Possible Involvement of the CCK Receptor in the Benzodiazepine Antagonism to CCK in the Mouse Brain

Kiminobu SUGAYA, Ikuko MATSUDA and Kazuhiko KUBOTA
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan
Accepted October 6, 1986

Abstract—In mice, intraperitoneally injected chlordiazepoxide and proglumide, both of which are regarded as cholecystokinin (CCK) receptor antagonists in the peripheral tissues, dose-dependently inhibited the satiety induced by 200 ng of intracisternally administered CCK octapeptide (CCK8). Intraperitoneally administered diazepam (1 mg/kg) and/or Ro 15-1788 (5 mg/kg), a benzodiazepine antagonist, both prevented the elevation in the pain threshold induced by 1 μg of CCK8. However, Ro 15-1788 did not antagonize the effect of diazepam that reversed the CCK-induced antinociception. Ro 15-1788 also inhibited the satiety induced by CCK8. From these results, it was considered that the antagonism, which was observed in the present work, of benzodiazepines and proglumide to CCK8 seemed to occur at the CCK receptor and not at the benzodiazepine receptor in the brain.

In 1980, the CCK receptor was identified through experiments using the homogenates, from rat, guinea-pig and mouse brains (1-3). Meanwhile, possible roles of CCK in the central nervous system have been suggested physiologically (4, 5) or pharmacologically (6, 7). In a series of previous papers, the authors reported that CCK action in the isolated guinea-pig gallbladder was antagonized by benzodiazepines in a competitive fashion (8, 9), and the competition was highly likely to occur at CCK receptors on the smooth muscle cells (10). Further, we demonstrated that the antinociceptive (11), hypothermic (12) and satiety action (13) of CCK, which was intracisternally administered to mice, could also be antagonized by benzodiazepines. However, the mechanism of the antagonism between CCK and benzodiazepines in the central nervous system remains unclear. In this paper, we show a possibility that the interaction of CCK and benzodiazepines may take place at the CCK receptor also in the central nervous system.

Materials and Methods
Male ddY strain mice weighing 20±2 g were used. The mice were housed in temperature- and humidity-controlled rooms (24±1 °C, 55±5%) with 12 hr light cycles for more than a week before use.

CCK8 was purchased from the Peptide Institute (Osaka). Chlordiazepoxide (CDP) hydrochloride and diazepam (DZP) were generous gifts from Yamanouchi Pharmaceutical Co., Ltd., and Ro 15-1788 and proglumide were from Nippon Roche K.K. and Eisai Co., Ltd., respectively. CCK8 was dissolved in distilled water containing 0.4% brilliant blue which served for monitoring proper intracisternal injection. Ten μl of the CCK8 solution containing 200 ng or 1 μg of CCK8 was injected into the cerebellomedullary oistern of mice by using a J-shaped needle according to the method described by Ueda et al. (14). CDP hydrochloride was dissolved in physiological saline solution. Ro 15-1788 was suspended in physiological saline solution or suspended in physiological saline solution with the addition of Tween 80 at a concentration of 0.5% when the concentration of Ro 15-1788 in the solution was high. Diazepam was suspended in physiological saline by using
Tween 80 in the same way. Proglumide was first dissolved in diluted sodium hydroxide solution, and then the solution was diluted with physiological saline solution. The pH of the final solution was adjusted to 7.0. The benzodiazepines, Ro 15–1788 and proglumide, were injected intraperitoneally to mice 10 min before the intracisternal injection of CCK8.

**Measurement of food intake:** Mice were fed on a liquid food (7% protein, 7.7% fat, 10.2% sugar, 1.6% ash, 73.5% water and 135 kcal/100 g) for a week and then fasted for 24 hr, allowing free access to water. The fasted mice were given an intraperitoneal injection of drugs or saline and an intracisternal injection of CCK or vehicle (brilliant blue solution) 10 min after the intraperitoneal injection, and then they were allowed to have the liquid food. The cumulative intakes of the liquid food by mice were measured. The food intake experiments were started at about 6 p.m.

**Measurement of pain threshold:** The pain threshold of mice was measured by the tail-flick method described in the previous paper (11). An intraperitoneal administration of drugs was made 10 min after an intracisternal administration of CCK.

### Results

**Effects of proglumide on CCK8-induced antinociception and satiety:** One μg of CCK8 administered intracisternally produced long lasting antinociceptive action in mice as reported previously (11). Intraperitoneally administered proglumide (200 mg/kg) caused no change in the pain threshold of mice by itself, but almost completely prevented the elevation in the pain threshold induced by CCK8 administration (Fig. 1). Intracisternal CCK8 (200 ng/mouse) caused significant inhibition of the food intake in mice, but the inhibition was dose-dependently antagonized by 30 to 100 mg/kg of intraperitoneal proglumide which revealed no appreciable effects on the food intake in mice (Fig. 2).

CDP also dose-dependently inhibited the satiety induced by CCK8. The dose-response relationships of CDP and proglumide on the reversal of CCK8-induced satiety are shown in Fig. 3. On the basis of the results shown in Fig. 3, ID50, the dose of CDP or proglumide that reverses the CCK8-induced depression of the food intake by 50%, was obtained. The ID50 for CDP was 0.28 mg/kg or 0.82 μmol/kg, and the one for proglumide was 34.5 mg/kg or 103.2 μmol/kg.

