The nociceptin system comprises the peptide nociceptin/orphanin FQ (N/OFQ) and the N/OFQ receptor (NOP). N/OFQ is a 17 amino acid opioid-like peptide first described in 1995\(^1\) with structural similarity to the endogenous \(\kappa\) (or KOP)-opioid dynorphin A, and is produced and released by neurones and other tissues from a 30 amino acid precursor ppN/OFQ. In humans, plasma concentrations of N/OFQ have been reported in a variety of conditions to be between 2.5 and 15 pg ml\(^{-1}\). NOP is distributed widely in the central,\(^3\) peripheral, and sensory nervous systems, and also in other peripheral tissues.\(^4\) Much of the original work on the N/OFQ-NOP system centred around its complex involvement in pain pathways, where it produces analgesia when administered supraspinally.\(^5\) However, there is now good evidence to support a role for this relatively new peptide-receptor system in regulating other physiological functions, for example, the cardiovascular, hypothalamic–pituitary–adrenal (HPA) axis, and immune systems. Binding of N/OFQ to NOP causes a variety of cellular actions, which in neurones reduces excitability and transmitter release (e.g. glutamate, serotonin, norepinephrine, and tachykinins).\(^6\)

A vast array of experimental NOP ligands are available of both peptide/non-peptide and agonist/antagonist nature. Of relevance to this review are the peptide and non-peptide antagonists UFP-101 and J113397, respectively.\(^6\)

There are a growing number of in vitro and in vivo studies showing that: (i) N/OFQ–NOP expression in cells of the immune system enables immunomodulation, and (ii) N/OFQ–NOP has important cardiovascular modulatory effects. Notably, there is growing evidence of the involvement of the N/OFQ system in sepsis and the inflammatory response. In this review article, we examine this evidence and attempt to link the immunomodulation and cardiovascular modulation effects with respect to sepsis.

**Overview of sepsis and multi-organ failure**

The pathophysiology of sepsis involves multiple inter-related pathways. One of the initial processes of cellular immunity involves activation of leucocytes, which trigger a sequence of events: neutrophil rolling and adhesion to activated endothelium; migration of leucocytes into the affected tissues by chemotaxis; recognition and binding to micro-organisms; and finally engulfment and phagocytosis of microorganisms. These processes are mediated by cytokines [tumour necrosis factor (TNF) and interleukins IL-1, IL-2, IL-6, IL-8, IL-18] released from macrophages, which maintain the ongoing chemotaxis and inflammatory response.\(^7\)
This inflammatory response is controlled by programmed cell death or apoptosis of leucocytes, which seems to be delayed in sepsis. If the leucocyte-mediated response is not limited to the zone of initial infection, a widespread inflammatory response occurs. Moreover, neutrophils also contain proteolytic enzymes and produce reactive oxygen species, which are necessary for the degradation of engulfed pathogens. If these substances, along with inflammatory cytokines, are released, then damage to the surrounding healthy tissue can occur. The mitochondrial-specific oxidative damage is a potential treatment target to prevent the inflammatory response and multi-organ failure.

Other consequences of sepsis include: (i) dysfunction of the macro- and micro-circulation which compromises tissue perfusion and organ function; (ii) hypotension and vascular dysfunction secondary to increased nitric oxide (NO) production, activation of vascular potassium channels and hormonal changes (e.g. vasopressin and cortisol); and (iii) metabolic dysfunction such as hyperglycaemia which leads to widespread cellular damage.

### The N/OFQ system in inflammation and sepsis

NOP and N/OFQ precursor (ppN/OFQ) mRNA are found in monocytes, lymphocytes, and polymorphonuclear cells (Table 1). This is not the case with the other classical opioid receptors (mu, MOP; delta, DOP; and KOP). In vitro studies show N/OFQ to be produced by immune cells and to act as an immunomodulator (Table 2). Its effects are mainly pro-inflammatory, for example, induction of chemotaxis and proliferation of immune cells. However, some studies showed reduced immune cell proliferation and reduced chemokine production. These differences could be attributed to differences in techniques used to activate immune cells, cell population studied, and the response analysed.

