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Intradermal application of nociceptin increases vascular permeability in rats: the possible involvement of histamine release from mast cells

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Abstract

Intradermal application of nociceptin was used to investigate its in vivo effect on the inflammatory response in rats. Intradermal nociceptin (5 pmol/site–5 nmol/site) increased vascular permeability in a dose-dependent manner. The increased vascular permeability by nociceptin (5 nmol/site) was dose-dependently inhibited by the histamine H_1 receptor antagonist pyrilamine (50 pmol/site–5 nmol/site). In rat peritoneal mast-cell preparation, nociceptin (10^{-8}–10^{-4} M) dose-dependently stimulated histamine release. The effect of nociceptin (10^{-5} M) occurred rapidly (within 30 s) and was inhibited by pertussis toxin, Ca^{2+}, but was not sensitive to naloxone, a classical opioid receptor antagonist. These characteristics are in agreement with features of the opioid-receptor-like$_1$ (ORL_1) receptor, a non-classical opioid receptor linked to a pertussis toxin-sensitive G protein. Taken together, these data suggest that nociceptin, likely acting via the ORL_1 receptor at the site of inflammation, might be critical for the enhancement of the inflammatory response by stimulating histamine release from mast cells.

Keywords: Nociceptin; Inflammatory response; Histamine; Mast cell; G protein; ORL_1 receptor; (Rat)

1. Introduction

The heptadecapeptide nociceptin (or orphanin FQ) has been identified as an endogeneous ligand for the opioid-receptor-like$_1$ (ORL_1) receptor from rat (Menuier et al., 1995) and porcine brain (Reinscheid et al., 1995). The ORL_1 receptor is coupled to a pertussis toxin-sensitive G protein (Fukuda et al., 1994; Mollereau et al., 1994) and possesses high homology with other classes of opioid receptors (Fukuda et al., 1994; Mollereau et al., 1994). ORL_1 receptor protein and mRNA are abundantly localized in the brain as well as the spinal cord (Bunzow et al., 1994; Fukuda et al., 1994; Wick et al., 1994; Lachowicz et al., 1995), thus suggesting a probable functional role in the central nervous system (CNS) (see review of Meunier, 1997).

ORL_1 receptor mRNA is also located in several non-nervous system tissues and organs (e.g., intestine, skeletal muscle, vas deferens and spleen) (Wang et al., 1994), as well as various populations of immune cells (Peluso et al., 1998; Pampusch et al., 1998). Thus, it is predicted that nociceptin will likely pharmacologically modulate features of ORL_1-positive populations, including the immune system, although to date, only a few reports have addressed this possibility (see review of Meunier, 1997). Interestingly, earlier in vitro studies have demonstrated that antisense oligonucleotides against the ORL_1 receptor inhibited polyclonal immunoglobulin production (Halford et al., 1995) and that the stimulation of human peripheral blood lymphocytes upregulates ORL_1 receptor mRNA expression (Wick et al., 1995), suggesting that nociceptin likely plays certain roles in the functional regulation of the immune system.

In addition to these in vitro findings, Nemeth et al. (1998) have described immune system influences of nociceptin using an in vivo paradigm. They reported that intraperitoneal injection of nociceptin inhibited plasma extravasation in denerved hindpaw induced by subplantar

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application of histamine and mast-cell-degranulating peptide. These results suggest that nociceptin likely has an anti-inflammatory effect. However, they did not address the issue of which component(s) is(are) important for the change in the inflammatory response at the local site. Namely, is there a direct anti-inflammatory effect of nociceptin on immune components and/or is there an indirect effect mediated by nociceptin modulation of the release of various neuropeptides [e.g., substance P and calcitonin gene-related peptide (CGRP)]? Indeed, considering nociceptin has been shown to inhibit the release of substance P and CGRP induced by capsaicin and bradikinin from sensory neuronal afferents (Helyes et al., 1997; Nemeth et al., 1998), and that it is possible that substance P and CGRP might work as pro-inflammatory factors (Schaffer et al., 1998; Sirinek and O'Dorisio, 1991; Rameshwar, 1997), the anti-inflammatory capacity of nociceptin may potentially involve modulation of the release of these and/or other neuropeptides.

Accordingly, it was deemed important to evaluate more precisely the nature of nociceptin in immune system modulation. In this study, we thus attempted to investigate the in vivo effect of the topical application of nociceptin in rats. Moreover, we investigated the effect of nociceptin on histamine release from rat peritoneal mast cells. Our data suggest that nociceptin is critical to regulate the immune response at the local site.