**Effects of Ro 15–1788 on CCK8-induced antinociception and satiety:** Although the data were omitted here, intraperitoneal administrations of 5 mg/kg of Ro 15–1788 and/or 1 mg/kg of DZP did not affect the pain threshold of mice. However, they suppressed the elevation in the pain threshold of mice produced by 1 μg/mouse of intracisternal CCK8 (Fig. 4).

An intraperitoneal administration of Ro 15–1788 (0.3 mg/kg) did not affect the food intake in mice, but it antagonized the satiety action of CCK8 as shown in Fig. 5.

### Discussion

In the present work, CCK8 was intracisternally administered to mice at doses of 200 ng/mouse in the satiety and 1 μg/mouse
Fig. 2. Effect of proglumide on satiety induced by CCK8 in mice. CCK8 was dissolved in a vehicle, 0.4% brilliant blue aqueous solution, and administered intracisternally to mice 10 min after an intraperitoneal injection (i.p.) of proglumide or saline. Ordinate, cumulative volume (ml) of liquid food taken by mice in the initial 20 min period. Saline and vehicle D, proglumide (10 mg/kg) and vehicle M, proglumide (30 mg/kg) and vehicle ®, proglumide (100 mg/kg) and vehicle ®, saline and 1 ug CCK8 ®, proglumide (10 mg/kg) and CCK8 ®, proglumide (30 mg/kg) and CCK8 ®, proglumide (100 mg/kg) and CCK8 ®. Vertical bars represent the S.E. of the mean. '"P<0.01, ++P<0.01, +P<0.05, in the comparison of saline and vehicle with saline and CCK8 and saline and CCK8 with proglumide (10–100 mg/kg) and CCK8 by Student’s t-test.

in the antinociception experiments. These small amounts of CCK8 caused significant effects, suggesting that CCK8 probably exerts its central effects through action on the CCK receptors of the brain. On the other hand, CDP and DZP that were intraperitoneally administered at low doses like 1 to 5 mg/kg suppressed the satiety (Fig. 3) and antinociception (Fig. 4) induced by CCK, suggesting that the benzodiazepines may act on the benzodiazepine receptor in the brain. Therefore, several possibilities on the mechanism of the antagonism between CCK8 and benzodiazepines in the brain can be considered. Firstly, the antagonism may occur either at CCK or at the benzodiazepine receptor. Secondly, physiological antagonism may also occur via an action on their own receptor. Bradwejn et al. suggested using an electrophysiological technique that in the CCK-benzodiazepine antagonism, benzodiazepines act on the benzodiazepine receptor because the central benzodiazepine antagonist, Ro 15–1788, counteracted the flurazepam and CDP with regard to their reversal of CCK8 action (15). In contrast, in our previous papers, we demonstrated that benzodiazepines antagonized CCK action in a competitive manner at the CCK receptor on the smooth muscle cell membrane of the guinea-pig gallbladder (8–10). Meanwhile, proglumide has also been well established to act as a competitive receptor antagonist of CCK in the peripheral organs (16).

In the present work, proglumide suppressed the satiety and antinociception induced by CCK8 in a manner similar to the benzodiazepines, and the dose-response lines for CDP and proglumide were parallel to each other, as shown in Fig. 3. These findings are indicative that both benzodiazepines and proglumide revealed their action to antagonize CCK through acting at the same site in the brain. In addition, 5 mg/kg of Ro 15–1788, failed to antagonize the effect induced by 1 mg/kg of DZP that reversed CCK8-induced antinociception (Fig. 4). According to
Fig. 4. Effect of Ro 15–1788 and diazepam on antinociception induced by CCK8 in mice. CCK8 was dissolved in a vehicle, 0.4% brilliant blue aqueous solution, and administered intracisternally to mice 10 min after an intraperitoneal injection of Ro 15–1788, diazepam or control solution (0.5% Tween 80 physiological saline solution). Ordinate: pain threshold of mice expressed as percent increase in latency when compared with that obtained before the i.p. injection. An i.p. injection of control solution and an i.c. injection of vehicle (○), i.p. Ro 15–1788 (5 mg/kg) and DZP (1 mg/kg) and i.c. vehicle (●), i.p. control solution and i.c. 1 μg CCK8 (∆), i.p. DZP (1 mg/kg) and i.c. 1 μg CCK8 (■), i.p. Ro 15–1788 (5 mg/kg) and i.c. 1 μg CCK8 (■), i.p. Ro 15–1788 (5 mg/kg) and DZP (1 mg/kg) and i.c. CCK8 (▲). Vertical bars represent the S.E. of the mean. **P<0.01, in the comparison of (A) with (A) by Student’s t-test.

