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INVITED REVIEW ARTICLE

Nociceptin system as a target in sepsis?

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Abstract The nociceptin system comprises the nociceptin receptor (NOP) and the ligand nociceptin/orphanin FQ (N/OFQ) that binds to the receptor. The archetypal role of the system is in pain processing but the NOP receptor is also expressed on immune cells. Activation of the NOP receptor is known to modulate inflammatory responses, such as mast-cell degranulation, neutrophil rolling, vasodilation, increased vascular permeability, adhesion molecule regulation and leucocyte recruitment. As there is a loss of regulation of inflammatory responses during sepsis, the nociceptin system could be a target for therapies aimed at modulating sepsis. This review details the known effects of NOP activation on leucocytes and the vascular endothelium and discusses the most recent human and animal data on the role of the nociceptin system in sepsis.

Keywords Nociceptin · N/OFQ · ppNoc · NOP · Sepsis

Introduction

The inflammatory response involves activation of several cell types in blood and tissues to initiate a cascade of events to remove pathogens and repair damaged tissue. Sepsis occurs when dysregulation of the inflammatory response occurs and localised inflammation becomes systemic (often referred to as a cytokine storm). This is accompanied by a compensatory anti-inflammatory response, which may lead to a state of immune paralysis [1]. The pathogenesis of sepsis involves a series of interacting neuroendocrine immune and inflammatory pathways that cause circulatory and cellular dysfunction (with impaired mitochondrial function and oxygen utilisation). These have widespread effects that can result in multiorgan failure. The reported annual incidence of severe sepsis is 50–90 per 100,000 population [2], and the incidence of sepsis is three to four times higher than this. Severe sepsis is increasing in incidence because of an ageing population with chronic morbidities, increasing medical interventions and the development of antimicrobial resistance [3]. Mortality from severe sepsis is estimated at 28–50 %, with recent overall hospital mortality rates of 36 % from European and 39.8 % from UK intensive care units (ICUs), respectively [4]. Sepsis affects all age groups, and with its incidence increasing, mortality, morbidity and economic costs of treatment are all poised to rise. Despite advances in understanding the mechanisms and pathways involved, global (e.g. improving oxygen delivery) or specific interventions targeted at components of these pathways [e.g. tumour necrosis factor (TNF) antagonists, antiendotoxin antibodies] have been unsuccessful. Possible reasons are:

1. Sepsis is a clinical syndrome comprising a biological response to a heterogeneous variety of insults rather than a distinct disease entity.
2. Exaggerated responses to inflammatory cytokines or other pathways may be adaptive and not necessarily harmful per se.

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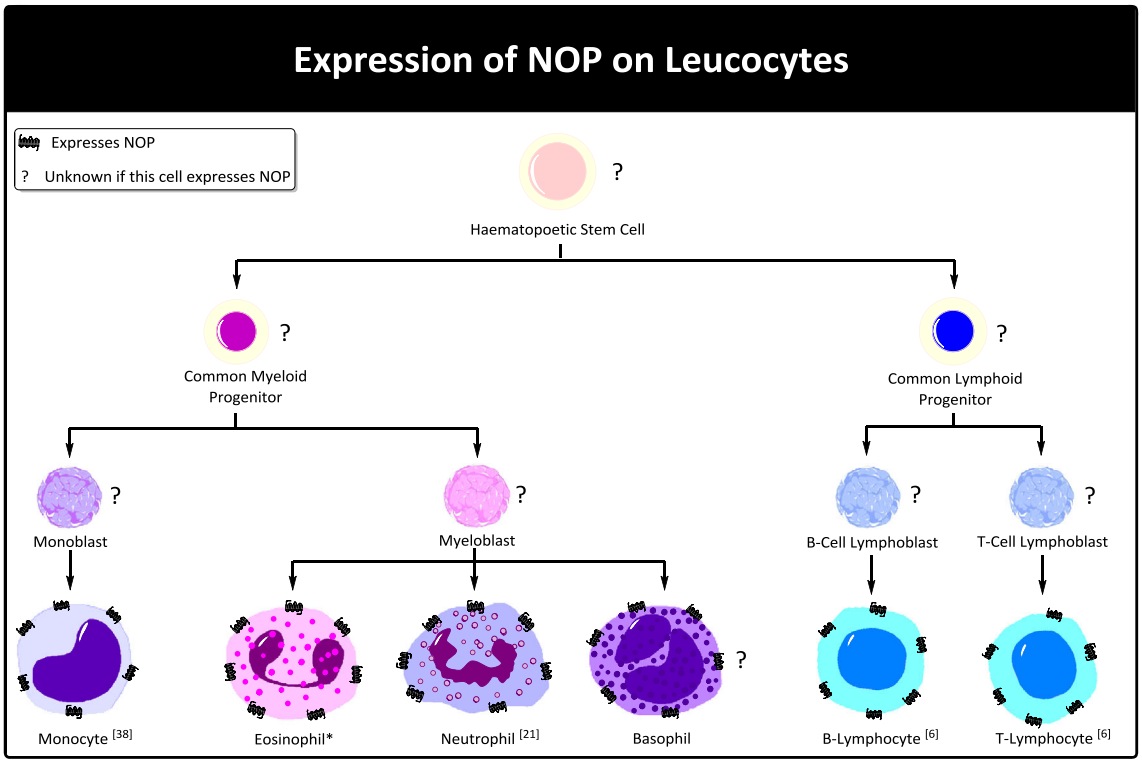

