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Adenosine stimulates nitric oxide synthesis in vascular smooth muscle cells

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Abstract

Objective: The aim was to investigate the effects of adenosine on nitric oxide (NO) synthesis in vascular smooth muscle cells. **Methods:** NO and cAMP synthesis was measured in confluent rat vascular smooth muscle cells in culture at passage 5–10, using Griess reagent and an enzyme immunoassay kit, respectively. The expression of inducible NO synthase mRNA was assayed by Northern blotting. **Results:** Incubation of cultures with interleukin-1β (10 ng/ml) for 24 h caused a significant increase in nitrite production. The interleukin-1β-induced nitrite production by vascular smooth muscle cells was significantly increased by adenosine or its stable analogue, 2-chloroadenosine, in a dose-dependent manner. The adenosine A_{2a} receptor antagonist, KF17837, but not the A_1 receptor antagonist, DPCPX, significantly inhibited 2-chloroadenosine-mediated nitrite production. The 2-chloroadenosine-induced nitrite production by interleukin-1β-stimulated cells was accompanied by increased inducible NO synthase mRNA accumulation. In the presence of dibutyryl-cAMP (1 mM), interleukin-1β-induced nitrite accumulation was further increased, but the effect of 2-chloroadenosine was not additive or synergistic. Addition of 2-chloroadenosine dose-dependently increased intracellular cAMP levels of vascular smooth muscle cells. **Conclusions:** These results indicate that adenosine acts on A_2 receptors and augments NO synthesis in interleukin-1β-stimulated vascular smooth muscle cells, at least partially through a cAMP-dependent pathway.

Keywords: Rat; Adenosine; Interleukin-1; Nitric oxide; cAMP; Vascular smooth muscle cell

1. Introduction

Nitric oxide (NO), the extensively characterized endothelium-derived relaxing factor, is a short-lived free radical. NO is synthesized from L-arginine by three isoenzymes expressed either constitutively (neuronal, type I cNOS; endothelial, type III cNOS) or following stimulation by cytokines (inducible, type II iNOS) [1,2]. Inducible NO synthase has been identified in endotoxin- and cytokine-treated macrophages, hepatocytes, endothelial cells and myocardium [1,3]. NO synthase activity is also induced in aortic rings and cultured vascular smooth muscles by cytokines and endotoxins [4,5]. It has been demonstrated that balloon injury induces NO synthase activity in rat carotid artery smooth muscle cells [6]. Therefore, NO synthase induction in vascular smooth muscle cells may play a role in vaso-occlusive disorders such as atherosclerosis, and restenosis after angioplasty and bypass surgery [7].

Vascular cells have many metabolic pathways responsible for generating adenosine [8,9]. Adenosine has several anti-vasoocclusive properties: e.g., it induces vasodilatation [10], inhibits platelet aggregation [11], prevents platelet adhesion [12], abrogates neutrophil-induced endothelial damage [12], blocks the synthesis of potent vaso-occlusive factors such as angiotensin II and norepinephrine [13,14], and inhibits growth of vascular smooth muscle cells [15]. The effects of adenosine are mediated via discrete membrane receptors. Thus far, 5 types of receptors for adenosine (A_1, A_{2a}, A_{2b}, A_3 and A_4 receptors) have been identified to date [16,17]. The A_1 and A_2 receptors mediate several vasoactive effects of adenosine within the vasculature and are coupled to adenylyl cyclase via guanine nucleotide-binding proteins (G proteins). Activation

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of the A_1 receptor stimulates G_i, which either inhibits adenylyl cyclase directly or reduces the effectiveness of G_s, and results in a fall in tissue levels of cAMP [18–20]. In contrast, stimulation of the A_2 receptor results in activation of G_s, which has a stimulatory effect on adenylyl cyclase, leading to a rise in tissue cAMP levels. Vascular smooth muscle cells were found to possess A_2 receptors capable of stimulating adenylyl cyclase [21–23]. Recently, it has been shown that adenosine induces relaxation of coronary and pulmonary arteries via endothelial NO production [24,25]. However, there have been no reports concerning the effects of adenosine on NO production by vascular smooth muscle. Therefore, in the present study, we investigated the effects of adenosine on NO synthesis in cultured rat vascular smooth muscle cells.

