Published in final edited form as:

J Surg Res. 2013 October ; 184(2): 973–980. doi:10.1016/j.jss.2013.03.076.

Nociceptin Receptor Signaling in Sympathetic Neurons from Septic Rats

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Abstract

Background—The endogenous opioid peptide, nociception (Noc) contributes to the regulation of systemic blood pressure and regional blood flow. Recent clinical and animal studies have reported that Noc and its receptor (NOP) are involved in inflammation and sepsis. The purpose of the present study was to examine the modulation of Ca²⁺ channels by Noc in acutely isolated stellate ganglion (SG) neurons from control and septic rats.

Materials and methods—Sepsis was induced in male Sprague-Dawley rats via cecal ligation and puncture (CLP). SG neurons were isolated 24 and 72 hr post-sepsis induction. Thereafter, the concentration-response relationships for the Noc-stimulated NOP receptor Ca²⁺ current inhibition were determined employing the whole-cell patch-clamp technique. In addition, the Noc precursor (PNOC) and NOP receptor mRNA levels were determined by quantitative RT-PCR, and PNOC protein levels were measured by Western blot analysis.

Results—Comparison of the Noc concentration-response relationships in SG neurons from control and septic rats 24 hr post-sepsis revealed similar potency and efficacy. Moreover, 72 hr post-sepsis, neurons from control and septic rats exhibited an increased potency when compared to both groups at the 24 hr time point—an effect that was more pronounced in neurons from septic rats. PNOC mRNA levels were significantly greater in SG neurons isolated from septic rats compared to control neurons but NOP receptor mRNA levels remained unchanged during the 72 hr period.

Conclusions—Our study demonstrates the CLP model-induced temporal upregulation of components within the NOP receptor signaling pathway in rat sympathetic neurons. As SG neurons provide the main sympathetic input to the heart, an increased Noc release and potency during sepsis may compromise cardiovascular function.

Keywords

NOP receptor; nociceptin; sepsis; Ca²⁺ channels; stellate ganglion; prepronociceptin

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Introduction

Sepsis is a severe medical condition that affects multiple systems including the immune, cardiovascular and autonomic nervous systems [1–2]. During sepsis, the immune system is initially overstimulated such that a considerable amount of cytokines are released ('cytokine storm') leading to an early excessive inflammatory response [1]. In this early septic stage, high levels of catecholamines are also released by sympathetic neurons that further aggravate the inflammatory response. During the late stages of sepsis, the adrenergic regulation of the heart and blood vessels is impaired [2–4]. As septic shock ensues, the cardiovascular system is unable to compensate for the systemic vasodilation and hypotension, contributing to the development of multiple organ failure.

Crosstalk between the autonomic nervous system and the immune system plays a major role during sepsis. Such stressful stimuli activate the hypothalamic-pituitary-adrenal (HPA) axis and increase the release of catecholamines and glucocorticoids [1]. The catecholamines released by sympathetic nerves innervating lymphoid organs (i.e. spleen, thymus, bone marrow) act as immunomodulators that stimulate adrenergic receptors present on the immune cells. A number of released sympathetic neurotransmitters and neuropeptides also function as chemoattractants for monocytes [5]. One study found that the neuropeptide, nociceptin (Noc), was capable of stimulating human monocyte chemotaxis [6]. Noc is the endogenous heptadecapeptide ligand that binds with high affinity to the nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptors. NOP receptors are G protein-coupled receptors (GPCR) whose activation results in inhibition of voltage-gated Ca^{2+} channels, activation of G protein inwardly rectifying K^{+} (GIRK) channels and negative coupling to adenylyl cyclase [7–9]. In sympathetic neurons, the actions of Noc are usually manifested as an inhibitory neuromodulation on transmitter release.

Recent studies have suggested that the NOP receptor:Noc signaling pathway may play a role in sepsis. For example, it was found that blocking NOP receptors decreased lethality in rats made septic with cecal ligation and puncture (CLP), while activating the NOP receptors with administered Noc increased lethality [10]. Furthermore, two clinical studies have reported the elevation of both Noc plasma levels [11] and mRNA in blood cells [12] when compared to nonseptic patients. Noc also plays a role in cardiovascular regulation [for review, see 9, 13]. In this regard, one study demonstrated that Noc decreases heart rate and mean arterial pressure in mice, an effect that was abolished in NOP receptor knockout animals [14]. Noc has also been reported to contribute to microvascular inflammation [15]. We have previously shown that NOP receptors are natively expressed in rat stellate ganglion (SG) neurons [16]. The sympathetic neurons within the SG innervate the heart and regulate both heart rate and contractility [17–18].

