Pharmacogenetic Study of Anxiolytic Effects of New Cholecystokinin Receptor Antagonists in Animals with Different Levels of Emotionality

L. G. Kolik, T. A. Gudasheva, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 5, pp. 519-523, May, 2003
Original article submitted October 30, 2002

Effects of two new peptide antagonists of central cholecystokinin receptors, GB-101 (0.05-0.40 mg/kg) and GB-115 (0.006-0.100 mg/kg), on the behavior of inbred animals differing by the reactions to emotional stress were studied in standard tests for evaluation of tranquilizers. The test drugs dose-dependently increased the total locomotor activity in the open field test in BALB/c mice and had no effect on the behavior of C57Bl/6 mice. GB-101 (0.4 mg/kg) and GB-115 (0.025 and 0.05 mg/kg) produced an anxiolytic effect on MR rats (but not on MNRA rats) in the elevated plus-maze and conflict tests. These data confirm anxioselective effects of the test compounds.

Key Words: *anxiolytic effect; emotional stress reaction; MR and MNRA rats; BALB/c and C57Bl/6 mice*

Behavioral characteristics of experimental animals under conditions of emotional stress are genetically determined [2]. Inbred animals differing by behavior during stress (C57Bl/6 and BALB/c mice, MR and MNRA rats) provide excellent possibilities for evaluation of the role of phenotypes of the emotional stress reaction (ESR) and for studies of the effects of anxiolytics on animals with initially different anxiety levels.

The studies of individual effects of benzodiazepine derivatives showed that predominance of anxiolytic or sedative action depends on genetic factors: under conditions of emotional stress benzodiazepine tranquilizers activated behavior of animals with hereditarily determined freezing reaction and, vice versa, caused sedation in animals with active reactions.

It was previously shown that central cholecystokinin receptor antagonist GV150013 (GLAXO-Wellcome), a 1,5-benzodiazepine derivative, considerably reduced anxiety of BALB/c mice with passive ESR phenotype and did not modify the behavior of C57Bl/6 mice with active reaction to stress [9]. Targeted search for agents with cholecystokinin-negative effect at the Institute of Pharmacology resulted in development of original peptide compounds. We investigated the effects of these compounds in animals with different reactions to emotional stress in standard psychopharmacological tests for evaluation of tranquilizers.

Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out on male C57Bl/6 and BALB/c mice weighing 18-20 g (Stolbovaya Breeding

TABLE 1. Effects of GB-101 and GB-115 on Spontaneous Motor Activity of BALB/c Mice ($M \pm m$, n=6)

Dose, mg/kg	GB-101	GB-115
Control	2393.2±346.4	2312.0±288.1
0.025	—	2321.8±270.4
0.2	2552.2±277.1	—

Note. n: number of animal groups. "—" these doses were not used for this compound.

0007-4888/03/1355-0440$25.00

Center) and male Maudsley Reactive (MR) and Maudsley Non-Reactive (MNRA) rats weighing 160-190 g (descendants from animals obtained from Winston Salem Breeding Center, USA, and bred at the Laboratory of Biological Models, Russian Academy of Medical Sciences, Svetlye Gory Breeding Center). Ten days before the experiment the animals were kept under standard vivarium conditions: 8-10 animals per cage, with free access to water and dry fodder and at 12:12 h day:night regimen. All experiments were carried out from 9.00 to 13.00.

The most active peptide compounds characterized as anxiolytics in screening studies GB-101 (Ph(CH2)4 COGlyTrpNH2) and GB-115 (Ph(CH2)5COGlyTrp NH2) were injected intraperitoneally in suspension with Twin-80 15 min before the test. Control animals received suspension with Twin-80.

At the first stage of the experiment we used pharmacogenetic methodology based on recording of the parameters of different behavioral phenotypes of C57Bl/6 and BALB/c mice in the open field (OF) test. In accordance with this methodology, the selective anxiolytic effect is characterized by activation of BALB/c mice behavior and the absence of sedation in C57Bl/6 mice. The total motor activity (TMA) determined as the sum of peripheral, central, and vertical activities in OF and the level of emotionality evaluated by the number of defecation acts during testing were recorded for 3 min.

