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Investigation of Gastrin-Releasing Peptide as a Mediator for 5'-Guanidinonaltrindole-Induced Compulsive Scratching in Mice

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Abstract

Gastrin-releasing peptide (GRP) has been implicated in the itch-scratch cycle. We investigated if this gut-brain-skin peptide plays a role in the compulsive, hindleg scratching of the neck of mice by 5'-guanidinonaltrindole (GNTI), the kappa opioid receptor antagonist, and in the antipruritic activity of nalfurafine, the kappa opioid agonist. Previously, we showed that GNTI (0.03–1 mg/kg, s.c.) elicits dose-related scratching and that nalfurafine (0.001–0.02 mg/kg, s.c.) inhibits this behavior in mice. Utilizing immunohistochemistry, GRP positive nerve fibers were detected in mouse skin and superficial layer of the dorsal horn of the spinal cord as well as GRP positive cells in the dorsal root ganglion. Pretreating mice with either a pseudopeptide GRP receptor antagonist, RC-3095 (10–30 mg/kg, s.c. at –15 min), or a peptide GRP receptor antagonist, [D-Phe⁶]bombesin(6–13) methyl ester (2–100 nmoles, i.t. at –10 min), did not suppress GNTI-induced scratching. However, pretreating mice with either antagonist inhibited scratching precipitated by the GRP receptor agonist, GRP_{18–27} (2 nmoles, i.t.). Pretreating mice with a muscarinic M₁ receptor agonist, McN-A-343 (1.5–15 µg/5 µl, i.t. at –10 min) antagonized GNTI-induced scratching. Norbinaltorphimine (20 mg/kg, i.p. at –18 to –20 h), a kappa opioid antagonist, countered the antiscratch activity of nalfurafine. We conclude that (a) the GRP receptor system does not mediate GNTI-induced scratching, and (b) the kappa opioid system is involved, at least in part, in the scratch suppressing activity of nalfurafine.

Keywords

Itch; Gastrin-releasing peptide; GNTI; Nalfurafine; Kappa opioid receptor; Muscarinic receptor

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1. Introduction

Sun and Chen [23] reported that the spinal receptor for gastrin-releasing peptide (GRP) mediates scratching behavior in mice induced by each of the following three chemically and pharmacologically diverse pruritogens: compound 48/80, chloroquine and a proteinase activating receptor 2 (PAR2) agonist. More recently, Sun et al. [24] showed that the selective ablation of lamina I neurons expressing gastrin-releasing peptide receptors (GRPR) in the spinal cord of mice led to a significant scratching deficit in response to chemically different itch stimuli (both histamine-dependent and histamine-independent). These findings raise the possibility of spinal GRP serving as a common itch neurotransmitter by relaying information to the somatosensory cortex in response to an array of pruritic stimuli. Such a contention would be strengthened by demonstrating an association between GRP, its receptor(s), and the pronounced pruritic effect of 5'-guanidinonaltrindole (GNTI), a well-established kappa opioid receptor antagonist [18, 21].

In this context, we have shown that subcutaneous (behind the neck) injection of GNTI (0.03–1 mg/kg) induces vigorous and compulsive, dose-related scratching in mice [4], probably by acting peripherally [11]. This strong behavior was nonetheless antagonized by pretreating (or post-treating) mice with low s.c. doses of nalfurafine, a kappa opioid receptor agonist [10]. Nalfurafine also antagonized scratching induced in mice by compound 48/80 [29], chloroquine [7], and agmatine [8]; secondary cholestasis due to chronic ethynylestradiol injections in rats [9]; and morphine in monkeys [13, 28]. Given the current interest in GRP as a likely common itch mediator, we now describe immunohistochemical and behavioral studies aimed at identifying possible links between the scratch-inducing effects of GNTI and GRP.

