British Journal of Pharmacology (2009), 157, 551–553

0007-1188/09
www.brjpharmacol.org

THEMED SECTION: ENDOTHELIUM IN PHARMACOLOGY

COMMENTARY

Vascular K_{ATP} channels: dephosphorylation and deactivation

Paolo Tammaro

Faculty of Life Sciences, University of Manchester, Manchester, UK

Vascular ATP-sensitive potassium (K_{ATP}) channels (Kir6.1/SUR2B) are regulated by both cell metabolism and chemical transmitters. They are the target for a number of vasodilators and vasoconstrictors whose mechanisms of action involve activation of protein kinase A (PKA) and protein kinase C (PKC), respectively. The article by Orie *et al.* in this issue of the *BJP* sheds new light on the (opposing) role of protein phosphatases in the regulation of this ion channel activity. Their data suggest that calcineurin, a Ca^{2+}-dependent protein phosphatase, modulates Kir6.1/SUR2B by inhibiting PKA-dependent phosphorylation of the channel. This novel mechanism may provide a modulation opposing the action of vasodilators on the K_{ATP} channel.
British Journal of Pharmacology (2009) **157,** 551–553; doi:10.1111/j.1476-5381.2009.00204.x

This article is a commentary on Orie *et al.*, pp. 554–564 of this issue and is part of a themed section on Endothelium in Pharmacology. For a list of all articles in this section see the end of this paper, or visit: http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: Kir6.1; SUR2B; ATP-sensitive potassium channel; protein kinase A; calcineurin

ATP-sensitive potassium (K_{ATP}) channels serve as metabolic sensors that convert changes in cell metabolism into changes in electrical activity. In vascular smooth muscle (VSM), they contribute to the resting membrane potential and regulate the vessel tone. Alterations in their activity in response to vasoactive agonists, exercise or hypoxia cause changes in arterial diameter that play an important role in blood pressure regulation (Quayle *et al.*, 1997). At present, little information is available on the contribution of endothelial K_{ATP} channels in vascular function. However, there is evidence that adenosine- and α_2-adrenoceptor mediated vasodilation (Kuo and Chancellor, 1995; Gendron *et al.*, 2004) may be dependent on endothelial K_{ATP} channels and a role for these channels in the regulation of coronary blood flow by modulation of the release of endothelin-1 has been proposed (Malester *et al.*, 2007). The pivotal role of K_{ATP} channels in the vascular system is emphasized by the observation that genetic deletion (knockout) of either of its two subunits, SUR2 (Chutkow *et al.*, 2002) or Kir6.1 (Miki *et al.*, 2002), causes coronary vasospasm in mice.

K_{ATP} channels are an octameric complex of four sulphonylurea receptor subunits (SUR) surrounding a central pore composed of four inwardly rectifying subunits (Kir6.1 or Kir6.2). The hallmark of K_{ATP} channels is their sensitivity to inhibition by intracellular ATP. Additionally, Mg-nucleotides open K_{ATP} channels via interaction with SUR. Thus, reciprocal changes in the intracellular concentrations of ATP and MgADP underlie the metabolic regulation of K_{ATP} channels.

A property of VSM K_{ATP} channels is that they are targets for vasoactive hormones. For example, they are inhibited by angiotensin II (via protein kinase C (PKC) activation) and opened by cyclic-AMP-elevating vasodilators such as calcitonin gene-related peptide, adenosine, prostacyclin and vasoactive intestinal peptide through a mechanism which requires protein kinase A (PKA) activation (Quayle *et al.*, 1997). PKA was shown to phosphorylate serine and threonine residues on both SUR2B and Kir6.1 (Quinn *et al.*, 2004; Shi *et al.*, 2008). Although the mechanisms of modulation of channel activity by PKA (and PKC) phosphorylation are well described, substantially less information is available on the opposing effects of protein phosphatases.

Previous studies have shown that calcineurin, a Ca^{2+}-dependent protein phosphatase, can modulate K_{ATP} channel activity in isolated VSM cells (Wilson *et al.*, 2000). Channel activity was inhibited as the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was increased within the nanomolar range. Furthermore, inhibition of calcineurin activity was proposed to underlie cases of a life-threatening syndrome ('potassium-channel syndrome') associated with excessive K_{ATP} channel

Correspondence: Dr Paolo Tammaro, Faculty of Life Sciences, University of Manchester, The Core Technology Facility, 46 Grafton Street, Manchester, M13 9NT, UK. E-mail: paolo.tammaro@manchester.ac.uk
Received 7 January 2009; accepted 20 January 2009

activation in response to drugs (Singer *et al*., 2005). However, neither the site of action nor the calcineurin isoform involved in these effects were established.

Calcineurin is a Ca^{2+}/calmodulin Ser/Thr phosphatase, consisting of a catalytic subunit (CnA), which interacts with calmodulin, and a regulatory subunit (CnB) bearing a Ca^{2+} binding site. Three isoforms of CnA (CnAα CnAβ, CnAγ) with high sequence homology but different tissue and cellular distribution, have been identified.

In this issue of the British Journal of Pharmacology, Orie and colleagues use an heterologous expression system (transfected HEK-293 cells) to explore the molecular mechanism of calcineurin modulation of K_{ATP} channels (Orie *et al*., 2009). Two possible mechanisms of action for calcineurin were considered: (i) direct channel dephosphorylation and (ii) a possible indirect effect via inhibition of PKA, since the type-2 regulatory (RII) subunit of PKA is a substrate for calcineurin.

