Loxiglumide, L-364,718 and L-365,260 Prevent the Inhibition of Spontaneous Acetylcholine Release from the Frontal Cerebral Cortex of Freely Moving Rat Peripherally Administered with Cholecystokinin-8S

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ABSTRACT—We examined the effect of peripheral administration of cholecystokinin (CCK)-8S on spontaneous acetylcholine (ACh) release from the frontal cortex and its prevention by loxiglumide, L-364,718 and L-365,260 in freely moving rats using intracerebral microdialysis. Subcutaneously (s.c.) administered CCK-8S at 10 and 30 μg/kg significantly decreased the release of ACh. The inhibitory effect of 10 μg/kg (s.c.) CCK-8S was prevented by loxiglumide, a mixed type of CCK-A and -B-receptor antagonist, at 1 mg/kg (intraperitoneal) and 40 μg/rat (intracerebroventricular, i.c.v.); L-364,718, a CCK-A-receptor antagonist, at 125 and 250 ng/rat (i.c.v.); and L-365,260, a CCK-B-receptor antagonist at 250 ng/rat (i.c.v.). These results demonstrate that peripherally administered CCK-8S inhibits spontaneous ACh release from the frontal cortex through both central CCK-A (mainly) and -B receptors.

Keywords: Cholecystokinin-8S, Loxiglumide, Spontaneous acetylcholine release (frontal cortex)

Cholecystokinin (CCK) has been reported to produce various central effects such as anti-nociception, feeding suppression, panic, anxiety, learning and memory (1). Peripherally administered CCK-8S (intraperitoneal, i.p.) activates the mechanisms for CCK acting as a co-transmitter in the brain and inhibits food intake (2). The pathway from the peripheral site to the brain site is considered to be through afferent vagus nerves. The memory-enhancing effect of CCK-8S (i.p.) is blocked by vagotomy (3).

CCK exists at high concentrations in several brain regions. In the cerebral cortex, CCK is found in microgram quantities (4). CCK-8S prevents the degeneration of cholinergic neurons in the cerebral cortex following basal forebrain lesion (5). CCK-8S modulates the release of acetylcholine (ACh) from the cerebral cortex in a biphasic manner (6, 7), where CCK-8S enhances ACh release at lower doses but decreases it at doses higher, than 10 μg/kg, i.p. The two types of CCK receptors are evidenced in the brain by autoradiography (8). CCK-B receptors are widely distributed in various areas of the rat brain, whereas CCK-A receptors are found in the area postrema, the solitary tract, nucleus interpedunclaris and the nucleus posterior thalami. The present aim is to investigate whether peripherally administered CCK induces the fronto-cortical spontaneous ACh release through the CCK-A or -B receptor using an intracerebral microdialysis with its antagonists.

Wistar male rats (9- to 10-weeks-old; Japan SLC, Shizuoka) were used two days after implanting a microdialysis tube. Animals were stereotaxically implanted with a transverse dialysis tube (9) under chloral hydrate anesthesia (400 mg/kg, i.p.). The tube was placed in the sites (coordinates, A: +2.7 mm and V: 2.5 mm) measured from the bregma. The polyacrylonitrile tube was made of a hemofilter (i.d.=0.2 mm, o.d.=0.3 mm; a molecular weight of more than 55,000 was the cut-off; AHF-UN, Asahi Medical, Tokyo) having an active surface 8.0-mm-long. The outer surface was covered with epoxy resins. Its extracranial portions were fastened with dental cement to the cranium. The stainless steel tube was connected to a syringe pump (CMA/100; Carnegie Medicine AB, Stockholm, Sweden) through a polyethylene tube (700×0.13 mm). The polyethylene tubings (100 mm, i.d.=0.4 mm and o.d.=0.8 mm) were placed subcutaneously (s.c.) or i.p. on the back of the rat for the injection of CCK-8S and CCK antagonists. A stainless steel cannula (21 gauge) for intracerebroventricular injection was implanted into the right lateral ventricle (coordinates from the bregma: A: −0.8 mm, L: 1.4 mm and V: 3.6 mm) and was fixed with dental cement to the skull. After surgery,

the rats were housed individually in cages (20×30×40 cm) at 24±1℃, 55±5% humidity, and with a 12-hr light-/dark cycle (light-on time, 7:30−19:30). Food and water were given ad libitum.