2. Materials and Methods

2.1. Animals

Male, Swiss Webster mice (25–30 g, Ace Laboratories, Boyertown, PA) were used. They were housed under a 12 h light/dark cycle with food and water available *ad libitum*. Experiments were carried out between 10:00 AM and 5:00 PM. Experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Mice were brought to the laboratory on the morning of the experiment and were placed in individual, shielded observation boxes (18 cm × 23 cm × 25 cm) for acclimation for at least 1 h before injections. We used standard, submaximal s.c. doses of GNTI (0.3 mg/kg) and nalfurafine (0.02 mg/kg) which were based on experience from our previous studies [4, 10].

2.2. Detection of immunoreactive (ir) GRP nerve fibers and cells in the skin, spinal cord and dorsal root ganglia (DRG) of mice

Mice (n = 3) were deeply anesthetized with urethane (1.2 g/kg, i.p.) and perfused intracardially with ice-cold 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS and 0.2% picric acid. The fixed cervical spinal cords, cervical DRGs and 0.5 cm × 0.5 cm of neck skin were removed and post-fixed in 4% paraformaldehyde solution overnight at 4°C. Tissue samples were transferred to 30%

sucrose solution for at least 3–4 days before sectioning. Using a cryostat, cervical spinal cords (C5–C7) were cut at 35 μm thickness, while DRG and skin sections were cut at μm thickness, all at -19°C . Free floating sections were kept in PBS solution at 4°C until immunohistochemistry was performed.

Tissues were processed for GRP ir by the avidin-biotin complex procedure as described previously [1]. They were first treated with 3% H_2O_2 to reduce endogenous peroxidase, washed two times for 10 min with PBS, and blocked with 20% normal goat serum (1:20 dilution) at room temperature for 2 h. The sections were then incubated on a shaker for 2 days at 4°C with rabbit anti-gastrin releasing peptide antiserum 1:1000 dilution (H-027-07, Phoenix Pharmaceuticals, Burlingame, CA). After thorough rinsing, sections were incubated in biotinylated anti-rabbit immunoglobulin G secondary antibody (1:300 dilution, Vector Laboratories, Burlingame, CA) for 2 h at room temperature. Following two 10 min rinses with PBS, sections were incubated in avidin-biotin-peroxidase complex at room temperature for 90 min (1:150 dilution, Vectastain ABC Elite kit, Vector Laboratories). Following three 10 min washes in Tris-buffered saline, sections were reacted with 0.05% diaminobenzidine (Sigma, St. Louis, MO)/0.001% H_2O_2 solution for 5–7 min and washed 3 times for 10 min with Tris-buffered saline. Sections were mounted on slides with 0.25% gel alcohol, air-dried, dehydrated with ascending grades of alcohol followed by xylene and coverslipped with Permount.

2.3. Effect of GRPR antagonists on GNTI-induced scratching behavior

We selected a pseudopeptide antagonist of GRPR, RC-3095 [19], which is bioavailable after systemic administration to rodents. Mice ($n = 5$) were pretreated with either saline or RC-3095 (s.c., flank area). The choice of dose of RC-3095 (10 and 30 mg/kg) was based on a previous report [16]. Fifteen min later, animals were given GNTI (0.3 mg/kg, s.c., behind the neck) and 1 min after the injections the number of scratches directed to the neck with hindlegs was counted for 30 min.

[D-Phe⁶]bombesin(6–13) methyl ester was chosen as the peptide GRPR antagonist. Sun and Chen [23] reported that pretreating mice with this antagonist (2 nmoles, i.t.) reduced the incidence of scratching associated with compound 48/80, PAR2 and chloroquine, respectively, in this species. Mice ($n = 6–8$) were injected i.t. with either [D-Phe⁶]bombesin(6–13) methyl ester (2–100 nmoles/5 μl) or saline (5 μl) and, 10 min later, the animals were challenged with either saline or GNTI (0.3 mg/kg, s.c., behind the neck). The number of scratching bouts was counted for 30 min. To include a positive control group, and to be certain that both antagonists blocked GRPRs, we conducted additional experiments as follows. Sun and Chen [23] showed that pretreating mice with [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles/5 μl , i.t.), decreased the number of scratches induced by GRP_{18–27} (2 nmoles/5 μl , i.t.), a peptide GRPR agonist. We injected mice ($n = 7$) with either saline or RC-3095 (10 mg/kg, s.c.) and then, 15 min later, animals were challenged i.t. with GRP_{18–27} (2 nmoles/5 μl). The number of scratching bouts was counted for 30 min. Additionally, we pretreated mice i.t. with either saline (5 μl) or [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles/5 μl) and, 10 min later, the animals were challenged i.t. with GRP_{18–27} (2 nmoles/5 μl). Again, the number of scratching bouts was counted for 30 min.

