Published in final edited form as:
Brain Res. 1999 August 21; 839(1): 194–198.

The effects of the phyllolitorin analogue [desTrp^3,Leu^8] phyllolitorin on scratching induced by bombesin and related peptides in rats

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Abstract

Bombesin along with several closely related neuropeptides elicit scratching behavior when administered centrally. The first part of the study was designed to determine the antagonistic effects of a novel phyllolitorin analogue wdesTrp^3,Leu^8]phyllolitorin (DTP) on scratching induced by three peptides (bombesin, neuromedin-C, and [Leu^8]phyllolitorin). In addition, the binding affinity of each peptide for the bombesin receptor site was determined. DTP (30 μg) inhibited scratching induced by these peptides, but unlike the peptides, DTP had no affinity for the bombesin site, thereby suggesting that DTP is displaying physiological antagonism through an unknown mechanism.

Keywords

Bombesin; Neuromedin-C; GRP(18-27); Phyllolitorin; Scratching behavior; Bombesin antagonist

Bombesin (BBN), originally isolated from amphibian skin, has a wide range of biological activities [1]; peripherally injected, BBN suppresses different types of behavior such as deprivation-induced feeding and sham feeding [11]. While centrally administered BBN has been shown to induce changes in thermoregulation [2], contraction of smooth muscle, and reduction in feeding [17], and drinking [6]. The most striking change in behavior upon central administration of BBN is an increase in “grooming”, specifically scratching [4,20]. A second group of peptides, the phyllolitorins, has more recently been isolated from amphibian skin. The phyllolitorin subfamily of BBN-like peptides share a similar C-terminal heptapeptide with BBN, and some have been shown to produce similar behavioral effects as BBN [3,13,22].

These compounds have not been identified within the mammalian population [23], but gastrin releasing peptide (GRP), a peptide found within the mammalian central and peripheral nervous systems, is similar in structure to BBN. Specifically, the peptide sequence GRP(18-27) or neuromedin-C (NMC), has been shown to be identical in structure to BBN at the C-terminal [15,16]. Also, NMC elicits similar behavioral changes as both BBN and the phyllolitorins [4,

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13]. It appears that the presence of tryptophan is necessary for the production of changes in behavior [18,19,22].

Scratching behavior has only recently been adopted as a method for evaluation of BBN-like compounds. Several BBN analogues have been synthesized and have shown strong antagonistic effects on some BBN-induced effects in vitro. Two such analogues, [D-Phe^{12},Leu^{14}]bombesin and [Leu^{13}-ψ-CH$_2$NH-Leu^{14}]bombesin, have been found to antagonize BBN-induced amylase release [4,10]. However, [Leu^{13}-ψ-CH$_2$NH-Leu^{14}] bombesin failed to disrupt grooming and scratching induced by NMC and BBN [5,14].

Recently, a synthetic phyllolitorin analogue, [desTrp^{3},Leu^{8}]phyllolitorin (DTP) has been developed and shown to inhibit scratching behavior induced by NMC [14]. This analogue is similar in structure to [Leu^{8}]phyllolitorin, except that the amino acid tryptophan at position three has been deleted (Table 1). Masui et al. [14] suggested that the compound may be binding to the same site because of the similarity in structure and its antagonistic character.

In the present study, we examined the ability of DTP to block the scratching behavior induced by central administration of three different peptides. The peptides used in the present study were BBN, NMC and [Leu^{8}]phyllolitorin. These compounds were chosen because they have previously been shown to produce a significant level of scratching behavior when administered centrally and also because of their similarity in structure (Table 1). The second part of this study was designed to test the relative binding affinity of BBN, NMC, [Leu^{8}]phyllolitorin and DTP for the BBN receptor site.

