Published in final edited form as:
Circ Res. 2007 November 26; 101(11): 1075–1077.

Adenosine Receptors and Angiogenesis

John A. Auchampach
Department of Pharmacology & Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226 U.S.A

Keywords

adenosine; angiogenesis; receptors; ischemia; heart

As originally hypothesized by Berne,[1] adenosine is formed in the heart during hypoxia or ischemia and serves as a negative feedback signal to maintain oxygen delivery within a normal physiological range. Adenosine is formed by the metabolism of ATP and transported into the extracellular space by various nucleoside transport proteins.[2] In a more localized manner that provides the opportunity for coordinated signaling with the diverse P_2 purinoreceptor family that recognizes ATP and ADP, adenosine is also formed from the extracellular metabolism of adenine nucleotides released from stored granules (sympathetic nerves, platelets, mast cells) or through hemichannels (neutrophils, endothelial cells, epithelial cells) by ecto-apyrases and ecto-nucleotidases.[3–5] Adenosine signals through activation of widely distributed cell surface G protein-coupled receptors, of which four different subtypes have been identified designated A_1, A_{2A}, A_{2B}, and A_3.[6] By activating A_{2A} receptors that are abundantly expressed in coronary vascular smooth muscle, adenosine generated under hypoxic conditions produces potent vasodilation thereby increasing nutrient and oxygen delivery.

Although less appreciated, adenosine also serves to maintain tissue oxygenation in response to chronic ischemic/hypoxic stress by increasing the formation of new blood vessels; that is, by stimulating angiogenesis.[7, 8] Using adenosine receptor agonists or modulators of adenosine metabolism, an important role for adenosine has been established in *in vivo* models of angiogenesis.[7, 8] In cell culture systems, adenosine stimulates endothelial cell migration, proliferation, and tube formation,[7–11] actions required for the formation of new capillary networks. This action occurs at concentrations of adenosine that are reached in hypoxic tissues. Adair [7] recently estimated that adenosine can contribute up to 50–70% of the angiogenic response in some cases.

Adenosine has direct mitogenic effects on vascular cells that may contribute to angiogenesis. However, the main pro-angiogenic actions of adenosine have been attributed to its ability to regulate the production of pro- and anti-angiogenic substances from vascular cells and immune cells within the microenvironment of hypoxic tissues. Adenosine stimulates the release of interleukin-8 (IL-8), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) from endothelial cells via A_{2B} receptors that are dually coupled to G_s and G_q proteins while inhibiting production of the anti-angiogenic factor thrombospondin-1, seemingly by the G_s protein-coupled A_{2A} receptor.[9, 10] Adenosine also stimulates the production of VEGF, IL-8, and angiopoetin-1 from mast cells via A_{2B} and A_3 receptors.[11] A unique mechanism has been identified by Lebovich and colleagues[12] in which adenosine

Address correspondence to: John A. Auchampach, Ph.D., Department of Pharmacology & Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, Phone: 414-456-5643, Fax: 414-456-6545, Email: jauchamp@mcw.edu.

Disclosures
Nothing to disclose.

activation of the A_{2A} receptor converts macrophages from a pro-inflammatory phenotype that generates large amounts of tumor necrosis factor-α and IL-6 to a pro-angiogenic phenotype that predominantly secretes VEGF. This particular mechanism is likely important in promoting tissue repair following wounding or microbial infection.