2.4. Muscarinic-1 (M₁) receptors and GNTI-induced scratching

Since both GNTI and nalfurafine have an affinity for the M₁ receptor (see Discussion), we included M₁ receptor ligands (agonist and antagonist) in our in vivo studies. We first examined if telenzepine, the M₁ receptor antagonist, would antagonize GNTI-elicited scratching. After acclimation in their individual observation boxes, mice were injected s.c. (flank area) with either saline or telenzepine (1–30 mg/kg). Thirty min later, the animals were challenged with GNTI (0.3 mg/kg, s.c.) and those scratches directed to the neck with hindlegs were counted for 30 min.

Next, we studied whether a M₁ receptor agonist, McN-A-343, would affect GNTI-induced scratching. Mice were administered i.t. either saline (5 µl) or McN-A-343 (1.5–15 µg/5 µl). Ten min later, each mouse received GNTI (0.3 mg/kg, s.c.). One min later, the number of scratching bouts was counted for 30 min.

The locomotor activity of mice was monitored using a Digiscan D Micro System (AccuScan, Columbus, OH) to establish if our top dose of McN-A-343 caused behavioral depression in the animals. Mice were acclimated for 1 h in individual cages (27 cm × 48 cm × 20 cm) placed in a quiet room. The animals were injected i.t. with either saline (5 µl) or McN-A-343 (15 µg/5 µl) and total distance traveled over 1 h was measured.

2.5. Effect of norbinaltorphimine pretreatment on the antiscratch activity of nalfurafine

Mice (n = 6) were treated i.p. with either saline or norbinaltorphimine (norBNI, 20 mg/kg), a selective kappa opioid receptor antagonist. At 18–20 h later, the animals were acclimated in individual observation boxes and injected (s.c., flank area) with either saline or nalfurafine (0.02 mg/kg). Twenty min later, they were challenged with GNTI (0.3 mg/kg, s.c., behind the neck). This experimental design involved the following four groups of mice: (saline + saline + GNTI); (norBNI + saline + GNTI); (saline + nalfurafine + GNTI); and (norBNI + nalfurafine + GNTI). One min after GNTI challenge, the mice were observed for 30 min and the number of scratching bouts was counted.

2.6. Compounds

GNTI dihydrochloride and norbinaltorphimine dihydrochloride were purchased from Tocris Bioscience (Ellisville, MO). [D-Tpi⁶, Leu¹³ ψ(CH₂NH)Leu¹⁴]bombesin(6–14) (RC-3095), GRP_{18–27}, telenzepine dihydrochloride and McN-A-343 (4-3-chlorophenylcarbamoyloxy-2-butynyltrimethyl-ammonium chloride) were obtained from Sigma (St. Louis, MO). [D-Phe⁶]bombesin(6–13) methyl ester was synthesized by Phoenix Pharmaceuticals (Burlingame, CA). Nalfurafine hydrochloride was a generous gift from Dr. Patrick J. Little of Adolor Corporation (Exton, PA). All compounds were dissolved in saline and, where appropriate, doses are expressed as the salt form.

2.7. Data analyses

Statistical comparisons were performed with one-way ANOVA followed by the Newman-Keuls test. The Mann Whitney U test was used where indicated. Data are expressed as the mean ± standard error of the mean (S.E.M.). *P* < 0.05 was considered statistically significant.

