported that CCK-8 immunoreactivity in the striatum is projected from the piriform cortex and claustrum (4) and that the release of CCK-8 is regulated by the activities of both D-1 and D-2 receptors (9). On the other hand, DA release in the striatum is modulated by CCK-8 (6, 7). Thus the interaction of CCK-8-like peptide with the monoamine system in the nigrostriatal pathway is complex; and in this study, we suggested that CCK-8 was related to the post-synaptic function of dopaminergic neurons in the nucleus caudatus.

We found from the present experiments that bilateral injection of CCK-8 into the nucleus caudatus reduced the locomotor hyperactivity and stereotyped behavior induced by a DA stimulant (methamphetamine) and an agonist (apomorphine), respectively. These results suggest that CCK-8 blocked the post-synaptic DA receptor in the striatum, but further experiments are needed to clarify the mechanism of action of CCK-8 in relation to dopaminergic and other neuronal systems.

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