In this issue of *Circulation Research*, Clark and colleagues[13] demonstrate for the first time that the A_1 adenosine receptor also participates in the angiogenic actions of adenosine. The A_1 receptor is a G_i protein-coupled receptor that is well-known in the cardiovascular system to mediate the negative chronotropic, negative dromotropic, and anti-adrenergic actions of adenosine responsible for reducing oxygen demand during metabolic stress.[14] Using the chick chorioallantoic membrane model, Clark *et al*[13] demonstrated that the selective A_1 receptor agonist N^6-cyclopentyladenosine (CPA) increased membrane vessel number by 40%. This effect produced by CPA was inhibited by the highly selective A_1 receptor antagonist WRC-0571 confirming involvement of the A_1 receptor. The authors[13] need to be commended for devoting substantial effort in cloning and pharmacologically characterizing three of the four chick adenosine receptors to verify the selectivity of the pharmacological agents used in their study, as the binding affinities of adenosine receptor ligands are well known to differ markedly among species.[15, 16] A_1 adenosine receptor expression has been reported previously in the vasculature,[17, 18] suggesting that CPA may functionthrough a direct mitogenic action. However, CPA did not promote capillary formation in an aortic ring model of angiogenesis, leading the authors to conclude that the actions of CPA are indirect involving non-vascular cells. Among cells known to be involved in the angiogenic response, monocytes express A_1 receptors.[19] Acting on this lead, the authors showed that: 1) CPA stimulated VEGF release from isolated peripheral human monocytes through an A_1 receptor-mediated mechanism, and 2) conditioned media from CPA-treated monocytes promoted vessel growth in the aortic ring model, which was blocked by the addition of an anti-VEGF antibody. Collectively, these results suggest that A_1 receptor-mediated angiogenesis may involve the release of VEGF from monocytes. This study is important to our understanding of mechanisms of angiogenesis and the identification of the A_1 receptor as a new therapeutic target. This is also the first study to demonstrate that A_1 receptor activation promotes the release of a pro-angiogenic substance from an immune cell population. Previously, the only known function of the A_1 receptor in the immune system is to promote migration of neutrophils and dendritic cells.[20]

Clark and colleagues[13] are careful to emphasize that the two major findings in their study may not be interrelated. That is, it is possible that A_1 receptor-mediated angiogenesis in the chick chorioallantoic membrane model may involve other mechanisms that do not involve the release of VEGF from monocytes. Considering that A_1 receptors facilitate migration of some immune cells, [8] one interesting possibility is that CPA increased vasculogenesis. Following wounding, adenosine has been implicated in mediating recruitment of endothelial progenitor cells to sites of neovascular formation. [21] The authors[13] also note that the importance of the A_1 receptor in angiogenesis needs to be demonstrated in mammalian models. This work will be facilitated by the availability of mice with global deletion of adenosine receptor genes as well as mice with tissue-specific deletion of adenosine receptors. If confirmed, the exciting possibility will exist that pharmacological therapies targeting A_1 receptor function may provide a basis for treating coronary artery disease, cancer, or other diseases in which the underlying basis is exaggerated or limited angiogenesis. Of note, the clinical use of direct A_1 agonists as pro-angiogenic agents would face several obstacles, such as the potential to produce undesirable cardiovascular effects including bradycardia, atrio-ventricular block, and hypotension. Small molecule allosteric enhancers of the A_1 receptor, which do not directly activate the receptor but instead enhance the actions of endogenous adenosine by increasing coupling efficiency to G proteins, may prove to be more useful.[22, 23] These agents could theoretically lead to angiogenesis in those tissue areas where hypoxia is greatest without affecting systemic hemodynamic parameters. Cardiovascular side-effects may also be minimized by using partial

A_1 receptor agonists that elicit submaximal responses even when administered at high doses.[24, 25] The use of A_1 receptor antagonists to reduce angiogenesis may be less problematic. Afzal and colleagues[26] recently reported that intraocular injection of a ribozyme specific for the A_{2B} receptor reduced preretinal neovascularization in a neonatal mouse model of oxygen-induced retinopathy, highlighting the feasibility of using genetic approaches to modulate adenosine-mediated angiogenesis.

In summary, Clark *et al*[13] have made the novel discovery that A_1 adenosine receptor activation can stimulate angiogenesis in an established experimental model. Combined with previous work conducted by others, it now appears that all four adenosine receptor subtypes participate in mediating the angiogenic actions of adenosine, working in a coordinated fashion involving indirect and direct actions on endothelial cells, smooth muscle cells, fibroblasts, and resident immune cells including monocytes, macrophages, and mast cells (see Figure 7 in Clark *et al*[13]). The relative importance of each adenosine receptor subtype awaits further investigation.

Acknowledgements

Sources of Funding

The author is supported by NIH R01 grants HL60051 and HL077707.

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