3. Results

3.1. GRP is detected in skin, DRG and spinal cord sections

GRP ir nerve fibers were detected in skin sections (Fig. 1). GRP ir expressed medium (20–30 μm) and small-sized (<20 μm) cell bodies in the DRG as well as cell processes in the superficial layer of the dorsal horn of the cervical spinal cord (Fig. 2).

3.2. Neither RC-3095 nor [D-Phe⁶]bombesin(6–13) methyl ester antagonize GNTI-induced scratching behavior

Pretreating mice with RC-3095 (10 or 30 mg/kg) had no significant effect on GNTI-induced scratching behavior (Fig. 3a). The number of scratches in 30 min was similar in mice pretreated with saline and in mice pretreated with RC-3095. In contrast, pretreating mice with RC-3095 (10 mg/kg) significantly ($U [12] = 8.00, p < 0.05$) antagonized GRP_{18–27}-induced scratching (Fig. 3b). Pretreating mice with [D-Phe⁶]bombesin(6–13) methyl ester (2–100 nmoles, i.t.), 10 min before GNTI (0.3 mg/kg), also did not markedly reduce scratching induced by the pruritogen (Fig. 4a). [D-Phe⁶]bombesin(6–13) methyl ester significantly ($U [14] = 6.00, p < 0.01$) reduced the incidence of scratching induced by GRP_{18–27} (Fig 4b). We conducted another set of experiments to examine if [D-Phe⁶]bombesin(6–13) methyl ester inhibits GRP_{18–27}-induced excessive grooming, as opposed to scratching, behavior. Mice were pretreated (i.t.) with either saline (5 μl) or [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles/5 μl) and then, 10 min later, challenged i.t. with either saline (5 μl) or GRP_{18–27} (2 nmoles/5 μl). Upon injection, each mouse was placed into its individual observation box and monitored, specifically, for grooming behavior for 20 min. Using a stopwatch, the time spent face and body grooming and tail licking was recorded. Mice treated with GRP_{18–27} significantly increased overall grooming behavior compared with mice injected with saline ($F [2, 21] = 7.04; p < 0.01$). [D-Phe⁶]bombesin(6–13) methyl ester did not markedly affect grooming behavior induced by GRP_{18–27} (Fig 5).

3.3. Effects of M₁ receptor ligands on GNTI-induced scratching

Pretreating mice (at –30 min) with telenzepine (1–30 mg/kg) had no statistically significant inhibitory effect on scratching induced by GNTI (Fig. 6). However, pretreating mice (at –10 min) with McN-A-343 (1.5–15 μg , i.t.) significantly decreased scratching bouts induced by GNTI in a dose-dependent manner (Fig. 7) ($F [3, 32] = 16.06; *p < 0.05$ and $**p < 0.01$ represent significance compared with saline group; $###p < 0.01$ represents significance of McN-A-343 at 15 μg compared with 1.5 and 5 μg).

We measured locomotor activity using the highest dose of McN-A-343 to establish if antiscratch activity of McN-A-343 is a consequence of behavioral depression. Figure 8 clearly shows that the suppressing effect of McN-A-343 is not likely due to behavioral depression.

3.4. Antiscratch activity of nalfurafine is mediated through kappa opioid receptors

Nalfurafine (0.02 mg/kg) significantly inhibited GNTI-induced scratching. Injecting mice with norBNI, 18–20 h before this dose of nalfurafine, significantly attenuated the antiscratch activity of the kappa agonist ($F [4, 23] = 23.12; p < 0.01$) (Fig. 9).

4. Discussion

The key finding of this study was that gastrin-releasing peptide receptors do not mediate the repetitive scratching induced by GNTI in mice. This conclusion runs counter to the possibility [24] that GRPR might serve as a common itch mediator and is based on our observation that (a) [D-Phe⁶]bombesin(6–13) methyl ester [22] and RC-3095 [5], two antagonists of GRPR, had no significant effects on scratching elicited by GNTI, and (b) the antiscratch action of nalfurafine is related to kappa opioid receptors.

