Psychotropic Activity of Cholecystokinine Tetrapeptide Analogs

I. P. Anokhina, Zh. D. Bespalova, T. V. Proskuryakova, N. V. Pankratova, M. E. Pal'keeva, and O. B. Petrichenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 6, pp. 647-650, June, 1998
Original article submitted May 29, 1997

Psychotropic activity of two cholecystokinin analogs Trp-R_1-Asp-Phe-NH_2 and R_2-Trp-R_3-Asp-Phe-NH_2 is studied using behavioral tests. Both analogs have no effect on motor activity. Trp-R_1-Asp-Phe-NH_2 in doses of 0.1 and 0.3 mg/kg exhibits anxiogenic activity and impairs learning (only in a dose of 0.1 mg/kg) of experimental animals. R_2-Trp-R_3-Asp-Phe-NH_2 possesses no anxiogenic activity and in doses of 0.1 and 0.3 mg/kg promotes learning, which attests to its nootropic activity.

Key Words: *cholecystokinin; psychotropic activity*

Bioactive fragments of cholecystokinin (CCK) have attracted great attention as modulators of the dopaminergic system [3] that plays an important role in pathogenesis of various psychopathological states [6] and in drug abuse [1].

Behavioral experiments have demonstrated neuroleptic-like effects of CCK octapeptide (CCK-8) in animals [8]. It was also shown that CCK-8 produces sedative and anticonvulsant effects, reduces tremor, induces catalepsia and ptosis [9-11], inhibits D-amphetamine-induced stereotyped behavior [12], and suppresses alcohol addiction [2].

These data allow us to consider CCK-8 as a potential drug for the treatment of psychopathological states and drug and alcohol abuse.

However, endogenous CCK-8 is unstable and interacts with both types of CCK receptors [7]. These limitations can be overcome by introducing spatial substituent into CCK-8 molecule. Analysis of the structure-activity relationships is very important for the development of new drugs. Such an analysis for large molecules is an involved procedure. Therefore, we used a tetrapeptide Trp-Met-Asp-PheNH_2, the smallest biologically active cholecystokinin fragment (CCK-4).

Research Center of Addiction, Russian Cardiology Research-and-Production Center, Ministry of Health, Moscow

In the present study on the basis of stereo-chemical structure of natural CCK-4 we synthesized two its analogs and compared their psychotropic activities.

MATERIALS AND METHODS

Two CCK-4 analogs were synthesized and used in the study: Trp-R_1-Asp-Phe-NH_2 [CCK-4(I)] and R_2-Trp-R_3-Asp-Phe-NH_2 [CCK-4(II)]. Radicals 1, 2, and 3 denote structural modifications. We assumed that modification of Trp residue and substitution of Met with other amino acid stabilize the peptide and affect its selectivity to CCK receptors.

CCK-4 analogs were synthesized according to classical liquid-phase protocol of peptide synthesis with maximum protection of amino acid functional groups. The obtained peptides were desalted by preparative high performance liquid chromatography; the final product was identified by thin-layer chromatography, nuclear magnetic resonance, analytic HPLC, and amino-acid analysis.

Psychotropic activity of CCK-4 analogs was assessed by modulation of motor activity and learning and by anxiogenic effect (intrinsic property of natural CCK-4 mediated through central CCK receptor). Model of morphine-induced analgesia was used to

0007-4888/98/0006-0573$20.00

find out whether test CCK-4 analogs possess the activity of central CCK receptor agonists.

Anxiogenic effect of CCK-4 analogs was studied in the two-compartment test [4] and Vogel conflict test using Lick suppression test chamber. Tetrapeptides were injected intraperitoneally in doses of 0.03 mg/kg (group 1), 0.1 mg/kg (group 2), and 0.3 mg/kg (group 3) 30 min before testing. Control animals were injected intraperitoneally with 0.9% NaCl.

Motor activity was assessed for 5 min before the two-compartment test, after which the animal was placed to the center of dark compartment, and the number of transitions between dark and illuminated compartments and the time spent in the illuminated compartment was counted.