BBN, NMC and [Leu^{8}]phyllolitorin were purchased from Peninsula Laboratories (USA). Peptidase inhibitors were purchased from Sigma (USA). DTP was synthesized at the University of Michigan. BBN and NMC were dissolved in saline. DTP and [Leu^{8}]phyllolitorin were dissolved in saline and 20% DMSO. Subjects used were male Wistar rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing between 275–350 g. The rats were group housed under a 12-h light/dark cycle, with light on at 0600 h and allowed food and water ad libitum. Three to 5 days prior to the behavioral study, subjects were stereotaxically implanted with stainless steel cannulas in the lateral ventricle. Rats were randomly selected for treatment groups (n = 6–8), injected i.c.v. (10 μl) with the selected peptide and placed in observation cages immediately. All peptides were studied over a range of doses to determine scratching response curves. For the antagonist study, DTP (30 μg) was injected in a volume of 10 μl immediately prior to the injection of either NMC, BBN, or [Leu^{8}]phyllolitorin. This concentration of DTP was the maximum concentration that was soluble in the vehicle and it was slightly larger than the dose (24 μg) that had been found to be effective in blocking NMC-induced scratching [14]. The behavioral measure was the number of scratches recorded every 5 min for 30 min. Only scratches using either hind leg to scratch the head and neck were counted by an observer who was blind to the experimental conditions. Duration of the behavioral effect was determined by analyzing the time course of the dose at which the most scratches were produced per 5 min of each peptide (data not shown). All subjects were used once and then euthanized in order to confirm placement of cannula by injecting dye. Only subjects exhibiting wide dye distribution in the ventricles were used for data analysis.

Binding of [^{125}I]bombesin to membranes prepared from whole rat brain (male Wistar, Harlan–Sprague Dawley, Indianapolis, IN) was performed according to the following methodology [9,21]. Briefly, membranes (100 μg protein, determined by the method of Lowry et al. [12]), were incubated with [^{125}I]bombesin (0.1 nM, New England Nuclear) in the presence of peptidase inhibitors (chymostatin 2 μg/ml, leupeptin 4 μg/ml, bacitracin 40 μg/ml, and phosphoramidon 2 μM) in Tris–HCl (50 μM, pH 7.4) containing 0.02% bovine serum albumin in a total volume of 0.5 ml. Nonspecific binding was defined with 1 μM BBN. For competition

assays at least six concentrations of competing ligand were used. After incubation for 60 min at 25°C assays were terminated by rapid filtration through glass fiber filters (Scheicher and Schuell, #32) pretreated with 0.1% polyethyleneimine, filters were washed three times and bound radioactivity was measured by gamma counting. Competition data were analyzed to provide IC_{50} values and 95% confidence intervals (95% CI) using Graphpad Prism (San Diego, CA). Behavioral data were analyzed by calculating the ED_{50} and 95% CI for each dose–effect curve. A rightward shift of the dose–effect curve was considered significant if the 95% CI did not overlap.

NMC produced maximal effects during the first 15 min after i.c.v. injection and by 20 min post injection scratching responses were reduced to those produced by saline. The effects of BBN and [Leu^8]phyllolitorin were found to be longer lasting with scratching behavior continuing to be elevated for up to 30 min (data not shown). These results are similar to those found by Masui et al. [13]. For the purpose of data analysis, only the results for the first 15 min of responding were analyzed. When administered alone, neither DMSO 20% nor DTP 30 μg was found to produce either scratching or toxic effects.

