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Nitric oxide in shock

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Refractory hypotension with end-organ hypoperfusion and failure is an ominous feature of shock. Distributive shock is caused by severe infections (septic shock) or severe systemic allergic reactions (anaphylactic shock). In 1986, it was concluded that nitric oxide (NO) is the endothelium-derived relaxing factor that had been discovered 6 years earlier. Since then, NO has been shown to be important for the physiological and pathological control of vascular tone. Nevertheless, although inhibition of NO synthesis restores blood pressure, NO synthase (NOS) inhibition cannot improve outcome, on the contrary. This implies that NO acts as a double-edged sword during septic shock. Consequently, the focus has shifted towards selective inducible NOS (iNOS) inhibitors. The contribution of NO to anaphylactic shock seems to be more straightforward, as NOS inhibition abrogates shock in conscious mice. Surprisingly, however, this shock-inducing NO is not produced by the inducible iNOS, but by the so-called constitutive enzyme endothelial NOS. This review summarizes the contribution of NO to septic and anaphylactic shock. Although NOS inhibition may be promising for the treatment of anaphylactic shock, the failure of a phase III trial indicates that other approaches are required for the successful treatment of septic shock. Amongst these, high hopes are set for selective iNOS inhibitors. But it might also be necessary to shift gears and focus on downstream cardiovascular targets of NO or on other vasodilating phenomena.

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SHOCK: CAUSES AND EPIDEMIOLOGY

Shock may be defined as the failure of the circulation to provide sufficient blood and oxygen to peripheral organs. Key symptoms of shock are severe hypotension and vasoplegia, ultimately resulting in the dysfunction of one or more vital organs, such as kidney, liver, gut, lung, and brain. Life-threatening shock may be caused by acute myocardial infarction (cardiogenic shock), severe fluid or blood loss (hypovolemic or hemorrhagic shock), severe infection (septic shock), or severe allergic reaction (anaphylactic shock). The most common type of shock is hemorrhagic shock; in children, elderly, and immunocompromized people, septic shock is the most common. In the first week after diagnosis, refractory hypotension is the leading cause of death; later on, death is generally caused by multiple organ failure as a result of prolonged hypotension and cytotoxicity. The history of clinical trials in septic patients extends back to 1963, when high-dose hydrocortisone was used.[1] But despite almost half a century of clinical trials, and more than two decades of extensive research, only two experimental approaches have survived the numerous clinical trials and have reached the septic patient: low-dose corticosteroids and recombinant human activated protein C.[1,2] Still, their beneficial effect on survival seems to depend on the severity of the illness and they may be rather harmful in patients with a lower risk of death.[2] In addition, recent trials failed to show any significant benefit of recombinant human activated protein C and indicated an increased risk of bleeding, making it unclear whether its alleged beneficial effects in fact outweigh its risks.[3] Thus, severe sepsis and septic shock are still associated with an unacceptably high mortality rate of 50–70%. Short-term mortality from septic shock has decreased in recent years. In one study, for example, mortality fell from 62% in the early 1990s to 56% in 2000.[4] Nevertheless, overall mortality is increasing, as the incidence of sepsis is growing by 9% each year.[4,5] Consequently, these days more people die annually from septic shock than from myocardial infarction, lung or breast cancer, stroke, or trauma.[6] Anaphylaxis can occur in response to any allergen, most commonly insect stings, food, and drugs such as antibiotics, contrast materials, and anesthetics. In general, about 1% of people with an allergic history are prone to anaphylaxis, but some authors consider up to 15% of the US population 'at risk'.[7] Overall, the frequency of anaphylaxis is increasing because of the

soaring incidence of allergies and the increased number of potential allergens to which people are exposed.

NITRIC OXIDE: HISTORY AND BACKGROUND

In 1980, Furchgott and Zawadzki[8] reported that endothelial cells release a labile factor that causes blood vessel relaxation. In 1986, it was suggested, and subsequently confirmed, that this endothelial-derived relaxing factor is the short-lived, gaseous, highly reactive radical nitric oxide (NO).[9–13]

NO is produced enzymatically by three different NO synthases (NOS). Neuronal NOS (nNOS) (NOS1) and endothelial NOS (eNOS) (NOS3) are constitutive enzymes important for homeostatic processes, such as neurotransmission and vascular tone, respectively. They produce small amounts of NO in response to increases in intracellular calcium. More recently, the constitutive nature of eNOS has achieved new dimensions, as it became clear that the enzyme's activity may be regulated, both transcriptionally and post-transcriptionally, with acylation, phosphorylation, subcellular localization, and protein interactions determining its activity.[14] The third enzyme, inducible NOS (iNOS) (NOS2), is normally not expressed, but is synthesized *de novo* in response to inflammation. It is calcium-independent and produces large amounts of NO over prolonged periods of time.[15] NOS enzymes make NO from L-arginine, and thus competitive L-arginine analogues may prevent them from producing NO. These analogues include N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA), and N^G-nitro-L-arginine methyl ester (L-NAME). As early as 1989, some of these compounds were already successfully used to demonstrate the important physiological role of NO in normal blood pressure homeostasis.[16,17]

NO IN SEPTIC SHOCK: CRITICAL MEDIATOR OF HYPOTENSION

Shortly after the discovery that NO is an important endogenous regulator of vascular tone, its fundamental contribution to inflammatory and septic shock became obvious as well. The NO metabolites nitrite and nitrate (collectively labeled NO_x^-), indicators of NO production, rise progressively in various animal shock models.[18] In small rodents, plasma concentrations of hundreds to even thousands micromolar may be detected. In larger mammals and humans, however, overproduction does not occur to the same extent and levels rarely increase above 100 μM, or more than 50% above background, despite major circulatory failure.[18] Nevertheless, the critical role of NO in shock has been clearly established, as NOS inhibitors prevent, revert, or at least minimize hypotension in shock induced by lipopolysaccharide (LPS), tumor necrosis factor (TNF), interleukin-1, interleukin-2, or hemorrhage.[19–24] NOS inhibition also successfully and rapidly elevates blood pressure and systemic vascular resistance in septic shock patients.[25–28]

The first studies on NOS inhibition immediately triggered great hopes for a new treatment of refractory hypotension in (septic) shock, but even the earliest studies already indicated the potential harm of NOS inhibitors, as they also caused a progressive fall in cardiac output, amplified organ dysfunction, and even increased mortality.[26,29–33] Exacerbated organ damage was first reported for the kidney,[31] but later studies revealed increased injury in other organs as well, including liver, lung, pancreas, and intestines.[18] Unfortunately, even a phase III clinical trial had to be prematurely terminated because of increased mortality in the septic patients treated with the NOS inhibitor, despite positive effects on blood pressure and vascular resistance.[28] Together, these observations clearly indicate that NO not only mediates hypotension in septic shock, but may also perform an important obligatory role in assorted beneficial pathways.

