PHARMACOLOGY AND TOXICOLOGY

Experimental Study of Antinociceptive Potency of Dipeptide GB-115 during Chemical and Thermal Stimulation

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The study examined the antinociceptive potency of dipeptide compound GB-115 (amide N-6-phenylhexanoyl-glycyl-L-tryptophan) during thermal and chemical noxious stimulation of mice. Peroral administration of GB-115 (0.1-20 mg/kg) decreased the incidence of abdominal contractions induced with intraperitoneal acetic acid (0.75%). This effect was comparable to that of sodium diclofenac (20 mg/kg); it was only partially antagonized with naloxone indicating the presence of significant non-opioid component in analgesic effect of GB-115. Ability of this dipeptide to moderate the nociceptive response in tail flick test under a non-selective blockade of the opioid receptors with naloxone and the absence of similar analgesic potency assessed in the hot plate test attest to predominant effect of GB-115 on spinal opioid receptors.

Key Words: *antinociception; dipeptide GB-115; acetic acid seizures; visceral pain*

In modern pharmacotherapy of pain syndromes of various etiologies, the most popular analgesics are agonists of opioid receptors, anti-inflammatory steroids, and nonsteroidal anti-inflammatory drugs. Despite high efficiency of these drugs, they cannot eliminate some types of pain, which prompt further search for analgesics with novel mechanisms of actions [13].

Most researchers agree that visceral pain arises during stimulation of the nociceptors located in a visceral organ and differs from somatic pain by diffuse spread, problematic localization, and the concurrent reflex reactions [16]. It is hypothesized that nociceptive sensations accompanying visceral diseases could be provoked by circulatory disturbances, spasmodic or convulsive contraction of smooth muscle vasculature, distension of the walls of hollow organs, and inflammatory alterations in the organs and tissues. However, the pathogenetic mechanisms of visceral pain are far from being clear, and the available methods to treat this type of pain are little efficient [14]. At present, clinical researchers examine the antagonists of 5-HT_3-, CRF_1-, or NMDA-receptors, the agonists of peripheral opioid κ-receptors, and probiotics [9].

A novel retropeptide analog of cholecystokinin, amide N-6-pheN-phenylhexanoyl-glycyl-L-tryptophan amide (GB-115) [3] was synthesized at V. V. Zakusov Research Institute of Pharmacology. GB-115 after intraperitoneal injection increased nociceptive threshold [4] and potentiated morphine-induced analgesia during thermal stimulation [5]. Examination of anti-inflammatory properties of this dipeptide showed that GB-115 (0.1-10.0 mg/kg intraperitoneally) significantly moderated inflammatory reaction provoked by concanavalin A in mice and pronouncedly decreased the degree of exudative edema induced by carrageenan in rats [7]. The pharmacokinetic parameters calculated from the experimental data attest to greater resistance of the dipeptide against the action of peptidases in the

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gastrointestinal tract in comparison with the natural neuropeptides [1].

Here we compared antinociceptive action of peroral GB-115 during chemical (visceral pain model) and thermal (somatic pain model) stimulation.

MATERIALS AND METHODS

The experiments were carried out on random-bred male mice weighing 24-29 g. Prior to experiments, the mice were maintained for 6 days under vivarium conditions (10 animals per case) with a 12-hour day-night cycle and *ad libitum* water and mouse food pellets. The experiments were performed from 9:00 to 15:00 during autumn-winter period.

The study examined GB-115, a retropeptide analog of endogenous tetrapeptide cholecystokinin [3]. Morphine hydrochloride (an agonist of opioid receptors, Sigma) and sodium diclofenac (Sigma) were employed as the comparative agents, while naloxone hydrochloride (Sigma) was used as a non-selective antagonist of opioid receptors. All substances were dissolved in distilled water with exception of GB-115, which was administered as suspension with Tween-80.

The test of acetic acid seizures was performed as described elsewhere [4]. The mice received: 1) intraperitoneal morphine (5 mg/kg for 20 min), 2) peroral GB-115 (0.05–20 mg/kg for 40 min), 3) subcutaneous naloxone (1 mg/kg injected 15 min prior to morphine and GB-115), and 4) peroral distilled water as a control agent (40 min prior to the test). Then 0.75% acetic acid was injected intraperitoneally. The number of postinjection lordoses (seizures) was calculated for each mouse during 15 min.

