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Short communication

Inhibition of nociceptin on sensory neuropeptide release and mast cell-mediated plasma extravasation in rats

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Abstract

Nociceptin (20 μg/kg i.p.) strongly inhibited cutaneous Evans blue accumulation in the chronically denervated hindpaw of the rat in response to mast cell degranulating peptide (MCDP, 0.25 μg in 100 μl) but it had no and marginal effect on plasma extravasation induced by 5-hydroxytryptamine (5-HT, 0.5 μg in 100 μl) and histamine (0.1 μg in 100 μl), respectively. Release of sensory neuropeptides such as substance P, calcitonin gene-related peptide (CGRP) and somatostatin from the rat isolated trachea in response to capsaicin (10^{-8} M) or bradykinin (10^{-7} M) were also attenuated by nociceptin (100 and 300 nM). It is concluded that chemically induced discharge of mediators from mast cells and from capsaicin-sensitive afferent nerve terminals are both inhibited by nociceptin that participates in the anti-inflammatory effect of the peptide. © 1998 Elsevier Science B.V.

Keywords: Nociceptin; Capsaicin-sensitive primary afferent neuron; Mast cell degranulating peptide; 5-HT (5-hydroxytryptamine, serotonin); Neuropeptide release; Bradykinin

1. Introduction

Meunier et al. (1995) and Reinscheid et al. (1995) isolated a novel neuropeptide structurally related to opioids named nociceptin or orphanin FQ. These terms refer to its ability to have hyperalgesic activity when injected intracerebroventricularly into mice (Meunier et al., 1995) and nanomolar binding affinity to ORL1 (opioid receptor-like protein 1) which was formerly an orphan receptor (Reinscheid et al., 1995). Morphine and enkephalines have an inhibitory effect on the release of substance P from the activated peripheral endings of the capsaicin-sensitive primary afferent neurones (Maggi, 1991, 1995) and inhibit in this way the development of neurogenic inflammation (Barthó and Szolcsányi, 1981; Barber, 1993). Recently, it has been shown that nociceptin exerts an inhibitory action on electrically and chemically induced neurogenic inflammation in vivo and on substance P and calcitonin gene-related peptide (CGRP) release from capsaicin-sensitive sensory fibres in response to electrical field stimulation in vitro (Helyes et al., 1997). The present study was to examine the effect of nociceptin on non-neurogenic inflammation evoked by mast cell degranulating peptide (MCDP) as well as on neuropeptide release in vitro provoked by chemical stimulants such as capsaicin or bradykinin.

2. Materials and methods

The experiments performed in the present study conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical committee of the Medical University of Pécs, Hungary.

2.1. Experiments on inflammation

Female Wistar rats (200–250 g) were anaesthetised with 40 mg/kg i.p. pentobarbital (Nembutal). For detection and quantification of plasma extravasation, Evans blue

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dye (50 mg/kg i.v.) was injected 10 min before subplantar application of MCDP (0.25 μg in 100 μl), 5-hydroxytryptamine (5-HT, 0.5 μg in 100 μl) or histamine (0.1 μg in 100 μl) into the hindpaw (Pintér and Szolcsányi, 1995). Nociceptin (20 μg/kg i.p.) or isotonic saline was also given 10 min prior to the induction of inflammation. In order to exclude neurogenic components from the inflammatory process the saphenous and sciatic nerves were cut 5 days before the experiment (Jancsó et al., 1967).

2.2. Measurement of neuropeptide release in vitro

After exsanguination, 2–2 removed tracheae were perfused (1 ml/min) in an organ bath (1.8 ml) at 37°C for 60 min with oxygenated (95% O_2 and 5% CO_2) Krebs solution of the following composition (in mM): NaCl 119; $NaHCO_3$ 25; KH_2PO_4 1.2; $MgSO_4$ 1.5; KCl 4.7; $CaCl_2$ 2.5; glucose 11. After stopping the flow, the solution was changed three times for 8 min (prestimulated–stimulated–poststimulated). Chemical stimulation was performed to induce peptide release by administration of capsaicin (10^{-8} M) or bradykinin (10^{-7} M) at the fifth min of the second fraction. Nociceptin (100 or 300 nM) was added into the incubation medium at the beginning of the second 8-min period. In control experiments, the stimulants were applied in the absence of nociceptin. The fractions were collected in ice-cold tubes and the wet weight of each trachea was measured. Concentrations of substance P, CGRP and somatostatin were determined by means of specific radioimmunoassay (RIA) methods developed in our laboratory, and were expressed as the released amount of peptide per tissue weight. Detection limits of the assays were 2 fmol/tube (substance P), 1 fmol/tube (CGRP), 2 fmol/tube (somatostatin) (Németh et al., 1996).