NO IN SEPTIC SHOCK: DETRIMENTAL VERSUS BENEFICIAL EFFECTS

Different explanations may be suggested for the dual personality of NO during septic shock. First of all, there is no doubt about the detrimental effect of excessive NO on vasorelaxation, hypotension, and shock. The NO-mediated hypotension leads to severe hypoxia in peripheral vital organs, resulting in progressive organ failure. NO may also directly contribute to tissue and organ injury by its direct, peroxynitrite-mediated cytotoxic effects. It is generally accepted that NO may cause blood vessel relaxation by activating the cyclic guanosine monophosphate (cGMP)-producing enzyme soluble guanylate cyclase (sGC), leading to activation of the cGMP-dependent protein kinases (PKGs). For smooth muscle contraction, calcium-dependent activation of the myosin light chain (MLC) kinase and subsequent phosphorylation of MLC are essential. Several PKG-dependent phosphorylations ultimately converge on the dephosphorylation of MLC and hence relaxation[34] (Figure 1). Important molecular targets of PKG include various pumps and channels involved in modulating intracellular calcium levels and membrane potential, leading to decreased cytosolic calcium and relaxation. In addition to changes in intracellular calcium levels and membrane potential, other important targets for PKG in smooth muscle are the pathways regulating the calcium-sensitivity of the contractile machinery, more particularly the regulatory subunit of MLC phosphatase, which may be directly activated by PKG or indirectly via PKG-mediated inactivation of the inhibitory RhoA pathway.[35,36] Nevertheless, NO may also contribute independently of sGC and PKG to lower cytosolic calcium levels, for instance via direct S-nitrosation of potassium channels,[37] via NO-dependent peroxynitrite-mediated S-glutathiolation of the sarco/endoplasmic reticulum calcium adenosine triphosphatase (ATPase) (SERCA) pump,[38] or via direct inhibition of cytochrome P450 (CYP). Enzymes of the CYP4A family are known to produce the vasoconstrictor 20-HETE, an inhibitor of BK channels.[39] Although sGC has long been regarded as the predominant target for NO in the vasculature, the notion and importance of sGC-independent actions has gained considerable interest lately. The sGC-independent pathways would be especially important in certain vascular beds (particularly in the renal and mesenteric

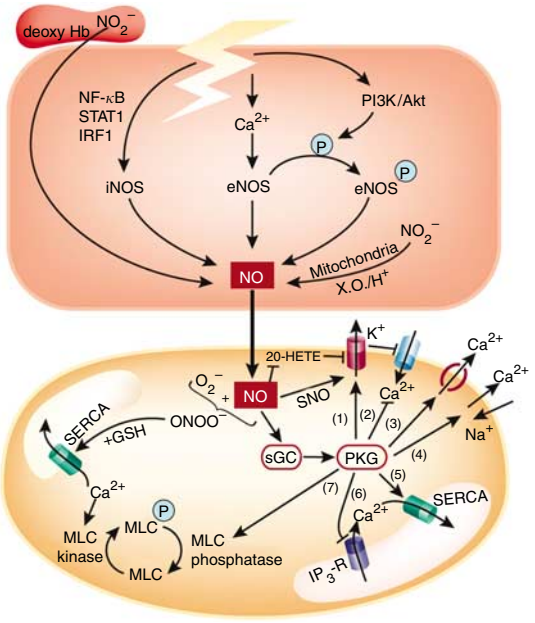

Figure 1 | Schematic of possible molecular mechanisms of NO-mediated vascular relaxation. NO may be enzymatically produced by eNOS, iNOS, or via nitrite (NO_2^-) reduction by deoxygenated heme-globins, xanthine oxidase, mitochondria, or acidic disproportionation. Vasoactive agonists normally elevate intracellular Ca^{2+} concentrations in endothelial cells, thus stimulating eNOS activity. In addition, fluid shear stress, estrogen, insulin, or inflammatory signals may cause PI3K/Akt-dependent phosphorylation of eNOS, resulting in its increased catalytic activity at basal Ca^{2+} levels. Various inflammatory stimuli, such as LPS or TNF, also trigger *de novo* transcription of the Ca^{2+}-independent inducible iNOS enzyme, resulting in the production of large amounts of NO for a prolonged period of time. For reasons of simplicity, the production of NO was depicted only in the endothelial cell, but may of course also occur in the smooth muscle cell, especially in inflammatory conditions. In the smooth muscle cell, NO may cause relaxation by a myriad of actions reducing cytosolic Ca^{2+} levels on the one hand and the sensitivity of the contractile apparatus for Ca^{2+} on the other. Independent of sGC, NO can directly S-nitrosate and activate K^+ channels, causing K^+ efflux, membrane hyperpolarization, and thus a decrease in voltage-dependent Ca^{2+} entry. NO may also sGC-independently inhibit the CYP4A-dependent production of 20-HETE, an inhibitor of BK channel activity. In addition, through its reaction with superoxide to form peroxynitrite, NO may cause glutathione (GSH) to bind to and activate the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), which takes up cytosolic Ca^{2+}. Nevertheless, many of the relaxing effects of NO are the result of its binding to Fe^{2+}-heme in sGC, resulting in a conformational change that activates the enzyme, increasing cGMP levels and PKG activity. PKG-mediated phosphorylation may cause (1) activation of K^+ channels and hyperpolarization; (2) inhibition of L-type Ca^{2+} channels and Ca^{2+} influx; (3) increased Ca^{2+} efflux through activation of the Ca^{2+} Mg^{2+} ATPase and (4) the Na^+/Ca^{2+} exchanger; (5) Ca^{2+} sequestration through SERCA activation; (6) reduction of Ca^{2+} mobilization through the inhibition of the sarcoplasmic reticulum IP_3 receptor or the phospholipase C-dependent formation of IP_3 (data not shown); or activation of the MLC phosphatase. (7) The latter may be achieved directly via phosphorylation of the MLC phosphatase or indirectly via inhibition of the inactivating RhoA pathway (data not shown), ultimately resulting in dephosphorylation of the vasoconstricting MLC.

vasculature), at high NO concentrations, and/or in the presence of disease,[40] suggesting that the sGC-independent mechanisms may be important targets for drug development.

Second, increased NO may also provide certain benefits to the patient during septic shock. Arterial vasodilation results in arterial underfilling, which is rapidly sensed by the baroreceptors, thereby leading to increased sympathetic outflow and the activation of the renin–angiotensin–aldosterone system. This leads to vasopressin release, renal vasoconstriction and kidney failure, an acute problem in most septic shock patients, which is associated with very high mortality.[41] In this context, increased NO release protects the kidney by causing local vasodilation and by inhibiting platelet aggregation and leukocyte adhesion. In addition, NO may also exert protective effects in other organs via its capacity to counteract oxidative stress, shut off apoptosis, prevent platelet aggregation and leukocyte adhesion, induce anti-inflammatory gene expression, and kill pathogens.

NO IN SEPTIC SHOCK: WHAT IS ITS SOURCE?