2. Methods

2.1. Materials

Human recombinant interleukin-1β (specific activity $\sim 2 \times 10^7$ units/mg) was a gift from Otsuka Pharmacy (Tokushima, Japan). Mouse inducible NO synthase cDNA was a gift from Dr. Y. Kawahara (Kobe University School of Medicine, Kobe, Japan). Adenosine, 2-chloroadenosine, N^2,2′-*O*-dibutyryl cAMP (db-cAMP) and isobutylmethyl xanthine (IBMX) were from Sigma Chemical Co. (St. Louis, MO). KF17837 was a gift from Kyowa Hakko Co. (Tokyo, Japan). 5′-*N*-Ethylcarboxyamidoadenosine (NECA), CGS21680, *N*-6-cyclopentyladenosine (CPA), N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA), and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were from Research Biochemicals Int. (Natic, MA).

2.2. Culture of cells

Primary cultures of vascular smooth muscle cells were obtained from the media of thoracic aortae of Sprague-Dawley rats (200–250 g), as described previously [26]. The cells were grown in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells grown to confluence were detached by treatment with 0.125% trypsin and 0.02% EDTA and reseeded in secondary cultures. The cells exhibited typical 'hill and valley' growth morphology of vascular smooth muscle cells, and almost all cells reacted with the anti-α-actin antibody, which selectively recognizes muscle forms of actin but does not react with endothelial cells or fibroblasts [26]. Typically, cultured cells were used at passage 5–10. Cells (3×10^4/ml) were plated in 24-well or 100-mm culture dishes in DMEM supplemented as described above, and allowed to grow to subconfluence for 24–48 h. Subsequently, they were preincubated in DMEM containing 0.5% fetal bovine serum and supplemented with insulin (5 μg/ml) and transferrin (5 μg/ml) for 24 h, and used for the experiments described below.

The investigation was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.3. Measurements of nitrite

NO production by the cultured cells was determined by measuring the nitrite content of the culture media. Accumulation of nitrite in the medium represents the summation of NO synthase activity during the time period studied, since NO secreted by cells is rapidly decomposed to the more stable products, nitrite and nitrate [27]. Vascular smooth muscle cells plated in 24-well dishes were incubated in DMEM containing 0.5% fetal bovine serum at 37°C. The nitrite content of culture media was determined by mixing 500 μl of medium with an equal volume of Griess reagent (1 part 0.1% naphthylethylene-diamine dihydrochloride to 1 part 1% sulfanilamide in 5% phosphoric acid) [28]. The absorbance at 550 nm was measured and the nitrite concentration was determined by interpolation of a calibration curve of standard sodium nitrite concentrations against absorbance. After washing, cells were dissolved in 0.2 ml of 1% sodium dodecyl sulfate and used for protein assay (Bio-Rad assay kit, Hercules, CA) with bovine serum albumin as a standard. Nitrite levels were corrected by protein measurement and data are shown as nmol per mg protein.

2.4. Northern blot analysis

Total RNA was extracted from vascular smooth muscle cells plated in 100-mm culture dishes by the acid guanidinium isothiocyanate–phenol–chloroform method, and 30 μg aliquots were subjected to electrophoresis on 1% agarose gels. After electrophoretic separation, RNA was transferred onto nylon filters, which were then hybridized with a random-primed ^{32}P-labeled mouse macrophage inducible NO synthase cDNA probe for 24 h [29], followed by washing twice with an aqueous solution of 150 mM NaCl, 15 mM sodium citrate, and 0.1% sodium dodecyl sulfate at 65°C. The filters were exposed to Kodak XAR-5 film for 1–2 days at −70°C using one intensifying screen.