Fig. 1 Nociceptin receptor (NOP) expression on white blood cells. NOP is expressed on all mature leucocytes. *Asterisk* unpublished data from our laboratory

3. Interventions aimed at specific downstream mediators of sepsis may be limited when upstream mechanisms are not addressed.
4. Inflammatory or immune responses vary between individuals and over the clinical course of sepsis. Initially exaggerated inflammatory responses are often followed by depression of aspects of immune function.
5. Inflammatory responses have significant genetic variability.
6. Extrapolations from data derived from animal or laboratory models may be limited, and translational research is needed.

Therefore, further research is required to understand the pathogenesis and define the differences between responses to infective and noninfective challenge. Because of innate variability in responses, it is logical to target endogenous systems, in particular, responses early after an inflammatory stimulus (upstream). One such endogenous system that has been implicated in modulation of the immune response in sepsis is the nociceptin/orphanin FQ (N/OFQ) system [5, 6]. The nociceptin system comprises the nociceptin receptor (NOP) and the ligand that binds to the receptor (N/OFQ). The ligand is a peptide of 17 amino acids in length and is defined as a non-classical member of the opioid family as it has a similar sequence to classical opioids but does not bind to their receptors [7]. N/OFQ has many other roles distinct from its role in the pain pathway (for which it is most well known), such as in depression, anxiety, stroke and heart failure [8].

Leucocytes, cytokines, and the N/OFQ system in sepsis

The first indication that the nociceptin system may be involved with regulation of immune responses arose in 1995 when NOP messenger RNA (mRNA) was detected on mouse and human lymphocytes and lymphocytic cell lines [6]. Subsequently, NOP mRNA was found in the thymus, lymph nodes, spleen and splenocytes of pigs [9] and additional types of human leucocytes (Fig. 1). Prepronociceptin (ppN/OFQ) mRNA, the precursor for nociceptin, was later also found to be expressed on human peripheral blood lymphocytes [10]. Further evidence of the involvement of the nociceptin system arose from studies examining the effects of activating or antagonising the NOP receptor, or the effect of changes in the system during conditions of inflammation or sepsis. Miller and Fulford showed that inflammatory stimuli can cause N/OFQ release in splenocytes. They incubated a crude splenocyte culture with 25 μg/ml lipopolysaccharide (LPS), a component of the cell wall in gram-negative bacteria responsible for initiating an immune response, and measured N/OFQ concentrations at regular intervals over a period of 48 h.

They found that N/OFQ concentrations rose at around 18 h poststimulation [11].

An increased peripheral leucocyte count is usually found as part of the inflammatory response in patients with sepsis [12]. Data have shown that N/OFQ has a role in the chemoattraction of leucocytes by mast cells [13]. Mast cells release histamine, which is also increased in sepsis [14] and increases vascular permeability [15] and upregulates adhesion molecules [16] (which also show upregulation in sepsis [17]). Kimura et al. found that intradermal injection of N/OFQ causes histamine release from rat peritoneal mast cells. This response could be inhibited by both calcium and pertussis toxin but not by naloxone, which provides evidence that this was due to NOP receptor activation. These authors also found that N/OFQ increased vascular permeability in rats in a dose-dependent manner, which could be prevented by using an H1 histamine receptor antagonist [13].

