Effects of Cholecystokinin Tetra and Octa Peptides on Locomotor Activity in Mice

Yasuo TAKEDA, Yukio TAKANO and Hiro-o KAMIYA*

Department of Pharmacology, School of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-01, Japan

Accepted June 21, 1986

Abstract—Intraperitoneal injection of cholecystokinin octapeptide (10 and 100 μg/kg, i.p.), but not the tetra-peptide (1 mg/kg, i.p.), into mice significantly reduced spontaneous locomotor activity in a dose-dependent manner. The increase in locomotor activity induced by methamphetamine (1 mg/kg, i.p.) and thyrotropin-releasing hormone (5 mg/kg, i.p.) was significantly reduced by cholecystokinin peptides. However, the inhibitory effects of these peptides differed. Neither the tetra nor octa peptide influenced the increased locomotor activity induced by nomifensine (5 mg/kg, i.p.), apomorphine (3 mg/kg, i.p.) or scopolamine (2 mg/kg, i.p.). Thus, these cholecystokinin peptides seem to selectively antagonize increased locomotor activity via the presynaptic dopaminergic system.

Cholecystokinin (CCK) is a gut hormone in the peripheral nervous system, and it is present in large amounts in the brain. Hökfelt et al. (1) found that CCK coexists with dopamine (DA) in a population of mesolimbic neurons with projections to the limbic regions. Behavioral studies showed that systemic injection of C-terminal CCK-octapeptide (CCK-8) caused analgesia, hypothermia, potentiation of hexobarbital-induced sleep, sedation and an anticonvulsant effect in mice (2). CCK-8 also suppresses locomotor activity when administered centrally or peripherally (3-5). These behavioral effects of CCK-8 were similar to those of neuroleptics, thereby suggesting that CCK-8 may modulate the neuronal activity of dopaminergic neurons, and it may even be involved in the pathogenesis of schizophrenia. CCK-tetrapeptide (CCK-4), a C-terminal fragment of CCK-8, also has an effect on locomotor activity (4, 6, 7). We now report the effects of CCK-8 and CCK-4 on increased locomotor activity induced by central acting drugs.

Male ddY mice, weighing 20–25 g, were obtained from Kyudo Animal Laboratory (Kumamoto, Japan). They were housed in a room at 20–25°C with a 12 hr light-dark cycle (light on at 7:00 a.m.) and given free access to commercial food (Kyudo) and tap water. Behavioral experiments were carried out between 9:00 a.m. and 6:00 p.m. Locomotor activity was studied by an open-field method (8).

The animals were allowed to adapt to the open-field apparatus for 30 min. Behavior was then observed 10, 20, 30, 40, 60, 90, 120, 240 and 360 min after injections of drugs. The locomotor score was expressed as the number of lines crossed for 1 min at each observation period. The apparatus was cleaned and deodorized between experiments.

CCK-4, CCK-8 and thyrotropin-releasing hormone (TRH) were purchased from Peptide Research (Osaka, Japan). Methamphetamine hydrochloride was from Dainippon Pharmaceutical Co., nomifensine from Hoechst, apomorphine hydrochloride from Sigma and scopolamine hydrochloride from Merck. CCK-4 was dissolved in 0.5 N NaHCO₃ and diluted with physiological saline. Other drugs were dissolved in physiological saline. All drugs were adminis-
tered intraperitoneally (i.p.) in a volume of 5 ml/kg. CCK-4 was pretreated 10 min before and CCK-8 was simultaneously treated with other drugs.

Statistical analysis of data was performed by the two-tailed Student's t-test.

CCK-4 (1 mg/kg) did not affect spontaneous locomotor activity between 10 min and 360 min after injection. However, CCK-8 (10 or 100 μg/kg) resulted in a significant and dose-dependent decrease in spontaneous locomotor activity compared with that in the saline-treated controls. This decrease persisted for at least 60 min (data not shown).

CCK-4 and CCK-8 significantly reduced the locomotor hyperactivity induced both by methamphetamine (1 mg/kg) and TRH (5 mg/kg) for 40 min. CCK-8 strongly inhibited the hyperactivity to less than that seen in the saline-treated controls, whereas CCK-4 reduced it to the control level (Fig. 1).