2.5. Measurements of cAMP

To determine intracellular cAMP levels, 0.5 mM IBMX, a cyclic nucleotide phosphodiesterase inhibitor, was added to each well 30 min before the addition of 2-chloroadenosine to prevent breakdown of accumulated cAMP. After incubation with 2-chloroadenosine for 2 h, cells were immediately immersed in 0.2 ml of 0.1N HCl to stop the reaction. Cells were then collected into glass tubes with a rubber policeman, boiled for 5 min, and then centrifuged at

$2500 \times g$ for 15 min at room temperature. The supernatants were decanted, and after addition of 0.05 ml of 50 mM sodium acetate to each tube, cells were kept at −70°C until they were assayed for cAMP content. The pellets were dissolved in 0.2 ml of 1% sodium dodecyl sulfate and kept at 4°C until protein assay. Intracellular cAMP content was measured with a commercial enzyme immunoassay kit using the manufacturer's high-sensitivity acetylation protocol (Amersham International, Bucks, UK). The values were normalized to the protein content of each well.

2.6. Statistical analysis

Data are expressed as means ± s.e.m. of 4 samples, which represented at least 3 separate experiments. Differences were analyzed by one-way ANOVA combined with Scheffé's test, and *P*-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of adenosine on nitrite production

First, we investigated the effects of interleukin-1β on the production of nitrite, a stable metabolite of NO, by vascular smooth muscle cells. Addition of interleukin-1β (10 ng/ml) stimulated nitrite production by vascular smooth muscle cells in a time-dependent manner (Fig. 1). The levels of nitrite increased within 6 h, and continued to increase for at least 24 h after exposure to interleukin-1β. The nitrite accumulation stimulated by interleukin-1β was significantly augmented by simultaneous treatment of the cells with adenosine (10^{-4} M).

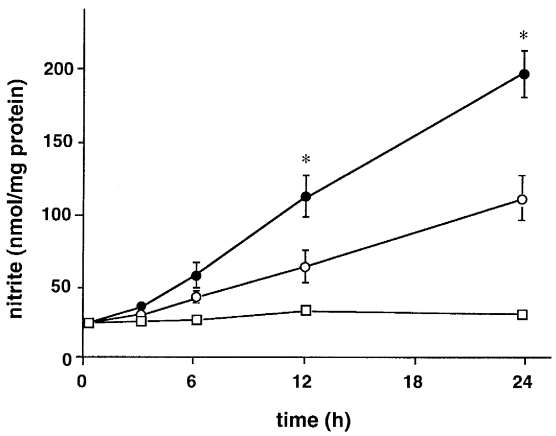

Fig. 1. Time-dependent effects of adenosine on nitrite accumulation. Vascular smooth muscle cells were incubated with interleukin-1β (10 ng/ml) (open circles), interleukin-1β + adenosine (10^{-4} M) (closed circles), or vehicle (open squares). Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$ compared with interleukin-1β-stimulated cells.

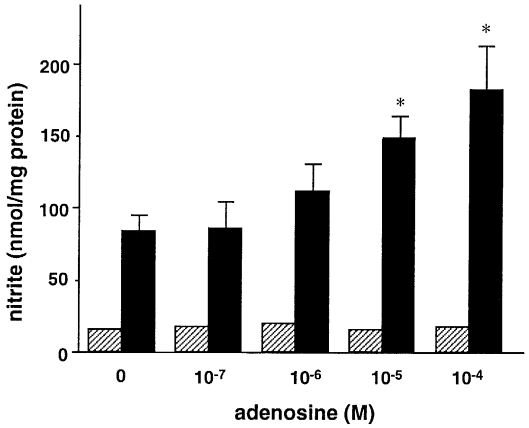

Fig. 2. Dose-dependent effects of adenosine on nitrite accumulation. Vascular smooth muscle cells were incubated for 24 h with (closed bars) or without (hatched bars) 10 ng/ml interleukin-1β in the presence of various concentrations of adenosine (10^{-7}–10^{-4} M) as indicated. Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$ compared with control cells without adenosine.