One end of a stainless steel tube was connected to a syringe pump through a polyethylene tubing (700×0.13 mm); from this tube to the other stainless steel tube (Igarashi Ika Kougyou, Tokyo), Ringer solution was perfused and collected into a teflon tube (1000×0.1 mm) that was connected to the sampling loop (100 μl; AS-10, Eicom, Tokyo). The samples (30 μl) were collected from freely moving rats and injected automatically with ethylhomocholine as an internal standard at 15-min intervals. The Ringer solution contained 147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$. The pH value was adjusted to 6.0 with 0.05 N NaOH. To collect detectable dialysate containing ACh, 1 μM neostigmine bromide (Sigma, St. Louis, MO, USA), a reversible cholinesterase inhibitor was added to the Ringer solution.

ACh was assayed by an HPLC-ECD system equipped with a precolumn, an analytical column (AC-gel; Eicom, Kyoto), an immobilized enzyme column (AC-Enzympac; Eicom, Kyoto) and an electrochemical detector. The mobile phase consisted of 0.1 M phosphate buffer solution (pH 8.0) containing 150 mg/l of sodium 1-decanesulfonate (Tokyo Kasei, Tokyo) and 65 mg/l of tetramethylammonium chloride (Nacalai Tesque, Kyoto) and flowed at a rate of 1 ml/min. An amperometric controller equipped with a platinum electrode (ECD-100; Eicom, Kyoto) was used for electrochemically monitoring the column eluates. The detector potential was maintained at 500 mV versus an Ag/AgCl reference electrode (10). ACh release was estimated as a percentage of basal release before the administration of CCK antagonists. The 100% basal release of ACh was the average of consecutive three data points just before drug application. ACh levels between the control and treatment groups at each time point were compared by one-way analysis of variance, and this was followed by the multiple Student's range test with $P=0.01$ or 0.05 as the criterion for significant difference. The drugs used were CCK-8S (Peptide Institute, Osaka) and loxiglumide (the gift by Kaken Seiyaku, Kyoto), a CCK-A and -B antagonist. CCK-8S and loxiglumide were dissolved in saline containing 3.3% benzylbenzoate solution just before use, respectively. CCK-8S (s.c.) and loxiglumide (i.p.) were administered at 1 ml/kg body weight, respectively. Loxiglumide (intracerebroventricular, i.c.v.) was also administered at 5 μl/rat. The CCK-A-receptor antagonist L-364,718 (3*S*(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide) (11) and the CCK-B-receptor antagonist L-365,260 (3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*N*′-(3-methylphenyl)urea) (12) (gifts by Dr. G. Katsuura, Shionogi Institute, Shiga) were solubilized in 2.5% propylene glycol plus 2.5% ethanol, and then each was diluted with warmed saline before use.