In the Vogel test, deprived animals were trained to find water 24 h prior to injection of tetrapeptide. Thirty minutes postinjection, the latency of the first approach and the number of approaches to water as well as the number of punished water takings were determined.

While studying the effect of CCK-4 analogs on morphine-induced analgesia in the tail flick test, the basal nociceptive threshold was determined as the mean of three measurements performed within the first 15 min of the experiment. The pain threshold was measured every 10 min for 1 h after injection of tetrapeptides and expressed as percent of the basal level.

The data were processed statistically using the Student *t* test.

RESULTS

None of CCK-4 analogs modulated motor activity of experimental animals (Table 1).

CCK-4(I) in doses 0.1 and 0.3 mg/kg reduced the time spent in illuminated compartment in the two-compartment test, while CCK-4(II) in all tested doses had no effects on this parameter.

In the Vogel conflict test, CCK-4(I) in a dose of 0.3 mg/kg reduced the number of electric shocks (punished drinking), while animals injected with CCK-4(II) did not differ from the control in this parameter. CCK-4(I) in doses of 0.03 and 0.1 mg/kg prolonged, while CCK-4(II) in doses of 0.1 and 0.3 mg/kg shortened the latency of the first approach to water dish (Table 1).

It is generally accepted that prolonged time spent in the illuminated compartment and increased number of shocks accepted by rats (punished drinking) suggest that test substance exhibits anxiolytic activity, while a decrease in these parameters indicates an anxiogenic effect.

Our experiments showed that CCK-4(I) in doses of 0.1 and 0.3 exhibits anxiogenic activity. CCK-4(II) was not anxiogenic in all tested concentrations. CCK-4(I) in a dose 0.1 mg/kg impairs, while CCK-4(II) in doses of 0.1 and 0.3 mg/kg promotes learning in experimental animas.

Pain threshold in animals injected with CCK-4 analogs did not differ from the control (Fig. 1). Being injected in combination with morphine, CCK-4(I) reduces its analgesic effects from the 20th to the 40th min postinjection, while CCK-4(II) has no effect of morphine-induced analgesia. Bearing in mind that agonists of central CCK receptors reduce and antagonists potentiate the analgesic effect of morphine [5], it can be concluded that CCK-4(I) acts as a central CCK receptor agonist.

Thus, our findings suggest that substitution of methionine residue in natural CCK tetrapeptide does

TABLE 1. Behavioral Parameters in Rats Intraperitoneally Injected with CCK-4(I) and CCK-4(II) (*M*±*m*)

Groups (*n*=10)	Motor activity (number of movements)	Two-compartment test (time spent in light compartment, sec)	Vogel test		
			latency of first approach	number of shocks	number of approaches
CCK-4(I)					
Control	317.5±28.4	50.4±5.8	19.2±5.5	39.2±5.3	4.5±1.1
First	391.6±18.9	43.0±7.3	54.6±11.3*	32.0±3.1	3.9±0.9
Second	346.2±25.6	34.1±5.2*	67.0±12.9*	41.3±4.9	3.8±1.2
Third	316.8±28.5	27.9±4.5*	28.2±6.1	26.6±4.4*	5.0±0.9
CCK-4(II)					
Control	339.8±65.8	26.1±3.0	40.8±4.0	21.0±5.8	5.8±1.2
First	446.4±18.3	28.0±6.0	35.6±5.0	19.1±4.5	6.9±0.9
Second	384.0±50.5	19.8±3.1	21.3±3.9**	29.2±3.9	6.5±1.2
Third	271.6±45.5	21.0±5.5	19.7±3.3**	25.3±3.2	7.0±0.8

Note. *$p<0.05$, **$p<0.002$ in comparison with the control.