A caecal ligation/perforation model of sepsis found that administration of parenteral N/OFQ in rats exacerbated the inflammatory process and increased mortality (Table 3). Animals treated with N/OFQ had 100% mortality, compared with 50% in those treated with NOP antagonist UFP-101 and with 70% in the control untreated group. N/OFQ treatment also increased plasma concentrations of TNFα and IL-1β. In addition, using anaesthetized (but non-septic) rats, Brookes and colleagues showed that N/OFQ produced an inflammatory response. In mesenteric vessels, there was vasodilatation, macromolecular leak, and leucocyte adhesion (Table 3).

Conversely, intracerebroventricular administration of N/OFQ led to reduced cytokine production by peritoneal macrophages in rats undergoing exploratory laparotomy. It is possible that there is a difference in the immune response to N/OFQ between peripheral and central administration, and this is an avenue for further investigation.

There are few data in humans. However, in a small study of 21 critically ill patients admitted to ICU with a diagnosis of sepsis, we measured plasma N/OFQ concentrations over four consecutive days. Plasma concentrations of N/OFQ at ICU admission were increased in patients who subsequently died (n=4) compared with those who survived (n=17) [median (IQR) concentrations 3.0 (2.5–5.0) and 1.0 (1.0–2.5) pg ml⁻¹, respectively]. More data are required to confirm these findings.

Further supporting evidence for the role of N/OFQ in the inflammatory response comes from gene NOP knockout

### Table 1 Distribution of the N/OFQ system in human peripheral blood immune cells; RT–PCR studies. PMN, polymorphonuclear cells; PBMC, peripheral blood mononuclear cells

<table>
<thead>
<tr>
<th>Cells studied</th>
<th>Target found</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes and monocytes</td>
<td>NOP</td>
<td>Peluso and colleagues3, Arjomand and colleagues4,9</td>
</tr>
<tr>
<td>PMN, lymphocytes, monocytes</td>
<td>NOP and ppN/OFQ</td>
<td>Fiset and colleagues10</td>
</tr>
<tr>
<td>PBMC</td>
<td>NOP and ppN/OFQ</td>
<td>Williams and colleagues11,12</td>
</tr>
<tr>
<td>PMN</td>
<td>NOP and ppN/OFQ</td>
<td>Young and colleagues50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrano-Gomez and colleagues51,52</td>
</tr>
</tbody>
</table>

### Table 2 In vitro studies of N/OFQ as pro- and anti-inflammatory in volunteer and animal samples. Different cell populations were treated with either N/OFQ or toxins. SEB, Staphylococcal enterotoxin B; LPS, lipopolysacharide; ConA, ConvalinA; IL-1β, interleukin 1β; TNFα, tumour necrosis factor; PHA, phytohemagglutinin

<table>
<thead>
<tr>
<th>Cell</th>
<th>Treatment</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td>SEB+N/OFQ</td>
<td>↓ cell proliferation with N/OFQ</td>
<td>Easten and colleagues13</td>
</tr>
<tr>
<td>PMN</td>
<td>N/OFQ</td>
<td>Chemotaxis of PMN</td>
<td>Serhan and colleagues14</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>SEB+N/OFQ</td>
<td>↑ cell proliferation with N/OFQ</td>
<td>Waits and colleagues15</td>
</tr>
<tr>
<td>Monocytes, PMN</td>
<td>N/OFQ</td>
<td>Monocytes chemotaxis. No effect on PMN</td>
<td>Trombella and colleagues16</td>
</tr>
<tr>
<td>PBMCs</td>
<td>PHA+N/OFQ</td>
<td>50% of samples showed ↓ cell proliferation. 50% no effect N/OFQ production</td>
<td>Peluso and colleagues17</td>
</tr>
<tr>
<td>Rat splenocytes</td>
<td>LPS, Con A, IL-1β, TNFα, dexamethasone</td>
<td>↑ N/OFQ production</td>
<td>Miller and Fulford18</td>
</tr>
<tr>
<td>Monocytes CD14+</td>
<td>N/OFQ and LPS+N/OFQ</td>
<td>↓ production of chemokines by monocytes</td>
<td>Kaminsky and Rogers19</td>
</tr>
</tbody>
</table>

