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EFFECTS OF A NOCICEPTIN RECEPTOR ANTAGONIST ON EXPERIMENTALLY INDUCED SCRATCHING BEHAVIOR IN MICE

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Itch and pain are two distressing sensations sharing a lot in common. In addition to the periphery, the central nervous system is proposed as a therapeutic target for the development of antipruritic drugs. The contribution of the most recently discovered opioid peptide, nociceptin/orphanin FQ (N/OFQ) and its receptor (NOP) in pain transmission is controversial. It seems to be pronociceptive when given supraspinally, but elicits antinociceptive action when injected spinally. Here, we examined whether the N/OFQ system plays a role in experimentally induced pruritus. Scratching behavior was produced by intradermal administration of serotonin (50 μg/50 μl/mouse) or nociceptin (30 nmol/50 μl/mouse) to Balb/c mice. JTC-801 (1, 3 or 10 mg/kg, i.p.), a NOP receptor antagonist, attenuated both serotonin- and nociceptin-induced scratches. When given intradermally, JTC-801 (100 nmol) significantly reduced serotonin-induced but not nociceptin-induced scratches. We propose that antagonizing NOP receptors either systemically or localy, may be a novel approach in the development of antipruritic agents.

Keywords: pruritus, scretching, serotonin, nociceptin; NOP receptors; JTC-801

INTRODUCTION

Pruritus, the general clinical term for itch, is a distressing but protective sensation that provokes the desire to scratch. Since inhibition of itch is beneficial for improving the quality of life, the importance of treating pruritus is increasingly gaining attention. Although itch and pain are distinct sensations, they share much in common. Itch sensation is transmitted from the skin to the brain by primary afferent C fibers and then by spino-thalamic pathways [1, 2]. Similarly to analgesic drugs, the spinal cord dorsal horn is very important in the modulation of itch [3, 4]. Moreover, descending inhibition plays important roles in both itch in pain [5]. Therefore, the central nervous system, especially the spinal cord, appears to be a pivotal target for the development of antipruritic agents [3, 6-8].

The endogenous ligand nociceptin/orphanin FQ (N/OFQ) and its receptor (N/OFQ peptide receptor, NOP) are the most recently discovered opioid receptor family. The N/OFQ system is implicated in many behavioral patterns, but especially in nociception [9]. Despite intense research, conflicting results have been obtained from studies of the effect of the N/OFQ-NOP receptor system on pain modulation; however, it seems that N/OFQ usually exerts an hyperalgesic action when administered supraspinally but analgesic effects when given spinally [9, 10]. Peripheral administration of N/OFQ, on the other hand, is suggested to exert NOP receptor-mediated local antinociceptive effects [11–14]. In contrast, peripheral NOP receptor antagonism has been indicated to attenuate inflammation-induced nociceptive behavior [15]. Not much is known with regard to the role of N/OFQ-NOP receptor system in itch. Of these, intradermal N/OFQ is suggested to elicit itch-associated responses through leukotriene B_4 in mice [16]. Additionally, intrathecal injection of N/OFQ small doses induces scratching, biting, and licking in mice via spinal NK_1 receptors [17].

Considering the discrepancies in the effects of N/OFQ and NOP receptors in the modulation of pain and the similarities between pain and itch, we aimed to observe whether local and systemic NOP receptor antagonism influences serotonin- and/or nociceptin-induced scratching behavior in mice.

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METHODS

Animals. Experiments were conducted on female Balb/c mice (Laboratory Animal Center, Trakya University) weighing 20-30 g. Mice were housed in groups of eight and maintained under 12/12 h light-dark cycles at the temperature of 21 ± 2°C; water and food were provided *ad libitum*.

Serotonin- and nociceptin-induced scratching behavior. Scratching behavior was induced by intradermal injections of 50 μg of serotonin or 30 nmol of nociceptin into the pre-shaved rostral part of the back of the mice. Immediately after intradermal serotonin or nociceptin administration, scratching of the injected site by the hind paws was videotaped and counted for 30 min under quiet circumstances. The mice usually produced several scratches per second, and such a manner was counted as one bout of scratching.

Study design and drugs. To evaluate the effects of the NOP receptor antagonist on serotonin- and nociceptin-induced scratches, different doses of JTC-801(1, 3, or 10 mg/kg), a NOP receptor antagonist, were given systemically (i.p.). JTC-801 (100 nmol) was also administered intradermally to determine its peripheral effect. Both systemic and intradermal JTC-801 was administered 30 min before serotonin and nociceptin injections. Doses and treatment times of the drugs were selected from previous reports [16, 18, 19].