2. Materials and methods

2.1. Animals and drugs

Male Wistar rats (300–400 g) were purchased from Japan SLC (Hamamatsu, Japan). Animals were kept in a temperature (22–24°C)-, humidity (55 ± 5%)- and light (12-h light–dark cycle, lights on at 07:00 h)-regulated room with food and water ad libitum for at least 3 days before surgery. The procedures involving animals and their care were conducted in accordance with "Guiding Principles for the Care and Use of Laboratory Animals" provided by Nagoya University, Japan. Nociceptin was purchased from Peptide Institute (Osaka, Japan) and the pertussis toxin, naloxone, pyrilamine and Evans blue dye were from Sigma (St. Louis, MO, USA). All other chemicals used were obtained commercially and were used without further purification. Evans blue dye (0.5%) and pyrilamine were dissolved in sterile saline. Nociceptin was dissolved in sterile saline or Ca^{2+}- and Mg^{2+}-free Tyrode's solution containing 20 mM HEPES buffer. Pertussis toxin was dissolved in Ca^{2+}- and Mg^{2+}-free Tyrode's solution containing 20 mM HEPES buffer.

2.2. Measurement of vascular permeability in rat skin

Under light ether anesthesia, male Wistar rats (300–400 g) were injected intravenously with Evans blue dye (1.5 ml/kg) into a lateral vein in the tail. Nociceptin with or without pyrilamine was injected intradermally in 50-μl volumes into the shaved dorsal skin 15 min after the Evans blue dye injection. The rats were killed with CO_2 35 min after dye injection, and the dorsal skin was immediately removed. The blue-stained area at each injection site was measured as described elsewhere (Hiramatsu et al., 1998). The doses of pyrilamine used were selected according to the previous reports (Wilsoncroft et al., 1994; Cardell et al., 1997) showing the involvement of histamine in increasing vascular permeability.

2.3. Crude peritoneal mast-cell preparation

Peritoneal mast cells were prepared from the peritoneal cavities of rats. Briefly, under light ether anesthesia, peritoneal fluid was collected by washing with Ca^{2+}-, Mg^{2+}-free Tyrode's solution containing 0.1% (w/v) bovine serum albumin and 10 U/ml of sodium heparin. After rinsing, peritoneal fluid was collected with the same Tyrode's solution three times by centrifugation (1200 rpm, 10 min) at 4°C. The pellets were then resuspended in ice-cold Ca^{2+}-, Mg^{2+}-free Tyrode's solution containing 20 mM HEPES buffer at a designated concentration (3×10^5 mast cells/ml). This cell preparation showed a viability of greater than 95% and contained approximately 30% mast cells throughout the experiments.

2.4. Cell incubation

Test tubes containing 0.9 ml of the mast-cell preparation (3×10^5 mast cells/ml) were preincubated for 10 min at 37°C and then 0.1 ml of nociceptin solution at designated concentrations (10^{-8}–10^{-4} M) was added and incubated for a further 10 min at 37°C, except for the time-course study (0–15 min). After finishing the experiments, all test tubes were immediately placed in an ice-cold bath and then centrifuged at 2500 rpm for 10 min and the supernatant was then collected. To determine the effects of extracellular calcium or naloxone on histamine release induced by nociceptin, the desired concentrations of Ca^{2+} or naloxone were added immediately before the preincubation period, after which, samples were incubated with 10^{-5} M nociceptin. To assess the effect of pertussis toxin, the mast-cell preparation (3×10^5 cells/ml) was pretreated with pertussis toxin (1–30 ng/ml) for 120 min at 37°C. After two washes, samples were resuspended in Ca^{2+}-, Mg^{2+}-free Tyrode's solution containing 20 mM HEPES buffer. Samples were then preincubated for 10 min, and then with nociceptin (10^{-5} M) for a further 10 min. Collected supernatants were kept at −80°C until analysis. In all in vitro experiments, the amount of lactate dehydrogenase in the supernatants was determined before and after the incubation in order to verify cell viability.

2.5. Measurement of histamine release from peritoneal mast cells

The amount of histamine in the supernatant solutions was determined by the method of May et al. (1970). The extracted histamine was taken up in a high-performance liquid chromatograph: the mobile phase was 40% methanol in water containing 0.042 M acetate buffer pH 4.0. The chromatography was performed on a YMC Packed Column A-302 (Yamamura Chemical Laboratories, Kyoto, Japan). The flow rate was 1 ml/min and the fluorescence was monitored at 460 nm with excitation at 360 nm. The total histamine release was determined in the fluid of intact peritoneal mast cells lysed by boiling for 5 min. Spontaneous histamine release was less than 7% throughout all experiments. Histamine release is expressed as percent of total mast-cell histamine after subtraction of values for spontaneous release from the value found.