Originally it was thought that the dual, Janus-faced effects of NO would relate to the NOS isoform responsible for its production, with eNOS providing the essential, protective NO and iNOS causing excessive vasodilation. For a long time, leukocyte iNOS was thus thought to be responsible for the production of shock-inducing NO. The reasons for this assumption are obvious: when iNOS is transcribed, it produces large amounts of NO for a long time.[15] In addition, rodent macrophages may be induced to produce large amounts of NO *in vitro*;[42] iNOS was originally identified as 'macrophage' NOS;[43] and although human macrophages do not seem to be capable of producing much NO *in vitro* or *ex vivo*,[44,45] neutrophils from septic patients display abnormally high amounts of iNOS mRNA activity.[46,47] More recently, however, it was demonstrated in mice that parenchymal cells, rather than blood cells, are required for the systemic production of NO during septic and endotoxic shock.[48] Tissues that do express high levels of iNOS during endotoxemia or bacteremia were identified as liver and intestines.[48,49] Whether hepatocytes, enterocytes, Paneth cells, or rather vascular cells are the predominant parenchymal source of the enhanced systemic NO, remains to be determined. Studying cell-specific iNOS-deficient or iNOS-reactivation mice may provide the answer to this question.

In well-oxygenated conditions, NOS enzymes may produce NO from L-arginine. Some of this NO reaches its targets, such as the smooth muscle cells where it triggers relaxation, but the majority is destroyed by rapid oxidation into nitrite and nitrate. Until recently, these metabolites were considered to be physiologically inert, stable end products, and an index of NO production. However, substantial proof is now emerging that plasma nitrite actually serves as an important vascular storage pool for NO.[50] Previously, it was suggested that S-nitrosated albumin and hemoglobin were the stable transporters of intravascular NO.[51,52] However, the levels of circulating S-nitrosothiols are either undetectable or in the lower nM range,

whereas nitrite is present in concentrations of 0.5–1 μM.[50,53] In addition, nitrite is more stable in blood and it seems that most of it is carried by erythrocytes. The reduction of nitrite back to the vasodilating NO occurs preferentially under hypoxic and/or acidic conditions and may be catalyzed by deoxyhemoglobin, xanthine oxidase, or mitochondrial enzymes[50] (Figure 1). In this way, nitrite derived from either dietary nitrite or nitrate (the latter further reduced by commensal bacteria) or from the oxidation of NOS-produced NO, may have an important function in the endocrine delivery of NO to hypoxic/acidic regions that need vasodilation.

NO IN SEPTIC SHOCK: SELECTIVE iNOS INHIBITORS AS THERAPEUTICS?

The dichotomous effects of NO present one of the biggest challenges to the development of potential therapeutic inhibitors. General inhibition of NOS enzymes improves hemodynamic functions but increases mortality.[28,30,32,33] Several arguments favor the development of selective iNOS inhibitors to treat septic shock patients.

(1) The protective capacity of eNOS-derived NO in septic conditions was underscored by the observation that transgenic overexpression of eNOS partly protected mice from LPS.[54] More recently, mice with a cardiomyocyte-specific overexpression of eNOS were also partially protected against both endotoxemia and polymicrobial sepsis.[55]
(2) Sepsis may cause an iNOS-dependent decrease in eNOS expression and activity,[56] causing endothelial dysfunction and impaired microvascular homeostasis, which may be particularly important in the kidney.
(3) In animals, most studies using selective iNOS inhibitors did not report deleterious effects like those observed with the nonselective NOS inhibitors; sometimes they even reported protective effects on organ failure or mortality. However, several models also failed to show beneficial effects of iNOS inhibition and, to be complete, a few reports even described deleterious effects on lung or liver injury.[18] Nevertheless, despite some conflicting results on the effects of iNOS inhibition on organ damage or outcome, circulatory failure was prevented or reverted in all studies.

However, we should not forget that, in contrast to iNOS-specific inhibitory drugs, iNOS-deficient mice are not at all protected against endotoxemia, sepsis, or TNF-induced shock. On the contrary, most studies even reported increased mortality,[57–61] suggesting that iNOS actually grants a survival advantage. This protective effect of iNOS might be due to the antiapoptotic or antioxidative activities of induced NO.[62,63] Indeed, both *in vitro* and *in vivo*, NO has been documented to efficiently interfere with lipid peroxidation.[61,64,65] The differences in the effects of iNOS inhibitors versus iNOS-deficiency may have several reasons.

(1) iNOS inhibitors could have additional pharmacological effects unrelated to iNOS inhibition. S-methyl-isothiourea, for example, also has antioxidative effects,[66] whereas aminoguanidine inhibits catalase activity.[67]
(2) It may be more important to merely downregulate (via pharmacological inhibitors), but not completely annul, iNOS activity, so that residual NO produced by iNOS may exert its necessary protective functions.
(3) Alternatively, the beneficial versus detrimental functions of iNOS might be linked to its induction in certain tissues or cells, which would warrant the development of cell-specific iNOS inhibitors. One could imagine that iNOS inhibitors injected systemically might reach selected tissues or cellular compartments more easily than others, such that iNOS activity in certain protective environments remains, while its activity in other locations, for example those that are more easily reached and that are also important for shock induction, is efficiently abrogated.

NO IN SEPTIC SHOCK: NO SCAVENGERS AS THERAPEUTICS?

Despite their preference for iNOS, most of the so-called selective iNOS inhibitors that are currently available still retain some activity against other NOS isoforms. In addition, NO may also be produced independently of NOS activity, for instance via the reduction of nitrite.[50] Therefore, the therapeutic use of compounds that selectively scavenge excessive NO, without interfering with NOS expression or activity, seems interesting. Theoretically, these scavengers might prevent toxicity and/or shock caused by excessive NO, while preserving some essential NO activities in the proximity of its area of production.

Various NO-scavenging compounds have been identified, ranging from endogenous proteins such as hemoglobin, to exogenous herbal substances, medications, nitronyl nitroxides such as carboxy-PTIO, and spin-trapping probes such as dithiocarbamate derivatives.[68] Some of these compounds have been proposed as efficient NO scavengers in various animal models of sepsis, reducing hypotension, organ dysfunction, bacterial translocation, and mortality.[69–73] Some NO scavengers have even entered clinical trials, such as the chemically modified human-derived hemoglobin conjugate pyridoxalated hemoglobin polyoxyethylene (PHP), which demonstrated its potential to increase systemic blood pressure and reduce vasopressor and ventilation needs without adverse effects on cardiac output, organ damage, or survival.[74,75] Based on these promising results, a phase III trial has recently been conducted, but was not yet published. However, just like the perfectly selective iNOS inhibitor, the optimal NO-scavenging compound (specific for NO radicals only, with appropriate solubility and half-life) has yet to be developed.[68]

NO IN SEPTIC SHOCK: TARGET DOWNSTREAM MEDIATORS?

Because even specific iNOS inhibition is not always associated with diminished organ damage and/or mortality in experimental endotoxic or septic shock, it might be necessary to shift gears in our approach to successful

therapeutics. A safer and more rational approach could be the selective modulation of certain specific downstream targets of NO that are known to perform an important role in its hypotensive effects. This way, only the shock-inducing effects of NO, and not its production, would be affected and its beneficial and antimicrobial effects could still carry on.

