Effect of Compound GB-115 on Morphine-Induced Analgesia

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Experiments on outbred mice showed that compound GB-115, a retropeptide analogue of the tetrapeptide cholecystokinin, produced a naloxone-dependent potentiating effect on morphine-induced analgesia in the hot-plate test, but did not modulate animal behavior in the tail-flick test in outbred mice. This potentiation of antinociceptive activity of morphine was probably related to the interaction of GB-115 with supraspinal opioidergic mechanisms.

Key Words: *antinociception; compound GB-115; morphine; naloxone; mice*

The cholecystokinin (CCK) system plays an important role in the regulation of pain sensitivity. CCK abolishes the antinociceptive effect associated with the release of endogenous opioids, but does not modulate opioid-independent analgesia [11]. CCK octapeptide (CCK-8) and its analogues produce a naloxone-reversible antinociceptive effect in the tail-flick test, writhing test, and hot-plate test [5]. Published data show that CCK receptor antagonists potentiate the antinociceptive effects of opioid peptides [7-9], which reflects the existence of functional antagonistic relationships between CCK and opioid systems. It was hypothesized that the effect of CCK analogues is realized via activation of central CCK_2 receptors, while CCK_2 receptor antagonists potentiate opiate-induced analgesia [3].

Taking into account the existence of functional interactions between CCK and opioidergic systems, we studied the effect of compound GB-115 (retropeptide CCK-4 analogue with selective anxiolytic activity [1]) on morphine-induced analgesia in mice.

MATERIALS AND METHODS

Experiments were performed on outbred male mice weighing 18-22 g. The animals were maintained in a vivarium (10-15 specimens per cage) under natural 12:12-h light/dark regimen and had free access to water and standard pelleted food for 10 days before the start of the study. The experiments were conducted in the autumn-winter period at 9.00-13.00.

Initial testing was performed to select animals for the study of their nociceptive response. The experiments were performed on mice whose latency in the tail-flick test and hot-plate test did not exceed 4 and 15 sec, respectively. The test compounds were administered 1 h after initial testing.

GB-115 ($Ph(CH_2)_5CO$-Gly-Trp-NH_2) was synthesized at the V. V. Zakusov Institute of Pharmacology and dissolved in distilled water with 1-2 drops of Tween 80. Morphine hydrochloride (Sigma) and naloxone (Sigma) were dissolved in distilled water.

Observation was performed 20 and 40 min after injection of distilled water (control), GB-115 (0.0125-0.5000 mg/kg), morphine hydrochloride (3 mg/kg), and naloxone (1 mg/kg). The test compounds were injected intraperitoneally (0.1 ml per 10 g body weight).

In the hot-plate test, the experimental animals were put on a copper plate heated to 55±0.5°C. This plate was the bottom of an organic glass cylinder with a diameter of 15 cm. The latency of the nociceptive response (limb licking or jump) was recorded. The latency of 30 sec (maximum time of exposure) was considered as 100% analgesia.

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The tail-flick test was performed on a Hugo Sachs Electronic analgesimeter (Analgesia test, tail flick type 812). The tail-flick latency was estimated after local heat application at a distance of 1 cm from tail base. The latency of 20 sec (maximum exposure) was considered as 100% analgesia. The doses and period of treatment with the test compounds in the tail-flick test were similar to those in the hot-plate test.

The nociceptive response was studied as follows: combined treatment with morphine hydrochloride and GB-115→20 min→first test→naloxone→15 min→repeated test.

The data were expressed as percentage of the maximum effect of the test compound [4].

The data were processed using one-way analysis of variance (ANOVA), post-hoc Fischer LSD test, and nonparametric Mann—Whitney *U* test.

RESULTS

GB-115 in the specified doses had no antinociceptive activity (Tables 1 and 2). After combined treatment with morphine and GB-115 (0.025 mg/kg), the response latency in the hot-plate test significantly exceeded that observed in experiments with individual administration of the opioid receptor agonist. The pharmacological effect of GB-115 in the hot-plate test was observed 20 min after administration of the test compounds (but not 40 min after treatment, Table 1). Naloxone completely abolished the antinociceptive effect of combined treatment with morphine and GB-115 (Fig. 1). Our results indicate that GB-115 potentiates the analgetic effect of morphine under these conditions, but not in the tail-flick test (Table 2). The tail-flick test showed that GB-115 in various doses had no effect on the degree and duration of morphine-induced antinociception.

Hence, compound GB-115 potentiates the antinociceptive effects of morphine. Under these conditions we revealed a tendency to the dome-shaped dose-response dependence, which is typical of various peptides (Table 1).

The specified effect of compound GB-115 was also observed in the hot-plate test. This test allows

TABLE 1. Effect of GB-115 on Mice with Morphine-Induced Antinociception in Hot-Plate Test (*M*±*m*)

Experimental conditions	Dose, mg/kg	*n*	Latency, %	
			after 20 min	after 40 min
Control	—	12	-2.4±2.9	-9.6±6.4
Morphine	3.0	12	23.8±7.5**	17.5±7.1**
GB-115	0.0125	9	3.4±2.2	3.5±3.3
	0.025	10	3.1±4.4	3.5±1.8
	0.5	10	2.2±2.8	1.9±2.5
Morphine+GB-115	0.0125	8	22.8±11.9*	26.3±11.1
	0.025	9	38.6±4.5***,+	24.3±2.6*
	0.5	10	20.0±5.6**	16.4±4.3

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control; +$p<0.05$ compared to the morphine group.

