AuPS/ASB Meeting - Canberra 2005

Symposium 2: Functional Roles of Potassium Channels in the Vasculature

Wednesday 28 September 2005

Chair: Mike Hill

Endothelium-dependent vasodilatation: Fundamental role of SK_{Ca} and IK_{Ca} potassium channels

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Activation of vascular potassium (K) channels underlies both the radial and axial spread of dilatation within the artery wall. Radial spread, or an endothelium-dependent hyperpolarizing factor (EDHF) response, is initiated by agonist activation of endothelial cells, while axial, or spreading dilatation, can follow local hyperpolarization in either the endothelial or the smooth muscle cells.

EDHF describes the endothelium dependent smooth muscle hyperpolarization persisting in the presence of inhibitors of nitric oxide (NO) synthase and cyclooxygenase, and causes smooth muscle relaxation by closing voltage-operated calcium channels. Originally assumed to reflect the action of a diffusible factor or factors, with analogy to EDRF or NO, the term is now also taken to encompass the possibility of passive spread of hyperpolarization from the endothelium (Busse *et al.*, 2002). Key to understanding this pathway is the observation that EDHF-evoked hyperpolarization and associated smooth muscle relaxation can be blocked with a combination of apamin (blocks small conductance calcium-activated K channels, SK_{Ca}) and charybdotoxin (blocks intermediate and large calcium-activated K channels, IK_{Ca} and BK_{Ca}, plus delayed rectifier channels, K_V). Alone, these toxins partially blocked EDHF responses, but in combination they totally abolished the response. Although initially taken to indicate that SK_{Ca} and BK_{Ca} on the smooth muscle were responsible for hyperpolarization (to a diffusible EDHF), iberiotoxin was unable to substitute for charybdotoxin (see Busse *et al.*, 2002 for review). Furthermore, direct membrane potential measurements from endothelial cells *in situ* revealed that apamin and charybdotoxin are acting on K channels in these cells (Edwards *et al.*, 1998). Pharmacological studies (using 1-EBIO and TRAM-34/39) then showed that the target for charybdotoxin is in fact the IK_{Ca} channel. Thus, agonist activation of the endothelium, leading to increases in $[Ca^{2+}]_i$, activates both SK_{Ca} and IK_{Ca} (which may be spatially separated, Crane *et al.*, 2003) causing hyperpolarization which is transfered by a diffusible factor or passive spread through myoendothelial gap junctions to the adjacent smooth muscle, where relaxation is evoked.

In addition to radial spread, axial spread of hyperpolarization is well described in the microcirculation (see Segal 2005). However, it seems to reflect an inherent property of resistance arteries as well (Takano *et al.*, 2004). In small mesenteric arteries, selective activation of endothelial cell hyperpolarization, or of the K_{ATP} channels localized in the smooth muscle, results in hyperpolarization which spreads along the endothelium causing distant upstream dilatation. Interestingly, spread of hyperpoarization is not associated with an increase in endotheial cell $[Ca^{2+}]$ (Takano, 2004). It also may in part reflect K_{IR} activity (Goto *et al.*, 2004).

Vascular potassium channels therefore play a crucial role in the spread of dilator signals through the artery wall, and disruption of this role may underlie alterations in vascular function in cardiovascular disease.

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Functional effects of vascular K_{IR} channels

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(Introduced by M. Hill)

Potassium ion (K^+) channel activity is one of the major determinants of vascular muscle cell membrane potential and thus vascular tone. Four types of K^+ channels are functionally important in the vasculature - Ca^{2+}-activated K^+ channels, voltage-dependent K^+ channels, ATP-sensitive K^+ channels, and inwardly rectifying K^+ (K_{IR}) channels. The latter type will be the subject of this review.

Recent advances in vascular K_{IR} channel research indicate that this channel: 1) is present in vascular muscle; 2) modulates basal arterial tone; 3) mediates powerful hyperpolarization and vasodilator responses to small but physiological increases in extracellular K^+; 4) may contribute to vasodilatation in response to flow-induced shear stress; 5) may be inhibited by protein kinase C activity; 6) may be involved in vasorelaxation mediated by endothelium-derived hyperpolarizing factor; and 7) may be functionally altered by gender and in cardiovascular diseases. Vascular effects of K_{IR} channels have so far been most extensively studied in the cerebral circulation where K_{IR} function may be important in coupling cerebral metabolism and blood flow. Despite the lack of selective inhibitors of K_{IR} channel subtypes, the use of gene knockout technology is beginning to enable more extensive insight to be gained regarding the functional role of these channels in blood vessels.