The average basal release of ACh from the frontal cortex was 3.24±0.35 pmol/15 min (mean±S.E.M., n=47). The s.c. administration of 10 and 30 μg/kg CCK-8S significantly decreased the release of ACh by 31% (Fig. 1A) and 25%, respectively. However, at the begining stage, CCK-8S at 30 μg/kg tended to increase spontaneous ACh release (data not shown). The inhibitory effect of CCK-8S reached the maximum value within 45 min and disappeared 1 hr after the administration. CCK-8S at 1.5 μg/kg (s.c.) did not affect the spontaneous ACh release. Loxiglumide (1 mg/kg, i.p.), which was administered 15 min before CCK-8S (10 μg/kg, s.c.), reversed the CCK-8S-induced decrease of spontaneous ACh release (Fig. 1A). The CCK-8S response was also prevented by loxiglumide (1 mg/kg, s.c.) and tended to be prevented by loxiglumide at 0.5 mg/kg (s.c.), but not 0.3 mg/kg (s.c.) (data not shown). These doses of loxiglumide did not affect the spontaneous ACh release when injected alone (data not shown). When loxiglumide was given at 40 μg/rat, and L-364,718 and L-365,260 were given at 250 ng/rat into the cerebroventricle, each CCK receptor antagonist prevented the inhibitory effect of CCK-8S on the ACh release (Figs. 1B, 2A and 2B). Although L-364,718 and L-365,260 (250 ng/rat, i.c.v.) did not affect the spontaneous ACh release by themselves, the CCK response tended to be increased by L-364,718 1 hr to 1.5 hr after CCK-8S application. The action of CCK-8S was neither affected by loxiglumide (12.5 μg/rat, i.c.v.) nor by L-365,260 (125 ng/rat, i.c.v.) (data not shown), but was prevented by L-364,718 at 125 ng/rat (i.c.v.) (data not shown) to the same extent as that at 250 ng/rat (i.c.v.) (Fig. 2A). These results demonstrated that the CCK-induced inhibition of ACh release was more sensitive to the CCK-A-receptor antagonist than the CCK-B-receptor antagonist. Loxiglumide (120 μg/rat, i.c.v.) itself increased ambulatory locomotion (data not shown).

Since CCK-8S does not pass the blood-brain barrier (10), the central action of CCK-8S is through stimulation of the peripherally located CCK-A receptors (13). CCK-8S (i.p.) modulates ACh release from the cortex in a biphasic manner (6, 7). In the present study, the decrease, not the increase, of spontaneous ACh release was observed in the same region and at the same dose. This may be due to the differences in methodologies and manipulation such as the use of the cortical cup, microdialysis technique, the anesthetics employed (6, 7) and the use of rats in the freely moving (the present study) state.

There are two pathways between peripheral and central CCK receptors modulating fronto-cortical ACh release.

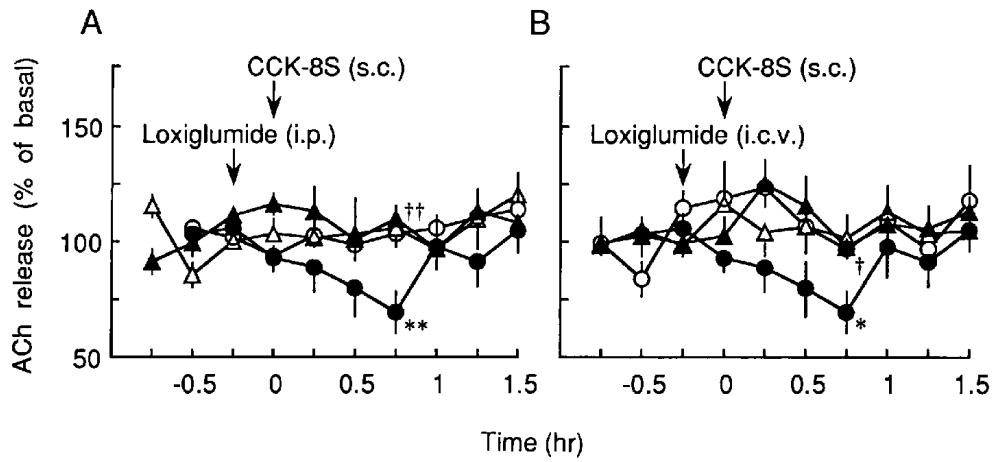

Fig. 1. Protective effects of loxiglumide (▲; A, 1 mg/kg, i.p. and B, 40 μg/rat) against the cholecystokinin (CCK)-8S (10 μg/kg, s.c.)-induced decrease of fronto-cortical spontaneous acetylcholine release. Loxiglumide was injected 15 min before CCK-8S administration. The injections are indicated by arrows. △: loxiglumide alone. Data are the mean values±S.E.M. of 6 rats presented as a percentage of the basal value (from three samples before the administration of loxiglumide). Significant difference between the control and the drug-treated group was analyzed by one-way ANOVA, followed by the multiple Student's range *t*-test. $^{*}P<0.05$ and $^{**}P<0.01$ versus vehicle (saline) (○) and $^{\dagger}P<0.05$ and $^{\dagger\dagger}P<0.01$ versus CCK-8S alone (●).

