Abstract

Sepsis is an infection-induced inflammatory syndrome responsible for ~10% of all deaths worldwide. While pathophysiological mechanisms remain to be fully unravelled, new insights and discoveries are yielding significant improvements in outcome, particularly in the high mortality conditions of shock and multi-organ failure. One potential target is the ATP-sensitive potassium (K\textsubscript{ATP}) channel, an ion channel critical to the cardiovascular stress response. Excessive activation of the vascular channel is now recognised as a major cause of hypotension and vascular hyporesponsiveness to catecholamines in septic shock. Some researchers advocate therapeutic blockade of these channels; however, outside the vasculature, channel opening may actually represent a protective mechanism against cellular damage. In this review we critically examine the role of the K\textsubscript{ATP} channel in sepsis.

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Keywords: K-ATP channel; Sepsis; Septic shock; Vascular hyporeactivity; Multi-organ failure; Mitochondria; Insulin

1. Pathophysiology of septic shock and multi-organ failure

Ill-defined genetic and cellular factors enable infectious insults to trigger an exaggerated and poorly regulated systemic inflammatory response. Components of the microorganism bind to membrane-bound and soluble proteins within plasma, activating white cells, endothelium, platelets and coagulation pathways (reviewed in Refs [1–3]). Binding of pathogen-associated molecular patterns (cell wall constituents, toxins, superantigens and bacterial DNA) to Toll-like receptors and other recognition receptors activate nuclear transcription factors such as NF-κB [2,3]. These upregulate expression of genes encoding for pro-inflammatory mediators such as the cytokines, tumor necrosis factor alpha (TNFα) and interleukins (IL-1 and IL-6) and enzymes like inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [3]. Stimulation of the arachidonic acid cascade within endothelial cells generates pro-inflammatory prostaglandins, thromboxanes and leukotrienes, while activated neutrophils produce and release large quantities of proteases, hydrogen peroxide, and reactive oxygen species [2,4]. The net result is increased vascular permeability, with enhanced passage of fluid, plasma proteins and activated neutrophils to extravascular compartments, microcoagulopathy and alterations in microvascular tone. Synergism between pro-inflammatory mediators and tissue hypoxia consequent to hypovolaemia, interstitial oedema and blood flow redistribution will result in decreased mitochondrial energy production and a metabolic shutdown which becomes clinically apparent as organ dysfunction [1,4].

Sepsis affects, to a greater or lesser degree, every major organ within the body, potentially leading to their failure. The most severe cardiovascular manifestation of septic shock, a condition of perfusion abnormalities in conjunction with hypotension unresponsive to adequate fluid resuscitation. This results from a combination of excessive vasodilatation, vascular hyporeactivity (i.e. decreased responsiveness to catecholamines) and variable degrees of
myocardial depression [1,2]. Mechanisms implicated in the development of vascular hyporeactivity include the nitric oxide (NO) pathway [5], excessive activation of potassium channels [6] and inappropriately low circulating levels of vasopressin [7]. Large dose catecholamine infusions can often be significantly reduced or even stopped after administering a NO synthase (NOS) inhibitor [5,8] or vasopressin [7,9]. Their actions are likely, at least in part, to result from closure of the vascular smooth muscle K\textsubscript{ATP} channel.