As mentioned before, sGC is generally regarded as the principal intracellular NO receptor in the cardiovascular system (Figure 1). Binding of NO results in a ~200-fold activation of sGC, leading to cGMP accumulation and cGMP-dependent cardiovascular changes, such as vascular relaxation, myocardial depression, and inhibition of platelet aggregation and adhesion.[34] One possibly safer therapeutic option could thus be the selective inhibition of sGC in shock. This approach seems especially attractive in view of the possibility that NO-independent activation of sGC could also contribute to shock.[76,77] On the other hand, there is also increasing evidence that sGC-independent mechanisms may contribute significantly, and in certain vascular beds, conditions, or diseases even predominantly, to NO-induced relaxation.[40] To inhibit sGC activation, methylene blue (MB), a chemical dye whose safety in humans has been proven, has been used in both experimental and clinical shock trials. Although MB can improve hemodynamics in both endotoxic and TNF-induced shock,[60,78,79] it can only provide protection in TNF-induced shock[60] and not against endotoxemia (Cauwels *et al.*, submitted). Analogously, MB infusion in humans suffering septic shock reverses hypotension, but does not change the overall course or the mortality rate.[80–84] It should be noted that MB not only prevents sGC activation, it also (partially) inhibits NOS enzymes and oxidative stress.[84–87] 1H-(1,2,4)oxadiazolo (4,3-α)quinoxalin-1-one (ODQ), a more selective and potent inhibitor of sGC [88–90] which may still inhibit NOS,[40,91] but has no effect on $O_2^{\bullet -}$ or $^{\bullet}OH$ production or scavenging,[92] is not capable of reverting endotoxic hypotension[92,93] or TNF-induced mortality (Cauwels *et al.*, unpublished data), in contrast to MB.[60,78,79] Hence, it seems possible that the protective hemodynamic effects of MB might be attributed to its non-sGC-dependent effects on oxidative stress. However, the inability of ODQ to prevent shock should be appraised with some caution, as the efficiency of ODQ to inhibit sGC activation *in vivo* is sometimes doubted because of its relatively rapid reaction with oxyhemoglobin.[89] Although the different effects of MB and ODQ might indicate a possible role for $O_2^{\bullet -}$ or $^{\bullet}OH$ in shock, these radicals have not really been implicated in vasorelaxation or shock so far.

Another possible target downstream of NO is the inhibition of K^+ channels, as NO may activate different K^+ channels both sGC-dependently and -independently[37,94–96] (Figure 1). The most important K^+ channel subclasses in the vasculature that are involved in the action of various endothelium-derived relaxing factors are the ATP-sensitive K_{ATP} channel and the large conductance calcium-activated BK channel. K_{ATP} channels have long been suspected of playing the most important role in septic shock because of their metabolic sensitivity.[97,98] They are activated by decreased ATP, increased lactate, or acidosis, all of which characterize sepsis. In addition, they may also be activated by NO, prostacyclin (PGI2), or the more recently identified vasodilator hydrogen sulfide (H_2S).[94,97,99] In many endotoxic animal models, K_{ATP} inhibition by parenteral glibenclamide could, at least partially, revert hypotension or vascular hyporesponsiveness.[100–103] However, glibenclamide did not restore responsiveness in all animal studies[104] or in a recent clinical trial.[105] Although the failure in the latter might have been due to administration of the drug by the enteral route or to the mild lactic acidosis in the enrolled patients,[106] the results imply that K_{ATP} inhibition might not be the appropriate treatment for septic shock.

Very recently, attention has also drifted towards the inhibition of BK channels to treat shock, as they are probably the most important channels involved in NO-dependent relaxation.[94,96] NO may activate BK channels not only sGC-dependently, through phosphorylation by cGMP-dependent PKG, but also sGC-independently via direct S-nitrosation and activation of BK channels[37,40,107,108] or via inhibiting the production of the BK inhibitor 20-HETE[39,40] (Figure 1). In addition, BK channels are also targeted by other potential vasodilators, including H_2O_2 and epoxyeicosatrienoic acids.[109–111] Studies with BK inhibitors are scarce and none of them have used specific BK-inhibiting drugs, but rather tetraethylammonium, a nonspecific inhibitor of BK, K_{ATP}, and certain voltage-gated K_v channels. Tetraethylammonium restored vascular responsiveness in one study,[104] but failed to improve blood pressure or mortality in another.[112] However, since tetraethylammonium could also restore vasopressor responses in an experimental human endotoxemia study,[113] high hopes are set for the modulation of BK channels as a potential therapy.

BK channels exhibit a very high conductance for K^+ ions, they are abundant in smooth muscle, and they are activated by the concerted influence of membrane depolarization and elevated intracellular Ca^{2+} levels.[114] However, they are not the only calcium-dependent K^+ channels. Small (SK) and intermediate (IK) conductance calcium-activated K^+ channels also exist. In contrast to the BK channels, they are not voltage-dependent. Because the BK channel is activated both directly and indirectly by NO, it has received most of the attention so far. Interestingly, however, the two other calcium-gated channels were implicated in the action and/or the effect of another vasodilating phenomenon: endothelium-derived hyperpolarizing factor (EDHF). EDHF is defined as the hyperpolarizing and relaxing effect that remains when NO and PGI2 production are inhibited and its contribution to vasodilation appears to be especially significant in smaller, resistance vessels.[111] Despite two decades of research, the molecular identity of EDHF remains controversial. Over the years, several candidates have been proposed, discussed, questioned, and refuted.[111] Despite that, it is universally accepted that the hyperpolarizing effect of EDHF depends strictly on SK channels, often assisted by IK channels.[115–117] A physiological, gender-dependent role for

EDHF in vascular tone was recently suggested,[118] but the question remains whether EDHF actively participates in physiological or pathological blood pressure regulation or whether it is merely a backup mechanism observed only when the production of NO and PGI2 is eliminated or fails. In addition, the involvement of EDHF in pathologies such as shock has not been suggested or studied so far. Intriguingly, specific SK channel inhibition by apamin can provide a substantial survival advantage in both TNF- and LPS-induced shock in mice, especially when combined with BK-channel inhibition and MB (Cauwels *et al.*, submitted), and may be well worth pursuing as a future therapeutic option.

SEPTIC SHOCK: OTHER VASORELAXING THERAPEUTIC TARGETS?

The ability of NOS inhibitors to prevent or revert hypotension in shock induced by LPS, TNF, interleukin-1, interleukin-2, hemorrhage, and sepsis is clear evidence for the pivotal and essential role NO plays in the development of hypotension during inflammation-associated shock.[19–28] However, some observations also indicate that NO might not be the only important shock-inducing factor. Indeed, the elevation of circulating NO_x^- is relatively modest in large mammals and humans[18] and seems not even obligatory in severe sepsis.[119] Moreover, in cancer patients receiving isolated limb perfusion with the anticancer agent TNF, complicated by leakage from the perfusion circuit to the general circulation, no systemic NO metabolites were found, despite substantial hypotension.[120] Therefore, other (still unknown?) vasodilating factors might be just as important as NO in causing systemic hypotension and shock.

