CLASSICAL PERSPECTIVES

Inward rectification and vascular function: As it was in the beginning

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A perspective on the classic papers of Edwards & Hirst (1988) and Edwards *et al.* (1988)

Potassium channels are known to play an important role in the regulation of vascular tone through their ability to hyperpolarize smooth muscle cells and produce vasodilatation, primarily by causing the closure of voltage-dependent calcium channels. Of the four different classes of potassium channels found in blood vessels (ATP-sensitive potassium channels, inwardly rectifying potassium channels (K_{IR}), voltage-activated potassium channels and calcium-activated potassium channels), K_{IR} channels have been implicated in a range of vasomotor functions, from vasodilatation of cerebral, coronary and skeletal muscle vascular beds in response to local release of potassium ions (K^+; for example, see Quayle *et al.* 1997; Armstrong *et al.* 2007) to augmentation and facilitation of the propagation of vasodilatory responses over distance along microcirculatory arterioles and feed arteries (Rivers *et al.* 2001; Goto *et al.* 2004; Jantzi *et al.* 2006). Finally, down-regulation or inactivation of these channels is increasingly seen as instrumental in the increased excitability of vascular smooth muscle found in pathological conditions such as hypertension, hypercholesterolaemia and diabetes (Chrissobolis & Sobey, 2003; Jackson, 2006; Matsushita & Puro, 2006).

K_{IR} channels are activated by hyperpolarization and most readily pass inward currents, selective for K^+, at potentials negative to the equilibrium potential for K^+ (E_K). However, at membrane potentials between the activation voltage and E_K, K_{IR} channels sustain small outward currents. Thus, although the channel name refers to the greater ability to pass inward than outward current, the involvement of K_{IR} channels in vasomotor function centres on the smaller outward currents activated at membrane potentials between about −40 and −80 mV. K_{IR} channels are blocked by low concentrations of barium ions (Ba^{2+}) and most importantly, their activation potential is shifted to more positive potentials by increases in extracellular potassium concentration ($[K^+]_o$) and to more negative potentials by decreases in $[K^+]_o$ (Quayle *et al.* 1997).

Over 60 years ago, it was known that small increases in $[K^+]_o$ could initiate vasodilatation, although the effect depended on the vascular bed, the level of vascular tone and the concentration of KCl (Dawes, 1941). While the underlying mechanism was unknown at the time, Dawes suggested that this phenomenon could explain the increased blood flow seen in contracting muscle, particularly if sufficient K^+ were released during muscular activity. Subsequent studies confirmed the link between small increases in perivascular $[K^+]$ and vascular tone in other vascular beds, including the cerebral circulation (Kuschinsky *et al.* 1972), underscoring the importance of activity-dependent alterations in $[K^+]_o$ in tuning blood supply to organ activity.

As potassium channels make a significant contribution to resting membrane potential, an increase in $[K^+]_o$ would be expected to shift E_K to more positive potentials and thus lead to depolarization and constriction. The fact that small increases in $[K^+]_o$ produced hyperpolarization and vasodilatation in certain arteries was seen as somewhat of a paradox. In 1988, although K_{IR} channels had been described in skeletal muscle fibres, starfish eggs and neurones of the olfactory cortex (Katz, 1949; Hagiwara & Takahashi, 1974; Constanti & Galvan, 1983), they had not been described in arterioles, nor were they considered to be activated at membrane potentials more positive to E_K. In earlier studies, Hirst & Neild (1978) had detected rectification in segments of guinea pig submucosal arterioles with resistances falling with membrane hyperpolarization, while Hirst & van Helden (1982) had observed that a reduction in $[K^+]_o$ resulted in depolarization and an increase in membrane resistance of similar arteriolar segments. Thus the aim of the first study by Edwards & Hirst in 1988 was to determine whether this hyperpolarization-induced rectification and the paradoxical effects of changing $[K^+]_o$ could be attributed to a K^+-selective inward rectifier.