TABLE 2. Effect of GB-115 on Mice with Morphine-Induced Antinociception in Tail-Flick Test (*M*±*m*)

Experimental conditions	Dose, mg/kg	*n*	Latency, %	
			after 20 min	after 40 min
Control	—	7	-0.6±1.6	-1.0±1.3
Morphine	3.0	12	18.7±6.7***	19.6±5.1**
GB-115	0.0125	9	1.8±1.5	2.0±1.4
	0.025	10	2.3±0.5	2.1±0.7
	0.5	10	1.9±1.7	2.4±1.8
Morphine+GB-115	0.0125	8	19.8±8.6***	20.9±5.9**
	0.025	17	19.5±4.7***	29.2±10.9**
	0.5	10	54.5±20.1**	55.4±21.5

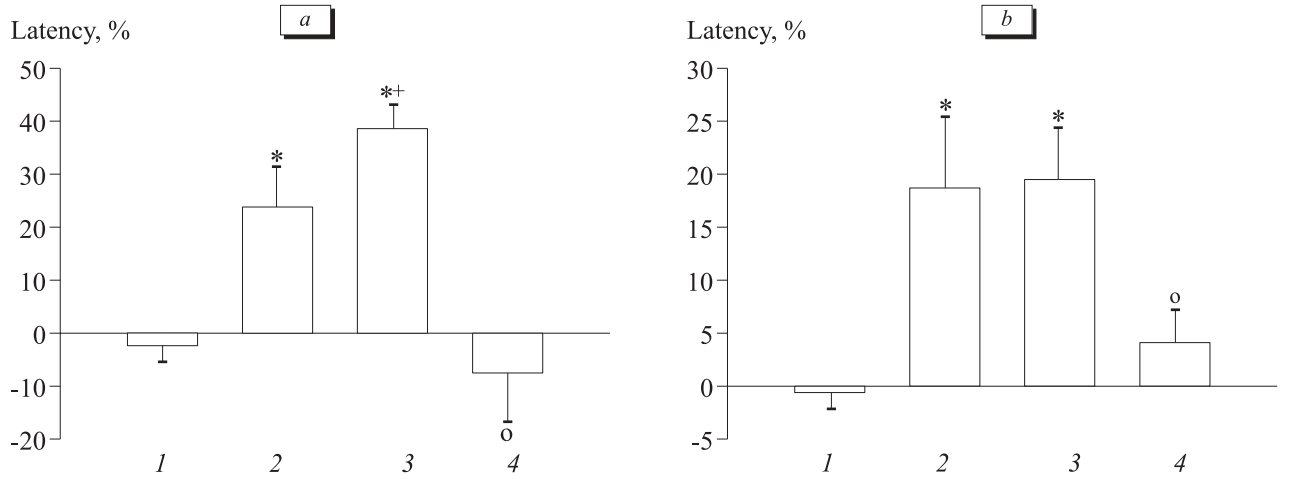

Fig. 1. Influence of naloxone on the analgetic effect of morphine in the hot-plate test (*a*) and tail-flick test (*b*). Control (*1*), morphine (*2*), morphine+GB-115 (*3*), and morphine+GB-115+naloxone (*4*). **p*<0.05 compared to the control; +*p*<0.05 compared to *2*; °*p*<0.01 compared to *3*.

us to evaluate the influence of neurotropic substances on supraspinal processes in the central nervous system [4]. At the spinal level (tail-flick test), compound GB-115 had little effect on the response latency after morphine administration. These data indicate that compound GB-115 potentiates the antinociceptive effect of morphine primarily at the spinal level.

Recent studies demonstrated that the animals with CCK_2 receptor deficiency are characterized by sensitization to morphine [2,10] and high activity of the endogenous opioid system [6]. Our results suggest that the antagonistic effect of CCK on opioid analgesia is partially realized via CCK_2 receptors. Receptors blockade with GB-115 probably abolishes this antagonism and contributes to the increased release of endogenous opioid peptides.

Compound GB-115 potentiates the antinociceptive effect of morphine, which probably depends on its interaction with supraspinal opioidergic mechanisms.

REFERENCES

1. L. G. Kolik, T. A. Gudasheva, and S. B. Seredenin, *Byull. Eksp. Biol. Med.*, **135**, No. 5, 519-523 (2003).
2. V. Dauge, F. Beslot, T. Matsui, and B. P. Roques, *Neurosci. Lett.*, **306**, Nos. 1-2, 41-44 (2001).
3. A. E. Friedrich and G. B. Gebhart, *J. Pharmacol. Exp. Ther.*, **292**, No. 2, 538-544 (2000).
4. D. Le Bars, M. Gozariu, and S. W. Cadden, *Pharmacol. Rev.*, **53**, No. 4, 597-652 (2001).
5. F. Noble, S. A. Wank, J. N. Crawley, *et al.*, *Ibid.*, **51**, No. 4, 745-781 (1999).
6. B. Pommier, F. Beslot, A. Simon, *et al.*, *J. Neurosci.*, **22**, No. 5, 2005-2011 (2002).
7. B. P. Roques and F. Noble, *Neurochem. Res.*, **21**, No. 11, 1397-1410 (1996).
8. L. Singh, R. J. Oles, M. J. Field, *et al.*, *Br. J. Pharmacol.*, **118**, No. 5, 1317-1325 (1996).
9. T. W. Vanderah, R. N. Bernstein, H. I. Yamamura, *et al.*, *J. Pharmacol. Exp. Ther.*, **278**, No. 1, 212-219 (1996).
10. A. Veraksits, K. Runkorg, K. Kurrikoff, *et al.*, *Psychopharmacology (Berl.)*, **166**, No. 2, 168-175 (2003).
11. Z. Wiesenfeld-Hallin, G. de Arauja Luca, P. Alster, *et al.*, *Brain Res.*, **848**, Nos. 1-2, 78-89 (1999).