As already mentioned, the possibility that the molecularly unidentified EDHF is implicated has not been evaluated yet. Until there is consensus on the molecular identity of EDHF, its synthesis cannot be specifically or selectively blocked and its role in shock cannot be easily studied. An alternative approach to investigate the possible contribution of EDHF could be the analysis of the importance of the various calcium-dependent K^+ channels. In this regard, the involvement of SK channels in TNF- and LPS-induced shock might be indicative of a possible contribution by EDHF (Cauwels *et al.*, submitted).

Another recently characterized endogenous vasodilator is the gaseous molecule H_2S, which causes vascular smooth muscle relaxation by acting on K_{ATP} channels and synergizes with NO to induce vessel relaxation.[99,121] In vascular smooth muscle cells, H_2S is formed by the hydrolysis of L-cysteine by cystathionine-γ-lyase. Inhibition of cystathionine-γ-lyase may prevent organ damage and mortality in experimental endotoxemia and sepsis,[122–124] but it cannot revert endotoxic hypotension.[123]

It is generally understood that therapeutic interventions to modulate oxidative stress in septic shock are worthwhile to be pursued, especially in terms of preventing tissue damage and organ failure caused by reactive radicals such as $O_2^{\bullet -}$, $^{\bullet}OH$, and $ONOO^-$.[125–127] As already alluded to earlier in this review, the conflicting effects of the sGC inhibitors MB and ODQ on LPS- or TNF-induced hypotension and shock[60,78,79,92,93] could indicate the involvement of oxygen radicals such as $O_2^{\bullet -}$ or $^{\bullet}OH$. The ability of these radicals to cause vasorelaxation, or to contribute to hypotension and shock, has not been extensively explored yet, but is definitely worth investigating.

NO IN ANAPHYLACTIC SHOCK

Anaphylaxis is a sudden and severe systemic allergic reaction that often occurs in the absence of a history of allergy. Allergens that commonly cause anaphylaxis include medications, such as penicillins, radiocontrast media and anesthetics, and foods such as nuts, fish, and shellfish as well as insect stings, exposure to latex, and exercise. Anaphylaxis is not as rare as generally believed and may affect as much as 1–15% of the population.[7] In addition, the prevalence of anaphylaxis is increasing significantly. During an anaphylactic reaction, severe cardiovascular or pulmonary dysfunction often leads to death, with acute hypotension as the most important clinical feature. Once an anaphylactic reaction has begun, the treatment of choice is an instantaneous injection of adrenaline, followed by emergency medical attention. To date, supportive adrenalin and large-volume intravenous fluid resuscitation are the only available treatments. Unfortunately, severe hemodynamic collapse during anaphylaxis is often resistant to this treatment.[128,129]

The possible contribution of NO to anaphylactic shock has been studied before. Although NOS inhibition partially reduced anaphylactic mortality in an early study,[130] it decreased survival time and rate in most other studies.[131,132] However, these studies were performed in animals under general anesthesia, which influences NO-mediated effects and blood pressure changes. More recently, the critical role of NO in anaphylactic shock was unequivocally demonstrated in conscious, nonanesthetized mice: pretreatment with the NOS inhibitor L-NAME successfully prevented both hypotension and mortality.[133] Surprisingly, this crucial NO was not produced by iNOS, but by the so-called constitutive isoform eNOS, which may be rapidly phosphorylated and activated via the PI3K/Akt pathway to produce large amounts of NO (Figure 1). In mice treated with inhibitors of PI3K, Akt, or NOS as well as in eNOS-deficient animals, anaphylaxis-induced hypothermia and hypotension were mild and transient and no deaths occurred. Despite the pivotal role for eNOS-produced NO, there seemed to be no significant involvement of sGC.[133] All in all, this study clearly indicated that PI3K/Akt-activated eNOS-derived NO is a pivotal vasodilator in anaphylactic shock and that selective and fast-acting inhibitors of any of these molecules may provide new, specific tools for the treatment of anaphylactic shock.

