PERSPECTIVE

Adenosine in Tissue Protection and Tissue Regeneration

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ABSTRACT
Adenosine promotes tissue protection and repair through four general modes of action: increased oxygen supply/demand ratio, preconditioning, anti-inflammatory effects, and stimulation of angiogenesis. A novel means by which adenosine stimulates angiogenesis is the topic of the article by Desai et al. in the April 2005 issue of Molecular Pharmacology. The report demonstrates that agonists of A2A adenosine receptors inhibit the release of the anti-angiogenic factor thrombospondin 1. Multiple cell types and all four adenosine receptors participate in these responses. Exploiting these effects of adenosine has great therapeutic potential.

Adenosine produced in hypoxic, ischemic, or inflamed environments reduces tissue injury and promotes repair by several receptor-mediated mechanisms. The intracellular production of adenosine is increased during hypoxia or ischemia and transported across cell membranes by various transporters (Thorn and Jarvis, 1996). In addition, adenine nucleotides are released from nerves, platelets, and other cell types in granules or through various channels (Dutta et al., 2004; Fabbro et al., 2004) and metabolized to adenosine primarily by ecto-apyrase (CD39) and ecto-5'-nucleotidase (CD73) (Koszalka et al., 2004). Adenosine signals through four widely expressed G protein coupled receptors: A1, A2A, A2B, and A3. There are four general modes of responses by which adenosine receptor activation protects tissues and facilitates tissue repair; these are summarized in Table 1: increased oxygen supply/demand ratio, preconditioning, anti-inflammatory effects, and stimulation of angiogenesis. A novel means by which adenosine stimulates angiogenesis is the topic of the article by Desai et al. (2005) in this issue. These new findings add to accumulating evidence that all four adenosine receptor subtypes participate in various ways to protect and facilitate regeneration of injured tissues.

Increases in Oxygen Supply/Demand Ratio
Tissue protection by adenosine was first attributed to its cardiovascular effects. Vascular smooth muscle of coronary arteries was found to have a high density of A2A adenosine receptors and to be very sensitive to adenosine partly because of spare receptors (Shryock et al., 1998). Adenosine produces vasodilation to increase oxygen delivery to the hypoxic heart. Blood vessels in other tissues are generally less sensitive to adenosine than coronary arteries but also dilate in response to adenosine through a combination of A2A and A2B receptors. Adenosine also acts to reduce oxygen demand in excitable tissues such as heart and neurons as a result of the activation of A1 receptors that increase neuronal conductance to potassium ions or decrease conductance to calcium ions (Dolphin et al., 1986). In addition, activation of A1 receptors on sympathetic nerve terminals reduces the release of norepinephrine, resulting in decreased cardiac output and decreased peripheral vascular resistance (Burgdorf et al., 2001, 2005). It has been shown recently that hypoxia increases the sensitivity of endothelial cells to adenosine in part by causing a rapid induction of A2B receptor mRNA (Eltzschig et al., 2003).

Ischemic Preconditioning and Postconditioning
Adenosine also protects tissues through its role in ischemic preconditioning—defined as a reduction in tissue infarct size during a prolonged ischemic episode as a result of a short prior episode of ischemia. Preconditioning has been most

ABBREVIATIONS: ZM241385, 4-{2-[7-amino-2-(2-furyl)[1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-ylamino]ethyl}phenol.
extensively studied in the heart but also occurs in other tissues. Adenosine produced in the ischemic heart participates in this response by activating A_2A and A_2B receptors, protein kinase C, and mitochondrial K_ATP channels (Miura et al., 2000; De et al., 2002). It has been demonstrated recently that “postconditioning”, a brief period of ischemia after prolonged ischemia, can reduce myocardial inflammation and infarct size (Zhao et al., 2003). It is likely that adenosine released during postconditioning contributes to inhibition of inflammation, probably as a result of activation of A_2A receptors on inflammatory cells.

**Anti-Inflammatory Responses.** Many recent studies indicate that adenosine acting on A_2A receptors can powerfully inhibit inflammation and reperfusion injury. The A_2A adenosine receptor is found on most bone-marrow–derived cells and produces cellular effects that in general inhibit inflammation. The cellular responses seem to be mediated predominately by cyclic AMP and result in inhibition of oxidative burst in neutrophils (Fredholm et al., 1996), reduced TNF\(\alpha\) release by monocytes (Link et al., 2000), reduced platelet activation (Hourani, 1996), and inhibition of lymphocyte activation (Lappas et al., 2005). In aggregate, these responses prevent the release of pro-inflammatory cytokines and oxygen radicals, prevent endothelial cell activation, and greatly reduce microvascular occlusion, which can exacerbate tissue injury during reperfusion of previously ischemic tissues.