BBN, NMC and [Leu^8]phyllolitorin produced increases in scratching (Fig. 1). Treatment with saline produced approximately 0–20 scratches during the 15-min trial. Co-administration of DTP significantly reduced scratching behavior produced by these peptides. The effects of DTP on BBN were insurmountable under these conditions, whereas this antagonism was surmountable for NMC and [Leu^8]phyllolitorin. The baseline ED_{50} (95% CI) for BBN was 0.59 μg (0.31–1.01 μg). The ED_{50} for the dose–effect curve of BBN co-administered with DTP could not be calculated because the effect was insurmountable. BBN doses of 10 μg or higher produced toxic effects when administered alone. Co-administration of DTP with BBN inhibited toxic effects at 10 μg, but not at 32 μg. The baseline ED_{50} for NMC was found to be 0.94 μg (0.49–1.82 μg). The ED_{50} for the dose–effect curve of NMC co-administered with DTP was found to be 6.83 μg (3.59–13.01 μg). The baseline ED_{50} for [Leu^8]phyllolitorin was found to be 0.78 μg (0.51–1.20 μg). The ED_{50} for the dose–effect curve of [Leu^8]phyllolitorin co-administered with DTP was found to be 17.36 μg (8.19–36.80 μg). The rightward shifts of NMC and [Leu^8]phyllolitorin dose–effect curves were considered to be significant because the 95% CI did not overlap.

The binding affinity of BBN and related peptides was determined by the percentage of specific [^{125}I]bombesin binding remaining across a range of concentrations. The peptides that elicited scratching showed different affinities for the BBN site in a descending order of BBN>NMC> [Leu^8]phyllolitorin. Bombesin was determined to have the highest affinity with an IC_{50} of 3.29 nM (2.43–4.45 nM). NMC was found to have the second highest affinity with an IC_{50} of 8.20 nM (5.76–11.68 nM) and [Leu^8]phyllolitorin the lowest affinity with an IC_{50} of 0.26 μM (0.19–0.34 μM). DTP was found to have no significant binding affinity for the BBN site (Fig. 2). After incubation with DTP (10 μM) the specific [^{125}I]bombesin binding remaining was found to be approximately 93%.

Many different compounds have been shown to elicit increases in the level of grooming behavior [7,8]. Scratching, perhaps, a specific type of grooming, appears to require a more selective type of agonist. Compounds, such as CRF and substance P, shown to elicit grooming have no effect upon scratching [13]. Peptides of the BBN family have been shown to reliably produce an increase in the level of scratching behavior while having minor effects on grooming [13]. This suggests that scratching may be mediated through some types of BBN or GRP receptors.

Previously it has been shown that DTP can block NMC-induced scratching behavior [14]. In part, the aim of this study was to evaluate whether DTP would block scratching induced by

other peptides closely related to NMC. It was shown that DTP reduced the potencies of these peptides (Fig. 1). The rightward shifts for NMC and [Leu^8]phyllolitorin dose–effect curves were 7- and 22-fold, respectively. In the case of BBN, the dose–effect shift produced by administration of DTP was found to be insurmountable because scratching responses were not restored by high doses of BBN.

The results of this study provide evidence that DTP has antagonistic effects for scratching induced by BBN-like compounds. Masui et al. [14] found that DTP inhibited NMC-induced scratching, therefore suggesting that DTP may be acting as an antagonist at BBN receptors. The results of the binding study demonstrate that DTP does not show binding affinity for the BBN site. This suggests that the antagonism produced by DTP is probably occurring through a different mechanism. It should also be noted that even though [Leu^8]phyllolitorin shows a lower binding affinity than BBN, it is equipotent to BBN at eliciting scratching. A possible explanation is that [Leu^8]phyllolitorin may be producing its effects though BBN receptor subtypes, whereas BBN may only be inducing these effects through one of these subtypes. Regardless, the results of the binding study did not show simple displacement curves for these peptides (Fig. 2), and the binding displacement functions suggest more than one binding site for BBN-like peptides. This result is similar to that reported by Guard et al. [9] in which a NMC preferring and a non-NMC preferring site were identified.

The structural similarity and common biological actions produced by these compounds provide further evidence that the amino acid tryptophan is required for these compounds to be active at the BBN receptor. This idea is supported by other observations in which the tryptophan is replaced with alanine at position four of NMC, after which the compound no longer shows biological activity [18]. The results presented herein provide support for the idea that tryptophan is required for binding and behavioral activation by BBN-like compounds. Nevertheless, a receptor-based antagonist of BBN-induced scratching continues to elude us.