The concept that N/OFQ can affect the vasculature by stimulating histamine release from mast cells was supported by work performed by our group. Using fluorescent intravital microscopy, we found that N/OFQ-induced macromolecular leak could be inhibited by H1 and H2 receptor antagonists or by using UFP-101 [18]. UFP-101 is a peptide antagonist that is selective for the NOP receptor [19]. We also found that N/OFQ elicited other inflammatory responses, such as vasodilation (also inhibited by the above antagonists) and increased rolling and adhesion of leucocytes on the endothelium [18]. More recently, we used fluorescent in vivo microscopy to demonstrate that NOP receptors are expressed on rat mesenteric arterioles and venules [20]. There is also evidence to suggest that N/OFQ may have the ability to recruit neutrophils directly. Serhan et al. [21] found that functional NOP receptors are expressed on human neutrophils and performed a microchamber migration assay with isolated human neutrophils, which demonstrated that N/OFQ is a potent chemoattractant for these cells.

In addition, N/OFQ may aid the infiltration of leucocytes via increasing endothelial expression of cellular adhesion molecules. This, in turn, can increase interactions between endothelial cells and leucocytes and hence increase the numbers of leucocytes infiltrating inflamed tissues [22]. Kato et al. [22] found that the number of neutrophils, lymphocytes and macrophages present in the colonic mucosa of dextran sulphate sodium-treated mice (a model of colitis) was lower in NOP-knockout mice, suggesting NOP is involved in the recruitment of all of these cell types; there was also significantly higher expression of mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) cellular adhesion molecule in wild-type mice compared with NOP-deficient mice. This suggests that N/OFQ is involved in the upregulation of adhesion molecules such as MAdCAM-1, which binds to lymphocytes and eosinophils. Evidence from our group shows that N/OFQ also upregulates the β2-integrin CD18/CD11b adhesion molecule on rat neutrophils [18]. Increased interactions of neutrophils and other leucocytes within the microvasculature could lead to vascular occlusion [23, 24]. Therefore, one of the mechanisms by which N/OFQ could contribute to hypoperfusion and hypoxia occurring in sepsis could be via upregulation of adhesion molecules. Unpublished data from our group show that NOP is expressed on eosinophils. The significance of this is not yet known, but these cells could also be contributing in a similar manner to that described for neutrophils.

N/OFQ may also be involved in triggering leucocyte effector functions. Trombella et al. found that N/OFQ promotes lysozyme release from neutrophils [25]. Lysozyme is an enzyme that hydrolyses glycosidic bonds in the cell wall of some micro-organisms, thereby killing them. Brown et al. suggest that the organ dysfunction that occurs in sepsis could be a result of excessive activation of neutrophils [26]. This could represent a mechanism whereby N/OFQ contributes to decreased plasma-circulating volume. N/OFQ (which is also expressed by neutrophils in patients with sepsis [27]) and inflammatory cytokine tumour necrosis factor alpha (TNF-α) could be working together to cause neutrophil activation; this could then lead to vascular damage and increased vascular permeability. A hypothetical mechanism illustrating this concept is outlined in Fig. 2.

TNF-α contributes to many of the pathophysiological processes involved in sepsis by initiating a cascade of events leading to inflammatory mediator release and upregulation of adhesion molecules on endothelial cells [28]. It has also been found that neutrophil apoptosis is delayed in patients with sepsis [26, 29], which may be due to the high concentrations of TNF-α found during sepsis. TNF-α is known to inhibit neutrophil apoptosis in vitro [30] and also to hinder neutrophil migration from the circulation into inflamed tissues [31].

