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

Ikuko Kimura, Satomi Wakasono and Masayasu Kimura

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan

Received October 17, 1994 Accepted March 11, 1995

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)
the rats were housed individually in cages (20 x 30 x 40 cm) at 24 ± 1°C, 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 x 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 x 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 ethyl-homocholine as an internal standard at 15-min intervals. The Ringer solution contained 147 mM NaCl, 4 mM KCl and 2.3 mM CaCl₂. 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-Enzymapac; 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 t-test with P=0.01 or 0.05 as the criterion for significance. The drugs used were CCK-8S (Peptide Institute, Osaka) and loxiglumide (the gift by Kaken Seiyaku, 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 beginning 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. 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 tP<0.05 and ttP<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 tP<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.

Acknowledgments

We thank Prof. Dr. H. Watanabe (Research Institute for Wakan-Yaku, Oriental Medicines, Toyama Medical and Pharmaceutical University) for his critical comments on this manuscript and technical advice, and Dr. G. Katsuura (Aburahi Lab., Shionogi & Co., Ltd., Shiga) for his gifts of CCK-A- and -B-receptor antagonists, L-364,718 and L-365,260, and Kaken Seiyaku (Kyoto) for the gift of loxiglumide.

REFERENCES

1 Hughes J and Woodruff GN: Neuropeptides. Arzneimittelforschung 42, 250–255 (1992)
2 Linden A and Södersten P: Relationship between the concentration of cholecystokinin-like immunoreactivity in plasma and food intake in male rats. Physiol Behav 48, 859–863 (1990)
3 Flood JF, Smith GE and Morley JE: Modulation of memory processing by cholecystokinin: dependence on the vagus nerve. Science 236, 832–834 (1987)
4 Crawley JN: Comparative distribution of cholecystokinin and other neuropeptides – Why is this peptide different from all other peptides? Ann NY Acad Sci 448, 1–8 (1985)
5 Sugaya K, Takahashi M and Kubota K: Cholecystokinin protects cholinergic neurons against basal forebrain lesion. Jpn J Pharmacol 59, 125–128 (1992)
6 Magnani M, Mantovani P and Pepeu G: Effect of cholecystokinin octapeptide and ceruletide on release of acetylcholine from cerebral cortex of the rat in vivo. Neuropharmacology 23, 1305–1309 (1984)
7 Magnani M, Florian A, Casamenti F and Pepeu G: An analysis of cholecystokinin-induced increase in acetylcholine output from cerebral cortex of the rat. Neuropharmacology 26, 1207–1210 (1987)
8 Moran TH, Robinson PH and Goldrich MS: Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res 362, 175–179 (1986)
9 Imperato A and Chiara GD: Dopamine release and metabolism in awake rats after systemic neuroleptics as studied by trans-striatal dialysis. J Neurosci 5, 297–306 (1985)
10 Potter PE, Meek JL and Neff NH: Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J Neurochem 41, 188–194 (1983)
11 Hamilton MH, Rose IC, Herberg LJ and de Belleruche JS: Effect of intracerebroventricular and systemic injections of caerulein, a CCK analogue, on electrical self-stimulation and its interaction with the CCK4 receptor antagonist, L-364,718 (MK-329). Psychopharmacology (Berlin) 101, 384–389 (1990)
12 Lotti VJ and Chang RSL: A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. Eur J Pharmacol 162, 273–280 (1989).
13 Moran TH, Norgren R, Crosby RJ and McHugh PR: Central and peripheral vagal transport of cholecystokinin binding sites occurs in afferent fibers. Brain Res 526, 95–102 (1990)
14 Setnikar I, Bani M, Ceredi R, Chiste R, Makovec F, Pacini MA, Revel L, Rovati LC and Rovati LA: Pharmacological characterization of a new potent and specific nonpolypeptidic cholecystokinin antagonist. Arzneimittelforschung 37, 703–707 (1987)