An Emerging Role for Adenosine in Angiogenesis

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The possibility that adenosine has an important role in cardiovascular function was introduced 75 years ago when Drury and Szent-Gyorgyi found that extracts from heart and other tissues produced vasodilation, hypotension, bradycardia, and a decrease in atrioventricular conduction velocity. In the early 1960s, Berne et al proposed the so-called retaliatory metabolite concept in which hypoxic tissues produce adenosine from ATP, and the adenosine in turn functions to restore a balance between oxygen demand and oxygen supply by causing vasodilation and increased blood flow. This has been shown to be true for heart, brain, and skeletal muscle in several different species. Adenosine thus serves as a negative feedback signal to maintain tissue oxygenation within a normal range.

Mounting evidence suggests that adenosine may also have a long-term role to increase tissue oxygenation by stimulating the growth of blood vessels. Physiological concentrations of adenosine or adenosine agonists cause a dose-related increase in the proliferation of endothelial cells obtained from bovine aorta, bovine coronary venules, and human umbilical veins, but they have no effect on the proliferation of human fibroblasts and rat myocardial myoblasts. Also, raising endogenous levels of adenosine by inhibition of adenosine kinase can stimulate the proliferation of human endothelial cells obtained from umbilical veins. Adenosine stimulates angiogenesis in the body and chorioallantoic membrane of the chick embryo and could mediate a portion of the hypoxia-induced angiogenesis in the same tissues. Early work has also shown that prolonged infusion of adenosine induces angiogenesis in rabbit heart and skeletal muscle. That adenosine could have a physiological role in the development of the vasculature is supported by the finding that prolonged administration of dipyridamole, an adenosine-reuptake inhibitor, stimulated angiogenesis in both the heart and skeletal muscle in rats; aminophylline, an adenosine-receptor blocker, produced a dose-related decrease in the size of the vasculature in chick embryos.

Although the mechanism by which adenosine induces angiogenesis is poorly understood, many studies have shown that the administration of adenosine or adenosine agonists as well as the upregulation of endogenous adenosine can increase the expression of vascular endothelial growth factor (VEGF) in a variety of different cells studied in vitro. VEGF is a key mediator of angiogenesis released from hypoxic tissues in both physiological and pathological conditions and may be subject to negative feedback regulation in the intact animal. Recent studies have also shown that adenosine can increase VEGF protein expression in humans and VEGF mRNA expression in chick embryos (unpublished observations). For these reasons, numerous investigators have postulated that this adenosine-mediated expression of VEGF might account for the angiogenic effects of adenosine; however, it is possible that adenosine can also stimulate angiogenesis via other secondary mediators or by way of an intracellular action.

In this issue of Hypertension, Feoktistov et al not only confirm previous work showing that adenosine agonists can induce VEGF expression by way of A2B receptors but also show that exposing human endothelial cells and smooth muscle cells to a hypoxic environment can induce an angiogenic phenotype and thereby amplify the VEGF response to adenosine. Both types of cells normally express A2A and A2B receptors, but A2A receptors predominate in these cells under normoxic conditions both in terms of mRNA expression and functional coupling to agonists. The study shows that exposing the cells to a hypoxic environment can downregulate the expression of A2A receptors and upregulate the expression of A2B receptors within a few hours, thereby greatly enhancing the adenosine-induced upregulation of VEGF. It is therefore conceivable that adenosine mediates a level of compensatory angiogenesis in hypoxic tissues far beyond what can be predicted from studies performed under normoxic conditions.

VEGF regulation by hypoxia occurs at both the transcriptional and post-transcriptional levels. Transcriptional regulation of VEGF is mediated by hypoxia-inducible factor-1 (HIF-1), which is thought to be the major stimulus for VEGF production under hypoxic conditions in most types of cells. HIF-1 accumulates under hypoxic conditions and activates VEGF transcription by binding to specific promoter sequences. In addition, hypoxia leads to stabilization of VEGF mRNA, which increases its steady-state levels. The mechanism by which adenosine induces VEGF is poorly understood, but the Feoktistov study shows that the mechanism is independent of HIF-1.