[Reference numbers correspond to the cited studies in the text.]
(NOP\textsuperscript{-/-}) mice where the gene for NOP is absent. A study of mice with induced colitis compared wild-type mice (non-genetically modified, NOP\textsuperscript{+/+}) with NOP\textsuperscript{-/-} mice.\textsuperscript{17} Administration of oral dextran sulphate sodium (DSS) caused bloody diarrhoea in the NOP\textsuperscript{+/+} group but normal stools in the NOP\textsuperscript{-/-} group. On histological examination, the colon of NOP\textsuperscript{+/+} mice had crypt distortion and increased number of lymphocytes, macrophages, and neutrophils (evidence of colitis), compared with normal crypts and reduced number of inflammatory cells in the NOP\textsuperscript{-/-} group. This demonstrated that the absence of NOP significantly reduced the inflammatory response to a known pro-inflammatory stimulus (DSS).

### Pain control by N/OFQ during inflammation: the neuroimmune axis

Pain is one of the cardinal features of inflammation and is mediated by prostaglandins, substance P, histamine, and other substances. Endogenous opioids provide analgesia by increasing the number of opioid receptors and the availability of endogenous opioid peptides at the site of inflammation. Opioid receptors are up-regulated in the terminal nerve endings at sites of inflammation; this can be attributed to increased synthesis of receptors in the dorsal root ganglia\textsuperscript{18} and increased intra-axonal transport of receptors to the terminal nerve endings (a process mediated by IL-1\beta).\textsuperscript{19} The highest concentration of opioid peptides available at the inflammatory site comes from lymphocytes, monocytes, PMN, and macrophages. These immunocytes have an increased expression of endorphin, met-enkephalin and dynorphin-A which is stimulated by endotoxins, viruses, IL-1, and corticotropin releasing hormone (CRH). When leucocytes migrate into the inflamed tissue, they release these opioids.\textsuperscript{20, 21} This mechanism of analgesia by endogenous opioids acting at peripheral sites has been suggested as being part of a physiological neuroimmune axis.\textsuperscript{22}

NOP has a similar distribution to classical opioid receptors in neuronal tissue and is also present in leucocytes.\textsuperscript{3} The antinociceptive effect of N/OFQ in an animal model of bowel inflammation has been examined by comparing peripherally and centrally administered N/OFQ.\textsuperscript{23} Intraperitoneal administration of N/OFQ reduced the painful response to colorectal distension in rats with induced colitis. In contrast, there was no effect when N/OFQ was administered by the intracerebroventricular route. Administration of the N/OFQ antagonist UFP-101 increased the painful response to colorectal distension and antagonized the anti-nociceptive effect of N/OFQ. An increased number of cells positive for NOP and myeloperoxidase activity (a marker of PMN granulation) were observed in a segment of distal colon, which confirmed the infiltration of immunocytes into the inflamed tissue. These findings support the hypothesis that N/OFQ may act peripherally as an analgesic at sites of inflammation. The classic modulation by N/OFQ of the pain response has been covered extensively.\textsuperscript{6, 24}

### N/OFQ and the HPA axis

The hypothalamus is involved in regulation of the stress response through the production of steroids and in particular cortisol via the feedback system of the HPA axis. CRH, produced in the hypothalamus, controls the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn regulates the release of glucocorticoids (mainly cortisol) from the adrenal cortex.\textsuperscript{25} Cortisol facilitates the normal response of catecholamines (epinephrine and norepinephrine), angiotensin, and vasopressin, up-regulates receptor expression, and has anti-inflammatory effects: (i) reduction of the function and number of lymphocytes, monocytes, neutrophils, and eosinophils at the site of inflammation; (ii) reduction of macrophage adhesion to the endothelium; and (iii) reduction of IL-2 production from macrophages.\textsuperscript{26}