When neutrophils are not active, they are in either a resting or a primed state. Priming occurs when a resting neutrophil encounters microbial-derived or proinflammatory molecules. These encounters then switch the resting neutrophil from an inactive state to a state of readiness. Further encounters with the molecules that led to the initial priming cause neutrophils to become active, resulting in responses such as degranulation. Degranulation should happen in tissue rather than in the vasculature; however, oxidative activity and enhanced expression of the transcription factor nuclear factor kappa B (NFκB) have been found in the circulating neutrophils of patients with sepsis [32–34], indicating an increase in primed neutrophils during sepsis. This could be related to the increase in N/OFQ

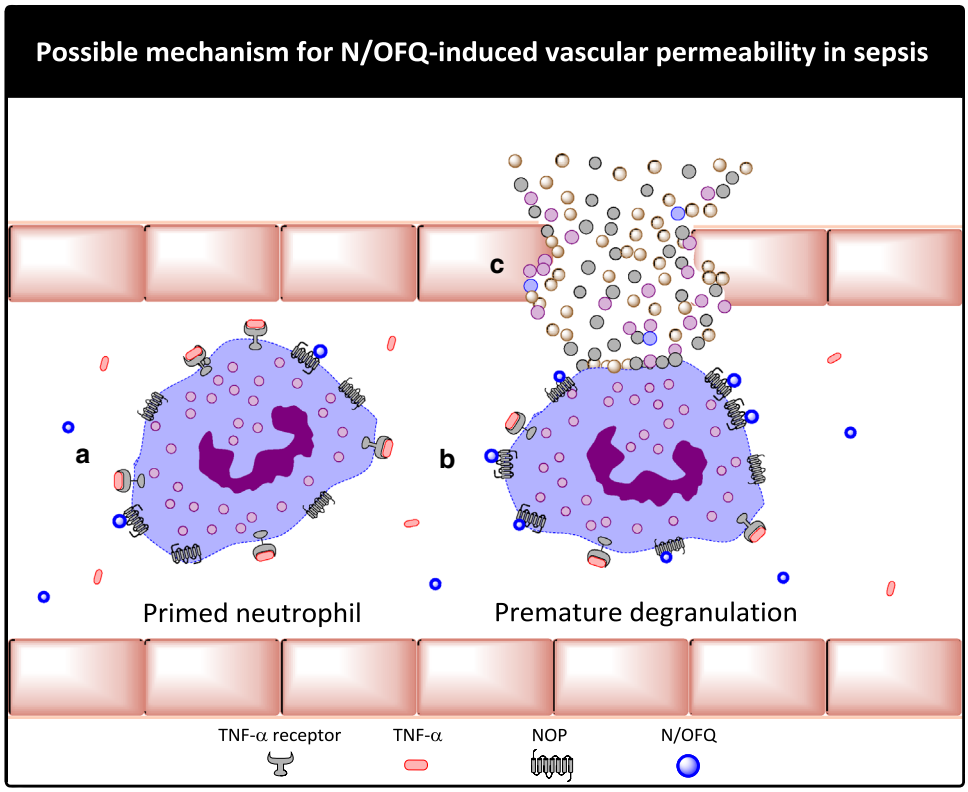

Fig. 2 Hypothetical mechanism of nociceptin/orphanin FQ (N/OFQ)-induced vascular permeability. **a** The high concentration of tumour necrosis factor alpha (TNF-α) delayed apoptosis of the neutrophil and prevented it from migrating to tissue. N/OFQ in plasma bound to the nociceptin receptor (NOP), leading to a primed state. **b** Increased plasma N/OFQ concentrations result in further encounters, which stimulate degranulation. **c** Neutrophil products damage the vascular endothelium, leading to increased permeability

observed in the plasma of patients who died from sepsis compared with patients who survived [35]. Neutrophil numbers rapidly increase during acute inflammation and peak after around 12 h [36]. As the inflammatory reaction progresses, the recruitment of neutrophils is downregulated; neutrophils begin to apoptose, and monocyte recruitment is upregulated. Monocytes are produced in bone marrow and circulate in blood before migrating to tissue, where they differentiate into macrophages and dendritic cells and continue pathogen eradication and contribute to inflammation resolution [37].