As shown in Fig. 2, the nitrite accumulation in interleukin-1β-stimulated vascular smooth muscle cells was significantly augmented by adenosine in a dose-dependent manner (10^{-7}–10^{-4} M). After a 24-h incubation, the level of interleukin-1β-stimulated nitrite accumulation in the presence of 10^{-4} M adenosine was about double that in its absence. Adenosine by itself did not affect the basal levels of nitrite production.

2-Chloroadenosine, a stable analogue of adenosine, also dose-dependently increased interleukin-1β-stimulated nitrite production by vascular smooth muscle cells, while 2-chloroadenosine by itself did not increase the basal levels of nitrite (Fig. 3). In contrast to adenosine, 2-chloroadenosine was more potent in increasing nitrite production,

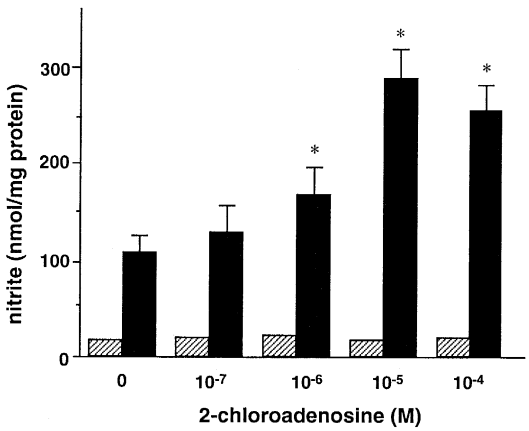

Fig. 3. Dose-dependent effects of 2-chloroadenosine on nitrite accumulation. Vascular smooth muscle cells were incubated for 24 h with (closed bars) or without (hatched bars) 10 ng/ml interleukin-1β in the presence of various concentrations of 2-chloroadenosine (10^{-7}–10^{-4} M) as indicated. Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$ compared with control cells without 2-chloroadenosine.

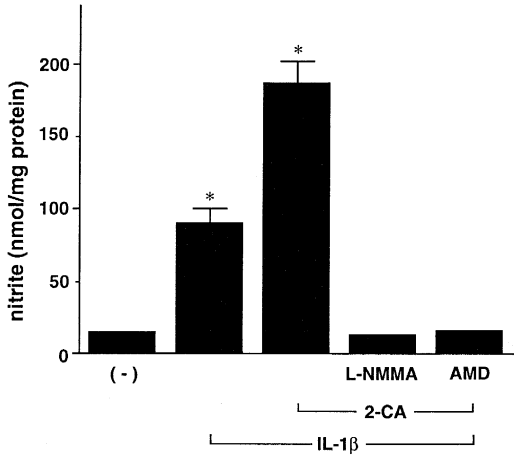

Fig. 4. Effects of L-NMMA and actinomycin D on nitrite accumulation. Vascular smooth muscle cells were exposed to 2-chloroadenosine (2-CA; 10^{-5} M) for 24 h with L-NMMA (1 mM) or actinomycin D (AMD; 5 μg/ml) in the presence of interleukin-1β (IL-1β; 10 ng/ml). * $P < 0.05$ compared with control cells, indicated as (−).