Table 1
Molecular structure, pharmacology and function of K\textsubscript{ATP} channels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Subtype</th>
<th>Function</th>
<th>Pathophysiology</th>
<th>Openers</th>
<th>Closers</th>
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</thead>
<tbody>
<tr>
<td>Brain (see Refs. [13,19,27] for review)</td>
<td>Kir6.2/SUR1 (neurones)</td>
<td>Neuronal activity</td>
<td>Ischaemic preconditioning/neuronal protection</td>
<td>Diazoxide</td>
<td>Glibenclamide Tolbutamide</td>
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<td></td>
<td>(Kir6.2/SUR2B) substantia nigra</td>
<td>Neurotransmitter release</td>
<td>Epilepsy and propagation of seizures</td>
<td>Insulin, leptin</td>
<td>Isoflurane</td>
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<td></td>
<td>(Kir6.1/SUR2) astrocytes</td>
<td>Hypoglycem-induced glucagon secretion</td>
<td></td>
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<td></td>
<td></td>
<td>Action potential shortening during metabolic stress</td>
<td></td>
<td>Nicorandil</td>
<td>Vasoactive relaxant and hypotension</td>
</tr>
<tr>
<td></td>
<td>*(Kir6.2/SUR2B) portal vein and mesenteric artery?</td>
<td>Regional tissue perfusion (e.g. coronary, cerebral and mesenteric circulation)</td>
<td>Ischaemic preconditioning/reactive hyperemia</td>
<td>Diazoxide</td>
<td>Vasopressin [33] Endothelin Angiotensin II</td>
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<td></td>
<td></td>
<td></td>
<td>Hyporeactivity and hypotension in sepsis and haemorrhagic shock [7]</td>
<td>Isoflurane</td>
<td></td>
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<tr>
<td>Smooth muscle (non-vascular) (see Ref. [13])</td>
<td>Kir6.2/SUR2B</td>
<td>Modulation of contractility in colon, bladder and airway smooth muscle</td>
<td>Unknown</td>
<td>Leveromakalim, Pinacidil</td>
<td>Glibenclamide</td>
</tr>
<tr>
<td></td>
<td>*(Kir6.1/SUR2B) urethra</td>
<td></td>
<td>Persistent hyperinsulimemia of infancy</td>
<td>Diazoxide, Leveromakalim, Pinacidil</td>
<td>Glibenclamide Tolbutamide Gliclazide</td>
</tr>
<tr>
<td>Pancreas (see Refs. [12,19] for review)</td>
<td>Kir6.2/SUR1</td>
<td>Insulin release</td>
<td>Persistent hyperinsulimemia of infancy</td>
<td>Diazoxide</td>
<td>Glibenclamide</td>
</tr>
<tr>
<td>Skeletal muscle (see Refs. [13,19] for review)</td>
<td>Kir6.2/SUR2A (Kir6.2/SUR1)</td>
<td>Glucose uptake</td>
<td>Protection against ischaemic reperfusion injury</td>
<td>Diazoxide</td>
<td>Glibenclamide</td>
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<td></td>
<td></td>
<td>Contractility during fatigue and recovery after fatigue</td>
<td>K\textsuperscript{+} channel syndrome [16]</td>
<td>Insulin resistance in sepsis?</td>
<td></td>
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<tr>
<td>Subcellular</td>
<td>Mitochondria MitoK\textsubscript{ATP} (see Refs. [13,36,92] for review)</td>
<td>Unknown</td>
<td>Metabolic regulation of mitochondrial membrane potential</td>
<td>Ischaemic preconditioning, Organ failure in sepsis</td>
<td>Glibenclamide 5-HD BMS-191095</td>
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</table>

References may be found in the text or as indicated. Potential subunit configuration or limited tissue expression is shown in brackets. *May underlie metabolically-sensitive channels in these tissues. †One of the few tissues to show K\textsubscript{N}\textsubscript{h}D-like current, but SUR1 may contribute.
2. KATP channel structure and function

2.1. What is a K+ channel?

Potassium (K+) channels are membrane-spanning proteins that selectively allow movement of K+ ions across cells through a water-filled permeation pathway (pore). A gating mechanism (driven by a voltage or ligand binding) switches the channel between open and closed conformations. Normally channel opening at the plasma membrane promotes K+ loss from the cell, resulting in membrane hyperpolarisation, while in mitochondrial membranes this produces depolarisation. Key roles for K+ channels include maintenance of cellular resting membrane potential, regulation of neuronal excitability, neurotransmitter release, control of heart rate and smooth muscle tone, hormone secretion and epithelial electrolyte transport. Several hundred genes encoding for K+ channels have been identified and these broadly fall into three families; voltage-gated (including Kv and calcium-activated), inward rectifiers (Kir1-7.0) and twin pore K+ channels [10].