The purpose of the present study was to examine the Noc-mediated modulation of Ca^{2+} channels in acutely isolated SG neurons from nonseptic and CLP-induced septic rats employing the whole-cell patch-clamp technique. We further employed biochemical and molecular tools to determine whether changes in NOP receptor and Noc expression levels were altered by sepsis.

Materials and Methods

Animals

Male, specific-pathogen free Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Cambridge, MA). At the time of surgery, rats weighed $216 \pm 34\text{g}$. The rats were acclimated for no less than 1 week at a constant temperature with *ad libitum* access to standard rat chow and water in a light controlled room (12 hr light/12 hr dark

cycle). All experiments were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State College of Medicine and National Institutes of Health (NIH) guidelines regarding the use of experimental animals and were followed precisely.

Animal Surgery

Rats were anesthetized with isoflurane. The abdomen of each rat was shaved with clippers and cleaned with betadine. Rats were maintained under general anesthesia using 3% vaporized isoflurane at 1L/min. Sepsis was induced by CLP. A 2 cm midline laparotomy incision was made and the cecum ligated at its base using 4-0 silk. Through-and-through puncture of the cecum was done with a 20 gauge needle, and patency of the puncture sites was verified by extruding a small amount of fecal matter. The patency of the intestinal lumen was maintained. The cecum was then returned to the peritoneal cavity. The abdominal wall was closed with 4-0 silk suture and the skin was closed using sterile wound clips. Time-matched non-septic sham-surgery animals were subjected to midline laparotomy with closure identical to that of the septic animals. All rats were resuscitated using 10 ml 0.9% normal saline subcutaneous injection. An additional control group received no surgery. Following the procedure, rats were provided water and standard rat chow *ad libitum*. Animals were sacrificed at 24 or 72 hr post-operatively. All animals sacrificed at 72 hr received two additional fluid boluses of 10 ml 0.9% normal saline at 24 hr and 48 hr post-operatively.

Following CLP, the rats showed overt signs of sepsis within 24 hr. It is well known that sepsis produces an initial hyperdynamic state with increased cardiac output, tachycardia, tachypnea, lethargy and decreased systemic vascular resistance [19–20]. After 72 hr of the initial septic insult, most animals are generally considered to be in the hypodynamic phase which is associated with impaired organ perfusion and increased mortality [21]. Evidence of illness was manifested by the presence of liquid stool, as well as subjective decrease in feeding, activity and grooming behavior. The septic animals also had periocular discharge and piloerection. The time point of 72 hr was selected for sacrifice to maximize the effects of sepsis on the nervous system. A longer time course was determined to be not feasible due to increasing mortality rate after 72 hr. The 24 hr septic model had 0% mortality, whereas a 25% mortality rate was observed at 72 hr.

Stellate Ganglion (SG) Neuron Isolation

SG neurons were isolated 24 or 72 hr post-sepsis employing the method previously described [16]. Briefly, rats were first anesthetized with CO₂ and then quickly decapitated with a laboratory guillotine. The SG tissue was then cleared of connective tissue in ice-cold Hank's balanced salt solution (Sigma Chemical, St. Louis, MO). Thereafter, the ganglia were enzymatically dissociated in a modified Earle's balanced salt solution containing 0.6 mg/ml collagenase (Roche Pharmaceuticals, Switzerland), 0.1 mg/ml DNase (Sigma Chemical) and 0.4 mg/ml trypsin (Worthington Biochemical, Lakewood, NJ) in a shaking water bath at 35°C for 60 min. The neurons were then dispersed by vigorous shaking, centrifuged twice for 6 min at 63 × g, and resuspended in minimal essential medium (MEM), supplemented with 10% fetal calf serum (MidSci, St. Louis, MO), 1% glutamine and 1% penicillin-streptomycin (both from Life Technologies, Carlsbad CA). The dissociated neurons were then plated onto 35 mm poly-L-lysine coated dishes and stored overnight in a humidified incubator (5% CO₂/95% air) at 37°C.