Experiments were carried out on short isopotential segments of arterioles in which it was possible to investigate the current–voltage relationships in either voltage or current clamp mode. In these short unpressurized segments the resting membrane potential was −70 mV. The results demonstrated membrane rectification, with the membrane resistance starting to fall at an activation voltage of around −50 mV and membrane conductance increasing with hyperpolarization. The rectifying current was blocked by Ba^{2+} and showed substantial changes in activation potential with only small changes in $[K^+]_o$, i.e. from 1.25 to 10 mM. More importantly, Edwards & Hirst (1988) showed that the activity of this channel could impact on arteriolar excitability, the very aspect now considered in pathological studies to be crucial. Thus, the time course of action potentials initiated from hyperpolarized potentials, at which K_{IR} channels would be active, were briefer than those initiated from more depolarized potentials. Similar effects were observed when the membrane potential was held at −70 mV and $[K^+]_o$ altered; time courses were shorter in 10 mM K^+ (more active K_{IR}) and longer in 2.5 mM K^+ (less active K_{IR}). The effect of the K_{IR} current in attenuating the decay of excitatory junction potentials following nerve stimulation could also be seen with summed excitatory junction potentials (more depolarized, less active K_{IR}) having larger time constants than single excitatory junction potentials (more hyperpolarized, more active K_{IR}).

In a back-to-back paper, Hirst and colleagues investigated the role of K_{IR} channels in cerebral arterioles (Edwards *et al.* 1988). Again current–voltage relationships in short arterial segments and the effect of alterations in $[K^+]_o$, from a control value of 5 mM, were tested. Both arteriolar segments located close to the middle cerebral as well as those located at some distance from this larger vessel were chosen, as previous studies had demonstrated loss of sympathetic fibres with increasing distance from the middle cerebral artery (Hill *et al.* 1986) and neuronal activity was considered to be capable of producing appropriate changes in $[K^+]_o$. Interestingly, the results showed that

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distal arterioles were more excitable than proximal arterioles, had more depolarized membrane potentials, often with superimposed oscillations, and little evidence of inward rectification. Moreover, regional differences in responses to increases in $[K^+]_o$ were found; distal segments hyperpolarized and now demonstrated inward rectification, while proximal segments depolarized and showed inward rectification at more positive potentials. Since Ba^{2+} abolished inward rectification of proximal segments, and of distal segments exposed to elevated $[K^+]_o$, it was clear that K_{IR} channels were expressed in both areas but that these channels differed in their sensitivity to $[K^+]_o$, i.e. higher concentrations were required to activate K_{IR} channels in distal than in proximal segments.

In both studies, inhibition of the K_{IR} channels with Ba^{2+} resulted in a large depolarization of submucous arterioles and proximal cerebral arterioles leading the authors to conclude that K_{IR} channels constituted the predominant resting potassium conductance. However, the resting membrane potential detected in these early studies was around −70 mV, representing recordings from unpressurized vessels. This value is considerably more hyperpolarized than that measured subsequently *in vivo* in systemic vessels at around −30 to −40 mV (Welsh & Segal, 1998; Emerson & Segal, 2000; Siegl *et al.* 2005). Although K_{IR} channels would be expected to be largely closed at these more physiological potentials, low concentrations of Ba^{2+} which selectively inhibit K_{IR} channels (Quayle *et al.* 1997) have been reported to reduce the diameter of skeletal muscle arterioles (Loeb *et al.* 2000) and large cerebral arteries *in vivo* and of other vessels *in vitro* (Chrissobolis & Sobey, 2003), suggesting perhaps a minor contribution at rest. Such a contribution seems especially surprising in the cerebral vessels since the $[K^+]_o$ of cerebrospinal fluid is around 3 mM and at this concentration the activation potential of the K_{IR} channels would be expected to be shifted to more negative potentials.

Irrespective of whether K_{IR} channels contribute to resting vascular tone, these two classical papers provided proof that these channels could influence vascular reactivity, especially in situations where $[K^+]_o$ was increased. They were the first to describe the existence of a potassium-sensitive inward rectifier in both cerebral and systemic arterioles, demonstrate the activation of this current at potentials more positive than E_K, show how the activation potential could be moved to more depolarized potentials by small increases in $[K^+]_o$ and illustrate how this current could modify arteriolar excitability. However, the original studies failed to take into account the fact that the preparations contained both smooth muscle and endothelial cells and so the precise cellular location of the channels was not determined. Although subsequent studies confirmed the existence of K_{IR} currents in smooth muscle cells isolated from cerebral arteries (Quayle *et al.* 1993), others have shown that K_{IR} channels are also expressed in isolated endothelial cells (Nilius *et al.* 1997) and in intact rat mesenteric arteries, they may even be confined to this layer (Doughty *et al.* 2001; Crane *et al.* 2003; Goto *et al.* 2004).