2.2. Basic channel characteristics

The KATP channel is an ubiquitously expressed protein (Table 1) with the particular characteristic of being blocked by intracellular ATP. In most tissues, inhibition by ATP occurs at micromolar concentrations but as cells usually contain millimolar ATP, channel openings will be rare. In contrast, nucleotide diphosphates (NDPs) such as adenosine diphosphate (ADP) activate the channel. Thus the ATP/NDP ratio is one of the major factors determining absolute channel activity [11-13]. These channel characteristics enable energy status within the cell to be directly linked to changes in membrane potential. During severe metabolic stress, ATP levels fall and NDP levels rise, thereby promoting substantial channel opening under these conditions. However, KATP channels do not simply respond to cell metabolism; other factors, including kinases, phosphatases, G-proteins and phospholipids regulate basal channel activity (Fig. 1). KATP channels can also be regulated both in vitro [14,15] and in vivo [16] through the Ca2+-dependent phosphatase, calcineurin, which indirectly regulates channel activity through an interaction with cyclic AMP-dependent protein kinase (PKA) [15]. This allows the channel to sense changes in intracellular Ca2+, being activated at resting levels and inhibited by high micromolar levels [14].

2.3. Channel structure

The KATP channel is a complex of at least two proteins: (i) the sulphonylurea receptor (SUR), and (ii) a pore-forming subunit belonging to the Kir6.0 family [11-13]. The stoichiometry consists of a tetramer of Kir6.0 subunits, which form the K+ selective pore-forming subunit, surrounded by four SUR proteins. These subunits have distinct functions. The SUR has high affinity binding sites for both sulphonylureas and K+ channel openers (KCOs) and has nucleotide (e.g. ADP) binding sites [11,12]. On the other hand, the pore-forming subunit controls the magnitude of K+ flow through the channel and is the site to which ATP binds to inhibit the channel. In addition, it possess low affinity binding sites for sulphonylureas and other types of channel inhibitors [11]. However, differing combinations of pore-forming and SUR subunits confer tissue-specific characteristics of the channel in the plasma membrane; the identity of KATP (mitoKATP) channels found in the inner mitochondrial membrane of different cell types is unknown (Table 1). Of note, the vascular smooth muscle KATP channel (synonymous with the KNDP channel) differs from the stereotypical KATP channel by being relatively insensitive to ATP. However, it is still activated by NDPs and inhibited by the sulphonylurea agent, glibenclamide (known in North America as glyburide) [13]. Importantly, key metabolically active proteins, including glycolytic enzymes involved in ATP production, exist in a macromolecular complex with the KATP channel and appear crucial in maintaining a high ATP/ADP ratio in the vicinity of the channel [17,18].

2.4. Physiological roles

The best characterised role of the KATP channel is to control insulin release from pancreatic β cells. During fasting, KATP channels are kept open by a low blood glucose (2–4 mmol/l). After a meal, glucose levels increase, causing intracellular ATP to rise, resulting in KATP channel closure and depolarization of the β cell membrane. This, in turn, opens voltage-gated Ca2+ channels, allowing Ca2+ to enter the cell. This triggers exocytosis of insulin-containing granules, thereby stimulating insulin release (reviewed in Ref. [12]).

KATP channels located in the hypothalamus are also involved in regulating glucose homeostasis. Glucose-sensing neurones in the ventromedial hypothalamus respond to

Fig. 1. Basic characteristics of KATP channels. Diagram illustrating factors that regulate the channel. PIP2: phosphatidylinositol-4,5-bisphosphate, KCOs: potassium channel openers, PKA: protein kinase A, Kir: inward rectifier subunit, SUR: sulphonylurea receptor subunit.
hyperglycaemia by increasing their firing rate, reducing glucagon secretion and thus lowering glucose [12,19]. Such regulation is mimicked by sulphonylureas and is impaired in Kir6.2 knockout mice, strongly suggesting a link between K\textsubscript{ATP} channels and the hypothalamic glucose-sensing mechanism, akin to that seen in pancreatic β cells [12]. K\textsubscript{ATP} channels also regulate glucose uptake in skeletal muscle; this mechanism is proposed to underlie glibenclamide-stimulated glucose uptake [12,13]. Thus K\textsubscript{ATP} channels from different tissues are involved in controlling glucose homeostasis in a co-ordinated manner [19].