We tested four i.t. doses of [D-Phe⁶]bombesin(6–13) methyl ester (2, 20, 50 and 100 nmoles) and two high s.c. doses of RC-3095 (10 and 30 mg/kg) in our failed attempt to “normalize” the robust scratching syndrome caused by GNTI. It is not known if the modest antagonizing action reported by Sun and Chen [23] for [D-Phe⁶]bombesin(6–13) methyl ester (2 moles, i.t.) is dose-related against scratching associated with compound 48/80, the PAR2 agonist and chloroquine. Would higher pretreatment doses of this peptide have completely suppressed the scratching precipitated by these pruritogens? At any rate, the biological significance of the antagonism must be questioned given that the three groups of mice still scratched excessively (~150, ~100 and ~75 times, respectively) during each of the 30 min observation periods despite pretreatment with the GRP antagonist. Further, GRPR knock out mice still scratched following histamine, endothelin-1 as well as serotonin injections [23]. It remains an open question as to the pharmacological basis of the substantial residual scratching.

Although inactive against GNTI, [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles, i.t.) nonetheless reduced the number of scratching bouts elicited by GRP_{18–27} in mice. (The amino acid sequence of GRP_{18–27} is identical to the carboxyl-terminal decapeptide of human GRP). This experiment served as a positive control for the octapeptide and is in accord with the findings of Sun and Chen [23]. There is a proviso, however, and it relates to a methodological issue that may complicate interpretation of the overt behavioral data. If the question is simply this: “Does [D-Phe⁶]bombesin(6–13) methyl ester antagonize GRP_{18–27}-induced scratching in mice?” then the answer is “yes”. If, on the other hand, the question is “Does the octapeptide antagonize the dermato-behavioral syndrome precipitated by GRP_{18–27} in mice?”, the answer is “yes and no”. The reason for the ambivalence is that GRP/bombesin agonists precipitate a range of behaviors in this species, all indicative of altered (aversive?) skin sensation and, potentially, of “itchiness”. These behaviors include incessant scratching of the flanks and neck area with the hindpaws, increased grooming/washing of the face with forepaws, and frequent preening of the tail with the mouth [2, 3]. Notably, [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles, i.t.) had no marked influence on the composite “grooming/washing/preening” behaviors.

Our demonstration of modest antagonism for RC-3095 against scratching precipitated by GRP₁₈₋₂₇ in mice represents a first for this compound and encourages further study in additional animal models of itch and, perhaps eventually, in humans. The knowledge that RC-3095 has already been subjected to a Phase I trial in patients with solid malignancies [20] may facilitate its evaluation against pruritic conditions.

GRPR is has been detected in the isocortex, hippocampal formation, amygdala, hypothalamus (arcuate, periventricular, paraventricular, supraoptic, and suprachiasmatic nuclei) and brain stem [12] as well as in lamina I layer of the dorsal horn of the spinal cord of mice [23]. In this report, we show GRP positive nerve fibers in the epidermis and dermis of the skin of drug-naïve mice. Recently, Tominaga et al. [27] reported GRP positive nerve fibers in NC/Nga mice, a strain of animal bred to model atopic dermatitis [25]. These mice do, in fact, scratch spontaneously and develop severe dermatitis [15]. Sun and Chen [23] showed that the density of GRP ir nerve fibers, located on the superficial layer of the dorsal horn of the spinal cord, was reduced after dorsal rhizotomy suggesting that these are primary afferents for GRP. Our finding of GRP positive nerve fibers in skin supports this result. These primary nerve endings may play a role in the transmission of itch sensation from the periphery to the spinal cord. Sun and Chen [23] also showed that GRP positive neurons are co-localized with TRPV1 receptors in the DRG of mice. Additionally, Tominaga et al. [27] reported the co-localization of GRP positive nerve fibers and TRPV1 positive fibers in mouse skin while Imamachi et al. [6] suggested that TRPV1 receptors are required for the transmission of histamine-induced itch sensation. Peripheral activation of histamine H₁ receptors leads to activation of pruritic GRP positive fibers and this activation is supported by the presence of TRPV1 receptors.

