Hypoxic Vasospasm Mediated by cIMP: When Soluble Guanylyl Cyclase Turns Bad

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Abstract: In a number of isolated blood vessel types, hypoxia causes an acute contraction that is dependent on the presence of nitric oxide and activation of soluble guanylyl cyclase. It is more pronounced when the preparations are constricted and is therefore termed hypoxic augmentation of vasoconstriction. This hypoxic response is accompanied by increases in the intracellular level of inosine 5′-triphosphate and in the synthesis of inosine 3′,5′-cyclic monophosphate (cIMP) by soluble guanylyl cyclase. The administration of exogenous cIMP or inosine 5′-triphosphate causes augmented vasoconstriction to hypoxia. Furthermore, the vasoconstriction evoked by hypoxia and cIMP is associated with increased activity of Rho kinase (ROCK), indicating that cIMP may mediate the hypoxic effect by sensitizing the myofilaments to Ca2+ through ROCK. Hypoxia is implicated in exaggerated vasoconstriction in the pathogenesis of coronary artery disease, myocardial infarction, hypertension, and stroke. The newly found role of cIMP may help to identify unique therapeutic targets for certain cardiovascular disorders.

Key Words: hypoxia, vasoconstriction, nitric oxide, soluble guanylyl cyclase, cIMP

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INTRODUCTION

In the presence of the endothelium, hypoxia causes a rapid constriction in a number of blood vessel types.1–11 Such a response also occurs in arteries without endothelium treated with exogenous nitric oxide (NO).8,11 It is prevented by the inhibition of soluble guanylyl cyclase (sGC) but appears not to be related to guanosine 3′,5′-cyclic monophosphate (cGMP) signaling.5,11 Recent studies suggest that inosine 3′,5′-cyclic monophosphate (cIMP) synthesized by sGC may act as the mediator of such hypoxic “vasospasm.”11,12 This brief review discusses the characteristics of the endothelium-dependent hypoxic vasospasm, the synthesis of cIMP by sGC, and the role of this cyclic nucleotide in augmented vasoconstrictions in response to hypoxia.

CHARACTERISTICS OF HYPOXIC SPASM

The phenomenon that hypoxia can cause an acute contraction in systemic blood vessels was first reported in 1976 when describing experiments in isolated canine veins.1 This phenomenon has subsequently been confirmed in a number of blood vessel types including the canine femoral,4,5 and coronary4,6 arteries, porcine coronary arteries,8,10,11 rat aortae and mesenteric arteries,9 and human and canine internal mammary arteries.6 The hypoxic constriction is more pronounced when the blood vessels are contracted. Therefore, it is more appropriate to be termed “hypoxic augmentation of vasoconstriction.”5 It occurs in arteries contracted with norepinephrine,2,3 phenylephrine,2,3 prostaglandin F2α,4,5,7 or the thromboxane A2 mimetic U46619.10,11 Thus, it is not related to a specific vasoconstrictor or restricted to a single blood vessel type.

The hypoxic augmentation of vasoconstriction occurs in arteries with endothelium but not in those without endothelium.2,4,5,8,11 It is not due to the release by the endothelial cells of a known endothelium-derived contracting factor, such as endothelin 1, constrictor prostanoids, or reactive oxygen species.8 The involvements of calcium-active potassium channels, inward rectifier potassium channels, L-type calcium channels, sodium–potassium ATPase, hyperpolarizing factors, gap junctions, adenyl cyclase, and adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) were excluded by studies with various pharmacological inhibitors.8

The endothelium-dependent hypoxic augmentation of vasoconstriction is abolished by the inhibition of endothelial NO synthase with nitro-L-arginine and by the inhibition of sGC with methylene blue, ODQ, or NS2028.2,5,8–11 It is restored in arteries without endothelium by exogenous NO donors or Bay 58-2667 (a heme-independent activator of sGC).5,8,11 These results indicate that activation of sGC is critically involved. Cyclic guanosine monophosphate is considered the only known functional molecule synthesized by sGC, but its action is to cause vasodilatation.13,15 Therefore, a suppressed formation of cGMP