Figure 2 shows results on the effects of CCK-4 and CCK-8 on the locomotor hyperactivity induced by various drugs. CCK-4 and CCK-8 had no effect on the locomotor hyperactivity induced by nomifensine (5 mg/kg), apomorphine (3 mg/kg) or scopolamine (2 mg/kg).

There is increasing evidence that CCK-8 reduces food intake and exploratory behavior in rats and mice (3, 4, 9). It was suggested that the inhibitory effect of CCK-8 on such behavior may relate to satiety (3). The effect of CCK-4 seems to differ from that of CCK-8. Intracerebroventricular injection of CCK-4 increased the spontaneous locomotor activity in rats (6, 7) or had no effect on this activity (10), whereas CCK-8 decreased the activity (7). The i.p. injection of CCK-4 did not have a significant effect on ambulation and rearing activity (10). Crawley et al. (4) reported that

---

Fig. 1. Time course of the effects of (A, C) CCK-4 (1 mg/kg, i.p.) and (B, D) CCK-8 (10 and 100 μg/kg, i.p.) on the increased locomotor activity induced by (A, B) methamphetamine (MP, 1 mg/kg, i.p.) and (C, D) TRH (5 mg/kg, i.p.). *P<0.05, **P<0.01 vs. control, #P<0.05, ##P<0.01 vs. MP or TRH alone. Number in parenthesis indicates the number of mice.
CCK-4 had no effect on exploratory behavior in mice over the dose range of $10^{-7}$ to $10^{-3}$ mol/kg. They found that the effect of i.p. administration of CCK-4 on exploratory behavior did not relate to the satiety syndrome. The present study indicates that intraperitoneal injection of CCK-4 and CCK-8 strongly inhibits increases in locomotor activity induced by methamphetamine and TRH (Fig. 1), but not those induced by nomifensine, apomorphine and scopolamine (Fig. 2). Methamphetamine and TRH are known to cause a release of DA (11). Makanjuola and Ashcroft (12) reported that the increase in locomotor activity is caused by activation of mesolimbic dopaminergic neurons, whereas stereotyped behaviors such as sniffing are related to activation of the nigro-striatal dopaminergic neurons. It has also been suggested that the increase in locomotor activity induced by TRH is related to presynaptic sites of dopaminergic neurons of the nucleus accumbens (13). On the other hand, nomifensine was shown to inhibit the
reuptake of DA (14), and a high dose of apomorphine, such as that used in this study, was found to stimulate the postsynaptic receptor of DA (15). These findings suggest that CCK-4 and CCK-8 interfere selectively with the locomotor hyperactivity response induced by DA release from presynaptic dopaminergic neurons. However, the inhibitory effect of CCK-4 on the locomotor hyperactivity induced by methamphetamine and TRH differed from that of CCK-8 (Fig. 1).

After intravenous injection of $^{125}$I-CCK-8, no radiolabelled CCK-8 was detected in the cerebrospinal fluid over a period of 30 min, thus CCK-8 does not penetrate the blood-brain barrier (16). Bilateral abdominal vagotomy completely blocked the effect of CCK administered peripherally on satiety and related exploratory behavior (17, 18). Radiofrequency lesions of the nucleus tractus solitarius also abolished the effect of acute administration of CCK on exploratory behavior (19). Therefore, all these results taken together suggest that the most possible neuronal pathway related to the satiety syndrome induced by peripheral injection of CCK-8, but not of CCK-4, is the afferent vagus and the nucleus tractus solitarius.

In conformity to the report of Cornford et al. (20), it is also conceivable that a small amino acid sequence such as CCK-4 may be able to penetrate the blood-brain barrier (BBB) or non-BBB (e.g., choroid plexus) and finally act on the central nervous system. However, whether CCK-4 administered peripherally can cross the blood-brain barrier in sufficient quantities is a matter of controversy.

In conclusion, these results can be well explained if it is assumed that the physiological mechanisms of action of CCK-4 and CCK-8 differ. CCK-4, but not CCK-8, antagonizes only the increased activity induced by compounds which will release DA.

**Acknowledgements:** We thank M. Ohara for reading the manuscript. This work was supported by a grant for Scientific Research (1984, No. 59480420) from the Ministry of Education, Science and Culture of Japan and by a Grant from the Research Foundation for Pharmaceutical Sciences, 1982. Nomifensine was a generous gift from Hoechst Japan Co. We thank Y. Ohmine and M. Nagano for technical assistance.