Hot plate test was performed to determine the latency of the response (licking of a hind leg or jump). The latency was determined in mice administered with: 1) intraperitoneal morphine (3 mg/kg for 20 min), 2) peroral GB-115 (10 mg/kg for 40 min), 3) subcutaneous naloxone (1 mg/kg injected 15 min prior to GB-115), and 4) peroral distilled water as a control agent (40 min prior to the test). The mice were selected 1 h prior testing by their baseline reactivity in the hot plate test excluding the animals that remained on the copper plate heated to 56.0±0.5°C for more than 15 sec. The latency of 30 sec was considered as 100% anesthesia.

In the tail-flick test, a Type 812 Algesimeter (Hugo Sachs Elektronik) was employed to fix the moment of tail flick after the start of local thermal stimulation. The examined substances were administered in the same doses and periods as in the hot plate test. The mice were selected 1 h prior testing by the baseline reactivity excluding the animals that demonstrated no response for more than 4 sec after the onset of stimulation. The latency of 20 sec was taken as 100% anesthesia.

All the agents were administered in a volume of 0.1 ml/10 g body weight.

The data were analyzed statistically with one-way ANOVA variation analysis software. Significance was assessed with Mann–Whitney non-parametrical *U* test for independent groups.

RESULTS

During chemical stimulation with acetic acid, GB-115 (0.1-20 mg/kg) demonstrated pronounced analgesic potency (Fig. 1, *a*). This effect was dose-dependent: the maximum inhibitory effect exerted by GB-115 on nociceptive reaction (decrease in the number of seizures by 76.3% in comparison with the control number, $p<0.0001$) was attained at a dose of 10 mg/kg, which is comparable with the effect of nonsteroidal anti-inflammatory drug sodium diclofenac applied in a dose

TABLE 1. Effect of GB-115 on Nociceptive Reactions in Mice during Thermal Stimulation (*M*±*SEM*)

Agent	Dose, mg/kg	Mode of administration	*n*	Latency (% of MPE)	
				hot plate	tail flick
Distilled water (control)	-	Peroral	14	3.9±5.6	1.6±0.5
Morphine hydrochloride	3.0	Intraperitoneal	11	51.3±9.7***	7.1±1.7***
Sodium diclofenac	20.0	Peroral	12	5.3±3.9	4.3±1.4
GB-115	10.0	Peroral	9	20.6±9.2	4.5±0.5***
GB-115+naloxon	10.0	Peroral	8	7.2±5.4	3.1±0.2*+
	1.0	Subcutaneous			

Note. *$p<0.05$, ***$p<0.001$ compared to the control; +$p<0.05$ compared to GB-115. "% of MPE"=(reaction latency after drug administration minus baseline latency)/(maximum exposure time minus baseline latency)×100%.