2.6. Statistical analysis

Results are expressed as means ± S.E. Statistical analyses were performed using a commercially available software package (StatView; Abacus Concept, Berkeley, CA, USA). Statistical analyses were calculated using the non-parametric Mann–Whitney *U* rank sum test. *P* values < 0.05 were considered statistically significant.

3. Results

As shown in Fig. 1(a), nociceptin (5 pmol/site–5 nmol/site) induced edema formation in rat skin in a dose-dependent manner, with an increase in vascular permeability of approximately 2.9-fold being associated with the highest nociceptin dose (Fig. 1(a)). In addition, nociceptin (5 nmol/site)-associated increased vascular permeability was significantly blocked by pyrilamine (50 pmol/site–5 nmol/site), a histamine H_1 receptor antagonist, in a dose-dependent fashion (Fig. 1(b)).

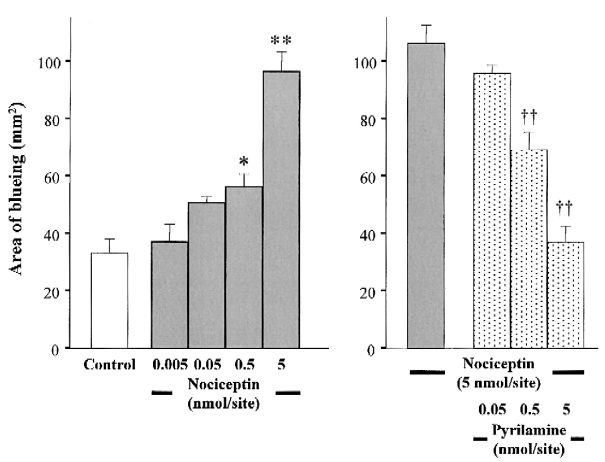

Fig. 1. (a) Dose-dependent effect of nociceptin on vascular permeability in rat skin. Each column represents the mean ± S.E. of five experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control (Mann–Whitney *U*-test). (b) Effect of pyrilamine on the increase of vascular permeability in rat skin induced by nociceptin (5 nmol/site). Each column represents the mean ± S.E. of four experiments. ††$P < 0.01$ vs. nociceptin (5 nmol/site) (Mann–Whitney *U*-test).

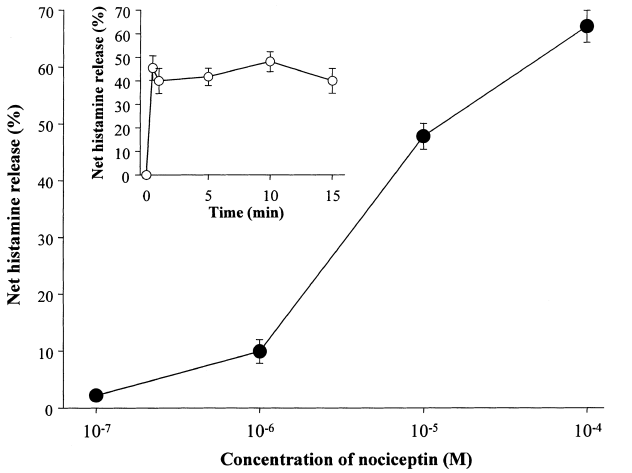

Fig. 2. Time-course effect on the release of histamine induced by nociceptin (10^{-5} M) (inset) and dose-dependent effects on the release of histamine induced by nociceptin (10^{-8}–10^{-4} M). Each point represents the mean ± S.E. of six experiments.

Fig. 2 (inset) shows the time-course of histamine release induced by nociceptin. Nociceptin-induced histamine release was very fast and reached its maximum effect within 30 s. Based on this result, we selected 10 min as an incubation period in the later part of the experiments. Nociceptin dose-dependently stimulated histamine release from rat peritoneal mast cells (Fig. 2). The threshold concentration was around 10^{-6} M and the release of 67% total histamine was found at 10^{-4} M. Nociceptin (10^{-4} M) did not alter the level of lactic dehydrogenase release into the supernatants from the mast cells (less than 8% above background, data not shown), indicating that noci-

Table 1
Effect of calcium and pertussis toxin on histamine release induced by nociceptin (10^{-5} M) from rat peritoneal mast cells