N/OFQ activates the HPA axis under resting and mild stress conditions. Intracerebroventricular injection of N/OFQ in rats led to increased plasma corticosterone\textsuperscript{27} and ACTH concentrations\textsuperscript{28} for up to 30 min under resting conditions. N/OFQ administered to rats under mild stress, enhanced the raised ACTH response to stress and prolonged the higher concentrations of corticosterone. Under a more stressful stimulus like restraint, intracerebroventricular N/OFQ did not affect the already elevated plasma concentrations of ACTH or

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**Table 3** In vivo animal and human studies showing the response to N/OFQ administration. i.v., intravenous; s.c., subcutaneous; i.c.v., intracerebroventricular; CLP, coecal ligation and perforation; UFP-101, NOP antagonist

<table>
<thead>
<tr>
<th>Model</th>
<th>Intervention</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthetized rats</td>
<td>i.v. N/OFQ</td>
<td>On mesenteric vessels: leucocyte rolling and adhesion, [macromolecular leak, [blood flow vasodilatation</td>
<td>Brookes and colleagues\textsuperscript{14}</td>
</tr>
<tr>
<td>Septic rats (CLP)</td>
<td>Group 1: s.c. N/OFQ</td>
<td>Group 1 mortality 100%</td>
<td>Carvalho and colleagues\textsuperscript{15}</td>
</tr>
<tr>
<td></td>
<td>Group 2: UFP-101</td>
<td>Group 2 mortality 50%</td>
<td>Zhao and colleagues\textsuperscript{16}</td>
</tr>
<tr>
<td></td>
<td>Group 3: control</td>
<td>Group 3 mortality 70%</td>
<td>Williams and colleagues\textsuperscript{16}</td>
</tr>
<tr>
<td>Rats undergoing</td>
<td>i.c.v. N/OFQ</td>
<td>[production of IL-1 and TNF\textsubscript{a} by peritoneal macrophages</td>
<td></td>
</tr>
<tr>
<td>exploratory laparotomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU septic patients</td>
<td>Plasma concentration of N/OFQ measured</td>
<td>N/OFQ concentrations higher in patients who died of sepsis compared with those who survived</td>
<td></td>
</tr>
</tbody>
</table>
corticosterone. Increased CRH mRNA expression in the paraventricular nucleus of the hypothalamus in rats after intracerebroventricular N/OFQ administration has also been observed.

These data show the potential effects of N/OFQ on HPA axis activity. Whether these effects occur during an episode of systemic inflammation and lead to maintenance of plasma hormone concentrations or to an immunosuppressive effect has yet to be determined. Further data are required.

**Effects of N/OFQ in the cardiovascular system**

Activation of the immune system during inflammation and sepsis can cause significant cardiovascular changes leading to hypotension, hypo-perfusion, and subsequent multi-organ failure. Endogenous and exogenous opioids have important cardiovascular effects. Similarly, N/OFQ causes hypotension and bradycardia when administered i.v. and intracerebroventricularly, as demonstrated by various in vitro and in vivo animal studies and this has been covered extensively.

Different mechanisms of action have been proposed and here we concentrate on the data which suggest an immunovascular link.

**Centrally mediated cardiovascular effects**

The central nervous system contributes to the cardiovascular response during inflammation via the HPA axis, the sympathetic nervous system, and the cholinergic anti-inflammatory pathway. Over the past 15 yr, it has been found that NOP, N/OFQ, and ppN/OFQ are present in areas of animal and human brains associated with cardiovascular regulation. ppN/OFQ mRNA has been isolated from brainstem in mice and brainstem of human fetus and adult rats, and N/OFQ is found in rat and human (neonatal deaths or fetal losses) brainstem.

In vivo, administration of intracerebroventricular N/OFQ in mice caused a dose-dependent decrease in arterial pressure and heart rate. These cardiovascular changes had a rapid onset (<1 min) with a peak effect at 20 min, and duration of 40–50 min. Pre-treatment with intracerebroventricular UFP-101 abolished the cardiovascular response to N/OFQ.