In vascular smooth muscle, K\textsubscript{ATP} channels contribute to the maintenance of resting membrane potential and local blood flow [13,20]. Such evidence is based upon observing significant effects of glibenclamide on vascular resistance, blood vessel diameter and membrane potential (Table 1). K\textsubscript{ATP} channels influence vascular tone because of the steep relationship between membrane potential and Ca\textsuperscript{2+} influx. A small depolarisation (3 mV) can generate large (two-fold) changes in Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels [20]. This, in turn, promotes contraction of the vascular smooth muscle cell. The extent to which K\textsubscript{ATP} channels regulate systemic blood pressure under physiological conditions is unclear. Glibenclamide had little effect on blood pressure in normal animals [21–23], though the vascular selective inhibitor, PNU-37883A was able to elevate blood pressure, albeit more in dogs than rats [24]. These differences could reflect glibenclamide’s ability to block all K\textsubscript{ATP} channel subtypes in vivo but also oppose the circulating effects of thromboxane, being an antagonist of its receptor [25]. Consistent with a role for this channel in regulating blood pressure, SUR2 was reported to be a susceptibility gene for essential hypertension in humans [26] and mice lacking SUR2 develop hypertension and coronary vasospasm [12]. Hypertension could also potentially result from effects outside the vasculature, since both SUR2B and the Kir6.1 subunit are widely expressed throughout the body [27].

2.5. Hormonal regulation

It is well established that vasoactive substances modulate K\textsubscript{ATP} channel activity (Table 1 and Fig. 2). Vasodilators elevating cyclic AMP, including calcitonin gene-related peptide (CGRP), adenosine and prostacyclin, activate K\textsubscript{ATP} currents and produce glibenclamide-sensitive relaxation and hypotension [20,28]. Channel activation involves PKA [13,20] and the co-ordinated phosphorylation of sites located on both SUR2B and Kir6.1 [29]. Agents that increase cyclic GMP can also activate K\textsubscript{ATP} channels in vascular smooth muscle (reviewed in Ref. [20]) and cardiomyocytes [30], which can involve cyclic GMP-dependent kinase [30] or Ras and mitogen-activated protein kinase kinase [31]. However, their contribution to NO-mediated vasodilatation is minimal under physiological conditions [20,21,23,25,32]. By contrast, vasoconstrictors (Fig. 2) such as vasopressin endothelin and angiotensin II inhibit K\textsubscript{ATP} currents in isolated myocytes via protein kinase C activation and increased ATP synthesis [13,20,33–35].

2.6. Role in pathophysiology

K\textsubscript{ATP} channels are strongly implicated in mediating systemic vasodilatation during tissue hypoxia [21], increasing blood flow to heart, brain and muscle [13,20]. The mechanism of channel activation involves hypoxia-induced release of vasodilators such as NO or adenosine, as well as a direct effect of hypoxia, either via an oxygen sensor and/or changes in cell
metabolism [13,20]. Excessive opening will significantly in-
crease plasma K⁺, as seen during strenuous exercise. Indeed,
we have described a ‘K⁺ channel syndrome’ where phar-
macological opening of the channel in the presence of critical
illness gave rise to life-threatening hyperkalaemia, which was
successfully treated with glibenclamide [16].

K<sub>ATP</sub> channels within the brain and heart are also strongly
implemented in the phenomenon of ischaemic precondition-
ing, whereby sublethal ischaemia protects against a subsequent
and otherwise lethal insult (reviewed in Refs. [12,13,36]).
Such a role is reinforced by the observation that message and
protein levels for Kir6.1 markedly increase during prolonged
myocardial ischaemia [37] or post-hypoxia [38]. A similar
protective effect can also be achieved with low-dose endotoxin
(lipopolysaccharide) [39,40]. Protection is thought to be
afforded, at least in part, by action potential shortening and/
or a decreased firing rate, resulting in reduced Ca<sup>2+</sup> overload
and a lower energy demand [12,36].