Peluso et al. [38] showed that monocytes express mRNA for NOP receptors, and Trombella et al. [25] found that N/OFQ is a potent chemoattractant for these cells. This is significant because patients with sepsis have an increased monocyte count in their plasma [39], to which N/OFQ could be contributing. Monocytes perform tasks such as antigen presentation and pathogen and apoptotic cell phagocytosis, and macrophage activation is believed to play a central role in mediating sepsis [40]. Another important function of monocytes is production of proinflammatory cytokines, such as TNF-α and interleukin-1 beta (IL-1β), both of which affect mRNA concentrations and N/OFQ secretion. TNF-α and IL-1β exposure causes N/OFQ mRNA concentrations to increase in rat astrocytes [41], and incubation of unstimulated rat splenocytes with IL-1β increases N/OFQ secretion [11]. The effects of TNF-α on neutrophils have been discussed previously, but TNF-α has other proinflammatory actions, such as upregulating expression of intracellular adhesion molecules, which are important for leucocyte extravasation [e.g. intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) [26] on endothelial cells]. IL-1β also upregulates adhesion molecules and induces chemokines [42], so recruitment of monocytes by N/OFQ leads to an increase in proinflammatory cytokines. The relationship between N/OFQ and proinflammatory cytokines was demonstrated by Goldfarb et al., who showed that TNF-α mRNA concentrations in the spleen and plasma increase significantly if N/OFQ is given before staphylococcal enterotoxin A challenge (a method of inducing inflammation). However, it was previously shown that there was a reduced expression of TNF-α and IL-1β in peritoneal macrophages when N/OFQ was administered intracerebroventricularly to rats [43]. Therefore, the route of administration (and thus the source of endogenous N/OFQ) seems to be important in the immune responses observed previously. Goldfarb et al. also found that concentrations of the proinflammatory cytokine interferon gamma (IFN-γ), which activates (and is secreted by) monocytes, also increased in the spleen in response to N/OFQ. These responses were diminished with ppN/OFQ knockouts [44]. Activated monocytes also produce chemokines, such as chemokine (c-c motif) ligand 2/monocyte chemotactic protein-1 (CCL2/MCP-1) (chemotactic for monocytes, T cells and dendritic cells) and CCL5/RANTES, (a

chemoattractant for T cells, eosinophils and basophils). Carvalho et al. [45] found increased CCL2/MCP-1 in septic rats that had been given N/OFQ. However, Kaminsky and Rogers investigated the effect of N/OFQ on expression of CCL2/MCP-1 and also CCL5/RANTES in vitro by monocytes and found that CCL2/MCP-1 production is suppressed in human monocytes [46]. This again highlights the complexity of the role of N/OFQ in modulating the immune system. N/OFQ can neither be seen as having anti-inflammatory nor proinflammatory effects on the immune system and is often referred to in the literature as having a modulatory effect [47].

In terms of the development of sepsis, the anti-inflammatory effects of N/OFQ could be just as important as the proinflammatory effects. Impaired lymphocyte responsiveness and decreased lymphocyte numbers are observed in sepsis [48], and N/OFQ suppresses IL-2 (a cytokine important for T-cell proliferation and differentiation) production and inhibits lymphocyte proliferation [11]. Lymphocyte suppression results in a decrease in proinflammatory cytokines and chemokines and therefore reduces leucocyte infiltration and activation. Other anti-inflammatory actions associated with N/OFQ include inhibited release of proinflammatory neuropeptides somatostatin [49], substance P and calcitonin-gene-related peptide (CGRP) [50] from sensory nerve terminals. A recent study further illustrates that the effects of N/OFQ on immune function are complex, indicating that different doses can produce opposite effects: Petrella et al. [51] administered N/OFQ by intraperitoneal injection to rats after inducing colitis by infusing trinitrobenzenesulfonic acid into the colon. At lower doses (0.02 and 0.2 nmol/kg), improvements in microscopic damage and myeloperoxidase activity (indicative of neutrophil infiltration), along with decreased IL-1β concentrations, were observed. Conversely, a high dose (20 nmol/kg) resulted in a worsening of colitis. Hence, in vivo effects of N/OFQ seem to be determined by both the source and the dose. Whilst colitis is not directly related to sepsis, these findings demonstrate the involvement of N/OFQ in immune modulation.