Acknowledgments

We would like to thank Brendan Hug, Kathy Lee, and John R. Omnaas for their technical support and hard work. This study is supported by USPHS Grant DA00254 and the Clinical Research Partnership Fund of the University of Michigan.

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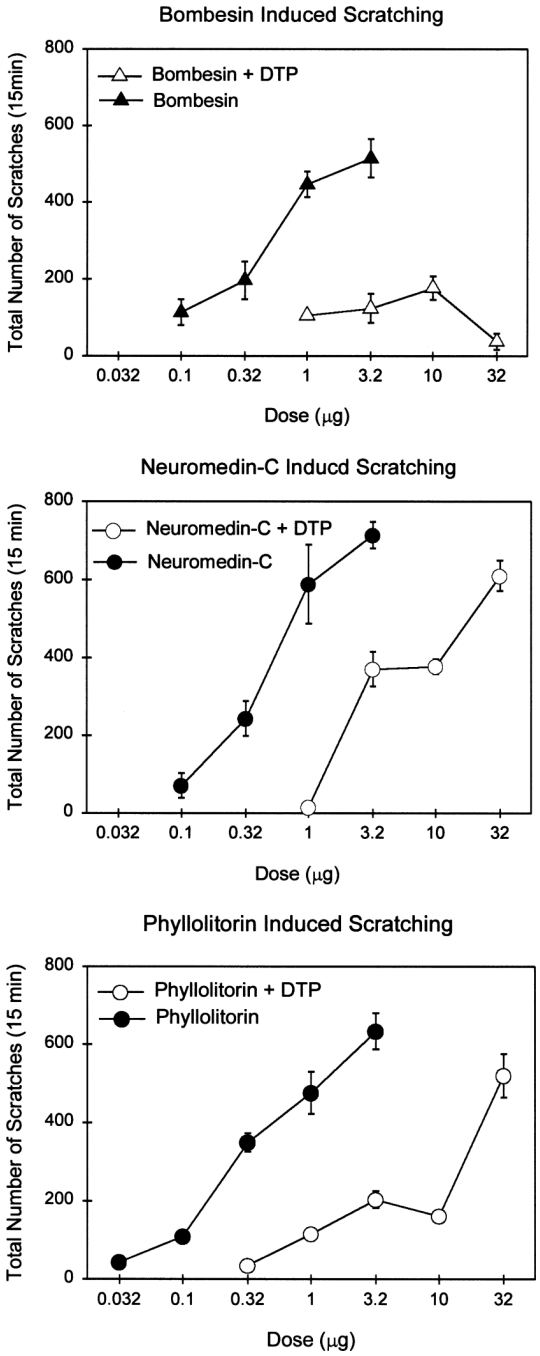

Fig. 1.

Dose–effect curves in the absence or presence of DTP for scratching induced by bombesin, neuromedin-C and [Leu^8]phyllolitorin, respectively. Data are expressed as mean scratches per 15 min±S.E.M. Dose–effect shifts were significant for both neuromedin-C, and [Leu^8] phyllolitorin. Each data point contains 6–8 subjects.