Spontaneous motor activity of BALB/c mice was studied on an Optovarimex device for 10 min, animal motions were recorded with infrared transducers. The resultant value was a sum for 3 tested animals put into the device in their home cages.

In experiments on rats the anxiolytic effects of the test compounds were studied in the elevated plus-maze test (the number of excursions and time spent in open and closed arms of the maze was recorded for 300 sec) and in the conflict test based on the conflict between drinking motivation and electric painful stimulation (experimental setup was developed at Institute of Pharmacology) [4]. Prolongation of the time spent in illuminated arms and increased number of punished responses attested to anxiolytic effect of the studied compounds.

Effects of GB-101 and GB-115 on animal motor coordination were studied in the rotarod test.

The results were statistically processed using Student's *t* test.

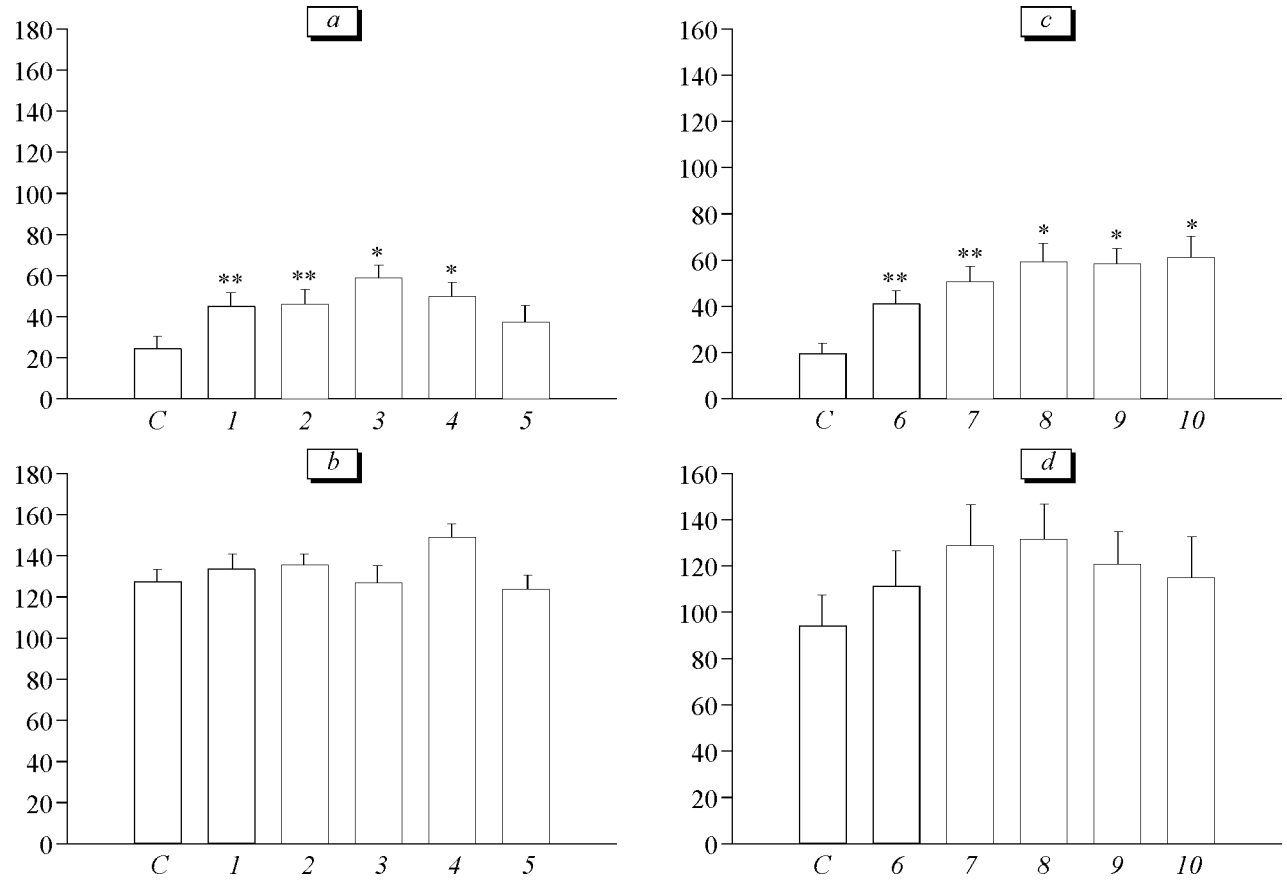

Fig. 1. Effects of GB-101 (*a*, *b*) and GB-115 (*c*, *d*) compounds on total motor activity of BALB/c (*a*, *c*) and C57Bl/6 mice (*b*, *d*) in the open field test (*M*±*SEM*). Ordinates: number of movements in OF. C: control. GB-101 doses (mg/kg): *1*) 0.05; *2*) 0.1; *3*) 0.2; *4*) 0.4; *5*) 0.5. GB-115 doses (mg/kg): *6*) 0.006; *7*) 0.01; *8*) 0.025; *9*) 0.05; *10*) 0.1. Here and in Fig. 2: *$p<0.01$, **$p<0.05$ compared to the control.