Notably, the administration of intracerebroventricular UFP-101 alone in mice also led to significant bradycardia for up to 2 h. A similar finding was also reported in conscious rats. This may be due to a partial agonist effect of centrally administered UFP-101 when NOP expression is high. N/OFQ has also been injected in more specific areas of the brain thought to be involved with the regulation of the cardiovascular system: the rostral ventrolateral medulla and the paraventricular nucleus of the hypothalamus. Direct administration into these areas caused dose-dependent bradycardia and hypotension in anaesthetized rats. Using doses comparable with those used in the intracerebroventricular studies, the cardiovascular effects also had a rapid onset and similar duration of 30–60 min. Pretreatment with the NOP partial agonist [Phe1,ψ(CH2-NH)Gly2]N/OFQ(1–13)NH2 blocked the cardiovascular response to N/OFQ.

In a comparison of the effects of intracerebroventricular N/OFQ in wild-type NOP+/+ and NOP knockout NOP−/− mice, the basal values for mean arterial pressure and heart rate were similar in both groups. Administration of intracerebroventricular N/OFQ produced significant hypotension and bradycardia in NOP+/+ mice, in contrast to no cardiovascular changes in NOP−/− mice. These data show that the cardiodepressor effect of N/OFQ is mediated by NOP when N/OFQ is administered centrally in healthy animals. It is unknown whether the expression of the N/OFQ system in the brain is altered during sepsis. However, current evidence suggests this may be an option worthy of study.

**Peripherally mediated cardiovascular effects**

Multiple mechanisms are involved in the cardiovascular response that occurs during inflammation and sepsis. The N/OFQ system is present in the immune cells and activated as a response to inflammatory stimuli. Activation of immunocytes and the release of N/OFQ possibly have an effect on vascular reactivity. N/OFQ causes vasodilatation and increased capillary leak from mesenteric vessels that could be a potential target during inflammation.

The peripheral actions of N/OFQ have been noted by the cardio-depressor response observed after i.v. administration of N/OFQ in anaesthetized rats, guinea pigs, and conscious mice. These studies used similar doses of N/OFQ, between 10 and 100 nmol kg⁻¹, and demonstrated dose-dependent bradycardia and hypotension of nearly 30%, which lasted around 10 min. These cardiovascular effects were antagonized by pre-treatment with UFP-101. On the basis of evidence from animal studies (Fig. 1), there are four potential mechanisms for the peripheral effects of N/OFQ: (i) pre-synaptic inhibition of noradrenergic neurotransmission; (ii) direct arterial vasodilatation with reduced blood flow; (iii) histamine mediated, and (iv) via the parasympathetic nervous system.

**Summary**

In summary, the N/OFQ system is present in multiple organ systems and has been attributed different roles in pain control, anxiety, depression, drug abuse, and heart failure. Because of the location of NOP and N/OFQ in immune cells, a number of studies have examined its involvement in inflammatory responses, and specifically how it affects the immune response, the cardiovascular system, and the HPA axis.

The evidence available that the N/OFQ-NOP system has a role in sepsis-inflammation is largely based on in vitro and in vivo animal studies, with only one observational study in human patients with sepsis. However, based on these studies, one could propose that the expression of the nociceptin system by leucocytes increases during sepsis. Circulating leucocytes could then become N/OFQ delivery vehicles.
releasing N/OFQ at the site of inflammation which may produce systemic hypotension and organ dysfunction. Indeed, this may form an immunovascular axis. As NOP activation is chemotactic the up-regulation of this receptor in sepsis may contribute, possibly by auto-activation, to the maintenance of the inflammatory response. However, these hypotheses require rigorous experimental validation as it remains unclear how N/OFQ and NOP alter in patients with an ongoing inflammatory process or systemic sepsis and whether the changes would correlate with pathophysiological effects. When we have this information, it should be possible to design appropriate clinical studies to evaluate NOP ligands in sepsis. On the basis of existing data,\textsuperscript{13, 16} we would predict that NOP antagonists might be useful in this situation as they may reduce the systemic effects of leucocyte-delivered peptide and reduce the chemotactic response and ongoing inflammation.

**Conflict of interest**

Dr J.P.T. is an editor of the *British Journal of Anaesthesia* and Prof. D.G.L. a Board member and former editor.

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