Electrophysiology and Data Analysis

Whole-cell Ca²⁺ currents were recorded employing the patch-clamp technique. The recording pipet electrodes, fabricated from borosilicate glass (Garner Glass Co., Claremont, CA), were pulled on a P-97 micropipette puller (Sutter Instrument Co., Novato, CA). The

currents were acquired with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), analog filtered at frequency 2 kHz (−3dB, 4-pole low-pass Bessel filter), and digitized with custom-designed S5 software (written by Stephen R. Ikeda, NIH/NIAAA) equipped with an 18-bit AD converter board (Instrutech Corp. Elmont, NY). Both cell membrane capacitance and pipette series resistance were electronically compensated (80–85%).

Ca²⁺ currents were evoked with the double-pulse voltage protocol [22] shown in Figure 1B (top right). The protocol consists of a test pulse to +10 mV (prepulse,) followed by a large depolarizing conditioning test pulse to +80 mV, a brief return to −80 mV and followed by a test pulse to +10 mV (postpulse,). The peak Ca²⁺ current amplitude was measured isochronally 10 ms following the initiation of the prepulse and postpulse. The Noc-mediated Ca²⁺ current inhibition was calculated as follows: (peak Ca²⁺ current (prepulse before agonist) – peak Ca²⁺ current (prepulse after agonist))/(peak Ca²⁺ current before agonist) × 100.

Data and statistical analyses were performed with Igor Pro 6.0 (Lake Oswego, OR) and Prism 6.0 (GraphPad Software, Inc., San Diego, CA) software packages with $P < 0.05$ considered statistically significant. Graphs and current traces were generated with Igor Pro and Canvas 8.0 (Deneba Software, Miami, FL) software packages. The Noc concentration-response relationships were determined by the sequential application of the receptor agonist in increasing concentrations. The results were then pooled and the concentration-response curves were fit to the Hill equation. The half-inhibition concentration (IC₅₀) values were obtained with Prism 6.0 software (GraphPad Software). All data are presented as mean ± SE.

Recording Solutions and Drugs

The external recording solution contained (in mM): tetraethylammonium hydroxide (TEA-OH) 145, methanesulphonic acid 140, HEPES 10, glucose 15, CaCl₂ 10 and TTX 0.0003, to pH 7.40 with TEA-OH, and an osmolality of 323–330 mOsm/kg. The pipette internal solution contained (in mM): NMG 90, TEA-OH 25, EGTA 11, HEPES 10, CaCl₂ 1, CsCl 20, CsOH 20, MgATP 4, Na₂GTP 0.3 and TRIS-creatine phosphate 14, to pH 7.2 and an osmolality of 308–313 mOsm/kg. A stock solution of Noc (Tocris Cookson, Ellisville, MO) was prepared in H₂O and diluted in the external solution to its final concentration prior to use. Noc was applied to the neuron under study with a custom-designed gravity-fed perfusion system that was positioned approximately 100 mm from the cell.

Quantitative real-time PCR (QRT-PCR)

Total RNA from SG tissue was isolated with the Nucleospin RNA/Protein Kit (Macherey-Nagel) using the standard protocol supplied by the manufacturer. Thereafter, equal quantities of RNA were DNase-treated and cDNA synthesis was performed employing the High Capacity cDNA RT Kit (Life Technologies Corp., Grand Island, NY). For the QRT-PCR assays, the TaqMan Gene Expression kits (Life Technologies) specific for rat NOP receptor, prepronociceptin (PNOC) and GAPDH were carried out according to the manufacturer's instructions. The assays were run on a 7900HT PCR system (Life Technologies) and analyzed using the comparative *Ct* method. Thereafter, the results were normalized to internal GAPDH mRNA controls.