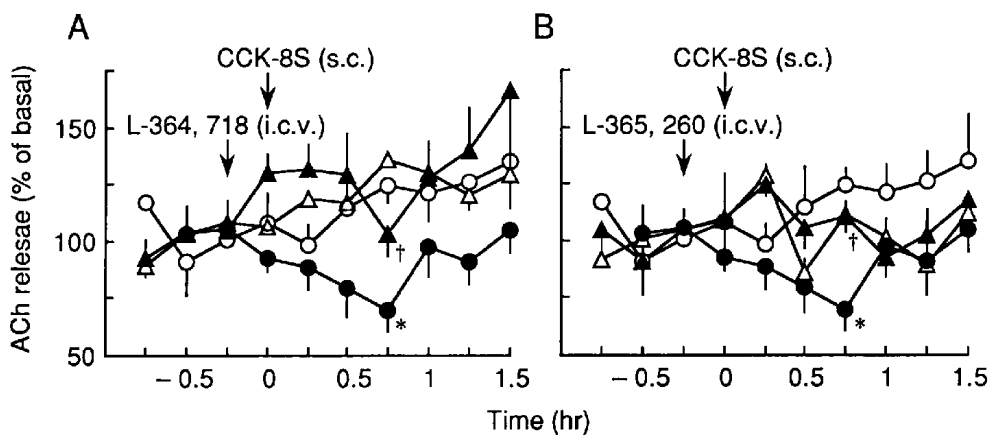

Fig. 2. Protective effects of L-364,718 (A, ▲: 250 ng/rat) and L-365,260 (B, ▲: 250 ng/rat) against the cholecystokinin (CCK)-8S (10 μg/kg, s.c.)-induced decrease of fronto-cortical spontaneous acetylcholine release. L-364,718 and L-365,260 were injected 15 min before CCK-8S administration. The injections are indicated by arrows. Data are the mean values±S.E.M. of 4–6 rats presented as the percentage of the basal value (from three continuous samples before the administration of CCK antagonists). △: CCK antagonists alone. Significant difference between the control and the drug-treated group was analyzed by one-way ANOVA, followed by the multiple Student's range *t*-test. $^{*}P<0.05$ versus vehicle (saline) (○) and $^{\dagger}P<0.05$ versus CCK-8S alone (●).

One is the afferent vagus nerve where CCK-binding sites are located (13), and the other is an unknown route since bilateral vagotomy does not abolish the inhibition of ACh release produced by high doses of CCK-8S (7). Loxiglumide is a potent and reversible CCK-A- and -B-receptor antagonist. Its affinity for peripheral CCK-A receptors is 30 times higher than that for central CCK-B receptors (14). In the present study, peripheral administration of loxiglumide completely inhibited the CCK-8S-induced decrease in ACh release in the frontal cortex. Central administrations of loxiglumide, a mixed type of CCK-A- and -B-receptor antagonist; L-364,718, a CCK-A-receptor antagonist; and L-365,260, a CCK-B-receptor antagonist, also antagonized the decrease of spontaneous ACh release by peripherally administered CCK-8S. Peripherally administered CCK-8S may stimulate afferently the central nervous system, release CCK to stimulate central CCK-A (mainly) and -B receptors, and consecutively inhibit ACh release from the frontal cortex.

In conclusion, peripherally administered CCK-8S in-

hibits ACh release from the frontal cortex through both central CCK-A (mainly) and -B receptors in freely moving rats.

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