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COMMENTARY

More PKA independent β-adrenergic signalling via cAMP: Is Rap1-mediated glucose uptake in vascular smooth cells physiologically important?

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The proteome characterising a specific cell type makes up a unique intracellular signalling network and signalling has to be studied in a cell specific manner. β-Adrenergic receptors are coupled to production of cAMP and PKA was initially believed to be the only protein activated by cAMP. However, cAMP-mediated signalling via Epac and Rap1 has emerged as an important contributor to cAMP signalling. In the current issue of the *British Journal of Pharmacology*, Kanda and Watanabe report that adrenaline stimulates glucose uptake in vascular smooth muscle cells. With pharmacological methods, supplemented with small interfering RNA against Rap1, the authors demonstrate that adrenaline increases glucose uptake via G_s, adenylate cyclase, cAMP and Rap1 activation. The authors could document neither PKA nor Epac as the receptor for cAMP mediating the effect. Although there is no doubt that Rap1 mediates adrenaline-stimulated glucose uptake in vascular smooth muscle cells, it may be too early to exclude PKA and Epac.
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Abbreviations: Epac, exchange protein directly activated by cAMP; PKA, cAMP-dependent protein kinase; VSMC, smooth vascular muscle cells

For a long time it was believed that all the effects of *β*-adrenergic stimulation were mediated via cAMP and activation of cAMP dependent protein kinase (PKA). However, the classical *β*-adrenergic signalling pathway via G_s, adenylate cyclase, cAMP and PKA has now been shown to be supplemented by other signalling pathways. In particular, β_2-adrenergic receptors have a rather diverse signalling with downstream signalling molecules like *β*-arrestin, mitogen-activated protein kinase, phosphoinositide 3-kinase and phospholipase A_2, all activated independently of cAMP production (Pavoine and Defer, 2005; Reiter and Lefkowitz, 2006).

Moreover, *β*-adrenergic receptors mediate signal via different intracellular cAMP receptors. In addition to the regulatory subunits of PKA, two other classes of proteins have cAMP binding domains; the cAMP-regulated ion channel and Epac (exchange protein directly activated by cAMP) (Bos, 2006). Epac is a GTP exchange factor that activates Rap proteins (Kawasaki *et al*., 1998; de Rooij *et al*., 1998). In particular, Epac-mediated activation of the small GTP-binding protein Rap1 has been shown to regulate a wide range of important physiological functions (Bos, 2006).

Signalling via cAMP varies to a great extent in different cell types and depends on the presence of other hormones (Brennesvik *et al*., 2005; Jensen *et al*., 2007). The complexities that can arise from cAMP signalling are shown in kidney cortical collecting duct where intermingled cells express *β*-adrenergic receptors or calcitonin receptors. Calcitonin and *β*-adrenergic receptors are both coupled to adenylate cyclase and cAMP production, but *β*-adrenergic receptors stimulate H^+,K^+-ATPase via cAMP and PKA whereas calcitonin stimulates H^+,K^+-ATPase via cAMP, Epac and Rap1 (Laroche-Joubert *et al*., 2002). Effects of cAMP, therefore, need to be defined on a cell to cell basis and in combination with activation of other signalling pathways (Laroche-Joubert *et al*., 2002; Brennesvik *et al*., 2005).

In this issue of the *British Journal of Pharmacology*, Kanda and Watanabe report that adrenaline stimulates glucose uptake in vascular smooth muscle cells via cAMP and activation of Rap1. The authors have systematically determined that the effect was via *β*-adrenergic receptors, G_s, adenylate cyclase and cAMP. However, the effect was independent of PKA. This finding would suggest that the effect was mediated via Epac. However, the Epac-specific cAMP analogue 8-(4-chlorophenylthio)-2′-*O*-methyl-cAMP (8-pCPT-2′-*O*-Me-cAMP) did not stimulate glucose uptake.