TABLE 2. Effects of Different Doses of GB-101 on the Behavior of MR and MNRA Rats in Elevated Plus-Maze Test (*M*±*m*, *n*=8)

Parameter	MR			MNRA		
	control	dose, mg/kg		control	dose, mg/kg	
		0.2	0.4		0.2	0.4
Time spent in closed arms, sec	27.5±6.9	86.2±28.8	190.1±36.0*	69.1±13.0^{+}	48.1±9.7	51.7±11.1
Number of entries into open arms/total number of excursions, %	30.1±8.4	43.6±5.0	68.8±6.1*	35.0±2.1	36.7±3.8	38.9±3.1
Motor activity	3.6±1.9	5.8±0.8**	3.4±0.7	7.5±1.3^{+}	6.9±1.2	4.8±0.7

Note. *p<0.01, **p<0.05 compared to the control, ^{+}p<0.01 compared to the MR control.

RESULTS

GB-101 in doses of 0.05-0.40 mg/kg increased TMA taken for the integral indicator of locomotor activity of BALB/c mice (Fig. 1). Injection of 0.2 mg/kg GB-101 significantly increased the number of central and vertical movements in OF, *i.e.* reduced anxiety and increased exploratory activity of animals. GB-101 in doses of 0.05-0.50 mg/kg did not modify the behavior of C57Bl/6 mice (Fig. 1).

GB-115 in doses of 0.006-0.100 mg/kg increased TMA (both the peripheral and central activity) in BALB/c mice (Fig. 1). Moreover, GB-115 in a dose of 0.025 mg/kg significantly decreased emotionality of experimental animals. GB-115 in the studied doses had practically no effect on the behavior of C57Bl/6 mice.

GB-101 (0.2 mg/kg) and GB-115 (0.025 mg/kg) did not change spontaneous motor activity of BALB/c mice (Table 1), which indicates the absence of psychostimulatory effects in these compounds.

