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COMMENTARY

A_{2b} adenosine receptors can change their spots

Michael V Cohen[1,2], Xiulan Yang[1] and James M Downey[1]

[1]*Department of Physiology, University of South Alabama, College of Medicine, Mobile, AL, USA, and* [2]*Department of Medicine, University of South Alabama, College of Medicine, Mobile, AL, USA*

Recently, a central role for the A_{2b} adenosine receptor in a variety of cardiovascular functions including inflammation, erectile function, coronary artery dilation, asthma and cardioprotection has been demonstrated. Despite this evidence, the low-affinity A_{2b} adenosine receptor is still poorly understood. This receptor appears to be very promiscuous in its coupling. In most tissues, it couples to G_s much like its cousin, the A_{2a} adenosine receptor, but in mast cells and now, most recently, in cardiac fibroblasts, the A_{2b} receptor also couples to G_q. Because of its low affinity, this receptor was originally thought unlikely to play any important physiological role. But the sensitivity of A_{2b} adenosine receptors can be greatly increased by interaction with protein kinase C (PKC) making this receptor, under various conditions, both an activator and a target of PKC. We have recently documented a third coupling involving G_i. This plasticity and versatility of A_{2b} adenosine receptors position them as potential triggers of signalling in multiple signalling cascades in many physiological responses, making this a most interesting receptor indeed.
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Abbreviations: IL-6, interleukin-6; NECA, 5′-(N-ethylcarboxamido) adenosine

Adenosine is a purine nucleoside that is widely distributed in all tissues and body fluids. Adenosine agonists and antagonists are being targeted for treatment of a variety of clinical conditions. These actions are attributed to binding of the purine to four distinct receptors: A_1, A_{2a}, A_{2b} and A_3. Although all four receptors have been characterized pharmacologically and have been cloned, our familiarity and appreciation of the A_{2b} adenosine receptor is still very limited because selective agonists and antagonists have only recently become available. And what we think we know about A_{2b} adenosine receptors may not be accurate.

All four receptors have been found in cardiac tissue, which is a mixed tissue composed of cardiomyocytes, fibrous tissue and fibroblasts, vascular endothelial and smooth muscle cells, mast cells, etc. A_1 and A_3 adenosine receptors have been shown to be present in cardiomyocytes (Grdeń *et al.*, 2005). A_{2a} and A_{2b} adenosine receptors are both present in the coronary vasculature (Mubagwa and Flameng, 2001). Message for A_{2a} receptor has been identified in cardiomyocytes (Xu *et al.*, 1996). The presence of A_{2b} receptors in cardiomyocytes has been controversial (Liang and Haltiwanger, 1995; Morrison *et al.*, 2002; Grdeń *et al.*, 2005; Yang *et al.*, 2006), although we have detected A_{2b} receptor message in both rabbit and rat ventricular myocytes (unpublished observation). Given this new evidence that A_{2b} adenosine receptors were expressed in cardiomyocytes, we were very surprised when A_{2a} receptors, but not A_{2b} receptors, were found on the sarcolemma in single cardiomyocytes in which cAMP was monitored (Xin *et al.*, 2009).

Binding of agonists to A_1 and A_3 adenosine receptors results in activation and cleavage of G_i leading to a decrease in cAMP and activation of PKC (Mubagwa and Flameng, 2001), whereas A_{2a} and A_{2b} receptors stimulate adenylyl cyclase and increase cAMP production by liberating components of G_s (Mubagwa and Flameng, 2001). The A_{2a} and A_{2b} adenosine receptors were first identified by their differential ability to stimulate cAMP production in brain slices at low (0.1–1 μM) and high (>10 μM) adenosine concentrations (Schulte and Fredholm, 2003). Thus the A_{2b} receptor became known as the low-affinity receptor. Using human embryonic kidney (HEK) 293 cells overexpressing human A_{2b} receptors Linden *et al.* (1999) found that A_{2b} receptors coupled not only to G_s but also to $G_{q/11}$ leading to activation of phospholipase C and mobilization of calcium in the transfected kidney cells. Although G_s coupling mediated vasodilation, the physiological significance of $G_{q/11}$ coupling was demonstrated in the

Correspondence: Michael V Cohen, Department of Physiology, MSB 3050, University of South Alabama, College of Medicine, Mobile, AL 36688, USA. E-mail: mcohen@usouthal.edu
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degranulation of mast cells. Thus the A_{2b} adenosine receptor could trigger at least two distinct signalling cascades.