Such differential cellular expression of K_{IR} channels may be a characteristic of different vascular beds, with vessel size perhaps contributing to further variation. Given that heterocellular coupling has been reported to play a critical role in vasodilatation evoked by endothelial-derived hyperpolarization and that the incidence of myoendothelial gap junctions varies along and between vascular beds (Sandow & Hill, 2000; Sandow *et al.* 2002, 2003), the specific cellular location of K_{IR} channels may differentially affect smooth muscle contractility. Indeed, a recent study has shown that K_{IR} channels expressed in vascular smooth muscle cells of cerebral or coronary arteries act as electrical amplifiers of hyperpolarizing responses activated in either the endothelium or the smooth muscle (Smith *et al.* 2007). However, this was not the case in mesenteric arteries in which K_{IR} channels were not highly expressed in the smooth muscle cells (Smith *et al.* 2007). If these vessels are analogous to those evaluated in previous studies of the mesenteric circulation, then one would expect the endothelium to express K_{IR} channels. In this case, the physiological role of these endothelial channels warrants further investigation.

The pioneering studies of Hirst and colleagues on arteriolar K_{IR} channels raised other questions which remain topics for future research. For example, what is the significance of the regional variation in the sensitivity of the K_{IR} channels to $[K^+]_o$? Interestingly, pericytes surrounding microvessels in the rat retina have also been shown to display a topographical heterogeneity in K_{IR} currents (Matsushita & Puro, 2006), while studies in the spiral modiolar artery of the ear have described a heterogeneously coupled population of smooth muscle cells with a bimodal distribution of membrane potentials resulting from the continuous activation or not of K_{IR} channels (Jiang *et al.* 2001). In the latter study, the two cell populations clearly differed in their sensitivity to $[K^+]_o$ in a manner analogous to that of the proximal and distal arteriolar cells. In the cerebral circulation, the distal arteriolar segments which demonstrated the less sensitive K_{IR} channels lacked a sympathetic innervation (Hill *et al.* 1986) leading the authors to suggest a possible trophic influence of the nerves on muscle properties (Edwards *et al.* 1988). It is not known whether such correlations exist in other vascular beds. Nevertheless, the end result is that the proximal cerebral vessels will hyperpolarize and relax to smaller increases in $[K^+]_o$ than will the distal vessels, a characteristic conducive to producing an effective increase in blood flow.

Future studies will need to address in more detail the differential regional and cellular expression of K_{IR} channels throughout the vascular system and the contribution of these channels to arteriolar excitability *in vivo*. The factors which might regulate the expression and biophysical characteristics of K_{IR} channels are also of great interest, particularly if these may vary with cell type or pathophysiological condition. In this respect it should be borne in mind that even a small change in the magnitude of fluctuations in perivascular $[K^+]_o$ could a have profound effect on the ability of K_{IR} channels to influence vascular excitability.

References

Armstrong ML, Dua AK & Murrant CL (2007). Potassium initiates vasodilatation induced by a single skeletal muscle contraction in hamster cremaster muscle. *J Physiol* **581**, 841–852.

Chrissobolis S & Sobey CG (2003). Inwardly rectifying potassium channels in the regulation of vascular tone. *Curr Drug Targets* **4**, 281–289.

Constanti A & Galvan M (1983). Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones. *J Physiol* **335**, 153–178.

Crane GJ, Walker SD, Dora KA & Garland CJ (2003). Evidence for a differential cellular distribution of inward rectifier K channels in the rat isolated mesenteric artery. *J Vasc Res* **40**, 159–168.

Dawes GS (1941). The vaso-dilator action of potassium. *J Physiol* **99**, 224–238.

Doughty JM, Boyle JP & Langton PD (2001). Blockade of chloride channels reveals relaxations of rat small mesenteric arteries to raised potassium. *Br J Pharmacol* **132**, 293–301.

Edwards FR & Hirst GD (1988). Inward rectification in submucosal arterioles of guinea-pig ileum. *J Physiol* **404**, 437–454.