3. K<sub>ATP</sub> channels and sepsis

3.1. In vivo studies

In vivo evidence for K<sub>ATP</sub> channel involvement in sepsis
comes mainly from anaesthetised, endotoxic rat [23,41], dog
[21] and pig [22,42] models. Immediate restoration of blood
pressure can be achieved with glibenclamide, resulting largely
from an increase in systemic vascular resistance rather than an
effect on cardiac output [21,42]. Glibenclamide also increased
vaspressor responses to α<sub>1</sub>-adrenoceptor agonists following
either short (3 h) [41] or long-term (24 h) [23] exposure to
endotoxin. Since glibenclamide had no effect in control
animals, this strongly suggests that K<sub>ATP</sub> channels preferen-
tially open during sepsis and are an important underlying cause
of hypotension and vascular hyporeactivity. This might also
explain the increased responsiveness to pharmacological
channel activators following endotoxin treatment [23,43].
Significantly, potentiation of K<sub>ATP</sub> channel function, vascular
hyporeactivity and the pressor effect of glibenclamide could be
prevented by pre-treatment with the corticosteroid, dexameth-
ason [43]. This may be related to dexamethasone either inhibiting
channel subunit expression and/or synthesis of a mediator regulating expression. Indeed, both mRNA and protein
levels for Kir6.1 were substantially increased in the
diaphragm of endotoxin-treated rats, with levels peaking over
24–48 h [44]. Similarly, Kir6.1 gene expression was up-
regulated 22-fold in experimental colitis [45]. Thus increased
K<sub>ATP</sub> channel expression is a common feature of metabolic
stress, though whether this is beneficial or detrimental in sepsis
remains to be determined. Based on animal studies one could
hypothesise that a reduction in channel expression may underlie the clinical benefit of glucocorticoid agents demon-
strated in septic patients [46].

The involvement of K<sub>ATP</sub> channels in the vascular
disturbances associated with sepsis was questioned in a
recent placebo-controlled study in septic shock patients
where glibenclamide had no effect on blood pressure or
norepinephrine requirements [47]. Although at first sight,
this would appear at odds with the animal literature, it
probably reflects the failure of SUR inhibitors at standard
therapeutic doses to adequately block the vascular channel,
particularly when opened during metabolic stress (see later).

3.2. Organ bath studies

Vascular hyporeactivity can also be demonstrated using in vitro organ bath models [6,23,32,41,48]. Here, arterial
rings (± an intact endothelium) are taken from blood vessels
of either septic/endotoxic animals (ex-vivo) or from healthy
animals which are incubated with endotoxin (in vitro).
Decreased contractility is readily shown as a flatter dose-
response curve to catecholamine vasoconstrictors, where
two potency and maximal tension are decreased (e.g. [48]).
The maintained ability of endotoxin to induce in vitro
vascular hyporeactivity in vessels without endothelium is not
surprising given that iNOS is expressed in all layers within
the blood vessel under these conditions [49,50].

Abnormal opening of K<sub>ATP</sub> channels and, to a lesser extent,
large conductance Ca<sup>2+</sup>-activated K⁺ (BK<sub>Ca</sub>) channels, is
responsible for the increase in membrane hyperpolarization
reported in mesenteric artery and aorta taken from rats ex-
posed to endotoxin for up to 6 h [51–53]. At the same
timepoint, drugs that close K<sub>ATP</sub> channels via pore inhibition
(PNU-37883A and barium) [24,54] almost fully restored ten-
sion to the α<sub>1</sub>-adrenoceptor agonist, phenylephrine [25].
Less reversal was achieved at 20 h, suggesting additional meche-
nisms underlying hyporeactivity and/or irreversible damage
to the contractile machinery. Likewise, relaxations to KCOs are
potentiated in both in vitro and ex-vivo models of endotoxin-
induced hyporeactivity [23,32,51], indicative of up-regulation
of K<sub>ATP</sub> channel function in vascular smooth muscle.

A somewhat surprising but consistent finding in in vitro
models is the lack of effect of SUR inhibitors on vascular
hyporeactivity and in some cases, contractions were even
further reduced [23,25,41,55]. An intriguing possibility is
that endotoxin alters K<sub>ATP</sub> channel pharmacology such that
channel inhibition via the SUR becomes dysfunctional. We
previously found that SUR inhibitors become significantly
less effective at inhibiting leveromakalim-or iNOS-induced
vascular relaxation in the presence of endotoxin [32]. In
contrast, the vascular selective pore inhibitor, PNU-37883A
remained effective. Perhaps less intuitive is why glibenclam-
ide is efficacious in in vivo animal studies. This may relate
to the much higher dosages used (10 to 100-fold compared to
in vitro studies), at which concentration glibenclamide may
be also acting on the pore-forming subunit [11]. Moreover,
these in vivo doses (10–40 mg/kg) are far in excess of those
given to humans (upper limit ~0.25 mg/kg) but within the
dose-range necessary to block the hypotensive effects of
pinacidil in rats [24]. Thus, the dose of glibenclamide
required to effectively block vascular K<sub>ATP</sub> channels would
probably never be given to patients.
4. What influences $K_{\text{ATP}}$ channel activation and function in sepsis?