Cardiovascular effects of nociceptin

Sepsis has profound effects on the cardiovascular system, and there is substantial evidence that N/OFQ modulates cardiovascular responses. When given peripherally intravenous, N/OFQ results in hypotension and bradycardia in rats [52], guinea pigs [53] and mice [54]. NOP is expressed in the human brain stem [55] and hypothalamus [52] (both important in cardiovascular regulation), and N/OFQ is expressed in the human brain stem [55]. Hypotension and bradycardia also occur in response to intracerebroventricular administration of N/OFQ [8, 52], responses that can be abolished by antagonising the NOP receptor with UFP-101 [56, 57]. N/OFQ administered directly into the rostral ventrolateral medulla and paraventricular nucleus (areas of the brain also involved in cardiovascular regulation) by microinjection also reduces blood pressure and heart rate compared with injection of the same volume of saline [58]. These responses are not seen in NOP knockout animals [57].

Studies of the nociceptin system during sepsis in animal models

Several studies examined the role of the nociceptin system in animal models of inflammation and sepsis. For example, Carvalho et al. [45] used a caecal ligation and puncture (CLP) model of sepsis to investigate effects on the inflammatory response of activating or antagonizing NOP using a range of doses of either N/OFQ or UFP-101. CLP involves ligation and puncture of the cecum in order to create fecal peritonitis. Rats were either killed after 12 h in order to carry out biochemical analysis or observed in order to measure differences in mortality over a period of 10 days.

Mortality in rats treated with UFP-101 in doses of 0.03 and 0.3 mg/kg was significantly reduced compared with rats that underwent CLP and received no treatment (50 and 70 % mortality, respectively), whereas mortality at 5 days was 100 % in rats treated with of N/OFQ 0.1 mg/kg alone. The reduction in mortality with UFP-101 treatment occurred during the first 2 days after CLP, and the authors proposed that the nociceptin system is involved in the early phases of the inflammatory response in sepsis [45]. In order to assess recruitment of inflammatory cells, fluid was collected from peritoneal and bronchoalveolar lavage, and cells were counted. Blood and peritoneal exudates were examined for differences in bacterial count, and blood cytokine and chemokine concentrations (TNF-α, IL-1β, IL-10 and MCP-1) were measured by enzyme-linked immunosorbent assay (ELISA). Carvalho et al. confirmed that leucocyte migration was significantly increased in septic rats compared with controls and that this could either be increased further by treatment with N/OFQ or inhibited by UFP-101. UFP-101 also substantially reduced bacterial spread. Treatment with N/OFQ led to increased plasma concentrations of CCL2/MCP-1, TNF-α and IL-1β, whereas UFP-101 decreased plasma concentrations of these cytokines (in comparison with septic rats). These results led the authors to conclude that antagonising NOP could be useful in treating sepsis by preventing both infiltration and bacterial spread.

In another CLP model of sepsis, Laufenberg et al. [59] found that ppN/OFQ mRNA is increased in stellate ganglion neurons of rats during sepsis and that the potency of

N/OFQ appeared to be increased after 72 h. Using whole-cell patch clamping, these investigators found that the dose of N/OFQ required to inhibit NOP-mediated voltage-gated calcium current by 50 % decreased from 78 nM in non-septic rats to 60 nM in septic rats. This is significant, because it suggests that the nociceptin system could contribute to decreased neurotransmission in these neurons during sepsis and thus decreased sympathetic innervation of the heart. Therefore, NOP antagonists have the potential to increase neurotransmission in the stellate ganglion and improve cardiac function during sepsis.

A further study in our laboratories demonstrated that vasodilation induced by N/OFQ is enhanced after LPS administration in rats [20]. Furthermore, we showed that antagonising NOP with UFP-101 during LPS-induced sepsis produced anti-inflammatory effects (such as decreased macromolecular leak and reduced leucocyte rolling) in postcapillary venules.

Studies of the nociceptin system during sepsis in humans

Data from rodents can only be used to inform clinical research, and studies in humans are clearly more relevant. We conducted a study involving patients with sepsis who had been admitted to the Intensive Care Unit (ICU), and we found that patients who subsequently died had higher plasma concentrations of N/OFQ than those who survived [35]. However, this was a small study, and further data are needed.