K_v as a target for nitroxyl anion (NO^-)-mediated vasodilatation

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Traditionally the vascular effects of nitric oxide (NO) have been attributed to the free radical form of NO ($NO^\bullet$) yet the reduced form of NO (NO^-) is also produced endogenously and vasodilates both large conduit and small resistance-like arteries (Irvine *et al.*, 2003). Interestingly, $NO^\bullet$ and NO^- have been shown to have distinct mechanisms of action in the cardiovascular system, particularly in the heart (Paolocci *et al.*, 2003). This study aimed to determine if the vasorelaxant effects of NO^- differed to those of $NO^\bullet$ in rat small mesenteric resistance arteries. Male Sprague-Dawley rats were killed *via* CO_2 sedation and cervical dislocation. Mesenteric arteries (~350μm diameter) were isolated, mounted in small vessel myographs and isometric force and intracellular membrane potential measured simultaneously. Cumulative concentration-response curves to $NO^\bullet$ (NO gas), the NO^- donor, Angeli's salt and the NO-independent soluble guanylate cyclase (sGC) activator, YC-1 were examined. Vasorelaxation to Angeli's salt (pEC_{50}=7.02±0.67 -log M; R_{max}=96.0±2.2%, n=4) was accompanied by simultaneous vascular smooth muscle cell hyperpolarisation (pEC_{50}=6.82±0.32, 10μM AS -17.8±4.4 mV, n=4). In contrast, maximal vasorelaxation to $NO^\bullet$ (pEC_{50}=6.82±0.39, 92.1±1.3%) was achieved before a small hyperpolarisation response was observed at 1μM $NO^\bullet$ (-4.9±2.3 mV, n=5). Both relaxation and hyperpolarisation responses to Angeli's salt were significantly attenuated ($P<0.05$, n=5) by the NO^- scavenger, L-cysteine (3mM) and virtually abolished by the sGC inhibitor, ODQ (10μM; $P<0.05$, n=4). In contrast, ODQ only decreased the sensitivity of $NO^\bullet$-mediated vasorelaxation approximately 10-fold ($P<0.05$, n=4) and failed to affect $NO^\bullet$-mediated hyperpolarisation. The K_v channel inhibitor, 4-aminopyridine (1mM) caused a 4-fold ($P<0.05$, n=4) decrease in sensitivity to Angeli's salt and abolished the hyperpolarisation response ($P<0.05$). Glibenclamide (K_{ATP} channel inhibitor) and charybdotoxin (BK_{Ca}/IK_{Ca} channel inhibitor) were without effect. YC-1 also induced smooth muscle hyperpolarisation (10μM YC-1 -43.0±6.3 mV, n=3) which was attenuated by 4-aminopyridine (10μM YC-1 -23.5±2.3 mV, $P<0.05$, n=3). In conclusion, in rat small mesenteric arteries, NO^- mediates relaxation in part *via* cGMP-dependent activation of K_v channels. In contrast, $NO^\bullet$-mediated vasorelaxation occurs independently of vascular smooth muscle hyperpolarisation and in part *via* cGMP-independent pathways. Thus, the redox siblings $NO^\bullet$ and NO^- have distinct mechanisms of vasorelaxation in resistance-like arteries.

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The smooth muscle BK_{Ca} potassium channel and its interaction with arteriolar myogenic tone

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Myogenic tone in arterioles, generated by intraluminal pressure, is important in autoregulation of blood flow and in determining the response of arterioles to vasodilator stimuli. The extent of arteriolar myogenic tone at a given intraluminal pressure varies among arterioles in different vascular beds; for example arterioles from skeletal muscle being relatively more constricted than those in the cerebral circulation at similar pressures. This may be due to differing expression or activity of large-conductance Ca^{2+}-sensitive K^+-channels (BK_{Ca}) in the smooth muscle cells, which are thought to play a key role in regulating pressure-induced myogenic tone (Wellman & Nelson, 2003). The activity of BK_{Ca} channels is also increased by cyclic nucleotides (cGMP, cAMP), suggesting BK_{Ca} may be involved in the actions of paracrine dilators such as nitric oxide (NO). The aims of our studies were to compare the roles of BK_{Ca} in regulating myogenic tone in cerebral and skeletal muscle arterioles and to examine the importance of BK_{Ca} in endothelium-dependent dilation in vessels possessing different levels of myogenic tone.

Functional studies in skeletal muscle arterioles from both rats and mice showed pressure-dependent vasoconstriction indicating the presence of myogenic tone. Over the pressure range 0 to 150 mmHg, a steep sigmoidal relationship was observed between the extent of myogenic tone (0 to 53.0 ± 7.8 %) and smooth muscle E_m (-55.3 ± 4.1 mV to -29.4 ± 0.7 mV). Compared with data from published studies in cerebral vessels the slope of this relationship was both steeper and shifted towards more depolarised values. The selective BK_{Ca} inhibitor iberiotoxin (0.1 μM) caused a slight but significant vasoconstriction and depolarisation. Iberiotoxin treatment did not, however, alter the fundamental relationship between myogenic responsiveness and E_m. Immunohistochemistry (IHC) demonstrated the presence of BK_{Ca} channels in smooth muscle cells of rat cerebral and cremaster muscle arterioles, without any difference in the expression pattern or levels. Real-time PCR, performed on mouse arterioles, demonstrated the expression of various Ca^{2+}-activated K^+-channels in the order sK_3 > IK > BK_{Ca}. There was no difference, however, in BK_{Ca} expression (normalized to actin) between cerebral and skeletal muscle vessels. The data suggest that while BK_{Ca} channels are expressed in skeletal muscle arterioles they are not as tightly coupled to myogenic responsiveness as has been suggested for cerebral vessels. This may relate to important differences in vessel function as skeletal muscle vessels under normotensive conditions typically exhibit a high vascular resistance whereas the cerebral circulation tends to maintain a lower vascular resistance to ensure continuity of blood supply.

