Role of the Cholecystokinin System in Anxiolytic Activity of Dipeptide GB-115
L. G. Kolik, T. A. Gudasheva, and S. B. Seredenin

We studied the effect of dipeptide GB-115, a retroanalogue of cholecystokinin-4 with anxiolytic properties, on the behavior of outbred rats and BALB/c and C57Bl/6 mice induced by cholecystokinin-4 receptor agonists and yohimbine. Anxiogenic agents were shown to cause anxiety in rats and C57Bl/6 mice (with an active response to stress) in the open field test and elevated plus maze test, but did not modulate the behavior of BALB/c mice exhibiting a freezing response to emotogenic exposure. Activation of cholecystokinin-4 type 2 receptors abolished the antianxiety effect of GB-115 in BALB/c mice. This dipeptide prevented the development of cholecystokinin-4-induced anxiety in C57Bl/6 mice and outbred rats. α₂-Adrenoceptor antagonist yohimbine did not modulate the effects of GB-115 in BALB/c mice. GB-115 did not prevent the development of yohimbine-induced anxiety in C57Bl/6 mice. Our results confirm the data on phenotype-specific activity of GB-115. We conclude that cholecystokinin-4 and GB-115 have a common pharmacological target.

Key Words: dipeptide GB-115; anxiolytic; cholecystokinin; cholecystokinin-4; anxiogenic agent

Neuropeptide cholecystokinin (CCK) plays a role in the neurobiological mechanisms of stress via the interaction with peripheral (CCK₁) and central receptors (CCK₂). Tetrapeptide CCK (CCK-4) activates CCK₂ receptors, which causes anxiety and panic response [7]. Previous experiments showed that stress and anxiogenic drugs increase the content of CCK in the frontal cortex of the brain [5]. Functional inactivation of the central CCK system holds promise for the prevention of mental symptoms, including anxiety and panic disorders. This mechanism mediates the anxiolytic effect of various chemical compounds.

A series of N-phenalkanoyl-substituted tryptophan-containing dipeptides with CCK-positive and CCK-negative properties was synthesized at the V. V. Zakusov Institute of Pharmacology (Russian Academy of Medical Sciences) on the basis of the Shemyakin–Ovchinnikov–Ivanov topochemical principle for construction of new peptide compounds [1]. An inverse structure of the peptide chain relative to the endogenous tetrapeptide CCK-4 (Trp-Met-Asp-Phe) can provide antagonistic (L-configuration of amino acid residues) or agonistic activity (D-configuration of amino acid residues). Pharmacological study of the series of more than 20 dipeptides has focused on glycyl-L-tryptophan amide-N-(6-phenylhexanoyl) (compound GB-115) exhibiting the highest anxiolytic activity [1].

It was hypothesized that the antianxiety effect of CCK₂ receptor antagonists requires the so-called “substrate” (i.e., high level of anxiety and fear) [15]. Taking into account these data, we studied the effect of a CCK receptor antagonist GB-115 on the behavior of outbred and inbred animals in the standard tests for anxiety after treatment with anxiogenic agents CCK-4, GB-104, and yohimbine.

MATERIALS AND METHODS
Experiments were performed on inbred male BALB/c and C57Bl/6 mice (20-22 g; Pushchino nursery) and...
outbred male rats (190-220 g; Stolbovaya nursery, Russian Academy of Medical Sciences). The animals were maintained in a vivarium of the V. V. Zakusov Institute of Pharmacology under standard conditions. They were housed in cages (10 mice per cage and 6 rats per cage) under natural light/dark regimen and had free access to water and standard pelleted feed for 10 days before the start of the study. Experiments were conducted in the fall-winter period at 9:00-13:00.