2.3. Drugs

Pentobarbital (Nembutal) from May and Baker (UK) was used for anaesthesia. Capsaicin (Sigma, St. Louis, MO) was dissolved in 10% ethanol, 10% Tween 80 (Reanal, Hungary) and 80% isotonic NaCl solution. Nociceptin, MCDP, rat α-CGRP, [Tyr^1]somatostatin-14 and Tyr–α-CGRP-(23–37) were purchased from Bachem (Bubendorf, Switzerland), bradykinin, somatostatin-14 and CGRP antiserum from Sigma, 5-HT-creatinine sulphate, histamine dichloride from Reanal (Budapest, Hungary) and substance P RIA tracer from Amersham (Amersham, UK). Substance P antiserum was provided by Prof. G.J. Dockray, University of Liverpool and somatostatin antiserum by Dr. T. Görcs, University Medical School of Budapest. ^{125}I-labelled Tyr–α-CGRP-(23–37) and ^{125}I-labelled [Tyr^1]somatostatin-14 were prepared in our laboratory.

2.4. Data analysis

The results are expressed as means ± S.E.M. Mann–Whitney *U*-test (for in vivo data) and Student's *t*-test for paired comparison (for in vitro data) were used for statistical evaluation, $P < 0.05$ was regarded as significant.

3. Results

3.1. Effect of nociceptin on plasma extravasation

Nociceptin (20 μg/kg i.p.) exerted 53.7 ± 4.55% and 19.5 ± 1.85% inhibitory action on plasma extravasation elicited by MCDP (0.25 μg in 100 μl s.c.) and histamine (0.1 μg in 100 μl s.c.), respectively 10 min later in the chronically denervated hindleg. On the contrary 5-HT-induced inflammation (0.5 μg in 100 μl s.c.) was not affected by nociceptin (Fig. 1).

3.2. Inhibitory effect of nociceptin on substance P, CGRP and somatostatin release in vitro

Capsaicin (10^{-8} M) evoked a significant increase in substance P (53.17 ± 7.12%), CGRP (114.28 ± 9.02%) and somatostatin (91.30 ± 4.65%) release from isolated rat tracheae in control samples. If chemical stimulation was performed by bradykinin (10^{-7} M), it also caused elevation in levels of substance P (53.33 ± 8.36%), CGRP (78.94 ± 11.76%) and somatostatin (68.03 ± 9.52%). In the presence of 100 and 300 nM nociceptin, the release of neuropeptides elicited by capsaicin were reduced to 41.17 ± 5.36 and 14.70 ± 3.22%, 52.38 ± 7.68 and 15.78 ± 3.63%, 40.90 ± 6.33 and 18.80 ± 7.68%, respectively. The corresponding values in response to bradykinin were 24.74

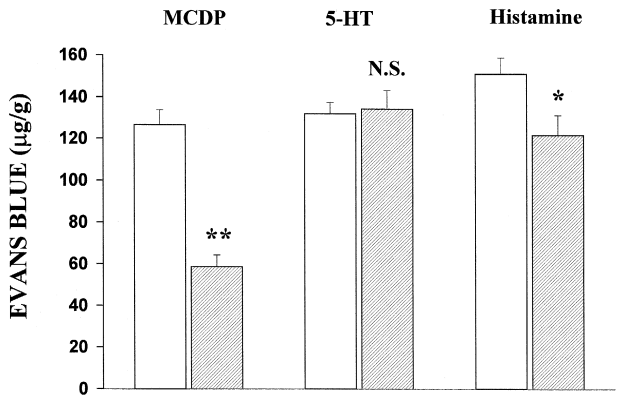

Fig. 1. Effect of nociceptin (20 μg/kg i.p.) administered 10 min before induction of plasma extravasation by MCDP (0.25 μg in 100 μl s.c., open), 5-HT (0.5 μg in 100 μl s.c., hatched) or histamine (0.1 μg in 100 μl s.c., hatched) in the skin of the chronically denervated rat hindpaw. Each value is mean ± S.E.M. obtained from six experiments. * $P < 0.05$; ** $P < 0.01$ vs. control group of animals (Mann–Whitney *U*-test).