Although the study has shown that exposure to a hypoxic environment converts human endothelial cells and smooth muscle cells to an angiogenic phenotype, one must be cautious in considering the quantitative importance of adenosine...
osine as a stimulus for VEGF production under hypoxic conditions. Studies by Gu et al.\(^8\) indicate that an adenosine A\(_2\) antagonist could not block \(\geq 20\)% of the VEGF expression induced by exposing myocardial vascular smooth muscle cells to a hypoxic environment (1% oxygen for 18 hours). Because the upregulation of adenosine A\(_{2B}\) receptors observed in the Feoktistov study\(^{16}\) occurred within 3 hours of exposure to hypoxia, it should be clear that sufficient time was allowed for similar conversion to an angiogenic phenotype in the Gu study.\(^4\) As pointed out by the authors,\(^{16}\) other studies indicate that the effects of adenosine on VEGF expression can be augmented under normoxic conditions in an additive manner when the cells are exposed simultaneously to cobalt chloride.\(^{19}\) This is pertinent because cobalt chloride can partially mimic hypoxic conditions by increasing the activity of HIF-1. Yet other studies indicate that VEGF mRNA is induced only slightly in mutant mouse hepatoma the activity of HIF-1. Yet other studies indicate that VEGF protein, is a maintenance factor (also called a survival factor) for the vasculature. The concept of a vascular survival factor implies that under normoxic conditions can be abolished by adenosine A\(_{2B}\) receptors to a hypoxic environment (1% oxygen for 18 hours). The upregulation of adenosine A\(_{2B}\) receptors observed in the Feoktistov study occurred within 3 hours of exposure to hypoxia, it should be clear that sufficient time was allowed for similar conversion to an angiogenic phenotype in the Gu study. As pointed out by the authors, other studies indicate that the effects of adenosine on VEGF expression can be augmented under normoxic conditions in an additive manner when the cells are exposed simultaneously to cobalt chloride. This is pertinent because cobalt chloride can partially mimic hypoxic conditions that do not express the HIF-1b (ARNT) subunit,\(^{17}\) which suggests a minor role for adenosine in these cells. Therefore, the available data indicate that the quantitative role of adenosine in the induction of VEGF under hypoxic conditions is poorly understood, especially because there are no studies in intact animals at the time of writing this commentary.

An interesting possibility is that adenosine, acting by way of VEGF protein, is a maintenance factor (also called a survival factor) for the vasculature. The concept of a vascular maintenance factor is based on the knowledge that the size of a capillary network is dependent on the metabolic activity of the tissues that it serves. For example, it is well-known that a long-term increase in metabolic activity can stimulate angiogenesis in skeletal muscle, whereas factors that decrease metabolic activity lead to a loss of capillaries.\(^2\) Therefore, a normal level of metabolic activity seems to be required to maintain the structural integrity of the capillary network. A recent study\(^{20}\) indicates that targeted skeletal muscle inhibition of VEGF expression in VEGFloxP/+/H11015 mice led to a 64% decrease in capillary density and capillary-to-fiber ratio in the VEGF-inactivated regions within 4 weeks, suggesting that VEGF is an essential vascular maintenance factor in skeletal muscle.

The possibility that adenosine is critical for establishing basal levels of VEGF under normoxic conditions and is therefore an essential maintenance factor for the vasculature is based on the following findings: (1) oxidative muscle has a relatively high metabolic rate, high basal level of adenosine, high basal level of VEGF, and high capillarity compared with glycolytic muscle\(^7,21,22\); (2) \(>60\)% of the VEGF protein secreted by myocardial vascular smooth muscle cells under normoxic conditions can be abolished by adenosine A\(_2\) antagonist\(^4\); and (3) adenosine deaminase (which metabolizes adenosine to inosine) can decrease the basal levels of VEGF by \(\approx 60\)% in the media of cardiomyoblasts.\(^5\) Also, when a glycolytic muscle is converted to an oxidative muscle by prolonged electrical stimulation of a motor nerve, \(\approx 2\)-fold increase in capillarity is associated with \(\approx 2\)-fold increase in basal levels of VEGF mRNA in the muscle tissues during resting conditions.\(^{13}\)

It is less likely that HIF-1 has a major role in establishing basal levels of VEGF under normoxic conditions for the following reasons: (1) the responsiveness of the HIF-1 system to changes in oxygen tension appears to be low under normoxic conditions, ie, large changes in oxygen tension cause relatively small changes in HIF-1 expression when oxygen tension is in a normal range\(^{23}\); and (2) HIF-1 levels may be insufficient to induce VEGF under normoxic conditions, ie, HIF-1 is often undetectable in the absence of hypoxia.\(^{17}\)

For these reasons, it is conceivable that adenosine plays an important role in establishing the basal levels of VEGF in a tissue under normoxic conditions. If this is true, and if adenosine serves to “fine-tune” the levels of VEGF in a tissue in accordance with its metabolic activity, then adenosine can be considered a vascular maintenance factor.

Overall, this article raises a number of interesting research questions. Will prolonged exposure to a hypoxic environment lead to development of an angiogenic phenotype in the intact animal in which the expression of A\(_{2B}\) receptors is downregulated and the expression of A\(_{2B}\) receptors is upregulated? Will an upregulation of A\(_{2B}\) receptors in cardiac fibroblasts and smooth muscle cells prevent cardiac remodeling associated with hypertension, myocardial infarction, and myocardial reperfusion injury and ischemia and at the same time stimulate blood vessel growth, as suggested by the authors?\(^{16}\) What is the quantitative importance of adenosine in inducing VEGF expression under normoxic and hypoxic conditions in the intact animal? By what mechanism does adenosine induce VEGF? Is adenosine a maintenance factor for the vasculature? Can modulation of the adenosine system be used as a therapeutic modality in angiogenic diseases characterized by too much or too little angiogenesis? Definitive answers to these questions will require long-term studies in real tissues of intact animals.

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**References**


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