Effect of N-Terminal Tripeptides of Bombesin, Litorin, and Their Analogue on Body Temperature and Vasomotor Responses

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Intranasal administration of bombesin caused hypothermia in rats maintained under cold conditions. N-terminal tripeptide of bombesin exhibits intrinsic vasomotor activity, while intranasal administration of its modified analogue produced a more potent hypothermic effect than intranasal bombesin.

Key Words: *bombesin; litorin; N-terminal fragments; body temperature; vasoconstriction*

Extensive use of hypothermia in neurosurgery, cardiosurgery, and resuscitation for cardiac arrest as well as increasing interest in the protective effect of hypothermia in pathophysiology of apoptosis [3,5,7, 9,11] necessitate the development of new bioactive compounds causing hypothermia. There are no drugs decreasing body temperature under conditions of normothermia. However, there are a variety of drugs decreasing body temperature during hyperthermia. Hibernation is an example of controlled hypothermia in nature. Peptide compounds, including opioids, bombesin (BN), delta sleep-inducing peptide, and ACTH, are involved in the regulation of hypobiosis. All natural peptide compounds are polyfunctional, which makes it difficult to synthesize substances with selective activity. Tetradecapeptide BN (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met) isolated from the skin of *Bombina bombina* frogs serves as a peptide with high hypothermic activity. However, this peptide does not cross the blood-brain barrier and is effective only after intracerebral administration [10]. BN induces dose-dependent and temperature-dependent hypothermic and vasomotor effects.

Structural and functional study of the BN family showed that physiological activity of BN-like peptides is related to the properties of the C-terminal nonapeptide fragment [10].

Nonapeptide litorin (LN, pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met) belongs to the family of BN peptides. The amino acid sequence of BN and LN includes N-terminal dipeptide pGlu-Gln and C-terminal heptapeptide Trp-Ala-Val-Gly-His-Leu(Phe)-Met-NH_2.

All peptide compounds in the organism are degraded with the formation of short fragments, many of which exhibit properties of the peptide. The mathematical model and computer software to study degradation of peptide molecules were developed at the Institute of Bioorganic Chemistry. Previous studies showed that the LN molecule undergoes degradation with the formation of the N-terminal tripeptide [1].

Here we studied the effect of intranasal treatment with BN, LN, N-terminal tripeptide fragments,

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and modified analogue of terminal fragments on temperature and vasomotor responses.

MATERIALS AND METHODS

Experiments were performed on 320 male outbred albino rats weighing 180-250 g. Each group included 8-14 animals (used once). We used BN and LN (Serva), N-terminal fragments pGlu-Gln-Arg-OMe (BN_{1-3}-OMe) and pGlu-Gln-Trp-OMe (LN_{1-3}-OMe), and analogue of pGlu-Gln-Glu(gOMe)-OH fragments (Institute of Bioorganic Chemistry). Aqueous solutions of peptides were administered intranasally (20 μl). BN was administered in doses of 0.05, 0.1, and 0.5 mg/kg. Other peptides were administered in a dose of 0.1 mg/kg. Control animals received an equivalent volume of distilled water. The study was performed as described elsewhere [4]. Variations in body temperature (ΔT_B) and temperature of tail skin (ΔT_T) that indirectly reflect the vasomotor state were calculated as follows: $\Delta T=T_i-T_{bas}$, where T_i is T_B or T_T in a certain period of time (*i*); and T_{bas} is basal temperature of the body or tail skin (arithmetic mean of 3 values estimated 30, 20, and 10 min before treatment).

The thermoregulatory effects of BN, LN, and their fragments were studied in a cold medium (4-6°C). The influence of pGlu-Gln-Glu(gOMe)-OH was evaluated under 3 temperature conditions (cold medium, comfortable temperature, 27-28°C; and hot medium, 31-32°C), which reflected various functional states of the thermoregulatory system [6].

The results were analyzed by nonparametric Wilcoxon *U* test (Microsoft Excel 2000 and Statistica 6.0 softwares).

RESULTS

Administration of BN to rats in a cold medium was followed by a dose-dependent decrease in T_B. These changes were most pronounced after treatment with BN in a dose of 0.1 mg/kg (ΔT_B=-1.0±0.07°C, Fig. 1). The dose dependence was described by a dome-shaped curve typical of the majority of peptide compounds. The dynamics of T_T remained unchanged.

Hence, intranasal administration of BN under cold conditions was followed by dose-depended hypothermia.

The dynamics of T_B and T_T remained unchanged after administration of LN and LN_{1-3}-OMe in a cold medium. T_T significantly decreased after injection of BN_{1-3}-OMe, which reflects the increase in vasoconstriction. The dynamics of T_B remained unchanged under these conditions. The analogue of pGlu-Gln-Glu(gOMe)-OH induced more significant (110%) and long-lasting hypothermia compared to BN. As differentiated from BN_{1-3}-OMe, this analogue did not have vasomotor activity. Evaluation of the effects of pGlu-Gln-Glu(gOMe)-OH on temperature and vasomotor responses in a thermoneutral and hot medium showed that the peptide had no effect on T_B under both temperature conditions (Fig. 2, *a*). However, pGlu-Gln-Glu(gOMe)-OH induced a long-term decrease in T_T that characterized peripheral vasoconstriction (Fig. 2, *b*).