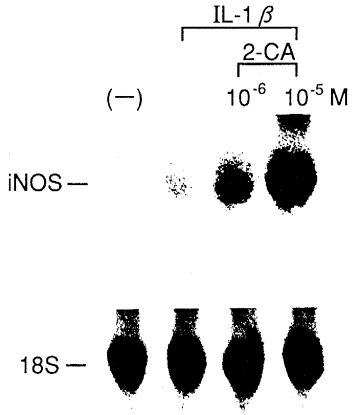

Fig. 5. Expression of inducible NO synthase mRNA in vascular smooth muscle cells. Cells were incubated for 24 h with interleukin-1β (IL-1β; 10 ng/ml) and 2-chloroadenosine (2-CA; 10^{-6}, 10^{-5} M). Total RNA was size fractionated by electrophoresis and transferred onto nylon filters. Filters were hybridized with ^{32}P-labeled mouse macrophage inducible NO synthase (iNOS; upper panel) and 18S rRNA (lower panel) cDNA probes. 18S signals show equal loading of total RNA onto each lane. Two independent experiments yielded identical results.

since 2-chloroadenosine is more slowly metabolized by adenosine deaminase than is adenosine [15].

As shown in Fig. 4, in the presence of the RNA synthesis inhibitor actinomycin D (5 μg/ml), or the NO synthase inhibitor, N^G-monomethyl-L-arginine (10^{-3} M), the effects of 2-chloroadenosine as well as interleukin-1β on nitrite production were completely abolished.

3.2. Effects of adenosine on inducible NO synthase mRNA levels

We then examined whether adenosine induced increases in inducible NO synthase mRNA levels in interleukin-1β-stimulated vascular smooth muscle cells. Unstimulated cells did not express inducible NO synthase mRNA. Incubation with interleukin-1β (10 ng/ml) for 24 h resulted in an induction of inducible NO synthase mRNA (Fig. 5). Co-incubation with 2-chloroadenosine (10^{-6}, 10^{-5} M) further augmented the interleukin-1β-induced increase in inducible NO synthase mRNA accumulation.

3.3. Effects of adenosine receptor agonists and antagonists

As shown in Fig. 6, both nonspecific adenosine receptor agonist NECA and the selective A_{2a} receptor agonist, CGS21680, dose-dependently increased interleukin-1β-stimulated nitrite production by vascular smooth muscle cells. On the other hand, both the A_1 receptor agonist, CPA, and the A_3 receptor agonist, IB-MECA, increased interleukin-1β-induced nitrite accumulation at high concentrations, but significantly inhibited its accumulation at low concentrations.

Further experiments were conducted using the adenosine receptor antagonists, DPCPX and KF17837, which inhibit the effects of adenosine by blocking A_1 and A_{2a} receptors, respectively. As shown in Fig. 7, KF17837 but not DPCPX significantly inhibited 2-chloroadenosine-mediated nitrite production.

3.4. Involvement of cAMP in the action of adenosine

We next investigated the mechanism of the stimulatory effect of adenosine on interleukin-1β-induced NO production. It has been shown that adenosine increases intra-

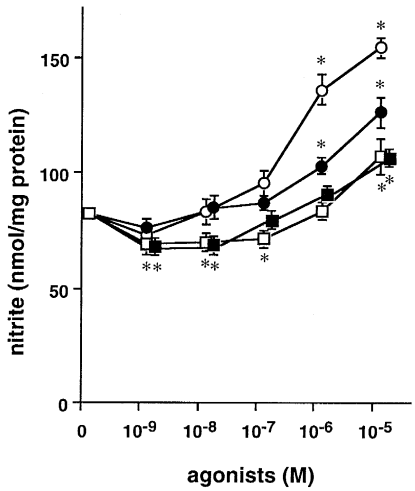

Fig. 6. Effects of the adenosine receptor agonists on nitrite accumulation. Vascular smooth muscle cells were incubated for 24 h with 10 ng/ml interleukin-1β in the presence of various concentrations (10^{-9}–10^{-5} M) of the adenosine receptor agonist NECA (nonspecific adenosine agonist; open circles), CGS21680 (A_{2a} agonist; closed circles), CPA (A_1 agonist; open squares) or IB-MECA (A_3 agonist; closed squares). Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$ compared with control cells without the agonists.