Edwards FR, Hirst GD & Silverberg GD (1988). Inward rectification in rat cerebral arterioles; involvement of potassium ions in autoregulation. *J Physiol* **404**, 455–466.

Emerson GG & Segal SS (2000). Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. *Circ Res* **87**, 474–479.

Goto K, Rummery NM, Grayson TH & Hill CE (2004). Attenuation of conducted vasodilatation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels. *J Physiol* **561**, 215–231.

Hagiwara S & Takahashi K (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J Membr Biol* **18**, 61–80.

Hill CE, Hirst GD, Silverberg GD & van Helden DF (1986). Sympathetic innervation and excitability of arterioles originating from the rat middle cerebral artery. *J Physiol* **371**, 305–316.

Hirst GD & Neild TO (1978). An analysis of excitatory junctional potentials recorded from arterioles. *J Physiol* **280**, 87–104.

Hirst GD & van Helden DF (1982). Ionic basis of the resting potential of submucosal arterioles in the ileum of the guinea-pig. *J Physiol* **333**, 53–67.

Jackson WF (2006). Silent inward rectifier K^+ channels in hypercholesterolemia. *Circ Res* **98**, 982–984.

Jantzi MC, Brett SE, Jackson WF, Corteling R, Vigmond EJ & Welsh DG (2006). Inward rectifying potassium channels facilitate cell-to-cell communication in hamster retractor muscle feed arteries. *Am J Physiol Heart Circ Physiol* **291**, H1319–H1328.

Jiang ZG, Si JQ, Lasarev MR & Nuttall AL (2001). Two resting potential levels regulated by the inward-rectifier potassium channel in the guinea-pig spiral modiolar artery. *J Physiol* **537**, 829–842.

Katz B (1949). Les constants electriques de la membrane du muscle. *Arch Sci Physiol (Paris)* **2**, 285–299.

Kuschinsky W, Wahl M, Bosse O & Thurau K (1972). Perivascular potassium and pH as determinants of local pial arterial diameter in cats. A microapplication study. *Circ Res* **31**, 240–247.

Loeb AL, Godeny I & Longnecker DE (2000). Functional evidence for inward-rectifier potassium channels in rat cremaster muscle arterioles. *Microvasc Res* **59**, 1–6.

Matsushita K & Puro DG (2006). Topographical heterogeneity of K_{IR} currents in pericyte-containing microvessels of the rat retina: effect of diabetes. *J Physiol* **573**, 483–495.

Nilius B, Viana F & Droogmans G (1997). Ion channels in vascular endothelium. *Annu Rev Physiol* **59**, 145–170.

Quayle JM, McCarron JG, Brayden JE & Nelson MT (1993). Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. *Am J Physiol Cell Physiol* **265**, C1363–C1370.

Quayle JM, Nelson MT & Standen NB (1997). ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev* **77**, 1165–1232.

Rivers RJ, Hein TW, Zhang C & Kuo L (2001). Activation of barium-sensitive inward rectifier potassium channels mediates remote dilation of coronary arterioles. *Circulation* **104**, 1749–1753.

Sandow SL, Bramich NJ, Bandi HP, Rummery NM & Hill CE (2003). Structure, function, and endothelium-derived hyperpolarizing factor in the caudal artery of the SHR and WKY rat. *Arterioscler Thromb Vasc Biol* **23**, 822–828.

Sandow SL & Hill CE (2000). Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ Res* **86**, 341–346.

Sandow SL, Tare M, Coleman HA, Hill CE & Parkington HC (2002). Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ Res* **90**, 1108–1113.

Siegl D, Koeppen M, Wolfle SE, Pohl U & de Wit C (2005). Myoendothelial coupling is not prominent in arterioles within the mouse cremaster microcirculation *in vivo*. *Circ Res* **97**, 781–788.

Smith PD, Brett SE, Luykenaar KD, Sandow SL, Marrelli SP, Vigmond EJ & Welsh DG (2007). Kir channels function as electrical amplifiers in rat vascular smooth muscle. *J Physiol*; DOI: 10.1113/jphysiol.2007.145474.

Welsh DG & Segal SS (1998). Endothelial and smooth muscle cell conduction in arterioles controlling blood flow. *Am J Physiol Heart Circ Physiol* **274**, H178–H186.