Caliper Life Sciences (Hanover, MD) screened receptors and mediators against which our sample of GNTI might have an affinity. Screening included neurotransmitters, steroids, ion channels, second messengers, prostaglandins, growth factors as well as hormones, brain-gut peptides and enzymes. Percent inhibition of receptor binding by GNTI (1 μM) was reported. GNTI decreased binding to kappa opioid receptors by 99% and to muscarinic M₁ receptors by 52%. Furthermore, Nakao et al. [17] reported that nalfurafine has affinity for the muscarinic M₁ receptor. Nalfurafine inhibited radioligand binding to M₁ receptors by 41% and 72% at 1 μM and 10 μM, respectively. Our studies revealed that a range of doses of telenzepine, the M₁ antagonist, had no marked effect on GNTI-induced scratching in mice. In contrast, McN-A-343, the M₁ agonist, attenuated the scratching associated with GNTI in a dose-related manner and this antagonism was not a consequence of behavioral depression. At present, this result defies interpretation but calls attention to an under-researched muscarinic M₁ link in current itch research and additional work is required to match McN-A-343 against such different pruritogens as compound 48/80, serotonin and GRPR agonists.

Kappa agonists, as exemplified by nalfurafine, antagonize GNTI-elicited scratching in mice [10]. Receptor binding studies would seem to rule out a direct interaction with bombesin receptors [17]. In this report we show that pretreatment of mice with norbinaltorphimine will counter the antiscratch activity of nalfurafine, suggesting a role for kappa receptors in mediating the robust GNTI behavior. Nalfurafine is the first in class drug against the itch associated with chronic renal failure in humans. Thus, Wikström et al. [30] and Kumagai et

al. [14] reported that intravenous and oral administration of nalfurafine, respectively, are effective in diminishing itch sensation in chronic renal failure. Takasaki et al. [26] showed that nalfurafine reduces inflammatory pain induced by herpes virus inoculation (suggesting postherpetic neuropathy) in mice. Nalfurafine may be helpful in diminishing the inflammation, pain and itch associated with postherpetic neuropathy in humans. A topical formulation is currently being developed (Maruho Company, Japan) for treating itching in patients suffering from atopic dermatitis.

In conclusion, our results indicate that a) the GRP receptor does not mediate GNTI-induced scratching in mice; b) the kappa opioid system is involved, at least in part, in the scratch suppressing activity of nalfurafine; and c) RC-3095 may eventually be exploited as an antipruritic agent against GRP-mediated itch.

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Abbreviations

DRG	dorsal root ganglia
GNTI	5'-guanidinonaltrindole
GRP	gastrin-releasing peptide
GRPR	gastrin-releasing peptide receptor(s)
IHC	immunohistochemistry
ir	immunoreactive
norBNI	norbinaltorphimine
PAR2	proteinase activating receptor
PBS	phosphate buffer saline

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Research Highlights

- GRP immunoreactive nerve fibers were found in mouse skin.
- RC-3095, a GRPR antagonist, does not inhibit GNTI-induced scratching.
- [D-Phe⁶]bombesin(6–13) methyl ester, a GRPR antagonist, has no marked effect on GNTI-induced scratching.
- McN-A-343, a muscarinic M₁ receptor antagonist, attenuates GNTI-induced scratching.

Figure 1.

A representative picture of a GRP positive nerve fiber in the epidermal and dermal layers of skin from a drug-naïve mouse (Scale A: 250, B: 100 and C: 50 μm).

Figure 2.

A representative picture of GRP immunoreactive small and medium sized cell bodies in the DRG (A); and cell processes located in the superficial layer of the dorsal horn of the cervical spinal cord (B) of a drug-naïve mouse (Scale: 100 μ m).

a

b

Figure 3.