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REFERENCES

1. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat Med* 2003; **9**: 517–524.
2. Deans KJ, Haley M, Natanson C *et al.* Novel therapies for sepsis: a review. *J Trauma* 2005; **58**: 867–874.
3. Eichacker PQ, Natanson C. Increasing evidence that the risks of rhAPC may outweigh its benefits. *Intensive Care Med* 2007; **33**: 396–399.
4. Annane D, Bellissant E, Cavaillon JM. Septic shock. *Lancet* 2005; **365**: 63–78.
5. Martin GS, Mannino DM, Eaton S *et al.* The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; **348**: 1546–1554.
6. Nguyen HB, Rivers EP, Abrahamian FM *et al.* Severe sepsis and septic shock: review of the literature and emergency department management guidelines. *Ann Emerg Med* 2006; **48**: 28–54.
7. Neugut AI, Ghatak AT, Miller RL. Anaphylaxis in the United States: an investigation into its epidemiology. *Arch Intern Med* 2001; **161**: 15–21.
8. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373–376.
9. Furchgott R. Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid-activatable inhibitory factor from retractor penis is inorganic nitrate the endothelium-derived relaxing factor is nitric oxide. In: Vanhoutte PM (ed). *Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium*. New York: Raven Press, 1988: 401–414.
10. Ignarro LJ, Burns R, Wood K. Biochemical pharmacological properties of EDRF its similarity to nitric oxide. In: Vanhoutte PM (ed). *Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium*. New York: Raven Press, 1988: 427–435.
11. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327**: 524–526.
12. Ignarro LJ, Buga GM, Wood KS *et al.* Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987; **84**: 9265–9269.
13. Ignarro LJ, Byrns RE, Buga GM *et al.* Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ Res* 1987; **61**: 866–879.
14. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R1–R12.
15. Morris Jr SM, Billiar TR. New insights into the regulation of inducible nitric oxide synthesis. *Am J Physiol* 1994; **266**: E829–E839.
16. Aisaka K, Gross SS, Griffith OW *et al.* NG-methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea pig: does nitric oxide regulate blood pressure *in vivo*? *Biochem Biophys Res Commun* 1989; **160**: 881–886.
17. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989; **86**: 3375–3378.
18. Feihl F, Waeber B, Liaudet L. Is nitric oxide overproduction the target of choice for the management of septic shock? *Pharmacol Ther* 2001; **91**: 179–213.
19. Thiemermann C, Vane J. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat *in vivo*. *Eur J Pharmacol* 1990; **182**: 591–595.
20. Kilbourn RG, Jubran A, Gross SS *et al.* Reversal of endotoxin-mediated shock by NG-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem Biophys Res Commun* 1990; **172**: 1132–1138.
21. Kilbourn RG, Gross SS, Jubran A *et al.* NG-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc Natl Acad Sci USA* 1990; **87**: 3629–3632.
22. Kilbourn RG, Gross SS, Lodato RF *et al.* Inhibition of interleukin-1-alpha-induced nitric oxide synthase in vascular smooth muscle and full reversal of interleukin-1-alpha-induced hypotension by N omega-amino-L-arginine. *J Natl Cancer Inst* 1992; **84**: 1008–1016.
23. Kilbourn RG, Fonseca GA, Griffith OW *et al.* NG-methyl-L-arginine, an inhibitor of nitric oxide synthase, reverses interleukin-2-induced hypotension. *Crit Care Med* 1995; **23**: 1018–1024.
24. Thiemermann C, Szabo C, Mitchell JA *et al.* Vascular hyporeactivity to vasoconstrictor agents and hemodynamic decompensation in hemorrhagic shock is mediated by nitric oxide. *Proc Natl Acad Sci USA* 1993; **90**: 267–271.
25. Petros A, Bennett D, Vallance P. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 1991; **338**: 1557–1558.
26. Petros A, Lamb G, Leone A *et al.* Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc Res* 1994; **28**: 34–39.
27. Avontuur JA, Tutein Nolthenius RP, van Bodegom JW *et al.* Prolonged inhibition of nitric oxide synthesis in severe septic shock: a clinical study. *Crit Care Med* 1998; **26**: 660–667.
28. Lopez A, Lorente JA, Steingrub J *et al.* Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. *Crit Care Med* 2004; **32**: 21–30.
29. Klabunde RE, Ritger RC. NG-monomethyl-L-arginine (NMA) restores arterial blood pressure but reduces cardiac output in a canine model of endotoxic shock. *Biochem Biophys Res Commun* 1991; **178**: 1135–1140.
30. Cobb JP, Natanson C, Hoffman WD *et al.* N omega-amino-L-arginine, an inhibitor of nitric oxide synthase, raises vascular resistance but increases mortality rates in awake canines challenged with endotoxin. *J Exp Med* 1992; **176**: 1175–1182.
31. Shultz PJ, Raij L. Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. *J Clin Invest* 1992; **90**: 1718–1725.
32. Teale DM, Atkinson AM. L-canavanine restores blood pressure in a rat model of endotoxic shock. *Eur J Pharmacol* 1994; **271**: 87–92.
33. Liaudet L, Rosselet A, Schaller MD *et al.* Nonselective versus selective inhibition of inducible nitric oxide synthase in experimental endotoxic shock. *J Infect Dis* 1998; **177**: 127–132.
34. Lucas KA, Pitari GM, Kazerounian S *et al.* Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol Rev* 2000; **52**: 375–414.
35. Schlossmann J, Hofmann F. cGMP-dependent protein kinases in drug discovery. *Drug Discov Today* 2005; **10**: 627–634.
36. Murthy KS. Signaling for contraction and relaxation in smooth muscle of the gut. *Annu Rev Physiol* 2006; **68**: 345–374.
37. Bolotina VM, Najibi S, Palacino JJ *et al.* Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* 1994; **368**: 850–853.
38. Adachi T, Weisbrod RM, Pimentel DR *et al.* S-glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. *Nat Med* 2004; **10**: 1200–1207.
39. Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002; **82**: 131–185.
40. Wanstall JC, Homer KL, Doggrell SA. Evidence for, and importance of, cGMP-independent mechanisms with NO and NO donors on blood vessels and platelets. *Curr Vasc Pharmacol* 2005; **3**: 41–53.
41. Schrier RW, Wang W. Acute renal failure and sepsis. *N Engl J Med* 2004; **351**: 159–169.
42. Stuehr DJ, Marletta MA. Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. *Proc Natl Acad Sci USA* 1985; **82**: 7738–7742.
43. Stuehr DJ, Cho HJ, Kwon NS *et al.* Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein. *Proc Natl Acad Sci USA* 1991; **88**: 7773–7777.
44. Denis M. Human monocytes/macrophages: NO or no NO? *J Leukoc Biol* 1994; **55**: 682–684.
45. Albina JE. On the expression of nitric oxide synthase by human macrophages. Why no NO? *J Leukoc Biol* 1995; **58**: 643–649.
46. Tsukahara Y, Morisaki T, Horita Y *et al.* Expression of inducible nitric oxide synthase in circulating neutrophils of the systemic inflammatory response syndrome and septic patients. *World J Surg* 1998; **22**: 771–777.
47. Goode HF, Howdle PD, Walker BE *et al.* Nitric oxide synthase activity is increased in patients with sepsis syndrome. *Clin Sci (Lond)* 1995; **88**: 131–133.
48. Bultinck J, Sips P, Vakaet L *et al.* Systemic NO production during (septic) shock depends on parenchymal and not on hematopoietic cells: *in vivo* iNOS expression pattern in (septic) shock. *FASEB J* 2006; **20**: 2363–2365.
49. Hickey MJ, Sihota E, Amrani A *et al.* Inducible nitric oxide synthase (iNOS) in endotoxemia: chimeric mice reveal different cellular sources in various tissues. *FASEB J* 2002; **16**: 1141–1143.
50. Lundberg JO, Weitzberg E. NO generation from nitrite and its role in vascular control. *Arterioscler Thromb Vasc Biol* 2005; **25**: 915–922.
51. Stamler JS, Jaraki O, Osborne J *et al.* Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proc Natl Acad Sci USA* 1992; **89**: 7674–7677.