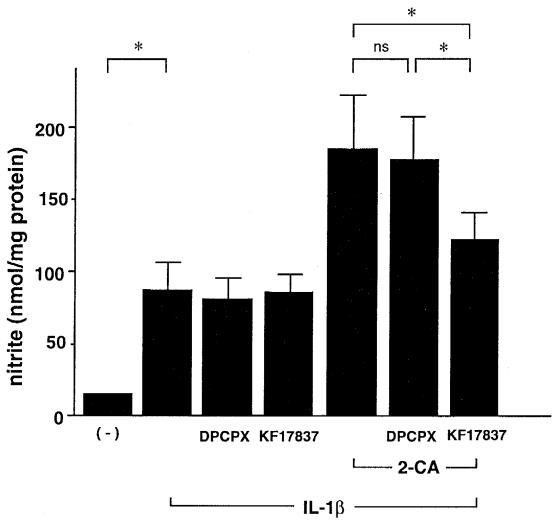

Fig. 7. Effects of adenosine receptor antagonists on nitrite accumulation in vascular smooth muscle cells. Cells were incubated for 24 h with 10 ng/ml interleukin-1β (IL-1β) and/or 10^{-5} M 2-chloroadenosine (2-CA) in the presence of the A_1 receptor antagonist, DPCPX (10^{-6} M), or the A_{2a} receptor antagonist, KF17837 (10^{-6} M). Nitrite accumulation in the culture medium was measured, and values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$; ns = not significant.

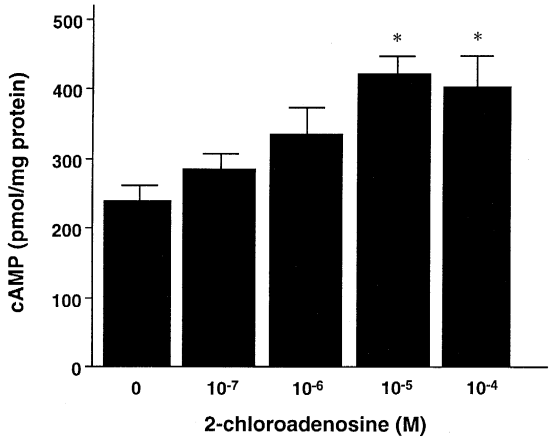

Fig. 9. Effects of 2-chloroadenosine on intracellular cAMP levels of vascular smooth muscle cells. Cells were incubated for 2 h in the presence of various concentrations (10^{-7}–10^{-4} M) of 2-chloroadenosine. Intracellular cAMP content was measured as described in Section 2, and the values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$ compared with control cells without 2-chloroadenosine.

cellular cAMP levels of vascular smooth muscle cells [21–23] and a cAMP-dependent pathway is involved in cytokine-induced NO production by vascular smooth muscle cells [29–31]. As shown in Fig. 8, in the presence of db-cAMP (10^{-3} M), a membrane-permeable analogue of cAMP, interleukin-1β-induced nitrite accumulation was significantly increased, but the effect of 2-chloroadenosine was not additive or synergistic, suggesting that the effect of adenosine is mediated through a cAMP-dependent pathway.

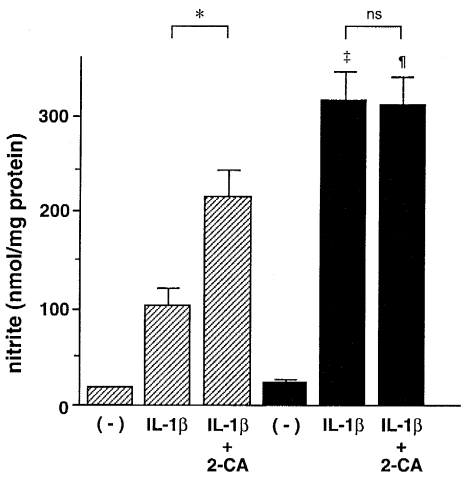

Fig. 8. Effects of dibutyryl-cAMP on nitrite accumulation in vascular smooth muscle cells. Cells were incubated for 24 h with 10 ng/ml interleukin-1β (IL-1β) and 10^{-5} M 2-chloroadenosine (2-CA) in the presence (solid bars) or absence (hatched bars) of 10^{-3} M dibutyryl-cAMP. Nitrite accumulation in the culture medium was measured, and values were normalized to the protein content per dish. Data represent means ± s.e.m. ($n = 4$). * $P < 0.05$; ns = not significant. ‡,¶ Significant difference ($P < 0.05$) from IL-1β and IL-1β + 2-CA, respectively.