Western blot assays and analysis

Total protein from SG tissue was also prepared with the Nucleospin RNA/Protein Kit. Protein concentrations were measured with the Qubit®2 Fluorometer (Life Technologies). Protein samples (30 µg) were electrophoretically separated on NuPAGE 10% Bis-Tris pre-cast gels (Life Technologies) employing 200 V for 55 min and then transferred to PVDF

membranes. The membranes were blocked with 5% (w/v) nonfat dry milk in Tris Buffered Saline-Tween 20 (TBS-T) solution overnight at 4°C and thereafter incubated with anti-PNOC (1 µg/ml; Catalog # NBP1-59307, Novus Biologicals, Littleton, CO) rabbit polyclonal antibody and mouse monoclonal antibody to actin (1/2500; Catalog # ab11003, Abcam, Cambridge, MA) in 5% (w/v) nonfat dry milk in TBS-T solution for 60 min at room temperature. Subsequently, the membrane was incubated with horseradish peroxidase conjugated anti-rabbit or mouse IgG antibody (both from GE Healthcare, Piscataway, NJ; 1/5000) for 1 hr. The MOR and actin protein bands were visualized employing the enhanced chemiluminescent (ECL) reagent SuperSignal West Femto (Thermo Scientific, Rockford, IL). The images were acquired with a ChemiDoc-It Imaging system (UVP, LLC, Upland CA) equipped with a 16-bit CCD camera and processed with VisionWorksLS software (UVP).

Results

Given the recent interest in the role of the NOP receptor in sepsis, inflammation, and cardiovascular regulation [9, 23], the aim of the present study was to determine whether Ca^{2+} channel modulation is altered by CLP-induced sepsis. In the first set of experiments, we examined the effect of acute sepsis (i.e. 24 hr post-sepsis induction) on the Noc-mediated modulation of Ca^{2+} currents in acutely isolated SG neurons. Three septic animals and three nonseptic (sham surgery) animals were used in this set. Figure 1A shows a representative time course of the prepulse and postpulse Ca^{2+} currents evoked every 10 sec with the double-pulse protocol (see Materials and Methods) recorded from SG neuron isolated from a nonseptic rat. The corresponding numbered traces are shown in Fig. 1B. Prior to Noc application, the activation phase of the prepulse (trace 1) was fast and reached a plateau within 5–10 msec. However, when the neuron was exposed to 0.001 µM Noc, the prepulse current (trace 3) was blocked by approximately 30%. After a recovery period, the application of 1 µM Noc led to a 70% inhibition of the Ca^{2+} currents (cf. traces 5 and 7, Fig. 1B). The representative time course illustrated in Figure 1C was obtained from an SG neuron isolated from a septic rat, and the corresponding numbered current traces are shown in Fig. 1D. The initial exposure to 0.1 µM Noc resulted in a 55% block of the current (trace 3), while application of 10 µM Noc inhibited the currents by approximately 70% (trace 7, Fig. 1D). The Noc concentration-response curves for nonseptic and septic rats are illustrated in Figure 1E. The data points were fit to the Hill equation and the IC_{50} values (measure of potency) are 106 and 98 nM for nonseptic and septic rats, respectively. The fits for both groups were not significantly ($P = 0.42$) different from each other. It should be noted that in this set of experiments and those described below, the maximum Noc-mediated Ca^{2+} current inhibition (measure of efficacy) was observed at concentrations > 1 µM (Fig. 1E and 2E). The measured IC_{50} values obtained for both groups of neurons were comparable to those previously observed in this neuron subtype [24]. These results indicate that the 24 hr septic period in rats does not produce significant changes in Ca^{2+} current modulation by NOP receptors in SG neurons.

We next examined whether extending the septic period from 24 to 72 hr in rats would have a greater effect on the Noc-mediated modulation of Ca^{2+} currents when compared to nonseptic animals. The control group for this set of experiments consisted of animals with no sham surgery. Although different from the sham surgery control group at the 24 hr time point, a no surgery control group was used in this study in an attempt to amplify any potential differences between control and septic rats. Again, three control rats and three septic rats were used. Figure 2A is a time course of peak Ca^{2+} currents in a control SG neuron isolated from a nonseptic rat 72 hr post-sham surgery. The corresponding superimposed current traces are shown in Figure 2B. Application of 0.1 µM Noc blocked the Ca^{2+} currents by approximately 45% (trace 3), while exposure to 10 µM Noc inhibited the

current amplitude 68% (trace 7). The time course in Figure 2C corresponds to an SG neuron isolated from a septic rat 72 hr post-septic induction. The superimposed current traces in Figure 2D show that 0.1 and 3 μ M Noc blocked the Ca^{2+} currents 66% (trace 3) and 77% (trace 7), respectively. The IC_{50} values for nonseptic and septic rats were 78 and 60 nM. The concentration-response curves shown in Fig. 2E also illustrate that maximal Ca^{2+} current inhibition (efficacy) was observed with Noc concentrations of 1 μ M and higher. While there was a leftward shift of the Noc dose-response curve, a statistical comparison of the fits for control and septic rats did not reach statistical significance ($P = 0.25$). Similarly, comparing the fits for the 24 and 72 hr septic groups did not reach statistical significance ($P = 0.24$). The leftward shift, however, suggests that the additional time for sepsis resulted in an increased Noc potency SG neurons from septic rats.

