large conductance calcium-activated potassium channels (BKCa) are abundantly expressed in smooth muscle cells (SMCs) lining the blood vessel wall. They are composed of an α-subunit (Slo) and a modulatory β1-subunit, which serves to maintain the normal high voltage- and Ca2+-sensitivity of the pore-forming α-subunit (reviewed in 1–2). In the vasculature, BKCa operate by limiting Ca2+ entry and arterial contraction by repolarizing SMCs and closing voltage-dependent Ca2+ channels previously opened by pressure or vasoconstrictor agents.1 BKCa can also mediate cellular hyperpolarization and vasorelaxation as a result of spontaneous transient outward currents (STOCs) activated by the localized release of micromolar concentrations of Ca2+ (Ca2+ sparks) from ryanodine receptors located in the sarcoplasmic reticulum (SR).1 Moreover, increased frequency of Ca2+ sparks may underlie activation of BKCa by endogenous vasodilators,1 though other mechanisms undoubtedly contribute.2,3 Genetic experiments also highlight BKCa as important regulators of vascular tone and blood pressure. Deletion of the α-subunit in mice results in membrane depolarization, a complete lack of STOCs, and attenuates cGMP relaxation in isolated blood vessels.4 On the other hand, deletion of β1 impairs the coupling of Ca2+ sparks to the activation of hyperpolarizing BKCa currents and enhances agonist-induced vasoconstriction without affecting nitric oxide (NO) mediated vasorelaxation.5 Knockout of both genes leads to systemic hypertension, though in BKCa β1−/− mice this is more pronounced6,7 suggesting physical interactions of the β1-subunit with other proteins, possibly other ion-conducting pores.5 Moreover, depending on the hypertensive model, β1-subunit expression can either increase6,8 or decrease.7 The latter might argue that β1 acts as a compensatory mechanism to limit development of hypertension. Consistent with this, a gain-of-function mutation in the human β1-subunit gene (KCNCMB1), involving an increase in the apparent Ca2+ and voltage sensitivity of the channel, protected patients against diastolic hypertension.8 Thus, while BKCa dynamically regulate vascular tone and blood pressure, the precise role/function of the pore-forming and β1 subunit requires further evaluation.

Role of BKCa in Shock

Shock is a condition of profound hemodynamic and metabolic disturbance characterized by failure of the circulatory system to maintain adequate perfusion of vital organs.9 This is largely attributable to the failure of blood vessels to constrict to catecholamines resulting in excessive vasodilatation. Several studies conclude that BKCa mediate, at least in part, SMC membrane hyperpolarization and vascular hyper-reactivity in experimental models of hemorrhagic and endotoxic shock.9–12 The mechanism of activation is largely unknown but probably involves NO9,10 which can phosphorylate the α-subunit of BKCa through cGMP-dependent protein kinase (PKG)2 or tyrosine protein kinase.13,14 In this issue, Zhao and colleagues investigate the mechanism by which BKCa are enhanced in acute hemorrhagic shock (HS).15 They tested the hypothesis that increased β1-subunit expression is responsible for enhanced coupling of Ca2+ sparks to BKCa and this contributes to vascular hyperreactivity and hypotension in HS.

Enhanced STOC Activity in HS

A number of interesting and related observations were made. First the authors show that STOC activity at depolarized potentials was enhanced in terms of amplitude, duration, and charge transfer in isolated mesenteric arterial SMCs (ASMCs) from HS rats. Frequency of Ca2+ sparks remained unchanged whereas mean amplitude was increased. In examining the relationship between current and Ca2+ spark amplitude (Figure 2C), the authors convincingly showed that Ca2+ spark-STOC coupling efficiency increased as did the Ca2+ sensitivity of BKCa at micromolar Ca2+ levels in isolated patches. This latter result is expected, given that high Ca2+ promotes a functional interaction between α- and β1-subunits.16,17 Coupled to Western blotting and immunostaining experiments showing β1 but not the α-subunit protein was elevated in HS, are all observations entirely consistent with their hypothesis. Another prediction is that the activation/deactivation kinetics of BKCa would be slowed if β1-subunit levels had a functional impact on the number of channels expressing both subunits at the membrane.16–18 This was indeed shown for STOC activation at depolarized potentials (Figure 1B).