Nociceptin (10^{-5} M)-induced net histamine release (%)			
(A) Calcium concentration (mM)			
0	0.5	1	2
50.0 ± 5.0	30.3 ± 4.5[a]	17.0 ± 4.3[b]	5.6 ± 0.8[b]
(B) Pertussis toxin concentration (ng / ml)			
0	1	3	10
32.5 ± 1.1	21.0 ± 2.5[b]	7.0 ± 2.2[b]	1.6 ± 0.5[b]

Values express the mean ± S.E. of six experiments.
[a] $P < 0.05$ vs. corresponding nociceptin (10^{-5} M)-induced net histamine release without drugs (Mann–Whitney *U*-test).
[b] $P < 0.01$ vs. corresponding nociceptin (10^{-5} M)-induced net histamine release without drugs (Mann–Whitney *U*-test).

ceptin induced histamine release from the mast cells without any concomitant cytotoxic effects.

The histamine release induced by nociceptin (10^{-5} M) was inhibited by adding calcium (Table 1(A)) as well as pertussis toxin in a dose-dependent manner (Table 1(B)). Naloxone up to 10^{-4} M (10^{-4} M: 97.4 ± 3.1% of control, no difference from control) failed to inhibit histamine release induced by nociceptin (10^{-5} M) (data not shown).

4. Discussion

The ORL_1 receptor is located on various immune cells including circulating lymphocytes and monocytes as well as T, B and monocytic cell lines (Peluso et al., 1998), suggesting the possible role of the ORL_1 receptor in the immune system. Earlier in vitro studies have demonstrated that antisense oligonucleotides against the ORL_1 receptor inhibit polyclonal immunoglobulin production (Halford et al., 1995) and that the stimulation of human peripheral blood lymphocytes upregulates ORL_1-receptor mRNA expression (Wick et al., 1995). However, there are few reports available that investigate the in vivo effect of nociceptin in the immune system function (Nemeth et al., 1998). Thus, the purpose of this study was to evaluate the role of the local application of nociceptin in the inflammatory response.

In the present study, intradermal (i.d.) injection of nociceptin dose-dependently increased the vascular permeability in the skin. The potency of nociceptin to increase vascular permeability is almost similar to that of other inflammatory mediators such as substance P (Wilsoncroft et al., 1994; Walsh et al., 1995) and bradikinin (Wilsoncroft et al., 1994; Newbold and Brain, 1995). The nociceptin-stimulated vascular permeability was inhibited by pyrilamine, suggesting the involvement of histamine at the local site, which probably originates from mast cells in the skin. Thus, it is likely that nociceptin itself may possibly potentiate the inflammatory response in skin, subsequently enhancing the pain stimuli. The effect of nociceptin against the inflammation might be varied depending on the organs. For example, Nemeth et al. (1998) have demonstrated that nociceptin inhibits the release of substance P, CGRP and somatostatin induced by capsaicin or bradikinin from isolated trachea. Therefore, further studies in detail should be necessary to investigate the involvement of other inflammatory mediators in the effect of nociceptin against the inflammation in the skin as well as in other organs.

The mRNA signal of the nociceptin precusor, prepronociceptin, has been found in human peripheral tissues such as the spleen, thymus and liver as well as blood leukocytes (Mollereau et al., 1996; Nothacker et al., 1996). In blood circulation, nociceptin seems to be more stable than other opioid peptides (Yu et al., 1996). Thus, it is likely that nociceptin might supply to the skin from blood circulation or from infiltrated leukocytes during inflammation. The mRNA for the ORL_1 receptor has been found in various immune cells including monocytic cell lines (Peluso et al., 1998; Pampusch et al., 1998). Thus, nociceptin, subsequently, could stimulate histamine release from skin mast cell. Taken together, these findings suggest that topical application of nociceptin potentiates hyperalgesia as well as allodynia in a non-neurogenic fashion.

In supraspinal CNS, it is well known that nociceptin acts as an "anti-opioid" (Meunier et al., 1995), such that intracerebroventricular (i.c.v.) injection of nociceptin in mice produces hyperalgesia (Meunier et al., 1995; Reinscheild et al., 1995, Rossi et al., 1996, Shimohigashi et al., 1996; Nishi et al., 1997) as well as allodynia (Okuda-Ashitaka et al., 1996; Hara et al., 1997). Nociceptin-induced hyperalgesia was not observed in mice lacking the ORL_1 receptor (Nishi et al., 1997), suggesting the facilitating effect of nociceptin on pain stimuli by altering CNS function. In a similar manner, nociceptin (i.c.v.) reversed the analgesia induced by stress as well as opioids (Mogil et al., 1996a,b). Interestingly, however, in many cases, intrathecal (i.t.) administration of nociceptin has effects similar to the classic opioids, including analgesia (Xu et al., 1996; Tian et al., 1997; Yamamoto et al., 1997). For example, Yamamoto et al. (1997) have demonstrated that nociceptin (i.t.) inhibited thermal hyperalgesia induced by carageenan injection into the rat paw. Thus, depending on the dose of systemic injection of nociceptin, its overall influence on pain might be varied, and could enhance (site of inflammation and/or supraspinal cord) or diminish (spinal cord) pain depending on its site of action.