The increased Noc potency (i.e. IC_{50} values) at 72 hr suggested that a change in expression levels of signaling elements within this pathway may have occurred. Thus, in the next set of experiments NOP receptor and the Noc precursor, prepronociceptin (PNOC) mRNA levels in nonseptic (sham surgery, $n=4$) and septic rats (72 hr, $n=10$) were measured. Figure 3 shows the relative mRNA levels of both NOP receptors and the Noc precursor. The plot illustrates that, while the NOP receptor mRNA levels were not acutely altered by sepsis, those of the precursor increased approximately 2.5-fold, ($P < 0.05$).

Given that mRNA levels for Noc precursor were elevated, we determined the PNOC protein content in both nonseptic (sham surgery, $n=2$) and septic (72 hr, $n=4$) SG tissue employing Western blotting analysis. The Western blot in Figure 4 shows that sepsis did not lead to greater PNOC levels in the septic group when compared to nonseptic rats. These results are difficult to reconcile with those described above for the increased PNOC mRNA levels (Fig. 3), discussed below.

Discussion

Research into the underlying mechanisms of pathophysiological derangements of sepsis is of clinical importance. It is necessary to elucidate the cellular and molecular mechanisms that contribute to the overall clinical manifestation of sepsis. In the present study, we examined the effect of CLP-induced sepsis on NOP-mediated Ca^{2+} current inhibition in acutely isolated SG neurons employing electrophysiological techniques. When compared to control SG neurons, the Noc concentration-response relationship of neurons isolated from septic rats showed a leftward shift that, while not reaching statistical significance, did trend toward an increase in Noc potency under septic conditions. Furthermore, there was an increase in Noc potency in both control and septic SG neurons at 72 hr vs. 24 hr, with a more pronounced shift in the septic rats (98 to 60 nM) than the control rats (106 to 78 nM). What factors could account for the difference between the 24 and 72 hr groups? An increase in NOP receptor levels would lead to greater effector response. It has been previously reported, for example, that NOP receptor mRNA was significantly elevated in blood samples from septic and cancer patients compared to healthy controls [12]. Similarly, in a rat model of inflammatory pain, NOP receptor immunoreactivity was enhanced in dorsal root ganglion (DRG) neurons [25]. Unlike both of these studies, our QRT PCR results indicated that the NOP receptor levels in SG neurons did not differ between control and septic rats.

Additionally, our QRT PCR results showed that mRNA levels of the Noc precursor, PNOC, were significantly higher in SG tissue from septic rats. PNOC is a polypeptide (181 and 176 amino acids in rats and humans, respectively) that is expressed highly in brain tissue and generates Noc, nocistatin and two small inactive peptides [26]. Nocistatin has been reported to exert inhibitory effects on the Noc-mediated allodynia and hyperalgesia [27]. A receptor for nocistatin and its role in sepsis have not been determined. Although we found greater

PNOC mRNA levels in SG tissue from septic rats 72 hr post-sepsis, the Western blotting analysis revealed no differences in PNOC protein expression between control and septic animals. This finding was surprising considering that Noc levels have been reported to be upregulated in isolated rat DRG neurons incubated for 24 hr with the proinflammatory agent, lipopolysaccharide (LPS) [28] and in neuropathic and inflammatory pain models [25, 29]. In contrast, a recent clinical study found that Noc mRNA levels in blood samples from both septic and cancer patients were lower than healthy controls in spite of augmented NOP receptor mRNA levels [12]. It is possible that the different model system and isolation conditions employed in our study could explain these discrepancies. We measured mRNA and protein levels from the entire SG tissue, which contains both neuronal and glial cells. Thus, it is likely that mRNA from glial cells may have contributed to the results. In future studies, it would be necessary to perform RNA isolation from single isolated SG neurons to confirm our current findings. Another possibility for the different observations may be that the NOP receptor signaling components in SG neurons are less susceptible to inflammatory agents than sensory neurons. Alternatively, the severity and nature of the inflammatory state produced by CLP and LPS may have yielded different results.