4.1. Nitric oxide/cyclic GMP pathway

Endotoxin-induced hypotension and vascular hyporeactivity are largely a consequence of over-production of iNOS-derived NO within the blood vessel wall [5,49]. Increased production of NO, albeit at much lower levels, is found in septic patients [5,49] and is reported to be inversely correlated with mean arterial pressure and systemic vascular resistance [56]. Compared to animals, induction of iNOS appears restricted in humans, occurring mainly in blood vessels at the very point of infection [56] or in neutrophils [57]. Taken together, this suggests NO derived from iNOS may not be the only source of NO in human sepsis [49]. However, a marked pressor response was still achieved in septic shock patients receiving a non-specific NOS inhibitor [8], suggestive of increased sensitivity to NO in humans. Once formed, NO can activate soluble guanylate cyclase and increase cyclic GMP, or can react with superoxide to form peroxynitrite, a process that occurs more readily during systemic inflammation [4]. Peroxynitrite is more reactive than NO, capable of nitrating tyrosine residues and reacting with thiols and iron-containing proteins, sometimes irreversibly.

In the short term (<6 h), the NO/cyclic GMP pathway is likely to account for $K_{\text{ATP}}$ channel activation. This is based on the observation that NOS or guanylate cyclase inhibitors fully reversed endotoxin-induced hyporeactivity or membrane hyperpolarisation, and that $K_{\text{ATP}}$ channel blockers had effects of near-similar magnitude [25,32,48,53]. An early indication that NO may activate $K_{\text{ATP}}$ channels in sepsis was the finding that extracellular L-arginine, which is required for continued cellular NO production by iNOS, caused persistent activation of $K_{\text{ATP}}$ channels in endotoxin-treated (24 h) but not control vascular smooth muscle cells [58]. Interestingly, persistent channel activation could not be mimicked by exogenous NO, strongly suggesting that the iNOS pathway yields additional or more stable factors that work in concert with NO. Likewise, the involvement of $K_{\text{ATP}}$ channels in NO-mediated relaxation requires prior endotoxin treatment [32] or the formation of peroxynitrite, which itself produces glibenclamide-sensitive relaxation in blood vessels [59,60]. In addition, prolonged NO and/or superoxide generation will inhibit calcineurin activity [61], the net effect might be to hyperphosphorylate the $K_{\text{ATP}}$ channel and cause substantial opening, even at normal ATP levels [14].

4.2. Calcitonin gene related peptide

This 37 amino acid peptide contained within sensory nerve endings acts as a neurotransmitter in vasodilator nerves scattered throughout most arterial beds. CGRP has been implicated in the pathophysiology of septic shock with plasma levels substantially rising in human and animal forms of shock, which correlated with disease severity [62,63]. In addition, administration of a receptor antagonist (hCGRP) transiently reversed tachycardia and hypotension in endotoxin-treated rats [64]. As mentioned previously, CGRP activates vascular $K_{\text{ATP}}$ channels through PKA-mediated phosphorylation [13,20]. This has physiological relevance since glibenclamide partially reverses relaxation of a variety of blood vessels exposed to CGRP both in vivo and in vitro [20,28]. There may also be contributions from other neurohormal activators of $K_{\text{ATP}}$ channels, including atrial natriuretic peptide and adenosine [20], which also rise in septic patients [65,66].

4.3. The actin cytoskeleton: more than scaffolding for $K_{\text{ATP}}$ channels?

The actin cytoskeleton consists of the protein actin, which exists as polymerised (F) or unpolymerized globular...
(G) actin and together represents ~50% of total protein content within the cell [67]. Both F and G actin are in a dynamic, energy-dependent flux and have been implicated in the control of contractility, cell motility and various ion channels [67,68], including the K\textsubscript{ATP} channel. In vascular muscle, actin cytoskeletal organization via the GTPase, RhoA constitutes a major pathway for sustaining contractions [68,69]. Since cyclic GMP inhibits RhoA, this pathway could become downregulated in prolonged sepsis and contribute to irreversible hypococontractility.