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by sGC under hypoxia would be expected to promote vasoconstriction. However, the hypoxic response is not accompanied by significant changes in cGMP levels. It is also not affected by the inhibition of cGMP-dependent protein kinase (PKG, the primary downstream effector of the cyclic nucleotide). In arteries without endothelium, the absence of hypoxic augmentation of vasoconstriction is not restored by the cell-permeable cGMP analog 8-Br-cGMP or by the elevation of the intracellular level of cGMP with atrial natriuretic peptide. Hence, cGMP is unlikely to be involved. Since the hypoxic response is enhanced by inhibition of phosphodiesterase, the phenomenon cannot be mediated by a degradation product(s) of cGMP but rather is due to a product of sGC different from cGMP.

**Cyclic Inosine 5’-Monophosphate Synthesized by sGC**

sGC is a heterodimer composed of an α (α1 or α2) and a β (β1) subunit, with the α1β1 dimer being the dominant isoform in most tissues including blood vessels. The β subunit of sGC contains a haem moiety as a prosthetic group. The binding of NO to the Fe²⁺ center of the haem moiety leads to a conformational change and a several hundred-fold increase in the rate of cGMP synthesis. Seifert et al, using a highly sensitive and specific high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS), demonstrated that, in addition to cGMP, purified recombinant rat sGC can synthesize cIMP, cAMP, and xanthosine 3’,5’-cyclic monophosphate (cXMP) when Mg²⁺ is used as a cofactor. If Mg²⁺ is replaced with Mn²⁺, 2 more cyclic nucleotides [uridine 3’,5’-cyclic monophosphate (cUMP) and cytidine 3’,5’-cyclic monophosphate (cCMP)] can also be synthesized. Among these nucleoside 3’,5’-cyclic monophosphates, the maximal rate of cIMP formation is substantially greater than that of the others except cGMP (with Mg²⁺ as the cofactor: cIMP > cGMP >> cUMP >>> cXMP; with Mn²⁺ as the cofactor: cGMP > cIMP > cUMP >> cAMP > cCMP ≈ cXMP)17. Regarding the affinity of sGC for the substrate, the K_M of inosine 5’-triphosphate ([ITP]), the substrate for cIMP, is considerably greater than that of guanosine 5’-triphosphate ([GTP], the substrate for cGMP) if Mg²⁺ serves as the cofactor. However, when Mg²⁺ is replaced with Mn²⁺, the K_M of ITP is lower than GTP.17 In intact HEK293 cells overexpressing sGC α1β1 and rat fetal lung fibroblast (RFL-6) cells endogenously expressing sGC, Mn²⁺ rather than Mg²⁺ may be the physiological cofactor for sGC.18

In isolated porcine coronary arteries, there is no difference in the basal levels of cIMP between arteries with or without endothelium whether treated or not with a sGC inhibitor, indicating a minor formation of the nucleotide by sGC under basal conditions. The cIMP content in cultured arteries without endothelium is replaced with Mn²⁺ to a conformational change and a several hundred-fold increase in the rate of cGMP synthesis.

When exposed to hypoxia (25–30 mm Hg), porcine coronary arteries with endothelium exhibit an increase in intracellular level of the order of 311 pmole/mg protein. The levels of cIMP are also elevated in arteries without endothelium stimulated with exogenous NO or in arteries with endothelium treated with ITPA, the substrate for cIMP formation. The elevation in cIMP under those conditions is further enhanced by hypoxia. These changes in cIMP are prevented by ODQ, indicating that cIMP is synthesized by sGC. In contrast to cIMP, in arteries with endothelium, the cIMP level is substantially greater in the absence than in the presence of ODQ, suggesting an active basal synthesis of cGMP by sGC. However, hypoxia has no significant effect on the cGMP levels.