Overall, our data contribute to a growing body of evidence that the NOP/Noc signaling system plays a role in sepsis, and raise questions for further study. It is known that Noc induces hypotension and bradycardia, as well as contributes to microvascular inflammation [13–15]. Such changes are known contributors to the pathophysiology of sepsis [9]. From our data it appears that Noc may be more of a late player in the septic inflammatory response. Noc plasma levels (normal and pathological) in humans range from 1 to 105 pg/ml [11, 30 and references therein], or ~ 1 to 60 pM, and are significantly lower than the IC₅₀ values determined in our study. Our single-cell model allowed us to assess both potency and efficacy in a relatively straightforward manner, but the true intrinsic Noc efficacy cannot be determined because the degree of receptor occupancy is not known.

Previous studies have reported that activation of NOP receptors, expressed in sympathetic neurons innervating cardiac muscle, leads to inhibition of neurogenic tachycardia [31]. However, it is well established that the cardiovascular effects mediated by Noc occur both centrally and peripherally [13,14]. The widespread distribution and effects of this peptide throughout the body imply that the modulation of NOP receptors required to affect a specific dysfunctional organ system in a clinical setting will be difficult to elucidate. Our data indicate an increased potency of Noc without an increase in NOP receptors. Possible therapeutic targets might be an NOP receptor antagonist or an agent to bind and deactivate Noc. Future application of our data might involve blocking Noc in early sepsis or blocking the transcription or translation of PNOC. Further studies are necessary to determine whether sepsis affects the NOP receptor/Ca²⁺ channel pathway in other sympathetic or sensory neurons and to further elucidate the clinical impact of increased NOP receptor activity, both in animal models of sepsis and cardiovascular disease, and in human clinical studies.

Furthermore, based on our 24 vs. 72 hr results, we recommend that investigators employing the CLP model remain cognizant that signaling moieties and protein expression may have significant temporal components. The increased mRNA of PNOC without a corresponding increase in PNOC protein content is most likely reconciled by citing temporal factors. Perhaps a sepsis model of 5–7 days would show an increased PNOC protein content.

Acknowledgments

We thank Jonathan Derr and Anne Pruznak for technical assistance with the QRT-PCR assays and assisting with animal surgery. This work was supported by NIH Grants DA-025574 to VR-V and GM-38032 to CHL.