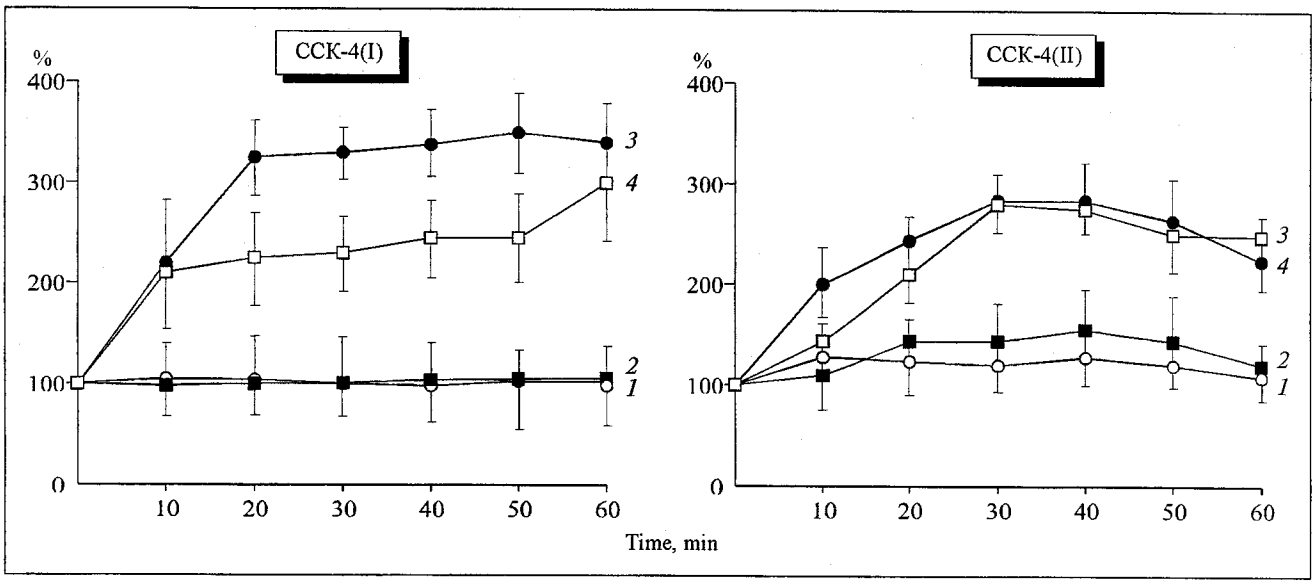

Fig. 1. Effect of cholecystokinin tetrapeptide analogs on morphine-induced analgesia. Ordinate: changes in pain threshold. Injection of 0.9% NaCl (*1*), CCK-4, 0.1 mg/kg (*2*), 5 mg/kg morphine (*3*), and morphine+CCK-4 (*4*).

not abolish its central CCK receptor agonist effects (anxiogenic activity and reduction of morphine-induced analgesia). Product of substitution of Met and modification of Trp possesses no anxiogenic activity and improves learning in experimental animals. These data allow us to propose CCK-4(II) as a basis substance for the development of new nootropics and encourage the search for new structural modifications of CCK-4 enhancing its psychotropic activity.

REFERENCES

1. I. P. Anokhina, *Vopr. Narkol.*, No. 2, 27-30 (1995).
2. I. P. Anokhina, N. N. Ivanets, E.V. Reshchikova, *et al., Ibid.*, No. 1, 14-17 (1988).
3. J. N. Crawley, *Trends Pharmacol. Sci.*, **12**, 232-236 (1991).
4. J. N. Crawley and F. K. Goodwin, *Pharmacol. Biochem. Behav.*, **13**, 167-170 (1980).
5. P. Gaudrean, G. J. Lavigne, and R. Quirion, *Neuropeptides*, **16**, 51-55 (1990).
6. M. F. Loconczy, M. Davidson, and L. Davis, In: Psychopharmacology: The Third Generation of Progress. Ed. H. Y. Meltzer, New York (1987), pp. 715-726.
7. F. Markovec, M. Bani, R. Cereda, et al., *Arzneimittel-forschung*, **37**, 1256-1268 (1987).
8. J. M. Van Ree, O. Gaffori, and D. Wied, *Eur. J. Pharmacol.*, **93**, 63-67 (1983).
9. G. Zetler, *Ibid.*, **65**, 297-300 (1980a).
10. G. Zetler, *Ibid.*, **66**, 137-139 (1980d).
11. G. Zetler, *Neuropharmacology*, **19**, 415-422 (1980c).
12. G. Zetler, *Ann. New York Acad. Sci.*, **448**, 448-469 (1985)