The study was performed with dipeptides GB-115 (Ph(Ch)2CO-Gly-L-Trp-NH2; 0.025 and 0.05 mg/kg; CCK-negative activity) and GB-104 (Ph(Ch)2CO-Gly-D-Trp-NH2; 0.02 mg/kg; CCK-positive activity). These compounds were synthesized at the V. V. Zakusov Institute of Pharmacology under standard conditions. We also used a benzodiazepine tranquilizer diazepam (1.0 mg/kg; Sigma), CCK, receptor agonist CCK-4 (4.0 μg/kg; Russian Cardiology Research and Production Complex), α2-adrenoceptor antagonist yohimbine (5.0 mg/kg; Sigma), and distilled water (control). The substances were injected intraperitoneally in a dose of 0.1 ml per 10 g body weight (for mice) or 0.1 ml per 100 g body weight (for rats). The scheme of treatment appeared as follows: (1) control; (2) anxiogenic agent; (3) GB-115 or diazepam (15 min after administration of the anxiogenic agent); and (4) GB-115 or diazepam. The doses were chosen from the results of experimental studies and published data on the efficiency of these agents.

The emotional stress exposure was modeled in the open field (OF) test as described elsewhere [2]. Before OF testing, all animals were maintained in darkness for 1 min and then placed into one of the peripheral squares. The behavior of animals was observed for 3 min. The following parameters were recorded: number of crossed peripheral segments (peripheral activity); number of crossed central segments and entries into the center (central activity); number of vertical rearing postures (vertical activity); and total locomotor activity (sum of the peripheral, vertical, and central activities).

Anxiety of animals was studied in the elevated plus maze (EPM) test [2,12]. The mice were tested in EPM with transparent walls, which allowed us to evaluate reliably the behavioral differences between the strains and anxiogenic effect of pharmacological agents [10]. The animals were placed into the central area. The main spatial and temporal parameters (time spent in the open arms; and number of entries into the open and closed arms) were recorded for 300 sec. Increasing the time spent in the open arms and number of entries into the open arms (with no change in locomotor activity; i.e., total number of entries into the open and closed arms) was considered as a criterion of the anxiolytic effect. The time spent in the open arms and number of entries into the open arms were calculated as follows:

\[
\text{Time spent in the open arms} = \frac{\text{time spent in the open arms (sec)}}{300 \text{ sec}} \times 100\% \ (1); \\
\text{and}
\]

\[
\text{Number of entries into the open arms} = \frac{\text{number of entries into the open arms}}{\text{number of entries into the open arms and number of entries into the closed arms}} \times 100\% \ (2).
\]

The results were analyzed by one-way analysis of variance (ANOVA) and nonparametric Mann–Whitney U test for independent samples. Intergroup differences were evaluated by Dunnett’s test (when the analysis of variance demonstrated a significant effect).

**RESULTS**

CCK-4 significantly decreased \( p<0.01 \) total locomotor activity of C57Bl/6 mice with active response to stress, but had no effect on this parameter in high-anxiety BALB/c mice exhibiting a greater freezing response as compared to C57Bl/6 mice \((21.2±3.6\ \text{and} \ 13.1±6.3 \ \text{sec, respectively;} \ p<0.001)\). Single administration GB-115 did not modulate the behavior of C57Bl/6 mice, but produced the anxiolytic effect on BALB/c mice. It was manifested in the increase of locomotor activity (criterion of anxiety), which is consistent with the results of previous experiments [2]. Preactivation of CCK receptors abolished the anti-anxiety effect of dipeptide GB-115 in BALB/c mice, which illustrates functional antagonism between CCK-4 and GB-115. These data suggest that anxiolytic activity of the CCK dipeptide analogue depends on its interaction with CCK receptors (Table 1).

Administration of CCK-4 to rats was followed by a significant decrease in the time spent in the open arms \( p<0.05 \) and number of entries into the open arms. These changes are typical of anxiety. The anxiolytic activity of CCK-4 was dose-dependent (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>BALB/c ( \pm ) SEM</th>
<th>C57Bl/6 ( \pm ) SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.0±3.6</td>
<td>133.0±6.3</td>
</tr>
<tr>
<td>CCK-4</td>
<td>19.0±3.1</td>
<td>80.0±7.7*</td>
</tr>
<tr>
<td>CCK-4+GB-115</td>
<td>22.0±2.2</td>
<td>130.0±8.9</td>
</tr>
<tr>
<td>GB-115</td>
<td>81.0±7.9*</td>
<td>127.0±8.3</td>
</tr>
</tbody>
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Note. *\( p<0.01 \) in comparison with the control (ANOVA; Dunnett’s test). Each group consists of 9-11 animals.