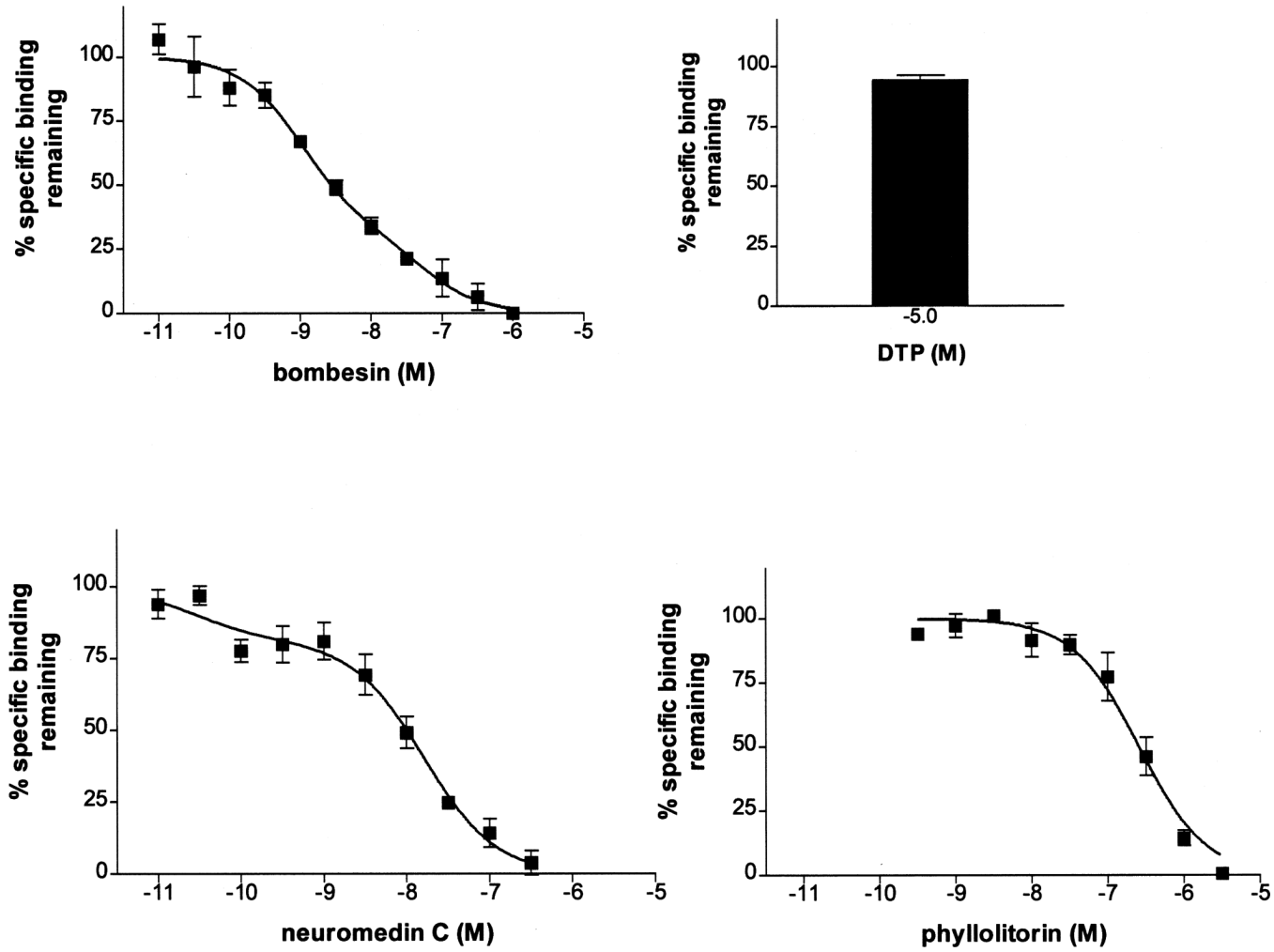

Fig. 2.

Percent specific binding remaining of [^{125}I]bombesin binding to rat brain membranes after incubation with bombesin, DTP, neuromedin-C and [Leu^{8}]phyllolitorin, respectively. Each data point represents three to four replications. S.E.M. was shown as a vertical bar for each data point.

Table 1

Amino acid sequences of bombesin and bombesin-related peptides

Amino acid sequences of bombesin-related peptides	
Bombesin	pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Neuromedin-C	Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
[Leu^8]phyllolitorin	pGlu-Leu-Trp-Ala-Val-Gly-Ser-Leu-Met-NH_2
DTP	pGlu-Leu- -Ala-Val-Gly-Ser-Leu-Met-NH_2