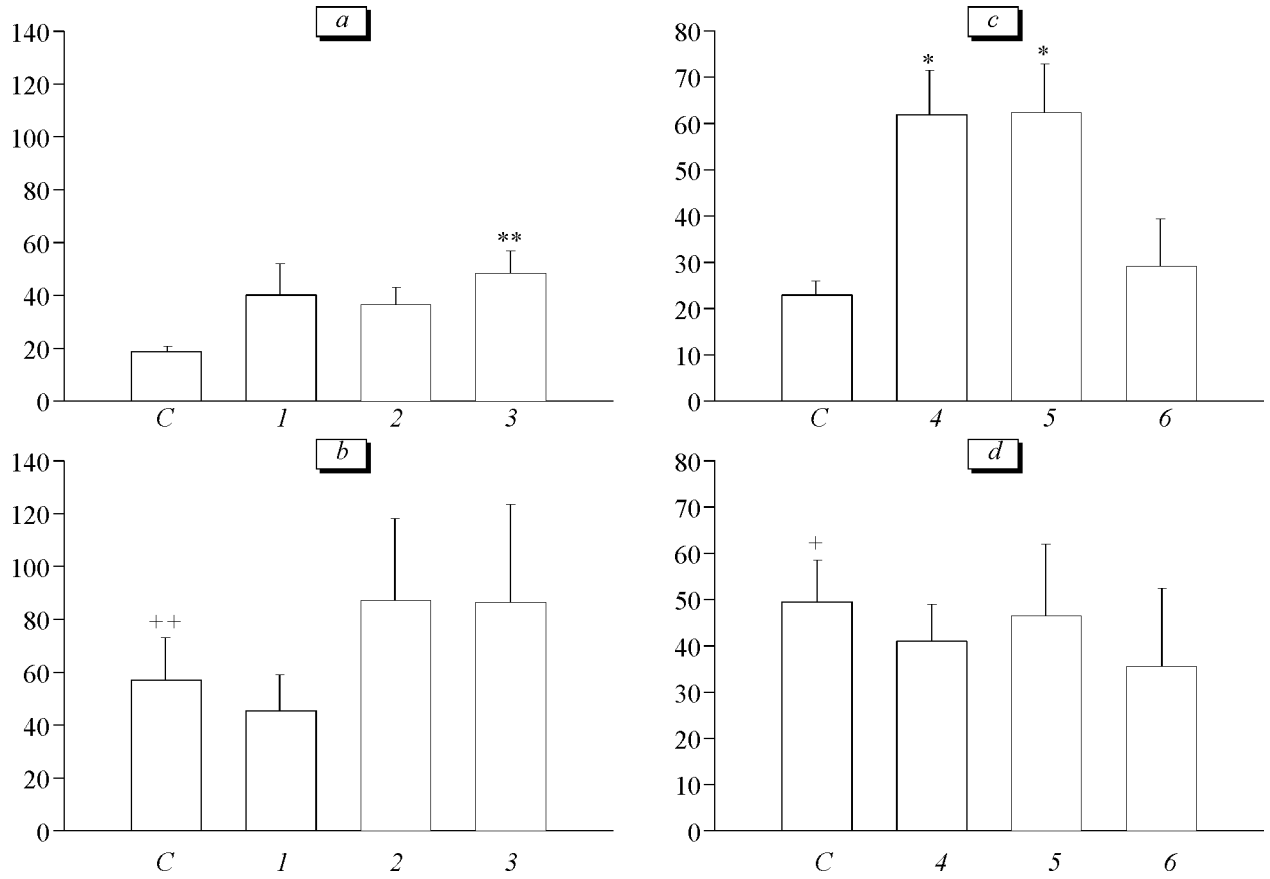

Fig. 2. Effects of GB-101 (*a*, *b*) and GB-115 (*c*, *d*) compounds on the behavior of MR (*a*, *c*) and MNRA rats (*b*, *d*) in the conflict situation test (*M*±*SEM*). Ordinates: number of punished responses. C: control. GB-101 doses (mg/kg): *1*) 0.1; *2*) 0.2; *3*) 0.4. GB-115 doses (mg/kg): *4*) 0.025; *5*) 0.05; *6*) 0.1. *p<0.01, **p<0.05 compared to the control; ^{+}p<0.01, ^{++}p<0.05 compared to MR control.

TABLE 3. Effects of Different Doses of GB-115 on the Behavior of MR and MNRA Rats in Elevated Plus-Maze Test (*M*±*m*)

Parameter	MR				MNRA			
	control (*n*=8)	dose, mg/kg			control (*n*=7)	dose, mg/kg		
		0.0125 (*n*=7)	0.025 (*n*=7)	0.05 (*n*=7)		0.0125 (*n*=7)	0.025 (*n*=7)	0.05 (*n*=7)
Time Spent in closed arms, sec	15.5±5.8	76.3±39.0	35.3±11.8	181.6±51.1*	129.7±38.1+	157.6±51.2	78.8±20.3	63.1±16.9
Number of entries into open arms/total number of entries, %	12.9±6.9	34.3±12.8	28.1±8.3	71.3±14.1*	51.5±9.7+	60.7±13.9	39.4±9.6	30.4±2.0
Motor activity	3.5±0.9	4.6±1.2	5.1±1.7	3.4±1.1	6.7±1.8	4.6±1.5	6.0±1.2	6.4±1.1

Note. *n*: number of animals. *$p<0.01$ compared to the control; +$p<0.01$ compared to MR control.