- a.** RC-3095 does not markedly affect GNTI-elicited scratching. Mice ($n = 7$) were injected s.c. with either saline or RC-3095 (10 and 30 mg/kg) and, 15 min later, challenged with GNTI (0.3 mg/kg). The animals were observed for 30 min and the number of scratches was counted.
- b.** RC-3095 inhibits GRP₁₈₋₂₇-induced scratching. Mice ($n = 7$) were injected s.c. with either saline or RC-3095 (10 mg/kg) and, 15 min later, administered GRP₁₈₋₂₇ (2 nmoles/5 µl, i.t.). Pretreating the animals with RC-3095 attenuated significantly GRP₁₈₋₂₇-induced scratching ($p < 0.05$, Mann Whitney U test).

a

b

Figure 4.

a. [D-Phe⁶]bombesin(6–13) methyl ester has no marked effect on GNTI-induced scratching. Mice (n = 7) were pretreated i.t. with either saline (5 µl) or [D-Phe⁶]bombesin(6–13) methyl ester (2–100 nmoles/5 µl). Ten min later, they were administered s.c. either saline (to control for a possible effect of 100 nmoles of peptidic antagonist per se) or GNTI (0.3 mg/kg) and the number of scratches was counted for 30 min.

b. [D-Phe⁶]bombesin(6–13) methyl ester attenuates GRP_{18–27}-induced scratching. Pretreating mice (n = 7) with [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles, i.t. at - min) significantly attenuated the scratching caused by GRP_{18–27} (2 nmoles, i.t.) (***p* < 0.01, Mann Whitney U test).

Figure 5.

[D-Phe⁶]bombesin(6–13) methyl ester does not attenuate GRP_{18–27}-induced grooming behavior. Mice (n = 7) were injected i.t. with either saline or [D-Phe⁶]bombesin(6–13) methyl ester (2 nmoles). Ten min later, they were given GRP_{18–27} (2 nmoles, i.t.) and the time the animals spent body grooming and tail licking was recorded for 20 min. Mice injected with GRP_{18–27} groomed significantly more than mice injected with saline (***p* < 0.01). Pretreating mice with [D-Phe⁶]bombesin(6–13) methyl ester did not affect GRP_{18–27}-induced grooming behavior (*p* > 0.05, one-way ANOVA followed by Newman-Keuls test).

Figure 6.

Telenzepine does not inhibit GNTI-elicited scratching. Mice ($n = 8$) were pretreated s.c. with either saline or telenzepine (1–30 mg/kg) and, 30 min later, they were challenged with GNTI (0.3 mg/kg, s.c.).

Figure 7.

McN-A-343 attenuates GNTI-induced scratching. Pretreating mice ($n = 8-10$) with McN-A-343 (1.5–15 μg , i.t.) significantly attenuated GNTI-elicited scratching ($F [3, 32] = 16.06; *p < 0.05$ and $**p < 0.01$ represent significance compared to the saline group and $##p < 0.01$ represents significance of McN-A-343 at 15 μg compared with 1.5 and 5 μg ; one way ANOVA followed by Newman-Keuls test).

Figure 8.

Mice ($n = 8$) were treated i.t. with either saline (5 µl) or McN-A-343 (15 µg) and locomotion was recorded for 1 h. McN-A-343 did not markedly affect locomotion ($p > 0.05$).

Figure 9.

NorBNI counters the antiscratch activity of nalfurafine. Mice ($n = 6$) were treated i.p. with either saline or norBNI (20 mg/kg).

The next day, 18–20 h later, the animals received s.c. either saline or nalfurafine (0.02 mg/kg) and, 20 min later, they were challenged with GNTI (0.3 mg/kg, s.c.). The number of scratches was counted for 30 min. Nalfurafine significantly inhibited GNTI-induced scratching and this inhibition was, in turn, antagonized by pretreatment with norBNI ($p < 0.01$, one way ANOVA followed by Newman-Keuls test).