We then measured intracellular cAMP levels of vascular smooth muscle cells. As shown in Fig. 9, addition of 2-chloroadenosine for 2 h dose-dependently (10^{-7}–10^{-4} M) increased intracellular cAMP levels of vascular smooth muscle cells.

4. Discussion

In this study, we investigated whether adenosine modulated NO production by vascular smooth muscle cells, and revealed that adenosine acts on A_2 receptors and augments NO synthesis in interleukin-1β-stimulated vascular smooth muscle cells.

The multiple biologic effects of adenosine are mediated via A_1, A_{2a}, A_{2b}, A_3, and A_4 receptors. However, participation of A_1 and A_2 adenosine receptors appears to be more important in vascular biology, particularly with regard to reducing the risk and consequences of vaso-occlusive events associated with hypertension and atherosclerosis [8–19]. In this study, CGS21680, an adenosine agonist that expresses its effects specifically via activation of A_{2a} receptors, was able to stimulate nitrite production. The selectivity of CGS21680 for A_{2a} versus A_1 receptors has previously been reported to be > 170-fold [32]. On the other hand, CPA and IB-MECA inhibited interleukin-1β-induced nitrite accumulation at low concentrations, while they increased the accumulation at high concentrations. CPA and IB-MECA express their effects specifically via A_1 and A_3 receptors, respectively, only at low concentrations (10^{-9}–10^{-8} M), whereas at high concentrations (> 10^{-7} M), they have other non-specific effects [16,33].

The observed inhibitory effects of CPA and IB-MECA on nitrite accumulation at low concentrations might be due to the inhibition of adenylyl cyclase via activation of A_1 and A_3 receptor stimulation, respectively [18,19,34]. Further experiments were conducted using the selective adenosine A_1 receptor antagonist, DPCPX, and the A_{2a} receptor antagonist, KF17837 [35]. KF17837 but not DPCPX significantly inhibited the effects of 2-chloroadenosine on nitrite accumulation. Taken together, our findings provide the evidence that adenosine increases nitrite production via the A_{2a} receptor but not via the A_1 or A_3 receptor.

On the other hand, evaluation of the A_{2b} receptor is more problematic in that no highly selective agonists have been identified to date. However, Gurden et al. [36] have recently demonstrated that the relative potencies of CGS21680 and NECA can be used as a reference to differentiate A_{2a} from A_{2b} receptors. When the effects of CGS21680 are as potent as those of NECA, the A_{2a} receptor is implicated. However, when CGS21680 is much less potent than NECA, it indicates that the observed effects are mediated via activation of the A_{2b} receptor subtype. In this study, CGS21680 was significantly less potent than NECA, which suggests that the effects of adenosine are also mediated via the A_{2b} receptor.

We obtained 3 pieces of evidence of a causal link between cAMP production and augmentation of NO synthesis by adenosine in vascular smooth muscle cells. First, adenosine augmented interleukin-1β-induced NO production, and this effect was accompanied by an increase in the cellular levels of cAMP. Second, the cAMP analogue db-cAMP increased interleukin-1β-induced NO production by vascular smooth muscle cells. Third, the effect of adenosine on interleukin-1β-induced NO production was not additive or synergistic in the presence of db-cAMP. These results suggest that adenosine augments interleukin-1β-induced NO production, at least partially through a cAMP-dependent pathway.