Another study in humans was performed on whole-blood samples from ICU patients with sepsis. Stamer et al. [60] found significantly higher expression of NOP receptor mRNA in ICU patients and also in patients with cancer compared with healthy controls. However, ppN/OFQ mRNA expression was lower than in controls. This could be due to more ppN/OFQ being cleaved into N/OFQ. In another study, Zhang et al. stimulated peripheral whole-blood samples from healthy volunteers with LPS, TNF-α, IL-1B, IL-10 or IFN-γ and then measured NOP and ppN/OFQ mRNA concentrations in leucocytes and cytokine concentrations in the supernatant. They found that NOP was suppressed by LPS, TNF-α, IL-1β, IL-10 and IFN-γ, whereas ppN/OFQ was suppressed only by LPS and IL-10 (an anti-inflammatory cytokine), suggesting the involvement of negative feedback mechanisms [61]. The same group also sought to elucidate the mechanism of changes in the NOP and ppN/OFQ expression under inflammatory conditions. This was investigated using anticytokine antibodies to determine whether NOP suppression and ppN/OFQ concentration were affected if individual cytokines were removed. LPS-induced NOP suppression was partially alleviated by incubation with either anti-TNF-α or anti-IL-1β antibodies, indicating that these cytokines are involved in NOP regulation. A potential mechanism could be that NOP induces TNF-α and IL-1β and they then feedback to prevent further NOP expression, thus preventing further TNF-α and IL-1β release. However, antibodies to TNF-α and IL-1β only partially prevented NOP suppression and made no difference to concentrations after 6 h, so the mechanisms of regulation of the system are clearly more complex and rely on more than just these two cytokines. Moreover, ppN/OFQ concentrations were not affected by anticytokine antibodies, indicating that ppN/OFQ is regulated in a different way.

Our group carried out a study of the effect of sepsis on the nociceptin system [27]. We assessed the relationship between the nociceptin system and clinical outcomes of patients who were either admitted to intensive care with sepsis or who represented a model of systemic inflammation (patients undergoing cardiac bypass, which induces a similar proinflammatory cytokine release profile as that observed in sepsis). The relationship between pro- and anti-inflammatory cytokines was also investigated. Concentrations of ppN/OFQ and NOP mRNA were measured by quantitative polymerase chain reaction (qPCR) in isolated polymorphonuclear leukocytes, a radioimmunoassay was used to determine concentrations of N/OFQ in plasma, and TNF-α, IL-8 and IL-10 in plasma were measured by ELISA.

Patients with sepsis showed an increase in plasma N/OFQ concentrations and both pro- and anti-inflammatory cytokines compared with controls, whereas ppN/OFQ and NOP mRNA concentrations in polymorphs decreased. A similar but lesser effect was observed in cardiac bypass patients, confirming upregulation in the nociceptin system in a nonseptic model of inflammation. The finding that ppNOFQ mRNA is decreased in sepsis is in agreement with the previously mentioned findings of Stamer et al. However, unlike their group, we found no differences between patients with and without a diagnosis of cancer, and we found a decrease as opposed to the increase in NOP mRNA expression in sepsis. Some discrepancies between these studies may be related to methodological differences, including the use of whole blood or polymorphonuclear cells isolated from blood samples for qPCR, or N/OFQ protein measurement rather than mRNA alone. In this recent study, we also found that cytokine concentrations were higher in nonsurvivors of sepsis [27]; however, in contrast to our earlier study, we found no relationship between N/OFQ plasma concentrations and patient survival. This illustrates the heterogeneity of clinical studies of sepsis in humans, the limitations of current clinical diagnostic criteria for sepsis and the potential effects on mortality of coexisting disease or clinical decisions