Our study confirmed previously demonstrated behavioral differences between MR and MNRA rat strains [5], which prompted the use of these strains in further pharmacogenetic studies.

The study of anxiolytic effect on inbred rats in the plus-maze test showed that GB-101 in a dose of 0.4 mg/kg and GB-115 in a dose of 0.05 mg/kg significantly prolonged the time spent in the open arms and the number of entries into illuminated arms without changing the total number of entries into maze arms (Tables 2, 3).

In the conflict test GB-101 (0.4 mg/kg) and GB-115 (0.025 and 0.05 mg/kg) significantly increased the number of punished responses in MR rats, but had no effect on the behavior of MNRA rats (Fig. 2). These findings confirm the selective anxiolytic effect of the test compounds in animals with high level of anxiety.

Experiments on random-bred mice showed that GB-101 (0.2 mg/kg) and GB-115 (0.025 and 0.1 mg/kg) did not impair motor coordination of animals during 2-min testing, which attested to the absence of myorelaxant effects of the test drugs in the studied anxiolytic dose range (in contrast to benzodiazepine tranquilizers).

Recent experimental studies demonstrated the involvement of the cholecystokinin system in the mechanisms of anxiety formation and the capacity of CCK-2 receptor antagonists to alleviate internal strain [9]. The tropism of GB-101 and GB-115 to CCK-2 receptors was proven, and hence, our results confirm the anxiolytic effects of peptide antagonists of CCK-2 receptors with the predominant effects on animals with passive reaction to emotional stress. GB-101 and GB-115 in anxiolytic doses do not modify the behavior of animals with active ESR phenotype in all standard psychopharmacological tests. The dipeptides did not modify spontaneous motor activity of BALB/c mice in the absence of emotional stress. We therefore conclude that activation of animal behavior in OF observed after injection of CCK-2 receptor antagonists can be explained by their effect on ESR parameters.

REFERENCES

1. I. P. Anokhina, Z. D. Bespalova, T. V. Proskuryakova, *et al.*, *Byull. Eksp. Biol. Med.*, **125**, No. 6, 647-650 (1998).
2. D. K. Belyaev, *Vestn. Akad. Med. Nauk SSSR*, No. 7, 9-14 (1979).
3. I. V. Viglinskaya, S. B. Seredenin, and L. G. Kolik, *Eksp. Klin. Farmakol.*, **60**, No. 3, 59-60 (2000).
4. G. M. Molodavkin, A. A. Karaev, T. A. Voronina, *et al.*, *Ibid.*, No. 1, 4 (1993).
5. S. B. Seredenin, I. V. Viglinskaya, and L. G. Kolik, *Eksp. Klin. Farmakol.*, **60**, No. 3, 3-5 (1997).

6. S. B. Seredenin, T. A. Voronin, G. G. Neznamov, *et al.*, *Vestn. Akad. Med. Nauk*, No. 1, 47-52 (1998).
7. J. De Vry and K. R. Jentzsch, *Eur. J. Pharmacol.*, **357**, 1-8 (1998).
8. H. Mohler, F. Crestani, and U. Rudolph, *Curr. Opinion Pharmacol.*, **1**, 22-25 (2001).
9. F. Noble and B. P. Roques, *Prog. Neurobiol.*, **58**, 349-379 (1999).
10. A. Rex, C. A. Marsden, and H. Fink, *Neurosci. Lett.*, **228**, 79-82 (1997).
11. A. Rex and H. Fink, *Peptides*, **19**, 519-526 (1998).