**References**

1. Hökfelt, T., Rehfeld, J.F., Skirboll, L., Iversen, B., Goldstein, M. and Markey, K.: Evidence for coexistence of dopamine and CCK in mesolimbic neurons. Nature 285, 476–477 (1980)
2. Zetler, G.: Analgesia and ptosis caused by caerulein and cholecystokinin octapeptide (CCK-8). Neuropharmacology 19, 415–422 (1980)
3. Crawley, J.N., Hays, S.E., Paul, S.M. and Goodwin, F.K.: Cholecystokinin reduced exploratory behavior in mice. Physiol. Behav. 27, 407–411 (1981)
4. Crawley, J.N., St.-Pierre, S. and Gaudreau, P.: Analysis of the behavioral activity of C- and N-terminal fragments of cholecystokinin octapeptide. J. Pharmacol. Exp. Ther. 230, 438–444 (1984)
5. Van Ree, J.M., Gaffori, O. and Wied, D.D.: In rats, the behavioral profile of CCK-8 related peptides resembles that of antipsychotic agents. Eur. J. Pharmacol. 93, 63–78 (1983)
6. Hsiao, S., Katsuura, G. and Itoh, S.: Cholecystokinin tetra peptide, proglumide and open-field behavior in rats. Life Sci. 34, 2165–2168 (1984)
7. Katsuura, G., Itoh, S. and Hsiao, S.: Specificity of nucleus accumbens to activities related to cholecystokinin in rats. Peptides 6, 91–96 (1985)
8. Takeda, Y., Kamiya, Y., Honda, K., Takano, Y. and Kameya, H.: Effect of injection of CCK-8 into the nucleus caudatus on the behavior of rats. J. Pharmacol. 40, 569–575 (1986)
9. Kádár, T., Penke, B., Kovacs, K. and Telegdy, G.: Depression of rat feeding in familiar and novel environment by sulfated and nonsulfated cholecystokinin octapeptide. Physiol. Behav. 34, 395–400 (1985)
10. Kádár, T., Penke, B., Kovacs, K. and Telegdy, G.: Inhibition of feeding by the C-terminal tetrapeptide fragment of cholecystokinin in a novel environment. Neuropeptides 7, 97–108 (1986)
11. Costa, E., Groppetti, A. and Naimzada, M.K.: Effects of amphibian on the turnover rate of brain catecholamines and motor activity. Br. J. Pharmacol. 44, 742–751 (1972)
12. Makanjuola, R.O.A. and Ashcroft, G.W.: Behavioral effects of electrolytic and 6-hydroxy-dopamine lesions of the accumbens and caudate-putamen nuclei. Psychopharmacology 76, 333–340 (1982)
13. Heal, D.J. and Green, A.R.: Administration of thyrotropin releasing hormone (TRH) to rats releases dopamine in N. accumbens but not N. caudatus. Neuropharmacology 18, 23–31 (1979)
14 Randrup, A. and Braestrup, C.: Uptake inhibition of biogenic amines by newer antidepressant drugs: Relevance to the dopamine hypothesis of depression. Psychopharmacology 53, 309–314 (1977)

15 Strombom, U.: Catecholamine receptor agonists. Naunyn Schmiedebergs Arch. Pharmacol. 292, 167–176 (1976)

16 Passaro, E., Jr., Debas, H., Oldendorf, W. and Yamada, T.: Rapid appearance of intraventricularly administered neuropeptides in the peripheral circulation. Brain Res. 240, 335–340 (1982)

17 Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R. and Simansky, K.J.: Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science 213, 1036–1037 (1981)

18 Morley, J.E., Gosnell, B.A. and Levine, A.S.: The role of peptides in feeding. TIPS 5, 468–471 (1984)

19 Crawley, J.N. and Schwaber, J.S.: Nucleus tractus solitarius lesions block the behavioral actions of cholecystokinin. Peptides 4, 743–747 (1983)

20 Cornford, E.M., Braun, L.D., Crane, P.D. and Oldendorf, W.H.: Blood-brain barrier restriction of peptides and the low uptake of enkephalins. Endocrinology 103, 1297–1303 (1978)