52. Stamler JS, Jia L, Eu JP *et al.* Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. *Science* 1997; **276**: 2034–2037.
53. Gladwin MT, Schechter AN. NO contest: nitrite versus S-nitroso-hemoglobin. *Circ Res* 2004; **94**: 851–855.
54. Yamashita T, Kawashima S, Ohashi Y *et al.* Resistance to endotoxin shock in transgenic mice overexpressing endothelial nitric oxide synthase. *Circulation* 2000; **101**: 931–937.
55. Ichinose F, Buys ES, Neilan TG *et al.* Cardiomyocyte-specific overexpression of nitric oxide synthase 3 prevents myocardial dysfunction in murine models of septic shock. *Circ Res* 2007; **100**: 130–139.
56. Chauhan SD, Seggara G, Vo PA *et al.* Protection against lipopolysaccharide-induced endothelial dysfunction in resistance and conduit vasculature of iNOS knockout mice. *FASEB J* 2003; **17**: 773–775.
57. Laubach VE, Foley PL, Shockey KS *et al.* Protective roles of nitric oxide and testosterone in endotoxemia: evidence from NOS-2-deficient mice. *Am J Physiol* 1998; **275**: H2211–H2218.
58. Nicholson SC, Grobmyer SR, Shiloh MU *et al.* Lethality of endotoxin in mice genetically deficient in the respiratory burst oxidase, inducible nitric oxide synthase, or both. *Shock* 1999; **11**: 253–258.
59. Cobb JP, Hotchkiss RS, Swanson PE *et al.* Inducible nitric oxide synthase (iNOS) gene deficiency increases the mortality of sepsis in mice. *Surgery* 1999; **126**: 438–442.
60. Cauwels A, Van Molle W, Janssen B *et al.* Protection against TNF-induced lethal shock by soluble guanylate cyclase inhibition requires functional inducible nitric oxide synthase. *Immunity* 2000; **13**: 223–231.
61. Cauwels A, Bultinck J, Brouckaert P. Dual role of endogenous nitric oxide in tumor necrosis factor shock: induced NO tempers oxidative stress. *Cell Mol Life Sci* 2005; **62**: 1632–1640.
62. Li CQ, Wogan GN. Nitric oxide as a modulator of apoptosis. *Cancer Lett* 2005; **226**: 1–15.
63. Cauwels A, Brouckaert P. Survival of TNF toxicity: dependence on caspases and NO. *Arch Biochem Biophys* 2007; **462** (doi:10.1016/j.abb.2007.1001.1021).
64. Rubbo H, Radi R, Trujillo M *et al.* Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 1994; **269**: 26066–26075.
65. Rubbo H, Radi R, Anselmi D *et al.* Nitric oxide reaction with lipid peroxyl radicals spares alpha-tocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha-tocopherol/ascorbate. *J Biol Chem* 2000; **275**: 10812–10818.
66. Afulukwe IF, Cohen RI, Zeballos GA *et al.* Selective NOS inhibition restores myocardial contractility in endotoxemic rats; however, myocardial NO content does not correlate with myocardial dysfunction. *Am J Respir Crit Care Med* 2000; **162**: 21–26.
67. Nilsson BO. Biological effects of aminoguanidine: an update. *Inflamm Res* 1999; **48**: 509–515.
68. Harbrecht BG. Therapeutic use of nitric oxide scavengers in shock and sepsis. *Curr Pharm Des* 2006; **12**: 3543–3549.
69. Yoshida M, Akaike T, Wada Y *et al.* Therapeutic effects of imidazolineoxyl N-oxide against endotoxin shock through its direct nitric oxide-scavenging activity. *Biochem Biophys Res Commun* 1994; **202**: 923–930.
70. Kaneda K, Yoshioka Y, Makita K *et al.* Effects of carboxy-PTIO on systemic hemodynamics, liver energetics, and concentration of liver metabolites during endotoxic shock in rabbits: a 31P and 1 H magnetic resonance spectroscopic study. *Crit Care Med* 1997; **25**: 1019–1029.
71. Bone HG, Fischer SR, Schenarts PJ *et al.* Continuous infusion of pyridoxalated hemoglobin polyoxyethylene conjugate in hyperdynamic septic sheep. *Shock* 1998; **10**: 69–76.
72. Dickinson E, Tuncer R, Nadler E *et al.* NOX, a novel nitric oxide scavenger, reduces bacterial translocation in rats after endotoxin challenge. *Am J Physiol* 1999; **277**: G1281–G1287.
73. Miura K, Yamanaka S, Ebara T *et al.* Effects of nitric oxide scavenger, carboxy-PTIO on endotoxin-induced alterations in systemic hemodynamics in rats. *Jpn J Pharmacol* 2000; **82**: 261–264.
74. Privalle C, Talarico T, Keng T *et al.* Pyridoxalated hemoglobin polyoxyethylene: a nitric oxide scavenger with antioxidant activity for the treatment of nitric oxide-induced shock. *Free Radic Biol Med* 2000; **28**: 1507–1517.
75. Kinasewitz G, Malcynski J, Steingrub J *et al.* Pyridoxalated hemoglobin polyoxyethylene (PHP) in distributive shock. *Crit Care Med* 2004; **32**(Suppl 12): A11.
76. Wu CC, Szabo C, Chen SJ *et al.* Activation of soluble guanylyl cyclase by a factor other than nitric oxide or carbon monoxide contributes to the vascular hyporeactivity to vasoconstrictor agents in the aorta of rats treated with endotoxin. *Biochem Biophys Res Commun* 1994; **201**: 436–442.
77. Wu CC, Chen SJ, Yen MH. Nitric oxide-independent activation of soluble guanylyl cyclase contributes to endotoxin shock in rats. *Am J Physiol* 1998; **275**: H1148–H1157.
78. Zhang H, Rogiers P, Preiser JC *et al.* Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock. *Crit Care Med* 1995; **23**: 1711–1721.
79. Evgenov OV, Sveinbjornsson B, Bjertnaes LJ. Continuously infused methylene blue modulates the early cardiopulmonary response to endotoxin in awake sheep. *Acta Anaesthesiol Scand* 2001; **45**: 1246–1254.
80. Schneider F, Lutun P, Hasselmann M *et al.* Methylene blue increases systemic vascular resistance in human septic shock. Preliminary observations. *Intensive Care Med* 1992; **18**: 309–311.
81. Preiser JC, Lejeune P, Roman A *et al.* Methylene blue administration in septic shock: a clinical trial. *Crit Care Med* 1995; **23**: 259–264.
82. Kirov MY, Evgenov OV, Evgenov NV *et al.* Infusion of methylene blue in human septic shock: a pilot, randomized, controlled study. *Crit Care Med* 2001; **29**: 1860–1867.
83. Donati A, Conti G, Loggi S *et al.* Does methylene blue administration to septic shock patients affect vascular permeability and blood volume? *Crit Care Med* 2002; **30**: 2271–2277.
84. Donati A, Preiser JC. Methylene blue: an old-timer or a compound ready for revival? *Crit Care Med* 2006; **34**: 2862–2863.
85. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. *Biochem Pharmacol* 1993; **45**: 367–374.
86. Salaris SC, Babbs CF, Voorhees III WD. Methylene blue as an inhibitor of superoxide generation by xanthine oxidase. A potential new drug for the attenuation of ischemia/reperfusion injury. *Biochem Pharmacol* 1991; **42**: 499–506.
87. Kelner MJ, Bagnell R, Hale B *et al.* Potential of methylene blue to block oxygen radical generation in reperfusion injury. *Basic Life Sci* 1988; **49**: 895–898.
88. Garthwaite J, Southam E, Boulton CL *et al.* Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol Pharmacol* 1995; **48**: 184–188.
89. Moro MA, Russel RJ, Cellek S *et al.* cGMP mediates the vascular and platelet actions of nitric oxide: confirmation using an inhibitor of the soluble guanylyl cyclase. *Proc Natl Acad Sci USA* 1996; **93**: 1480–1485.
90. Schrammel A, Behrends S, Schmidt K *et al.* Characterization of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one as a heme-site inhibitor of nitric oxide-sensitive guanylyl cyclase. *Mol Pharmacol* 1996; **50**: 1–5.
91. Feelisch M, Kotsonis P, Siebe J *et al.* The soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a] quinoxalin-1-one is a nonselective heme protein inhibitor of nitric oxide synthase and other cytochrome P-450 enzymes involved in nitric oxide donor bioactivation. *Mol Pharmacol* 1999; **56**: 243–253.
92. Zacharowski K, Berkels R, Olbrich A *et al.* The selective guanylate cyclase inhibitor ODQ reduces multiple organ injury in rodent models of Gram-positive and Gram-negative shock. *Crit Care Med* 2001; **29**: 1599–1608.
93. Preiser JC, Sun Q, Hadj-Sadok D *et al.* Differential effects of a selective inhibitor of soluble guanylyl cyclase on global and regional hemodynamics during canine endotoxic shock. *Shock* 2003; **20**: 465–468.
94. Waldron GJ, Cole WC. Activation of vascular smooth muscle K+ channels by endothelium-derived relaxing factors. *Clin Exp Pharmacol Physiol* 1999; **26**: 180–184.
95. Ahern GP, Klyachko VA, Jackson MB. cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO. *Trends Neurosci* 2002; **25**: 510–517.
96. Tanaka Y, Koike K, Toro L. MaxiK channel roles in blood vessel relaxations induced by endothelium-derived relaxing factors and their molecular mechanisms. *J Smooth Muscle Res* 2004; **40**: 125–153.
97. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. *N Engl J Med* 2001; **345**: 588–595.
98. Buckley JF, Singer M, Clapp LH. Role of KATP channels in sepsis. *Cardiovasc Res* 2006; **72**: 220–230.
99. Bhatia M. Hydrogen sulfide as a vasodilator. *IUBMB Life* 2005; **57**: 603–606.
100. Landry DW, Oliver JA. The ATP-sensitive K+ channel mediates hypotension in endotoxemia and hypoxic lactic acidosis in dog. *J Clin Invest* 1992; **89**: 2071–2074.
101. Wu CC, Thiemermann C, Vane JR. Glibenclamide-induced inhibition of the expression of inducible nitric oxide synthase in cultured