LN and LN_{1-3}-OMe did not modulate T_B and vasomotor tone in a cold medium. BN_{1-3}-OMe had vasomotor activity, which correlates with the fact that intracerebroventricular administration of the BN molecule potentiates peripheral vasoconstriction in a cold medium. The analogue of N-terminal tripeptide pGlu-Gln-Glu(gOMe)-OH caused a long-term decrease in T_B, which exceeded the hypothermic effect of BN. The tripeptide caused long-lasting vasoconstriction in thermoneutral and hot medium (more than 180 min), which was directed toward the maintenance of T_B.

Published data show that mammals have 6 types of temperature-sensitive ion channels of the TRP family (transient receptor potential), which are characterized by different temperature activation thresholds. The range of receptor activity is 0-50°C [2,8]. The effect of peptides on T_B depends on environmental temperature, which is probably related to their interaction with these receptors. pGlu-Gln-Glu(gOMe)-OH exhibits activity in a narrow temperature range, which is probably mediated by one type of these receptors.

Our results show that BN_{1-3}-OMe has vasomotor activity, which is typical of the whole molecule

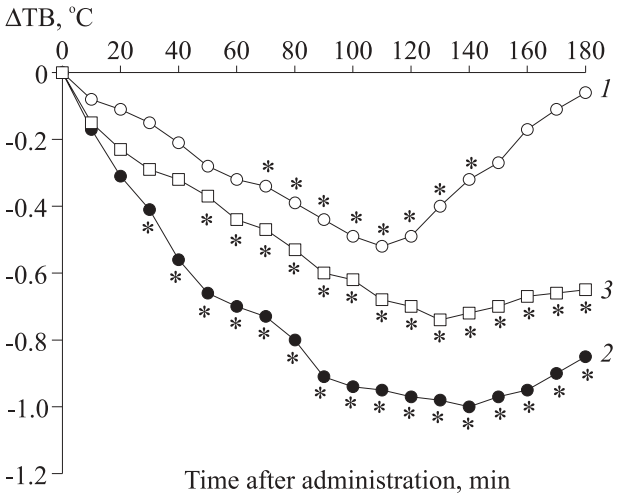

Fig. 1. Effect of BN on the dynamics of T_B in rats in a cold medium (4-6°C): 0.05 (*1*), 0.1 (*2*), and 0.3 mg/kg (*3*). Here and in Fig. 2: *$p<0.05$ compared to the control (0°C).

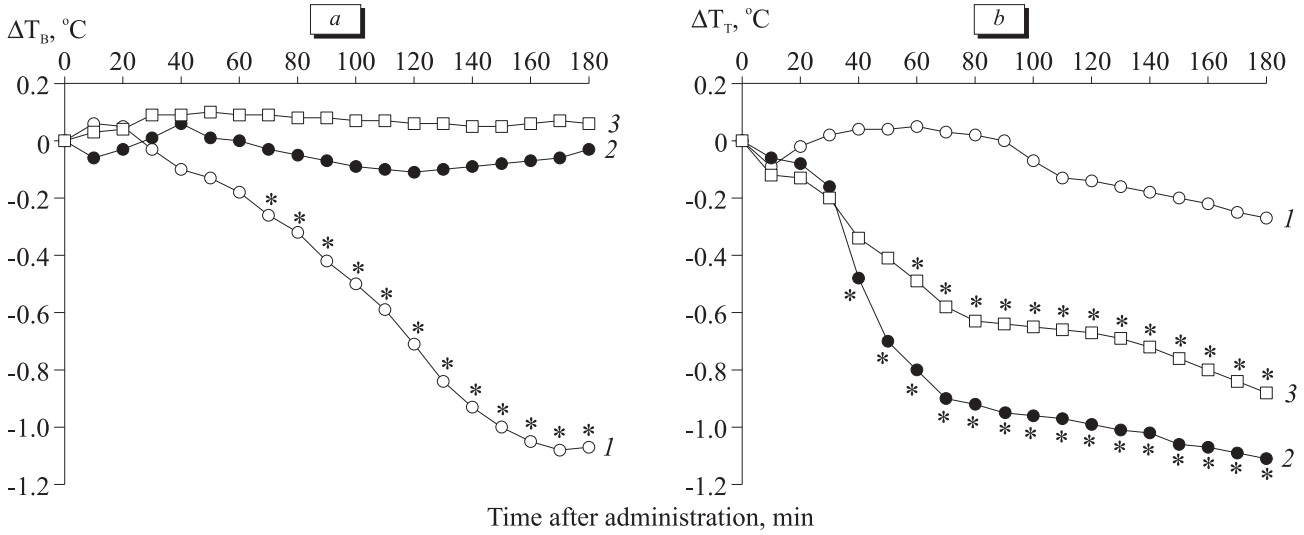

Fig. 2. Effect of intranasal treatment with pGlu-Gln-Glu(gOMe)-OH in a dose of 0.1 mg/kg on the dynamics of T_B (*a*) and T_T (*b*) in rats in a cold (4-6°C, *1*), comfortable (27-28°C, *2*), and hot medium (31-32°C, *3*).

of BN. Its modification allowed us to obtain a short peptide with selective hypothermic activity. Hence, BN_{1-3}-OMe and pGlu-Gln-Glu(gOMe)-OH may be used to synthesize highly active analogues.

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