The stimulatory effect of hypoxia on the synthesis of cIMP by sGC in porcine coronary arteries is associated with an elevated intracellular level of ITP. Hence, an increased availability of the substrate is involved. ITP is primarily derived from ATP deamination. ATP is the most abundant nucleotide in cells, with an intracellular concentration in the low millimolar range. It has been estimated that approximately 10%–25% of the ATP pool can be deaminated to ITP. The intracellular level of ITP is increased also by exogenous ATP. Under normal conditions, ITP is largely degraded by inosine triphosphatase and the intracellular level of ITP is very low. However, if hypoxia were to increase ATP deamination and/or inhibit inosine triphosphatase, the ITP level may increase which would facilitate the increased synthesis of cIMP by sGC (Fig. 1).

**Cyclic Inosine 5’-Monophosphate in Hypoxic Vasospasm**

In porcine coronary arteries with endothelium, the hypoxic augmentation of vasoconstriction is further enhanced by ITP in a manner sensitive to inhibition of sGC.11 In arteries without endothelium, the administration of cIMP restores the hypoxic augmentation. Therefore, it appears that cIMP derived from sGC is causally related to the response.

![FIGURE 1. Possible mechanism for increased synthesis of cIMP by sGC in response to hypoxia. The intracellular ITP is primarily derived from the deamination of ATP, which is the most abundant nucleotide inside the cell. Under normal conditions, ITP is mostly converted to IMP by ITPA and the cytosolic level of ITP is negligible. Under hypoxia, ATP deamination is stimulated and/or ITPA is inhibited. This leads to elevated ITP level, increased formation of cIMP by sGC, and hence enhanced cIMP action. Cyclic IMP is degraded by phosphodiesterase (PDE). Full arrows indicate activation and dotted arrows indicate inhibition. IMP, inosine 5’-monophosphate; ITPA, inosine triphosphatase.](image-url)
Cyclic inosine 5'-monophosphate or ITP has little effect on the basal tension of coronary arteries but markedly influences hypoxic facilitation of vasoconstriction. This observation suggests that cIMP ultimately may act by increasing the sensitivity of myofilaments of the vascular smooth muscle cells to Ca²⁺. In those cells, Ca²⁺ sensitization results from the activation of protein kinase C (PKC) or Rho kinase (ROCK), which results in reduced activity of myosin light-chain phosphatase, thus decreased dephosphorylation of phosphorylated myosin regulatory light chain (MLC₂₀) and augmented vasoconstriction. The hypoxic augmentation of contraction in the porcine coronary artery is attenuated by inhibitors of ROCK (Y27632 and HA1077), suggesting the involvement of ROCK in this process. ROCK (Y27632 and HA1077) may counteract the effect of ROCK by stimulating the activity of MLCP through cGMP-dependent protein kinase (PKG). Because hypoxia has no significant effect on cGMP formation, it appears not to play a significant role in hypoxic augmentation of vasoconstriction. Full arrows indicate activation and dotted arrows indicate inhibition (Modified from Gao and Vanhoutte⁹ by permission of Naunyn-Schmiedeberg’s Archiv). IMP, inosine 5'-monophosphate; MLCP, myosin light chain phosphatase; GMP, guanosine monophosphate. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

FIGURE 2. Proposed mechanism involving cIMP as a mediator in hypoxic augmentation of vasoconstriction. Hypoxia may stimulate the synthesis of cIMP by sGC, in part, by increasing the intracellular level of cGMP. Cyclic IMP can activate Rho kinase (ROCK), resulting in reduced activity of MLCP, thus decreased dephosphorylation of phosphorylated myosin regulatory light chain (MLC₂₀) and augmented vasoconstriction. Cyclic GMP may counteract the effect of ROCK by stimulating the activity of MLCP through cGMP-dependent protein kinase (PKG). Because hypoxia has no significant effect on cGMP formation, it appears not to play a significant role in hypoxic augmentation of vasoconstriction. Full arrows indicate activation and dotted arrows indicate inhibition (Modified from Gao and Vanhoutte⁹ by permission of Naunyn-Schmiedeberg’s Archiv). IMP, inosine 5'-monophosphate; MLCP, myosin light chain phosphatase; GMP, guanosine monophosphate. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

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