macrophages and in the anaesthetized rat. *Br J Pharmacol* 1995; **114**: 1273–1281.
102. Vanelli G, Hussain SN, Aguggini G. Glibenclamide, a blocker of ATP-sensitive potassium channels, reverses endotoxin-induced hypotension in pig. *Exp Physiol* 1995; **80**: 167–170.
103. Sorrentino R, d'Emmanuele di Villa Bianca R, Lippolis L *et al.* Involvement of ATP-sensitive potassium channels in a model of a delayed vascular hyporeactivity induced by lipopolysaccharide in rats. *Br J Pharmacol* 1999; **127**: 1447–1453.
104. da Silva-Santos JE, Terluk MR, Assreuy J. Differential involvement of guanylate cyclase and potassium channels in nitric oxide-induced hyporesponsiveness to phenylephrine in endotoxemic rats. *Shock* 2002; **17**: 70–76.
105. Warrillow S, Egi M, Bellomo R. Randomized, double-blind, placebo-controlled crossover pilot study of a potassium channel blocker in patients with septic shock. *Crit Care Med* 2006; **34**: 980–985.
106. Oliver JA, Landry DW. Potassium channels and septic shock. *Crit Care Med* 2006; **34**: 1255–1257.
107. Robertson BE, Schubert R, Hescheler J *et al.* cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. *Am J Physiol* 1993; **265**: C299–C303.
108. Archer SL, Huang JM, Hampl V *et al.* Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc Natl Acad Sci USA* 1994; **91**: 7583–7587.
109. Ellis A, Triggle CR. Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. *Can J Physiol Pharmacol* 2003; **81**: 1013–1028.
110. Archer SL, Gragasin FS, Wu X *et al.* Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation* 2003; **107**: 769–776.
111. Feletou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* 2006; **26**: 1215–1225.
112. Clayton NP, LeDuc BW, Kelly LJ. Effect of potassium channel and cytochrome P450 inhibition on transient hypotension and survival during lipopolysaccharide-induced endotoxic shock in the rat. *Pharmacology* 2005; **73**: 113–120.
113. Pickkers P, Dorresteijn MJ, Bouw MP *et al. In vivo* evidence for nitric oxide-mediated calcium-activated potassium-channel activation during human endotoxemia. *Circulation* 2006; **114**: 414–421.
114. Vergara C, Latorre R, Marrion NV *et al.* Calcium-activated potassium channels. *Curr Opin Neurobiol* 1998; **8**: 321–329.
115. Feletou M, Vanhoutte PM, Weston AH *et al.* EDHF and endothelial potassiun channels: IKCa and SKCa. *Br J Pharmacol* 2003; **140**: 225 (author reply 226).
116. Feletou M, Vanhoutte PM. EDHF: new therapeutic targets? *Pharmacol Res* 2004; **49**: 565–580.
117. Gluais P, Edwards G, Weston AH *et al.* Role of SK(Ca) and IK(Ca) in endothelium-dependent hyperpolarizations of the guinea-pig isolated carotid artery. *Br J Pharmacol* 2005; **144**: 477–485.
118. Scotland RS, Madhani M, Chauhan S *et al.* Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double-knockout mice: key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure *in vivo*. *Circulation* 2005; **111**: 796–803.
119. Pastor CM, Suter PM. Evidence that humans produce less nitric oxide than experimental animals in septic shock. *Crit Care Med* 1998; **26**: 1135.
120. Zwaveling JH, Maring JK, Moshage H *et al.* Role of nitric oxide in recombinant tumor necrosis factor-alpha-induced circulatory shock: a study in patients treated for cancer with isolated limb perfusion. *Crit Care Med* 1996; **24**: 1806–1810.
121. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 1997; **237**: 527–531.
122. Li L, Bhatia M, Zhu YZ *et al.* Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J* 2005; **19**: 1196–1198.
123. Collin M, Anuar FB, Murch O *et al.* Inhibition of endogenous hydrogen sulfide formation reduces the organ injury caused by endotoxemia. *Br J Pharmacol* 2005; **146**: 498–505.
124. Zhang H, Zhi L, Moore PK *et al.* Role of hydrogen sulfide in cecal ligation and puncture-induced sepsis in the mouse. *Am J Physiol Lung Cell Mol Physiol* 2006; **290**: L1193–L1201.
125. Cuzzocrea S, Riley DP, Caputi AP *et al.* Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacol Rev* 2001; **53**: 135–159.
126. Thiemermann C. Membrane-permeable radical scavengers (tempol) for shock, ischemia-reperfusion injury, and inflammation. *Crit Care Med* 2003; **31**: S76–84.
127. Victor VM, Rocha M, De la Fuente M. Immune cells: free radicals and antioxidants in sepsis. *Int Immunopharmacol* 2004; **4**: 327–347.
128. Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. *J Allergy Clin Immunol* 2002; **110**: 341–348.
129. Brown SG. Cardiovascular aspects of anaphylaxis: implications for treatment and diagnosis. *Curr Opin Allergy Clin Immunol* 2005; **5**: 359–364.
130. Amir S, English AM. An inhibitor of nitric oxide production, NG-nitro-L-arginine-methyl ester, improves survival in anaphylactic shock. *Eur J Pharmacol* 1991; **203**: 125–127.
131. Mitsuhata H, Saitoh J, Hasome N *et al.* Nitric oxide synthase inhibition is detrimental to cardiac function and promotes bronchospasm in anaphylaxis in rabbits. *Shock* 1995; **4**: 143–148.
132. Bellou A, Lambert H, Gillois P *et al.* Constitutive nitric oxide synthase inhibition combined with histamine and serotonin receptor blockade improves the initial ovalbumin-induced arterial hypotension but decreases the survival time in brown Norway rats anaphylactic shock. *Shock* 2003; **19**: 71–78.
133. Cauwels A, Janssen B, Buys E *et al.* Anaphylactic shock depends on PI3K and eNOS-derived NO. *J Clin Invest* 2006; **116**: 2244–2251.