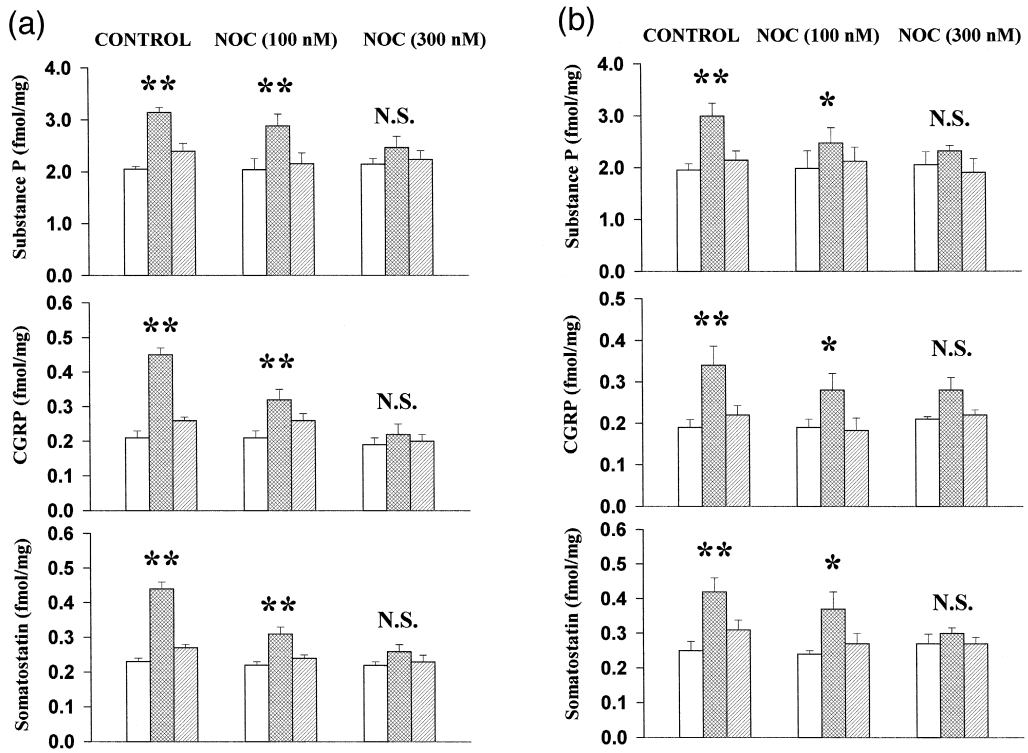

Fig. 2. Effect of nociceptin (100 and 300 nM) on substance P, CGRP and somatostatin release from the rat tracheae in vitro in response to (A) capsaicin (10^{-8} M) or (B) bradykinin (10^{-7} M). Columns indicate prestimulated (open), stimulated (cross-hatched) and poststimulated (hatched) values. Results are shown as means ± S.E.M. obtained from six experiments. $*\ P < 0.05$; $**\ P < 0.01$ (Student's *t*-test for paired comparison).

± 3.94 and 13.17 ± 4.31%, 47.36 ± 5.06 and 33.33 ± 3.84%, 54.56 ± 6.73 and 11.11 ± 3.33% (Fig. 2A,B).

4. Discussion

It has recently been described that nociceptin reduces both orthodromic and antidromic neurogenic inflammation, but it has no effect on non-neurogenic inflammatory reaction evoked by bradykinin. Since nociceptin (100 nM) diminished the release of substance P and CGRP evoked by electrical field stimulation in vitro, it was suggested that the anti-inflammatory effect of this peptide was due to a selective inhibition of the release of pro-inflammatory neuropeptides from the sensory nerve terminals (Helyes et al., 1997).

The present experiments show that non-neurogenic plasma extravasation after chronic denervation in response to mast cell degranulation is also inhibited by nociceptin. Nevertheless, the permeability increasing direct vascular effects of bradykinin (Helyes et al., 1997) or 5-HT are not inhibited by this opioid peptide. In mast cells, Ca^{2+} entry has been considered to play significant role in elevating cytosolic free Ca^{2+} concentrations during stimulus-secretion coupling to induce exocytosis of stored granular substances (Lloret and Moreno, 1995). MCDP is a highly cationic 22 aminoacid-containing peptide of the venom of the European honey bee (*Apis mellifera*) which activates mast cells by opening Ca^{2+} permeable channels via GTP-binding second messengers and by blocking K^+ channels (Kuno et al., 1989; Ziai et al., 1990; Koch et al., 1992). Morphine and methionin-enkephaline also inhibit neurogenic inflammation without any direct vascular effect but they do not diminish plasma extravasation evoked by mast cell degranulation (Barthó and Szolcsányi, 1981; Barber, 1993).

Substance P and CGRP are partly co-localized, while somatostatin is stored in another subclass of the capsaicin-sensitive afferents, from where they are released by capsaicin or bradykinin in a Ca^{2+}-dependent manner (Maggi, 1995; Szolcsányi, 1996). The present results revealed that bradykinin- or capsaicin-induced release of all three mediators is also inhibited by nociceptin in vitro. Opiate receptor agonists (δ and μ) do not inhibit chemically-evoked release of these sensory neuropeptides, although they inhibit the release evoked by electrical stimulation of the afferent fibres (Barthó et al., 1987; Maggi, 1995). Nociceptin increases K^+ conductance in rat dorsal raphe (Vaughan and Christie, 1996) and locus coeruleus (Connor et al., 1996a) and, unlike morphine, inhibits G-protein-independent low voltage T-type Ca^{2+} currents of neurones (Connor et al., 1996b; Abdulla and Smith, 1997). As discharge of vasoactive mediators from mast cells and neurotransmitter release from nerve terminals are both dependent on increased intracellular Ca^{2+} level, these data can give possible explanation for the inhibitory effect of

nociceptin on neurogenic and non-neurogenic inflammation elicited by mast cell degranulation.

It is concluded that a synthetic nociceptin analogue as an ORL1 receptor agonist is an effective anti-inflammatory agent with novel pharmacological profile acting on both mast cells and on nociceptive nerve terminals without affecting direct vascular responses to bradykinin and 5-HT.

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