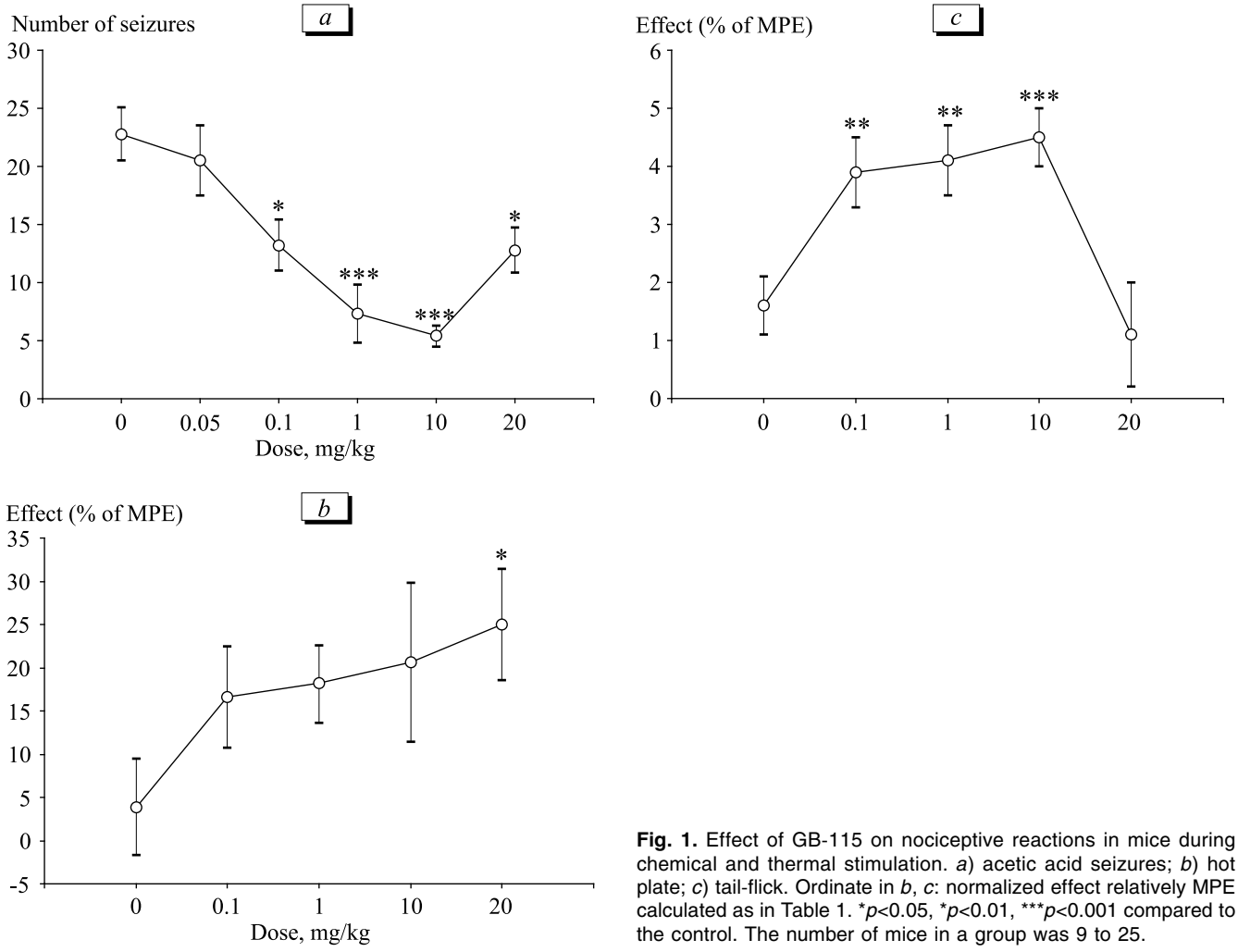

Fig. 1. Effect of GB-115 on nociceptive reactions in mice during chemical and thermal stimulation. *a*) acetic acid seizures; *b*) hot plate; *c*) tail-flick. Ordinate in *b*, *c*: normalized effect relatively MPE calculated as in Table 1. **p*<0.05, ***p*<0.01, ****p*<0.001 compared to the control. The number of mice in a group was 9 to 25.

of 20 mg/kg (75.9%, $p<0.0001$). Further elevation of GB-115 dose to 20 mg/kg diminished analgesia to 43.8% ($p<0.05$). In acetic acid test, the routine opioid analgesic morphine (5 mg/kg) inhibited the nociceptive response by 95.2% ($p<0.001$), and this effect was completely prevented by naloxone (Fig. 2). In contrast, preliminary nonselective block of opioid receptors with naloxone only partially (by 29.8%) antagonized the antinociceptive effect of GB-115 and non-opioid analgesic sodium diclofenac (Fig. 2).

In the hot plate test, GB-115 significantly moderated the nociceptive reaction only in the maximum tested dose of 20 mg/kg (Fig. 1, *c*) demonstrating far less potency than morphine used in the same test ($p<0.001$).

In the tail flick test, GB-115 increased the latency of the response in a dose-dependent manner (up to 281.2% at 10 mg/kg as compared to the control) which nevertheless was smaller than that induced by morphine (443.7%, Fig. 1, c; Table 1). Preliminary block of opioid receptors prevented analgesic action of GB-115 by 87.5% in comparison with the control (Table 1). However, GB-115 did not exert analgesic effect in this test when used in a greater concentration of 20 mg/kg (Fig. 1, *c*).