Keeping with our present results, Nemeth et al. (1998) have demonstrated that nociceptin marginally, but significantly, inhibited plasma extravasation induced by subplantar application of histamine to the denervated rat hindpaw. They have also demonstrated that nociceptin also suppressed plasma extravasation induced by subplantar application of mast-cell-degranulating peptide, suggesting that nociceptin may act as an anti-inflammatory peptide by inhibiting mast-cell-mediated plasma extravasation at the local site. In their studies, they administered nociceptin intraperitonealy whereas we injected intradermally. In addition to having the potential to act at the level of the skin, intraperitoneal injection of nociceptin could also have a neurogenic effect at the level of the spinal cord and/or supraspinal CNS, although there are currently no reports directly demonstrating this in rodents. Thus, there is a possibility that nerve afferents in the entire body and/or surviving neurons at local sites respond to nociceptin with subsequent alteration of neuropeptide levels in the hind paw and has been shown to inhibit the release of neuropeptides from sensory neuronal afferents (Helyes et al., 1997; Nemeth et al., 1998). Further studies thus remain in order to elucidate more fully the underlying mechanisms.

The studies using rat-peritoneal mast cells revealed that histamine release induced by nociceptin occurred rapidly

and reached its maximum effect within 30 s after the challenge, whereas Ig-E-mediated histamine release requires several minutes to reach its maximum (Church et al., 1991; Mousli et al., 1994). Nociceptin-induced histamine release was inhibited by the application of pertussis toxin and Ca^{2+}. These results suggest that nociceptin may bind to pertussis toxin-sensitive-G-protein-coupled receptors, and that extracellular Ca^{2+} may diminish the effect of nociceptin. Indeed, these data are consistent with the characterization of ORL_1 receptors in CHO cells expressing the ORL_1 receptor (Fawzi et al., 1997; Hawes et al., 1998). Furthermore, considering our present result suggesting that the effect of nociceptin was insensitive to naloxone (up to 10^{-4} M), the classical opioid-receptor antagonist, it is likely that ORL_1 receptors located on skin mast cells, but not classical opioid receptors, contribute to this action. However, other classical opioid receptors, also coupled to pertussis toxin-sensitive G protein, possess the intracellular loops having high sequence homology with the ORL_1 receptor (Menuier et al., 1995). Indeed, it has been reported that other classes (μ, κ and δ) of opioid peptides, such as β-endorphin (Shanahan et al., 1984; Sydbom, 1988) and dynorphin (Sydbom and Terenius, 1985) also induce histamine release from the mast cell. Although our experimental design is consistent with action via the ORL_1 receptor, further studies are necessary to clarify fully this issue.

Nociceptin, a 17-amino-acid peptide, most likely stimulates histamine release via the ORL_1 receptor. However, it should be noted that certain peptides such as neuropeptide Y (Mousli et al., 1995), substance P (Mousli et al., 1990) and a wasp peptide venom, mastoparan (Hirai et al., 1979; Mousli et al., 1989) might stimulate histamine release from mast cells via non-receptor-mediated mechanisms involving pertussis toxin-sensitive G proteins (Higashijima et al., 1990; see review of Mousli et al., 1994). For example, Grundemar et al. (1994) have demonstrated that the rank order of potency of neuropeptide Y and its fragments to stimulate histamine release from mast cells did not agree with that of any previously known or postulated affinity for neuropeptide Y receptors. Moreover, the effect of mastoparan was abolished by pretreatment with neuralminidase, suggesting that the effect of mastoparan requires cell-surface sialic-acid residues (Chahdi et al., 1998). It will thus be of interest to further elucidate whether or not nociceptin acts on mast-cell ORL_1 receptors to stimulate histamine release.

In summary, nociceptin appears to be involved in releasing histamine from mast cells in vivo as well as in vitro. Thus, it is hypothesized that nociceptin potentiates the inflammatory response at local sites. ORL_1-receptor antagonists acting at supraspinal CNS and/or peripheral tissues could inhibit the nociceptin-mediated pain response, potentially decreasing pain thresholds and/or inhibiting inflammation.

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