References

1. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol*. 2008; 8:776. [PubMed: 18802444]
2. Smeding L, Plötz FB, Groeneveld ABJ, Kneyber MCJ. Structural changes of the heart during severe sepsis or septic shock. *Shock*. 2012; 37:449. [PubMed: 22301606]
3. Annane D, Trabold F, Sharshar T, Jarrin I, Blanc AS, Raphael JC, Gajdos P. Inappropriate sympathetic activation at onset of septic shock: a spectral analysis approach. *Am J Respir Crit Care Med*. 1999; 160:458. [PubMed: 10430714]
4. Piepoli M, Garrard CS, Kontoyannis DA, Bernardi L. Autonomic control of the heart and peripheral vessels in human septic shock. *Intensive Care Med*. 1995; 21:112. [PubMed: 7775691]
5. Straub RH, Mayer M, Kreutz M, Leeb S, Scholmerich J, Falk W. Neurotransmitters of the sympathetic nerve terminal are powerful chemoattractants for monocytes. *J Leukoc Biol*. 2000; 67:553. [PubMed: 10770289]
6. Serhan CN, Fierro IM, Chiang N, Pouliot M. Cutting edge: nociceptin stimulates neutrophil chemotaxis and recruitment: inhibition by aspirin-triggered-15-epi-lipoxin A4. *J Immunol*. 2001; 166:3650. [PubMed: 11238602]
7. New DC, Wong YH. The ORL1 receptor: molecular pharmacology and signalling mechanisms. *Neurosignals*. 2002; 11:197. [PubMed: 12393946]
8. Chiou LC, Liao YY, Fan PC, Kuo PH, Wang CH, Riemer C, Prinssen EP. Nociceptin/orphanin FQ peptide receptors: pharmacology and clinical implications. *Curr Drug Targets*. 2007; 8:117. [PubMed: 17266536]
9. Lambert DG. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. *Nat Rev Drug Discov*. 2008; 7:694. [PubMed: 18670432]
10. Carvalho D, Petronilho F, Vuolo F, Machado RA, Constantino L, Guerrini R, Calo G, Gavioli EC, Streck EL, Dal-Pizzol F. The nociceptin/orphanin FQ-NOP receptor antagonist effects on an animal model of sepsis. *Intens Care Med*. 2008; 34:2284.
11. Williams JP, Thompson JP, Young SP, Gold SJ, McDonald J, Rowbotham DJ, Lambert DG. Nociceptin and urotensin-II concentrations in critically ill patients with sepsis. *Br J Anaesth*. 2008; 100:810. [PubMed: 18430746]
12. Stamer UM, Book M, Comos C, Zhang L, Nauck F, Stuber F. Expression of the nociceptin precursor and nociceptin receptor is modulated in cancer and septic patients. *Br J Anaesth*. 2011; 106:566. [PubMed: 21324928]
13. Malinowska B, Godlewski G, Schlicker E. Function of nociception and opioid OP₄ receptors in the regulation of the cardiovascular system. *J Physiol Pharmacol*. 2002; 53:301. [PubMed: 12369730]
14. Burmeister MA, Kapusta DR. Centrally administered nociceptin/orphanin FQ (N/OFQ) evokes bradycardia, hypotension, and diuresis in mice via activation of central N/OFQ peptide receptors. *J Pharmacol Exp Ther*. 2007; 322:324. [PubMed: 17452419]
15. Brookes ZLS, Stedman EN, Guerrini R, Lawton BK, Calo G, Lambert DG. Proinflammatory and vasodilator effects of nociception/orphanin FQ in the rat mesenteric microcirculation are mediated by histamine. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2977. [PubMed: 17766480]
16. Ruiz-Velasco V, Puhl HL, Fuller BC, Sumner AD. Modulation of Ca²⁺ channels by opioid receptor-like 1 receptors natively expressed in rat stellate ganglion neurons innervating cardiac muscle. *J Pharmacol Exp Ther*. 2005; 314:987. [PubMed: 15937148]
17. Mo N, Wallis DI, Watson A. Properties of putative cardiac and non-cardiac neurons in the rat stellate ganglion. *J Auton Nerv Syst*. 1994; 47:7. [PubMed: 7514622]
18. Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HA, Fishbein MC. Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. *Cardiovas Res*. 2001; 50:409.
19. Lang CH, Bagby GJ, Ferguson JL, Spitzer JJ. Cardiac output and redistribution of organ blood flow in hypermetabolic sepsis. *Am J Physiol Regulatory Integrative Comp Physiol*. 1984; 246:R331.
20. Alexander HR, Sheppard BC, Jensen JC, Langstein HN, Buresh CM, Venzon D, Walker EC, Fraker DL, Stovroff MC, Norton JA. Treatment with recombinant human tumor necrosis factor-