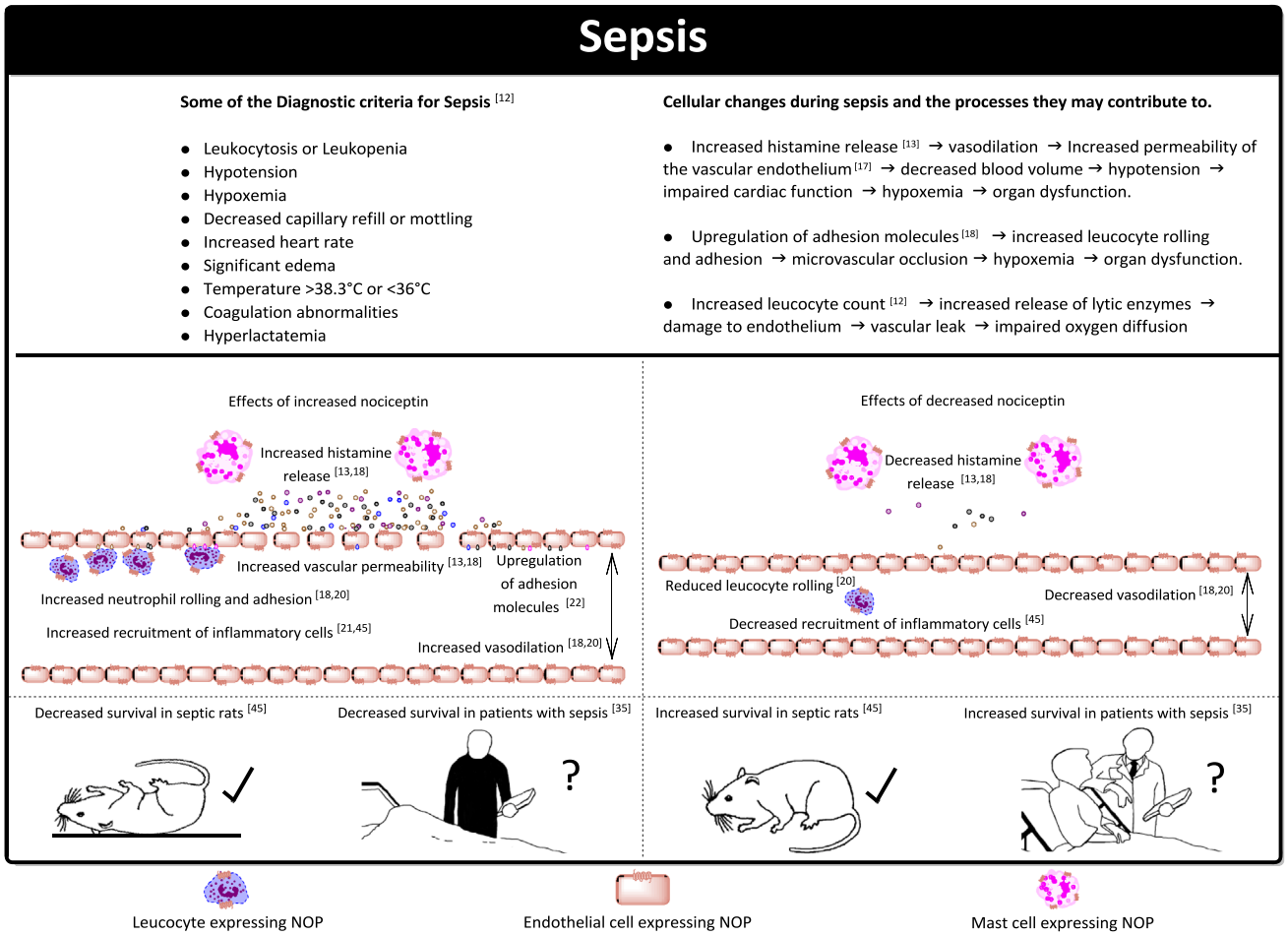

Fig. 3 Sepsis and the effects of increased and decreased nociceptin. *Top*: some of the diagnostic criteria, changes and processes of sepsis. *Middle*: effects of nociceptin/orphanin FQ (N/OFQ), which may be related to sepsis. *Bottom left*: effects of N/OFQ on survival in rats. *Bottom right*: possible effects of N/OFQ on survival in humans

regarding treatment withdrawal in clinical practice. Our findings of significant increases in inflammatory cytokines linked to increased plasma N/OFQ concentrations do, however, indicate that there may be a link with nociceptin system upregulation and increased morbidity. Further studies will be necessary to attempt to address the question of whether this is related to an increase in deaths from sepsis.

Taken together, data from the studies discussed above suggest that nociceptin system upregulation may be detrimental to health, whereas a decrease could be advantageous (Fig. 3).

Summary

Modulation of the immune system by the nociceptin system is extremely complex, and details are not fully elucidated. Further studies are needed to better understand the effects of activating or antagonising the system and to explain the conflicting results in terms of up- or down-regulation during inflammation and sepsis. However, available data indicate that a substantial number of processes are affected by the nociceptin system in inflammation and sepsis; sepsis has a high mortality rate, and there may be substantial benefits in therapeutic modulation of the nociceptin system.

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