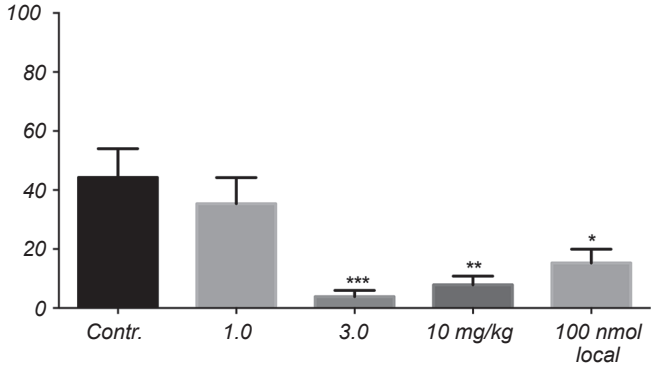
Serotonin hydrochloride and JTC-801 were purchased from Sigma (USA), and nociceptin from Tocris (Great Britain). Nociceptin was dissolved in distilled water, while JTC-801 was given in 20% DMSO, 5% Tween-80, 5% ethanol, and 70% saline.

Statistical analysis. To determine if there were significant differences ($P<0.05$) between groups, analysis of variance (ANOVA), followed by the Bonferroni *t*-test, was carried out. All numerical data are expressed as means ± s.e.m. for eight mice per group.

RESULTS

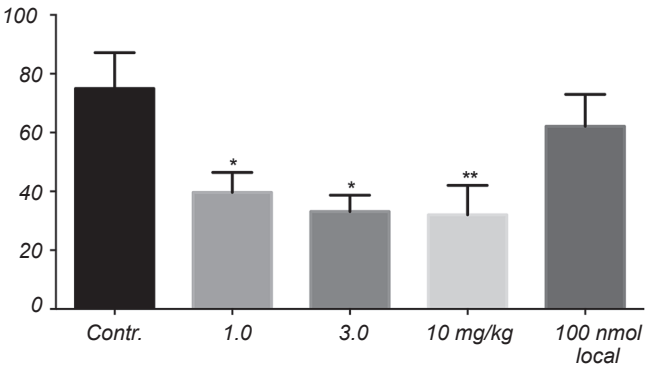
Effects of the NOP receptor antagonist JTC-801 on serotonin-induced scratches. Intradermal injection of 50 μg serotonin elicited rather intense scratching of the injection site (Fig. 1). Both systemic (1, 3, or 10 mg/kg) and local (100 nmol) administration of the NOP receptor antagonist JTC-801 reduced serotonin-induced scratches ($P < 0.05$; Fig. 1). JTC-801-induced reduction in scratching counts was highly significant at 3 and 10 mg/kg doses ($P<0.005$ and $P<0.01$, respectively); the effect of local application was also considerable ($P<0.05$; Fig. 1).

Effects of the NOP receptor antagonist JTC-801 on nociceptin-induced scratches. Intradermal injection of 30 nmol of nociceptin also produced intense scratching at the injection site (Fig. 2). Systemic administration of the NOP receptor



F i g. 1. Effects of systemic (1, 3, or 10 mg/kg) and local (100 nmol) administration of the NOP receptor antagonist JTC-801 on serotonin-induced scratches. (ANOVA, followed by the Bonferroni *t*-test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, $n = 8$ for each group)

Р и с. 1. Вплив системних (1, 3 або 10 мг/кг) або локальних (100 нмоль) уведень антагоніста NOP рецепторів JTC-801 на індуковану серотоніном «свербіжну» поведінку.



F i g. 2. Effects of systemic (1, 3, or 10 mg/kg) and local (100 nmol) administration of the NOP receptor antagonist JTC-801 on nociceptin-induced scratches. Designations are similar to those in Fig. 1.

Р и с. 2. Вплив системних (1, 3 або 10 мг/кг) або локальних (100 нмоль) уведень антагоніста NOP рецепторів JTC-801 на індуковану ноцицептином «свербіжну» поведінку.

antagonist JTC-801 (1, 3, or 10 mg/kg) attenuated nociceptin-induced scratching at all doses used ($P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively; Fig. 2), whereas it did not alter significantly scratching-related behavior when injected intradermally (100 nmol; Fig. 2).