Whether similar effects occur at more physiological resting potentials (ie, ≈−40 mV) and can account for enhanced depolarizing and contractile effects of iberiotoxin in HS remains to be demonstrated. These are important considerations given that Ca2+ spark frequency was actually decreased in resting SMCs in HS and associated with only a marginal increase in amplitude. Without these crucial experiments it remains unclear whether in resting cells there would be
significant increases either in the charge transfer of a single 
STOC or spontaneous transient hyperpolarizations, produced 
by STOCs and readily recorded in single cells in current 
clamp mode.4 Also the mechanism underlying enhanced 
spark amplitude is intriguing. That the Ca²⁺ load of the SR 
was not altered, and deletion of α or β1 had no effect on the 
kinetics of Ca²⁺ sparks³,⁴ is indicative of a long-term alter-
ation in ryanodine receptor function induced by HS. Conceiv-
able, reactive oxygen species, which are good activators of 
Ca²⁺ release from these channels, may contribute.

How the β1-subunit enhances the apparent Ca²⁺ sensitivity 
of BKCa is not well understood, although most studies 
conclude that it does not intrinsically alter Ca²⁺ binding.¹⁷–¹⁹
Single channel analysis have shown that the main effect of β1 
is to increase the length of time that BKCa spends in bursting 
states, an effect preserved over a wide range of voltages or 
Ca²⁺ concentrations (1 nmol/L to 10 μmol/L).¹⁸,¹⁹ The other 
less significant consequence of β1 expression is to increase 
the gap between bursts, an effect observed at low Ca²⁺ but 
negated at higher levels. Based on these results, it has been 
proposed that β1-subunits act to shift the equilibrium for 
voltage-sensor activation to more negative potentials as well 
as to reduce the work that Ca²⁺ binding must do to open the 
channel. From the initial inspection of their data, both types 
of kinetic changes appear to occur (Figure 4E), though 
increased burst duration at low Ca²⁺ was not evident. Thus a 
detailed analysis of dwell-time distributions is required to 
demonstrate that these β1-related changes in channel kinetics 
are taking place. Such analysis has already been performed in 
mesenteric SMCs isolated from rats with HS.¹¹ In these 
studies, enhanced BKCa activity was characterized by a 
dramatic decrease in mean close times and the slow close 
time constant, effects not attributable to β1-subunit func-
tion.¹⁸ These changes were best described by α-subunit 
modulation, most likely through NO-mediated sulphydryl 
activation of cSrc leading to phosphorylation of the Tyr766 
residue in the C terminus.¹¹,¹⁴ Interestingly, this form of 
channel modification leads to enhanced Ca²⁺ sensitivity of 
BKCa.¹⁴ This is also in keeping with genetic data showing the 
major downstream target for NO is the α-subunit.³,⁴ How-
ever, one cannot rule out that small changes in β1-subunit 
expression could facilitate the opening of the pore and either 
mask or mimic channel effects predicted by β1 alone. For 
example, like β1, methionine oxidation of hSlo1 slows 
deactivation kinetics, an effect dramatically potentiated by 
coexpressing β1.²⁰ Nevertheless, β1 was reported to confer a 
novel outcome of oxidation not observed with hSlo1 alone, 
amely a distinct acceleration of current activation. Together 
these effects shifted channel activation within the physiolog-
ical range of membrane potential even in the absence of Ca²⁺.

Contrary to what increased β1-subunit expression would 
do, the open channel probability (NPo) was found to be 
decreased at low nanomolar Ca²⁺ (Figure 4F). This is sur-
prising, given that voltage-sensitivity of BKCa are not altered 
by the β1-subunit below 100 nmol/L.¹⁶,¹⁷ This argues that 
channel expression at the membrane might be lower in HS. 
Recently, the human β1-subunit was reported to contain an 
endocytic signal in its C terminus that results in a reduction of 
surface expression of Hslo but not in total protein.²¹ Whether 
the β1-subunit modulates trafficking in ASMCs is unknown, 
though no increase in active channel number was reported in 
cells from hypertensive animals where reduced β1 expression 
was demonstrated.³,²²

Concluding Remarks
Although the new findings of Zhao and colleagues elegantly 
support a role for the β1 subunit in HS,¹⁵ we would modify 
their scheme to include that Slo probably also enhances 
Ca²⁺-sensitivity of BKCa (see Figure above). On a cautionary 
note, whether these mechanisms translate into the in vivo 
situation remains inconclusive because endothelial-derived 
factors or circulating hormones could well alter how BKCa.
open. The ultimate test would be to delete α and β1-subunits separately to see how each impacts on vascular hyporeactivity and blood pressure responses in HS. Finally, given that ATP-sensitive K⁺ channels are also important contributors of the cardiovascular collapse in shock⁹,¹⁰ adds to the growing body of evidence that K⁺ channels are important regulators of vascular reactivity in shock. Which channel, if either, assumes a greater role in the clinical scenario remains a moot point.

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Stoking Up \( \text{BK}_{\text{Ca}} \) Channels in Hemorrhagic Shock: Which Channel Subunit Is Really Fueling the Fire?

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