**A_2B Receptors are Proinflammatory.** Both A_2A and A_2B receptors couple to the heterotrimeric G protein, Gs; in many cell types, however, A_2B receptors have been found to be dually coupled to Gs and Gq. Signaling through Gq results in calcium mobilization and activation of phospholipase C and mitogen-activated protein kinase (Gao et al., 1999). This signaling pathway seems to be responsible for pro-inflammatory actions of adenosine mediated by A_2B receptors that results in facilitation by adenosine of antigen-induced deregulation of canine or human but not rodent mast cells (Feoktistov and Biaggioni, 1995; Auchampach et al., 1997). In addition, activation of A_2B receptors has been shown to increase the release of interleukin 6 from epithelial cells, astrocytes, and fibroblasts (Schwaninger et al., 1997; Sitaraman et al., 2001; Zhong et al., 2005). Hence, the activation of adenosine receptors is not invariably protective of tissues. In fact, A_2B blockers may be useful as anti-inflammatory agents.

**Angiogenesis**

In addition to protecting tissues, adenosine has long been known to stimulate angiogenesis, which is necessary for tissue repair (Teuscher and Weidlich, 1985; Dusseau et al., 1986). As is the case for tissue protection by adenosine, multiple adenosine receptor subtypes participate in the stimulation of angiogenesis. Activation of the A_1 receptors powerfully stimulates angiogenesis in vivo by an as-yet-undefined mechanism (Linden et al., 2003). Stimulation of angiogenesis also is caused in part by activation of A_2B receptors on endothelial cells (Feoktistov et al., 2002) and A_2B or A_3 receptors on mast cells (Feoktistov et al., 2003) that induce the release of angiogenic factors including VEGF. The report by Desai et al. (2005) in this issue describes another means by which adenosine receptors on human umbilical vein endothelial cells stimulate angiogenesis. Adenosine inhibits the release of the anti-angiogenic protein thrombospondin 1, resulting in increasing vascular tube formation. Blockade of this response by the A_2A-selective antagonist ZM241385 is not surmountable by high doses of A_2A agonists, but despite this peculiarity, the pharmacological profile of the response suggests that it is mediated by the A_2A receptor. Desai et al. (2005) also show that in the presence of antibodies to TSP1 or CD36, the receptor for TSP1, A_2A agonists fail to stimulate vascular tube formation. These results indicate that vascular tube formation by adenosine A_2A receptor activation is largely mediated by suppression of TSP1 secretion.

**Conclusion**

Tissue protection and regeneration by adenosine is mediated by multiple different cells types and involves participation of all four adenosine receptor subtypes. Fully understanding and exploiting these protective and regenerative mechanisms has great clinical potential.

**TABLE 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in oxygen supply/demand ratio</td>
<td>Vasodilation</td>
<td>Belardinelli et al., 1998; Ngai et al., 2001</td>
</tr>
<tr>
<td>A_2A, A_2B</td>
<td>Decreased heart rate</td>
<td>Dhall et al., 2003</td>
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<tr>
<td>A_1</td>
<td>Decreased neuronal activity</td>
<td>Dunwiddie and Masino, 2001</td>
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<td></td>
<td>Decreased sympathetic nerve activity</td>
<td>Burgdorf et al., 2005a</td>
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<tr>
<td>Ischemic preconditioning and postconditioning</td>
<td>Preconditioning</td>
<td>Zhao et al., 2003</td>
</tr>
<tr>
<td>A_1, A_3</td>
<td>Postconditioning</td>
<td>De et al., 2002b</td>
</tr>
<tr>
<td>Anti-Inflammatory Responses</td>
<td>Heart</td>
<td>Lasley et al., 2001; Glover et al., 2004</td>
</tr>
<tr>
<td>A_2A</td>
<td>Kidney</td>
<td>Day et al., 2003</td>
</tr>
<tr>
<td>A_2A</td>
<td>Liver</td>
<td>Ohita and Sitkovsky, 2001; Day et al., 2004</td>
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<tr>
<td></td>
<td>Spinal cord</td>
<td>Reece et al., 2004</td>
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<td></td>
<td>Skin</td>
<td>Peirce et al., 2001</td>
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<td></td>
<td>Lung</td>
<td>Ross et al., 1999</td>
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<tr>
<td>Angiogenesis</td>
<td>Decreased endothelial cell thrombospondin</td>
<td>Desai et al., 2005</td>
</tr>
<tr>
<td>A_2A</td>
<td>Chorioallantoic membrane</td>
<td>Linden et al., 2003</td>
</tr>
<tr>
<td>A_2B</td>
<td>Increase endothelial cell release of angiogenic factors</td>
<td>Feoktistov et al., 2002</td>
</tr>
<tr>
<td>A_2B, A_3</td>
<td>Increased mast cell release of angiogenic factors</td>
<td>Feoktistov et al., 2003</td>
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References