This receptor fickleness was confirmed by Feng *et al.* (2009) in the current edition of the *British Journal of Pharmacology*. They examined production of the proinflammatory cytokine interleukin-6 (IL-6) by mouse cardiac fibroblasts following exposure to 5′-(N-ethylcarboxamido) adenosine (NECA), a potent, albeit not selective, A_{2b} receptor agonist. Interestingly these fibroblasts expressed all four adenosine receptor subtypes. In response to NECA these cells produced significant amounts of IL-6, which was not mimicked by selective A_1, A_{2a} or A_3 receptor agonists. And only the selective A_{2b} receptor antagonist MRS 1754 could interfere with the response to NECA. Furthermore silencing of A_{2b} receptors with siRNA also suppressed NECA-induced IL-6 secretion. Clearly IL-6 production by cardiac fibroblasts was greatly influenced by stimulation of A_{2b} receptors. When Feng *et al.* (2009) studied the downstream signalling they found involvement of G_q and not G_s. Pretreatment with either the cAMP-competitive analog Rp-cAMPS or 1 of two protein kinase A (PKA) inhibitors, H-89 or KT 5720, before exposure to NECA failed to block NECA-induced IL-6 production. Nor could a cAMP analogue trigger IL-6 release. Therefore, the G_s-cAMP-PKA pathway was not involved in the regulation of IL-6 production by A_{2b} receptors. Although the authors did not directly test for G_q coupling, it was presumed to be responsible. These data confirm the plasticity of A_{2b} adenosine receptors, and demonstrate their complicated biology. This evidence of G_q coupling of A_{2b} receptors is probably as important as the observation that the heart can be its own source of inflammatory cytokines.

Interest in adenosine in the heart was stimulated when we found that adenosine released by a transient period of ischaemia (preconditioning) was a trigger for a signalling cascade that resulted in protection of myocardium from a subsequent more prolonged coronary occlusion (Liu *et al.*, 1991). Although A_1 adenosine receptor agonists were powerful preconditioning agents, they had little clinical value. For treatment of patients with acute myocardial infarction, an intervention was needed that could be applied at the time of reperfusion. We first reported that A_{2b} receptors were critical to signalling at the onset of reperfusion in the preconditioned heart (Philipp *et al.*, 2006). Thus both ischaemic preconditioning (Solenkova *et al.*, 2006) and post-conditioning (repeated episodes of very brief coronary reocclusion in the first minutes of reperfusion) (Philipp *et al.*, 2006) were aborted if A_{2b} receptors were blocked at reperfusion. But these observations posed a significant theoretical quandary. Under basal conditions, the extracellular concentration of adenosine ranges from 30 to 300 nM. Although the concentration increases substantially during ischaemia to 1–4 μM, it rarely exceeds 10 μM (Schulte and Fredholm, 2003). Yet the K_i of A_{2b} receptors for adenosine may be as high as 24 μM (Gao and Jacobson, 2007). Therefore, how could endogenous adenosine released from ischaemic heart muscle bind significantly to A_{2b} receptors? And why only in preconditioned hearts? In the study by Feng *et al.* in which the end point was IL-6 production, they also found that A_{2b} receptors activated PKC. However, in investigations of cardioprotection in which the end point was anatomical infarct size, Philipp *et al.* (2006) showed that in the cardioprotective signal transduction pathway, PKC is actually upstream of A_{2b} adenosine receptors, the reverse of the sequence reported by Feng *et al.* in cardiac fibroblasts. It is well known that PKC activity can sensitize A_{2b} receptor signalling, although the mechanism has never been elucidated nor has a physiological function been attributed to it (Feoktistov and Biaggioni, 1997). We (Kuno *et al.*, 2007) tested whether this sensitization phenomenon might be involved in preconditioning. We found that a subthreshold dose of NECA, which, by itself could not increase phosphorylation of the survival kinases Akt and ERK in rabbit myocardium could activate them if the PKC activator phorbol 12-myristate 13-acetate (PMA) had been previously administered. This same effect was reproduced by a preconditioning cycle of ischaemia/reperfusion that would also have activated endogenous PKC. Therefore, we proposed that, in a preconditioned heart, PKC increased the sensitivity of A_{2b} receptor signalling to the point that endogenous adenosine released during ischaemia could now bind to A_{2b} receptors at reperfusion and initiate protective intracellular signalling. This observation again emphasized the versatility of A_{2b} receptors and justified the impression that this adenosine receptor could play an important physiological role in the myocardium.

Most recently Kuno *et al.* (2009) further examined A_{2b} receptor coupling to survival kinases in HEK 293 cells stably transfected with human A_{2b} receptors. As in the heart NECA caused a dose-dependent phosphorylation of Akt and ERK. Furthermore NECA increased cAMP consistent with the previously reported G_s-coupling of A_{2b} receptors. Yet when G_s was inhibited with cholera toxin or the G_s antagonist NF449, the ability of NECA to phosphorylate Akt and ERK was actually enhanced. On the other hand, *Pertussis* toxin abolished NECA-triggered kinase phosphorylation indicating either a G_i or G_o coupling. These studies have now been repeated with the A_{2b} adenosine receptor selective agonist BAY 60-6583, and the observations are identical (submitted for publication). So here we have yet a third coupling pathway for cardiac A_{2b} receptors. The biology of the A_{2b} receptor is much more complicated than that of the other adenosine receptors and may be the key to a host of clinically relevant therapies. Unlike the leopard, A_{2b} adenosine receptors can change their spots.

Of course, additional questions must still be addressed. Where are the A_{2b} receptors located if they are not on the sarcolemma? Are they associated with some intracellular organelle? Are they functional? Does the cardioprotective signalling cascade occur in the cardiomyocyte, or does A_{2b} receptor signalling occur on outer cell membranes of another tissue, perhaps vascular endothelium, with production of a diffusible messenger that then targets cardiomyocytes?

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