With respect to the possible role of BK_{Ca} in endothelium-mediated dilation, responses to the endothelium-dependent dilator acetylcholine (ACh) were measured at differing levels of intraluminal pressure (50 and 120 mmHg) in isolated arterioles from the rat cremaster muscle. Dilation to ACh was significantly inhibited at the higher pressure, yet the magnitude of the ACh-induced hyperpolarization was not altered. In vessels maintained at 50 mmHg, EDHF made a substantial contribution to endothelium-dependent dilation with minor role for NO. At the higher intraluminal pressure (120 mmHg) the relative contribution of EDHF was reduced however, with a corresponding increase in the importance of NO/cGMP-mediated dilation. Further studies showed dilation to cGMP alone was enhanced at the higher pressure, suggesting an increased sensitivity to cGMP. We suggest this is due to a cGMP-induced increase in activity of BK_{Ca} channels (Schubert & Nelson, 2001), which is more pronounced with increased pressure-induced myogenic tone and, in part, counteracts the inhibitory effect of increased intraluminal pressure and membrane potential on K^+-channel activity.

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Potassium channels in vascular dysfunction

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Intermediate and small conductance calcium-activated potassium channels, IK_{Ca} and SK_{Ca} respectively, play a critical role in the regulation of endothelium-derived hyperpolarizing factor (EDHF)-mediated endothelium-dependent vasodilatation (EDV). Connexins (Cx) 37, 40, 43 and 45 are expressed in vascular tissue and also contribute to EDHF-mediated EDV in a tissue dependent manner. In the wild type control (WT) C57BL/6J mouse the contribution of EDHF increases, relative to NO, from 1st to 2nd and greatest in 3rd order vessels. Changes in the contributions of nitric oxide (NO) and EDHF have also been reported in disease states, such as diabetes, and may reflect an important contribution to the pathophysiology (Pannirselvam *et al.*, 2002). In this study we have compared EDV initiated by acetylcholine (ACh) in resistance vessels (small mesenteric arteries – SMA) from male eNOS-null mouse (eNOS-/-), that present with a hypertensive and insulin resistant phenotype, to the hypertensive, insulin resistant and hyperglycaemic type 2 diabetic db/db mouse and the type 1 diabetic apoE-null- streptozotocin (STZ) mouse. In SMA from the eNOS-/- mouse EDV, initiated by ACh, is mediated entirely by EDHF and similarly in the db/db, leptin receptor mutant type two diabetic mouse. Despite the absence of a contribution from NO to EDV in the db/db mouse no difference was found in either mRNA or protein levels of eNOS. In the STZ-induced type 1 diabetic apoE-null mouse the contribution of EDHF to EDV is reduced and the expression of eNOS is increased. The combination of the IK_{Ca} channel blockers, charybdotoxin (ChTx) or TRAM-34, and the SK_{Ca} blocker apamin inhibits a large portion of the contribution of EDHF to ACh-mediated EVD in eNOS-/-, db/db, and the STZ-apoE-/- mice with a small component remaining that is sensitive to iberiotoxin, IbTx. The data with IbTx indicates a role for the large conductance BK_{Ca} channel and this, likely, reflects an action on the vascular smooth muscle cells mediated by a cytochrome P450 metabolite. The presence of the putative myoendothelial gap junction (MEGJs) inhibitor, β-glycyrrhetinic acid (β-GA), produced a significant inhibition of EVD in the eNOS-/- but not in the WT mouse. These data suggest that a component of the EDHF-mediated EDV in the eNOS-/-, but not the WT, is mediated by MEGJs. Real time PCR was also conducted to determine mRNA expression for the K_{Ca} channels: the large conductance BK_{Ca}, IK_{Ca}, and the SK_{Ca} SK1, SK2 and SK3 subtypes in 1st, 2nd and 3rd vessels from eNOS-/- and WT mice; however, no difference, relative to the housekeeping gene β-actin was found. Similarly for the expression of mRNA for Cx 37, 40, 43 and 45 – no differences in expression levels were found. In contrast, in SMA from the STZ-apoE mouse, expressions levels of SK2, SK3 and Cx37 were significantly reduced as was the functional contribution of EDHF to EDV, whereas eNOS levels were increased We conclude that type 1 and type 2 diabetic states have different effects on EDV with type 1 decreasing the contribution of EDHF and type 2 decreasing the bioavailability of NO. Western blots to determine protein levels have not been consistently successful for interpretation reflecting the low protein yield from the SMA.

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