- alpha protects rats against the lethality, hypotension, and hypothermia of gram-negative sepsis. *J Clin Inv.* 1991; 88:34.
21. Tucker JJ, Wilson MA, Wead WB, Garrison RN. Microvascular endothelial cell control of peripheral vascular resistance in sepsis. *Arch Surg.* 1998; 133:1335. [PubMed: 9865652]
 22. Ikeda SR. Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. *J Physiol.* 1991; 439:181. [PubMed: 1654413]
 23. Serrano-Gomez A, Thompson JP, Lambert DG. Nociceptin/orphanin FQ in inflammation and sepsis. *Br J Anaesth.* 2011; 106:6. [PubMed: 21138900]
 24. Mahmoud S, Margas W, Trapella C, Calo G, Ruiz-Velasco V. Modulation of silent and constitutively active nociception/orphanin FQ receptors by potent receptor antagonists and Na⁺ ions in rat sympathetic neurons. *Mol Pharmacol.* 2010; 77:804. [PubMed: 20159949]
 25. Chen Y, Sommer C. Nociceptin and its receptor in rat dorsal root ganglion neurons in neuropathic and inflammatory pain models: implications on pain processing. *J Periph Nerv Syst.* 2006; 11:232.
 26. Nothacker HP, Reinscheid RK, Mansour A, Henningsen RA, Ardati A, Monsma FJ, Watson SJ, Civelli O. Primary structure and tissue distribution of the orphanin FQ precursor. *Proc Natl Acad Sci USA.* 1996; 93:8677. [PubMed: 8710930]
 27. Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, Kimura T, Ito S. Nocistatin, a peptide that blocks nociceptin action in pain transmission. *Nature.* 1998; 392:286. [PubMed: 9521323]
 28. Acosta C, Davies A. Bacterial lipopolysaccharide regulates nociceptin expression in sensory neurons. *J Neurosci Res.* 2008; 86:1077. [PubMed: 18027846]
 29. Andoh T, Itoh M, Kuraishi Y. Nociceptin gene expression in rat dorsal root ganglia induced by peripheral inflammation. *Neuro Report.* 1997; 8:2793.
 30. Lambert DG. Editorial III: Nociceptin/orphanin FQ peptide-receptor system: are we any nearer the clinic? *Br J Anaesth.* 2004; 93:626. [PubMed: 15472142]
 31. Malinowska B, Piszcz J, Koneczny B, Hryniewicz A, Schlicker E. Modulation of the cardiac autonomic transmission of pithed rats by presynaptic opioid OP₄ and cannabinoid CB₁ receptors. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2001; 364:233. [PubMed: 11521166]

Figure 1.

Time course of Ca^{2+} current amplitude for prepulse (●) and postpulse (○) acquired from the application of nociceptin (Noc) in SG neurons from nonseptic (A) and septic (B) rats 24 hr post-sepsis induction by CLP. Currents were evoked every 10 sec with the 'double-pulse' voltage protocol (B, top right). The numbered current traces in A and C are shown to the right (B and D, respectively), where 1–2 and 5–6 (black) represent control currents before Noc exposure, 3–4 and 7–8 (gray) represent inhibition at the end of Noc exposure. E, Noc concentration-response relationships of SG neurons isolated from nonseptic (●) and septic (▲) rats. Each data point represents the mean ($\pm$ SE) prepulse Ca^{2+} current inhibition ($n=3-10$). The smooth curves were obtained by fitting the data points to the Hill equation and the calculated IC_{50} values are shown.

Figure 2.

Time course of Ca^{2+} current amplitude for prepulse (●) and postpulse (○) acquired from the application of nociceptin (Noc) in SG neurons from nonseptic (A) and septic (B) rats 72 hr post-sepsis induction. Currents were evoked every 10 sec with the ‘double-pulse’ voltage protocol (Figure 1B, top right). The numbered current traces in A and C are shown to the right (B and D, respectively), where 1–2 and 5–6 (black) represent control currents before Noc exposure, 3–4 and 7–8 (gray) represent inhibition at the end of Noc exposure. E, Noc concentration-response relationships of SG neurons isolated from nonseptic (●) and septic (▲) rats. Each data point represents the mean ($\pm$ SE) prepulse Ca^{2+} current inhibition ($n=5-15$). The smooth curves were obtained by fitting the data points to the Hill equation and the calculated IC_{50} values are shown.

Figure 3.

Quantitative assessment of NOP receptor and prepronociceptin mRNA expression by QRT-PCR in SG tissue 72 hr post-sepsis induction. The delta-CT values were determined for nonseptic and septic rats and both NOP receptor and prepronociceptin mRNA levels were measured relative to GAPDH mRNA levels. The mRNA levels are expressed as fold-change compared to SG tissue from nonseptic rats, * $P < 0.05$ employing Student's t test analysis.

Western blot analysis

Figure 4.

Prepronociceptin (PNOC) detection by Western blot analysis in SG tissue from nonseptic and septic rats. Western blot experiment in A shows the results with anti-PNOC and anti-actin (loading control). Each lane was loaded with 30 μ g of protein isolated from SG tissue from a different rat and the lines indicate the approximate molecular weights (kDa). The intensity analysis of the Western blot for actin and PNOC (normalized to actin) are shown in B. The Western blots were quantified by measuring the area density (i.e. intensity).