Actin depolymerisation is known to increase K\textsubscript{ATP} channel opening in isolated cardiac myocytes and attenuate the inhibitory action of sulphonylureas [70,71]. It also abolishes high-affinity glibenclamide binding to the SUR in vascular smooth muscle [72,73]. Likewise, metabolic stress reduces SUR binding, probably through Mg\textsuperscript{2+}, ADP [13]. This pattern is remarkably similar to that seen with the SUR inhibitor, glibenclamide in both in vitro [25,32] and in vivo [74] sepsis, but not with the specific pore blocker, PNU-37883A. Such a mechanism may help to explain the differences observed between K\textsubscript{ATP} channel inhibitors having different sites of action (SUR versus pore) (Fig. 3). Cytoskeletal disruption in sepsis may occur through a decrease in energy status (see later), as normal assembly and maintenance of the actin cytoskeleton is reliant upon polymerization, an ATP-dependent process [67]. NO, which inhibits mitochondrial respiration, disrupts the actin cytoskeleton in various cell types, including kidney, liver and lamina propria of the small intestine [75–77], through either nitration [75,77], nitrosylation [78] or inhibition of RhoA kinase [69]. The actin cytoskeleton is also disrupted in bovine aortic and pulmonary vascular endothelial cells following endotoxin or TNF\alpha treatment [79,80], though whether this occurs in smooth muscle remains unknown.

4.4. Changes in energy status

K\textsubscript{ATP} channels may be opened by a fall in [ATP], pH or oxygen tension, or by a rise in intracellular lactate and NDPs [6,7]. These may facilitate NO activation of the channel or act independently. Many of these factors operate in sepsis though the extent to which these play a role will depend on the degree of perturbation of the individual variable as well as severity and duration of the septic insult. Lactic acidosis is consistently reported in animal and human sepsis (e.g. [21,81]) and probably results from increased activity of skeletal muscle Na\textsuperscript{+}/K\textsuperscript{−} ATPase rather than tissue hypoxia [81]. Likewise, raised tissue oxygen tensions are reported, for example, in skeletal muscle [82] and bladder epithelium [83] during sepsis. This finding, in conjunction with decreased global oxygen consumption, suggests adequate availability but decreased cellular utilisation of oxygen, giving rise to a state of ‘cytopathic dysoxia’ related to mitochondrial dysfunction, increased generation of NO and decreased ATP production [4,84]. The ensuing ‘energy failure’ is implicated in the pathophysiology of organ failure. Of note, ATP levels are preserved in the skeletal muscle of eventual survivors of septic shock but fall in non-surviving patients [85]. This fall in ATP has been replicated in other tissues in various animal models [86,87]. Thus, opening of K\textsubscript{ATP} channels due to metabolic failure may only be significant in severe sepsis although, in vascular smooth muscle, this is likely to depend to a greater extent on the change in NDP levels because of the main subtype (SUR2B/Kir6.1) expressed in this tissue [13].

4.5. The mitoK\textsubscript{ATP} channel and organ dysfunction

In patients and animal models of sepsis, mitochondria are swollen and distorted, with disruption of the matrix [88–90]. These changes are associated with a decrease in cellular oxidative capacity [90]. Matrix volume is considered an important regulator of mitochondrial metabolism [91]. Under physiological conditions, continual K\textsuperscript{+} influx across the inner membrane is accompanied by the passage of anions and water. To maintain mitochondrial integrity, movement of K\textsuperscript{+} out of the matrix is achieved through the K\textsuperscript{+}/H\textsuperscript{+} exchanger. MitoK\textsubscript{ATP} channels are thought to contribute to this mitochondrial K\textsuperscript{+} influx (reviewed in Refs. [36,92]). Observations that KCOs increase matrix volume in a K\textsuperscript{+}-dependent fashion, while glibenclamide and ATP inhibit swelling, support this view [92,93]. Increases in matrix volume can protect against ischaemic damage by preserving structure and function of the intermembrane space and the low permeability of the outer membrane to ADP and ATP [91]. However excessive, uncompensated mitoK\textsubscript{ATP} opening may cause significant depolarisation with rupture of the outer membrane, opening of the mitochondrial permeability transition pore, loss of cytochrome C [91,93] and triggering of cell death pathways. However, a lesser degree of membrane depolarisation may afford protection from cell damage and death by reducing ROS production, thereby preventing mitochondrial Ca\textsuperscript{2+} overload and opening of the transition pore [92].