The above data showed that dipeptide GB-115, an agent synthesized on the basis of endogenous tetrapeptide cholecystokinin [3], possesses pharmacological activity in peroral administration, which agrees with the results of the pharmacokinetic studies of this agent [1]. The revealed bell–shape dose-dependence corresponds to the data on pharmacological properties of the peptide preparations [15]. This study validated the approaches employing structural analysis of the natural regulatory peptides and creating the corresponding shorter active amino acid sequences with proteolytic stability [2].

GB-115 demonstrated the most pronounced analgesic properties in the model of peritoneal pain induced by intraperitoneal injection of acetic acid. Hypothetically, generation of abdominal contractions is partially originates from activation of local peritoneal receptors [8], so the results of acetic acid test are explained by activation of prostanoids, namely by the rise of prosta-

glandins E and F concentrations in the peritoneal fluid [10] and by activation of lipoxygenase products [11]. The test of acetic acid seizures is widely used to assess peripheral antinociceptive activity. The results of this study suggest involvement of lipoxygenase and/or cyclooxygenase in analgesic effect of GB-115.

During thermal stimulation, GB-115 demonstrated a week analgesic potency in the hot plate test (extensive heating) and somewhat greater potency in tail flick test (local heating), where GB-115 (10 mg/kg) elevated the nociceptive threshold by 2.5 times, which agrees with earlier findings on partial realization of the intrinsic antinociceptive properties of this dipeptide via spinal opioidergic pathways [4].

Comparison of the antinociceptive properties of GB-115 under similar conditions with the effects of narcotic analgesics showed that the profile of antinociceptive activity of this dipeptide includes a non-opioidergic component, because analgesia produced by GB-115 is only partially prevented by naloxone, an antagonist of opioid receptors.

The studies on chronic toxicity of GB-115 in peroral doses of 0.1 and 10 mg/kg showed that this dipeptide produced no damaging effect to the blood system, induced no morphological or histological alterations in tissues and organs of the experimental animals, and demonstrated no local irritant or ulcerogenic properties [6], which attests to its advantage over diclofenac [12]. Thus, the novel dipeptide analog of cholecystokinin can be considered as a promising agent to develop a tool to eliminate pain with the inflammatory component.

Thus, the available data conclude that peroral GB-115 produces a pronounced analgesic effect against visceral pain, which is no less than that of diclofenac, and demonstrate a moderate antinociceptive potency against somatic non-inflammatory pain. The analgesic effect of GB-115 is based on the opioidergic and non-opioidergic mechanisms.

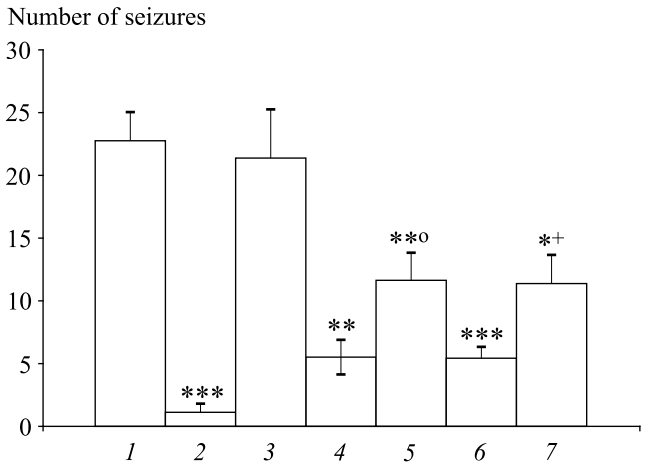

Fig. 2. Effect of GB-115 (10.0 mg/kg), morphine hydrochloride, and sodium diclofenac on the number of abdominal seizures in the test of acetic acid seizures in mice. *1*) control; *2*) morphine hydrochloride, 5 mg/kg; *3*) morphine hydrochloride, 5 mg/kg+naloxone, 1 mg/kg; *4*) sodium diclofenac, 20 mg/kg; *5*) sodium diclofenac, 20 mg/kg+naloxone, 1 mg/kg; *6*) GB-115, 10 mg/kg; *7*) GB-115, 10 mg/kg+naloxone, 20 mg/kg. *$p<0.05$, ***$p<0.001$ compared to the control; $p<0.05$ compared to $^{+}$GB-115 and ^{o}diclofenac group. The number of mice in a group was 9 to 21.

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