Bonetti et al. (17) and Polo et al. (18), the dose of 5 mg/kg of Ro 15–1788 is considered good enough to antagonize the behavioral effects induced by 1 mg/kg of DZP. On the other hand, 0.3 mg/kg of Ro 15–1788 prevented the satiety induced by CCK8 (Fig. 5). In previous papers, we also demonstrated that Ro 15–1788 behaved as if it were a potent benzodiazepine-like agonist on the prevention of CCK8-induced central effects. Therefore, a discrepancy exists between our results and those obtained by Bradwejn regarding the central antagonism between Ro 15–1788 and benzodiazepines. Our results give rise to the tentative conclusion that the antagonism between CCK8 and benzodiazepines seems to occur at CCK receptors in the brain and not at benzodiazepine receptors. However, whether such benzodiazepine-CCK antagonism as was observed in the present work can be directly related to the central effects of benzodiazepine such as antianxiety and muscle relaxation is unclear at present.

Acknowledgments: This work was supported by a Grant-in-Aid from the Tokyo Biochemical Research Foundation.

References
1. Saito, A., Sankaran, H., Goldfine, I.D. and Williams, J. A.: Cholecystokinin receptors in the brain: Characterization and distribution. Science 208, 1155–1156 (1980)
2. Hays, S.E., Beinfeld, M.C., Jensen, R.T., Goodwin, F.K. and Paul, S.M.: Demonstration of a putative receptor site for cholecystokinin in rat brain. Neuropeptides 1, 53–62 (1980)
3. Innis, R.B. and Snyder, S.H.: Distinct cholecystokinin receptors in brain and pancreas, Proc. Natl. Acad. Sci. U.S.A. 77, 6917–6921 (1980)
4. Ishibashi, S., Oomura, Y., Okajima, T. and Shibata, S.: Cholecystokinin, motilin and secretion effects on the central nervous system. Physiol. Behav. 23, 401–403 (1979)
5. Phillips, J.W. and Kirkpatrick, J.R.: The actions of motilin, luteinizing hormone releasing hormone, cholecystokinin, somatostatin, vaso-
active intestinal peptide, and other peptides on rat cerebral cortical neurons. Can. J. Physiol. Pharmacol. 58, 612–623 (1980)

6 Gibbs, J., Young, R.C. and Smith, G.P.: Cholecystokinin elicits satiety in rat with open gastric fistulas. Nature 245, 323–325 (1973)

7 Jurna, I. and Zetler, G.: Antinociceptive effect of centrally administered caerulein and cholecystokinin octapeptide (CCK-8). Eur. J. Pharmacol. 73, 323–331 (1981)

8 Kubota, K., Sunagane, N., Sugaya, K., Uruno, T. and Matsuoka, Y.: Competitive antagonism of cholecystokinin and some benzodiazepines at cholecystokinin receptors of smooth muscle. Japan. J. Pharmacol. 33, Supp. 87P (1983)

9 Kubota, K., Sugaya, K., Sunagane, N., Matsuda, I. and Uruno, T.: Cholecystokinin antagonism by benzodiazepines in the contractile response of the isolated guinea-pig gallbladder. Eur. J. Pharmacol. 110, 225–231 (1985)

10 Kubota, K., Sugaya, K., Fujii, F., Itonaga, M. and Sunagane, N.: Inhibition of cholecystokinin response in the gallbladder by dibenamine and its protection by benzodiazepines. Japan. J. Pharmacol. 39, 274–276 (1985)

11 Kubota, K., Sugaya, K., Matsuda, I., Matsuoka, Y. and Terawaki, Y.: Reversal of antinociceptive effect of cholecystokinin by benzodiazepines and a benzodiazepine antagonist. Ro 15-1788. Japan. J. Pharmacol. 37, 101–105 (1985)

12 Sugaya, K., Matsuda, I. and Kubota, K.: Inhibition of hypothermic effect of cholecystokinin by benzodiazepines and a benzodiazepine antagonist, Ro 15-1788, in mice. Japan. J. Pharmacol. 39, 277–279 (1985)

13 Kubota, K., Matsuda, I., Sugaya, K. and Uruno, T.: Cholecystokinin antagonism by benzodiazepines in the food intake in mice. Physiol. Behav. 36, 175–178 (1986)

14 Ueda, H., Amano, H., Shiomi, H. and Takagi, H.: Comparison of the analgesic effects of various opioid peptides by a newly devised intracisternal injection technique in conscious mice. Eur. J. Pharmacol. 56, 265–268 (1979)

15 Bradwejn, J. and Montigny, C.: Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurons. Nature 312, 363–364 (1984)

16 Kaplita, P.B. and Roebuck, B.D.: Proglumide antagonizes the stimulation of rabbit gallbladder by cholecystokinin. Arch. Int. Pharmacodyn. Ther. 269, 271–276 (1984)

17 Bonetti, E.P., Pieri, L., Cermin, R., Schaffner, R., Pieri, M., Gamzu, E.R., Muller, R.K.M. and Haefely, W.: Benzodiazepine antagonist Ro 15-1788: neurological and behavioral effects. Psychopharmacology (Berlin) 78, 8–18 (1982)

18 Polc, P., Laurent, J.P., Scherschlichts, R. and Haefely, W.: Electrophysiological studies on the specific benzodiazepine antagonist Ro 15-1788. Naunyn Schmiedebergs Arch. Pharmacol. 316, 317–325 (1981)