Evidence suggests that mitoK\textsubscript{ATP} channels may offer protection against apoptosis [92,94], possibly by acting as an upstream antagonist of p53 [95]. This could explain why apoptosis is not a widespread phenomenon in sepsis and appears limited to gut epithelia, lymphocytes, splenocytes and neurons in the cardiovascular autonomic centres [2,96]. The extent to which these mitoK\textsubscript{ATP} channel-related mechanisms operate in sepsis is unknown. Progress is hindered by the unknown molecular identity of these channels as well as the likely contribution from plasma K\textsubscript{ATP} channels to cellular protection [36].

4.6. Glucose homeostasis and insulin

During sepsis, disordered cell metabolism undoubtedly contributes to the development of MOF. Hyperglycaemia associated with insulin resistance is a common feature [2]. Tight control of glucose levels with additional infusion of insulin and glucose reduces mortality and morbidity in
Critically ill surgical [97] and medical ICU patients [98]. Regulation of blood sugar has been proposed to involve a reciprocal interaction between pancreatic beta cells and the ventromedial hypothalamus, with K_{ATP} channel activity controlling both insulin and glucagon secretion [19]. Thus, a consequence of excessive channel activation would result in high circulating levels of glucagon, decreased insulin release, and reduced glucose uptake in skeletal muscle, all of which will contribute to insulin resistance in sepsis.

Persistent hyperglycaemia has a number of toxic effects, promoting inflammation, oxidative stress and apoptosis [99]. In the context of ischaemic heart failure, many of these processes can be reversed by insulin treatment [99]. This is independent of blood glucose concentration but involves actin cytoskeleton-dependent activation of K_{ATP} channels [100,101]. Improved outcomes in critical illness with ‘tight glycaemic control’ appear due to a combination of prevention of direct glucose toxicity and insulin-independent effects on glucose control [102]. Of note, intensive insulin therapy in critically ill patients preserved hepatic mitochondrial ultrastructure and respiratory function [90].

4.7. K^{+} channels as key regulators of immune function

Circulating neutrophils play a pivotal role in the killing of a range of pathogens. In human neutrophils, activation of the BK_{Ca} channel is an absolute requirement for microbial killing, which functions to provide a potassium-rich environment necessary for activation of proteases within the phagocytic vacuole [103]. In macrophages, endotoxin-induced cytokine release [104–106] and NO production [41,107] needs prior activation of a number of K^{+} channels, including BK_{Ca}, Kv and K_{ATP} channels. This released NO has an important cytotoxic function in the killing of microorganisms. Thus K^{+} channels appear to play an essential role in the early activation of immune cells.

4.8. Clinical implications and summary

Sepsis still carries a high mortality rate and a comprehensive understanding of the underlying pathophysiology remains elusive. The significance of many mechanisms elucidated in laboratory models remains to be confirmed in patients. Animal studies are usually reductionist in nature, employ very aggressive insults and are of short duration. They may not be representative of a long-term, fluid-resuscitated, anaesthetised patient, often presenting with comorbidities, who is receiving multiple pharmacological and mechanical organ support.

The above caveats certainly apply to the K_{ATP} channel. Excessive activation of the K_{ATP} channel is clearly implicated in a number of crucial mechanisms in sepsis, including vascular hyporeactivity and insulin resistance. However, channel opening may afford a degree of cellular protection. Arguably, the balance between good and detrimental effects will depend critically on concurrent energy status, as low ATP and high NDP levels will induce greater channel opening, with the consequences of decreased insulin release and subsequent hyperglycaemia, disruption of the mitochondrial matrix with a further reduction in energy production, leading to profound, if not irreversible, vascular hyporesponsiveness (Fig. 4).

Therapeutic intervention with channel inhibitors still remains an attractive option, though targeting of the vasculature with specific pore rather than SUR inhibitors would appear more appropriate in sepsis. Clearly further research is needed.

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**Fig. 4.** Cartoon illustrating potential mechanisms by which endotoxin opens sarcolemmal or mitoK_{ATP} channels. Depending on the severity of sepsis, channel opening can lead to cellular protection or organ failure.
needed to better delineate the protective and harmful roles of the K\textsubscript{ATP} channel in sepsis. This may lead to development of tissue-specific, targeted modulators of channel function, which may benefit not only the septic patient, but also other pathological states.

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References