The authors employed a combined pharmacological and biochemical approach in their study. By using chemically unrelated inhibitors of either PKA or calcineurin, they demonstrated the involvement of both these enzymes in regulating K_{ATP} channel function. Specifically, calcineurin inhibitors enhanced K_{ATP} current whereas blockers of PKA activity reduced resting K_{ATP} currents and the responses to calcineurin inhibitors. The latter observations are in accord with the hypothesis that calcineurin exerts its effects, at least in part, indirectly via PKA inhibition.

Activation of calcineurin, obtained by increasing $[Ca^{2+}]_i$ (up to 36 nmol·L^{-1}) in the pipette solution, however, abolished the stimulatory effect of a constitutively active form of PKA, which lacks the RII and is therefore insensitive to calcineurin inhibition. This supports the notion that calcineurin acts directly on the channel and that PKA and calcineurin can compete for the same phosphorylation site on the channel. Furthermore, *in vitro* phosphorylation assays provided direct evidence that the catalytic subunit, CnAα, can dephosphorylate a serine residue in Kir6.1, previously phosphorylated by PKA.

Two other additional important observations were made: (i) CnAα, but not CnAβ, modulates Kir6.1/SUR2B; (ii) surprisingly, neither PP1, a protein phosphatase that is activated by calcineurin, nor PKC are involved in this modulation.

In conclusion, the authors show that calcineurin is likely to be the major player in the Ca^{2+}-dependent inhibition of Kir6.1/SUR2B and that this phosphatase suppresses channel activity by antagonising PKA-dependent phosphorylation.

The findings of this paper raise a number of important questions and no doubt will stimulate further research into how K_{ATP} channel activity is regulated by protein phosphatases. For instance, it will be important to determine if calcineurin can dephosphorylate those residues on SUR2B that have been found to be important for channel activation (Quinn *et al*., 2004; Shi *et al*., 2007).

The elucidation of the precise physiological significance for this novel regulatory mechanism will require further studies. A working model is that an increase of $[Ca^{2+}]_i$ during agonist stimulation at G-protein coupled receptors would result in calcineurin activation and K_{ATP} channel inhibition. In native cells, this effect may occur in combination with the well-described inhibitory effect of PKC on K_{ATP} channel activity. It needs to be clarified whether these effects coexist in native VSM cells, which one is predominant and whether their contribution differs in the various vascular beds. Answering these questions is likely to require the use of knockouts or siRNA directed against CnAα or various PKC isoforms in combination with patch-clamp experiments on permeabilized native cells.

Kir6.1/SUR2B channels are also present in the endothelium. It remains to be seen if calcineurin modulates K_{ATP} channels in this cell type and what is the potential physiological consequence of this modulation.

Perhaps the most important clinical question arising from this work is whether calcineurin plays a role in those pathological conditions associated with Ca^{2+} mishandling, such as sepsis or pulmonary hypoxia, and whether K_{ATP} channels are regulated by calcineurin under those conditions.

Finally, It is noteworthy that calcineurin is an important modulator of other VSM channels, including, but not limited to, calcium-activated chloride-channels and voltage-gated Ca^{2+} channels. We do not know which of these targets for calcineurin contribute most to the global electrical activity of the cell and whether their contributions vary in the various vascular beds. Elucidation of this will provide exciting research opportunities for the future.

Acknowledgement

P.T. holds a Research Council UK fellowship.

References

Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF *et al*. (2002). Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. *J Clin Invest* **110**: 203–208.

Gendron ME, Thorin E, Perrault LP (2004). Loss of endothelial K_{ATP} channel-dependent, NO-mediated dilation of endocardial resistance coronary arteries in pigs with left ventricular hypertrophy. *Br J Pharmacol* **143**: 285–291.

Kuo L, Chancellor JD (1995). Adenosine potentiates flow-induced dilation of coronary arterioles by activating K_{ATP} channels in endothelium. *Am J Physiol* **269**: H541–549.

Malester B, Tong X, Ghiu I, Kontogeorgis A, Gutstein DE, Xu J *et al*. (2007). Transgenic expression of a dominant negative K_{ATP} channel subunit in the mouse endothelium: effects on coronary flow and endothelin-1 secretion. *Faseb J* **21**: 2162–2172.

Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K *et al*. (2002). Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* **8**: 466–472.

Orie NN, Thomas AM, Perrino BA, Tinker A, Clapp LH (2009). Ca^{2+}/calcineurin regulation of cloned vascular K_{ATP} channels: crosstalk with the protein kinase A pathway. *Br J Pharmacol* **157**: 554–564.

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

Quinn KV, Giblin JP, Tinker A (2004). Multisite phosphorylation mechanism for protein kinase A activation of the smooth muscle ATP-sensitive K^+ channel. *Circ Res* **94**: 1359–1366.

Shi Y, Wu Z, Cui N, Shi W, Yang Y, Zhang X *et al*. (2007). PKA phosphorylation of SUR2B subunit underscores vascular K_{ATP} channel activation by beta-adrenergic receptors. *Am J Physiol Regul Integr Comp Physiol* **293**: R1205–1214.

Shi Y, Chen X, Wu Z, Shi W, Yang Y, Cui N *et al*. (2008). cAMP-dependent protein kinase phosphorylation produces interdomain movement in SUR2B leading to activation of the vascular K_{ATP} channel. *J Biol Chem* **283**: 7523–7530.

Singer M, Coluzzi F, O'Brien A, Clapp LH (2005). Reversal of life-threatening, drug-related potassium-channel syndrome by glibenclamide. *Lancet* **365**: 1873–1875.

Wilson AJ, Jabr RI, Clapp LH (2000). Calcium modulation of vascular smooth muscle ATP-sensitive K^+ channels: role of protein phosphatase-2B. *Circ Res* **87**: 1019–1025.