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Unfortunately, Rap1 activation was not studied after 8-pCPT-2′-*O*-Me-cAMP treatment, which raises several questions. How can cAMP activate Rap1 if neither PKA nor Epac is involved? Is Epac expressed in smooth vascular muscle cells (VSMC)? Or maybe 8-pCPT-2′-*O*-Me-cAMP did activate Rap1. In interpreting Kanda and Watanabe's data, we have to take into account that even though Rap1 activation is required for adrenaline-stimulated glucose uptake, Rap1 activation alone may not be sufficient.

Most kinase inhibitors, like the PKA inhibitors H89 and KT5720 used by Kanda and Watanabe, are ATP competitors and the high specificity stated by many authors is not always correct. Importantly, H89 inhibits Rho-kinase nearly as effectively as it inhibits PKA (Davies *et al*., 2000). Since Kanda and Watanabe performed their study in VSMC where Rho-kinase plays a key role in the regulation of contraction (Somlyo and Somlyo, 2000), particular attention has to be paid when these data are interpreted. In addition, the inhibitor of GTPase geranylgeranylation used by Kanda and Watanabe (GTI-298) to support Rap1-mediated glucose uptake also inhibits Rho activation. Therefore, I would find it particularly interesting to know whether adrenaline activates Rho in VSMC. Having said all that, the siRNA experiment clearly shows the pivotal role of Rap1 in adrenaline-stimulated glucose uptake in VSMC. However, I find it too early to exclude PKA and/or Epac from playing any role in adrenaline-stimulated glucose uptake in VSMC.

The present paper is a good example of the type of paper that raises more questions than it gives answers, but this is not necessarily a bad thing. The major question is of course: does adrenaline-stimulated glucose uptake in VSMC have a physiological role? There is evidence that glucose uptake via GLUT4 is required for the response of vascular smooth muscle to noradrenaline (Park *et al*., 2005). Skeletal muscles also express GLUT4, but adrenaline inhibits glucose uptake in these muscles (Aslesen and Jensen, 1998). Moreover, although we also find adrenaline-mediated signalling via cAMP and independent of PKA in skeletal muscles (Brennesvik *et al*., 2005), as reported in VSMC by Kanda and Watanabe, we have so far not been able to connect Epac signalling to regulation of carbohydrate metabolism in skeletal muscles.

How is glucose metabolised in VSMC during adrenaline stimulation? It is known that metabolism of glucose participates in intracellular signalling. For example, metabolism of glucose via mitochondria-associated hexokinase is required for growth-factor-mediated prevention of apoptosis (Gottlob *et al*., 2001; Majewski *et al*., 2004). Therefore, it will be interesting to know whether metabolism of the glucose taken up in VSMC during adrenaline stimulation has physiological roles.

Adrenaline-stimulated activation of Rap1 in VSMC may have interest beyond stimulation of glucose uptake. Rap1 is a small GTPase belonging to a superfamily also including Rho. Rho and Rho-kinase have central roles in agonist-induced vascular contraction and seem to participate in the pathogenesis of hypertension and atherosclerosis (Budzyn *et al*., 2006). Furthermore, Rho-kinase inhibitors have already shown potential for treatment of cardiovascular diseases (Budzyn *et al*., 2006). Maybe Rap1 will become a new drug target for cardiovascular disease.

Vascular smooth muscle cells certainly play a central role in the development of cardiovascular diseases, and epidemiological studies have documented mental stress (such as work stress) and the metabolic syndrome as major risk factors (Rosengren *et al*., 2004; Yusuf *et al*., 2004, 2005). Mental stress activates the sympathetic nerve system and hyperglycemia plays a key role in the development of cardiovascular disease in patients with metabolic syndrome and diabetes. After reading the paper by Kanada and Watanaba, I wondered whether mental stress and diabetes might have a common mechanism in the development of cardiovascular diseases, one in which glucose metabolism is an important participant.

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