DISCUSSION

The overall effects of the N/OFQ-NOP system on behavioral patterns is not clearly elucidated; it seems to be more complicated than the information given. Its effects on nociception are widely researched (although not understood thoroughly yet), but little is known about its effect on itch. Here, as shown before [16], we confirm that intradermal N/OFQ elicits scratching behavior. Moreover, we suggest, for the first time to our knowledge, that antagonizing NOP receptors either peripherally or systemically attenuates scratching behavior in mice.

As mentioned earlier, studies on the involvement of NOP receptor signalling in pain modulation generated contradictory results. In general, N/OFQ appears to have pronociceptive effects when given supraspinally and antinociceptive action when administered spinally; the site of administration and the dose of the drug may differentially influence this modulation [9, 10]. Nociceptin has been shown to prevent the antinociceptive action of paracetamol on the rat hot-plate test [20]. In contrast, we observed that blocking NOP receptors attenuates dipyrone-induced analgesia [21]. These totally opposite findings are in line with those indicating that both NOP receptor agonists and antagonists could potentially become useful treatments for chronic pain [22]. JTC-801, the NOP receptor antagonist we used in our experiments, has been shown to elicit potent antinociceptive effects both in acute and chronic pain states after systemic administration [23–25]. In the case of itch, we propose that systemic use of JTC-801 alleviates both serotonin- and nociceptin-induced scratching behavior. However, since NOP receptor signalling produces conflicting results in modulation of nociception, this anti-scratching activity of the NOP receptor antagonist must be strengthened with further experiments.

The NOP receptors are widely distributed not only in the central nervous system but also in the periphery, especially in sensory nerve terminals [26, 27]. Contradictory results have been also obtained from investigations with respect to the role of the N/OFQ-NOP system in the periphery. N/OFQ is shown to elicit local antinociceptive effects via NOP receptors when injected peripherally [11-14]. In a recent work, on the other hand, a peripheral NOP receptor antagonist exerted significant anti-allodynic and anti-hyperalgesic effects in an inflammatory pain model [15]. These results show that, similarly to its systemic effect, opposite findings can be seen when the N/OFQ-NOP system is modulated peripherally. It is suggested that N/OFQ produces nociception at low doses and antinociception at high doses, pointing to a dose-related opposite modulation by N/OFQ [28]. Our data indicate that, when applied locally, the NOP receptor antagonist JTC-801 reduces intradermal serotonin- but not nociceptin-induced scratches. This discrepancy may result from the complex action of the N/OFQ-NOP receptor system, but a peripheral pharmaceutical interaction may also be the reason.

The present findings suggest that blocking NOP receptors either peripherally or systemically attenuates experimentally induced scratches in mice, and that the N/OFQ-NOP receptor system could play an important role in itch transmission. NOP receptor antagonists are among potential new therapeutic targets for the treatment of pruritus.

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All procedures were performed according to the international ethical principles and guidelines for the use of animals for scientific purposes and were approved by the Animal Use Committee (Faculty of Medicine, Trakya University, Turkey).

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ВПЛИВ АНТАГОНІСТА НОЦИЦЕПТИВНИХ РЕЦЕПТОРІВ НА ЕКСПЕРИМЕНТАЛЬНО ВИКЛИКАНЕ ЧУХАННЯ У МИШЕЙ

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Р е з ю м е

Свербіж та біль є двома негативними почуттями, і в їх механізмах є багато спільного. ЦНС розглядається як можлива

терапевтична ціль для дії протисвербіжних ліків. Відомості про внесок нещодавно відкритого опіоїдного пептиду ноцицептину/орфаніну FQ (N/OFQ) та його рецепторів (NOP) у передачу болю є неоднозначними. В даній роботі ми дослідили питання, чи відіграє N/OFQ-система якусь роль у розвитку експериментально викликаного свербежу. Відповідна поведінка викликалася внутрішньошкірною ін'єкцією серотоніну (50 мкг) або ноцицептину (30 нмоль) мишам лінії Balb/c. Антагоніст рецепторів NOP JTC-801 (1, 3 або 10 мг/кг, внутрішньоочеревинно) зменшував інтенсивність «свербіжної» поведінки, викликаної ін'єкціями як серотоніну, так і ноцицептину. У разі внутрішньошкірних ін'єкцій JTC-801 (100 нмоль) істотно пригнічував свербіж, викликаний серотоніном, але не ноцицептином. Ми вважаємо, що антагоністи NOP рецепторів при їх системному або локальному введенні можуть слугувати основою для розробки нових протисвербіжних агентів.

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