The findings presented here do not address the molecular mechanism by which adenosine or cAMP alters the inducible NO synthase mRNA levels in interleukin-1β-stimulated vascular smooth muscle cells. Changes in the transcription and/or in mRNA stability may account for the observed changes in mRNA levels. From the potent inhibitory action of actinomycin D and the lag period of several h before the onset of inducible NO synthase activity, transcriptional activation of inducible NO synthase expression seems a likely explanation for our observations. Recently, Oddis et al. [37] revealed that cAMP enhanced inducible NO synthase mRNA stability in rat cardiac myocytes following cytokine exposure; however, Imai et al. [30] reported that the enhanced expression of inducible NO synthase mRNA in rat vascular smooth muscle cells by cAMP was not caused by increased mRNA stability. Nuclear run-on experiments will be necessary to directly assess rates of transcription of the inducible NO synthase gene.

The concentration of adenosine required to stimulate nitrite production effectively was 10^{-6}–10^{-4} M, whereas the physiological baseline concentration of adenosine within the circulation is 10^{-8}–10^{-6} M [15]. Since adenosine is synthesized in a differential manner by several cell types including endothelial cells and vascular smooth muscle cells, it is possible that adenosine levels may be much higher locally within the blood vessel walls than the levels measured in the blood.

Inducible NO synthase activity is induced in blood vessel walls and cultured vascular smooth muscle cells by endotoxins and cytokines [5]. Joly et al. [38] demonstrated that balloon injury induced NO synthase activity in rat carotid arteries, even in the absence of endothelium. Hansson et al. [6] detected inducible NO synthase gene expression in neointimal but not medial smooth muscle cells 1–14 days after balloon-induced rat carotid artery injury. Recently, Buttery et al. [39] also revealed that inducible NO synthase mRNA and protein were present within human arteriosclerotic lesions. NO production by vascular smooth muscle cells may in part compensate for the absence of endothelial NO synthesis by inhibiting smooth muscle cell proliferation, as well as by limiting thrombus formation by preventing platelet adhesion and aggregation [40,41]. This hypothesis is supported by the observation that, in animals, L-arginine attenuates neointimal formation after balloon injury [42].

Taken together, the present findings suggest that adenosine may increase NO production by interleukin-1β-stimulated vascular smooth muscle cells under various pathological conditions and modulates contractility and cellular proliferation of the vascular tissue. However, further studies are required to clarify the role of adenosine in NO synthesis in the vascular tissue and in the pathogenesis of vascular lesions.

Acknowledgements

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References

[1] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 1991;43:109–140.
[2] Nathan C, Xie Q. Regulation of biosynthesis of nitric oxide. J Biol Chem 1994;269:13725–13728.
[3] Ikeda U, Maeda Y, Kawahara Y, Yokoyama M, Shimada K. Angiotensin II augments cytokine-stimulated nitric oxide synthesis in rat cardiac myocytes. Circulation 1995;92:2683–2689.
[4] Koide M, Kawahara Y, Tsuda T, Yokoyama M. Cytokine-induced expression of an inducible type of nitric oxide synthase gene in

cultured vascular smooth muscle cells. FEBS Lett 1993;318:213–217.
[5] Dinerman JL, Lowenstein CJ, Snyder SH. Molecular mechanisms of nitric oxide regulation. Circ Res 1993;73:217–222.
[6] Hansson GK, Geng Y, Holm J, Hårdhammar P, Wennmalm Å, Jennische E. Arterial smooth muscle cells express nitric oxide synthase in response to endothelial injury. J Exp Med 1994;180:733–738.
[7] Bobik A, Campbell JH. Vascular derived growth factors: cell biology, pathophysiology and pharmacology. Pharmacol Rev 1993;45:1–42.
[8] Gordon EL, Pearson JD, Dickinson ES, Moreau D, Slakey LL. The hydrolysis of extracellular adenine nucleotides by arterial smooth muscle cells: regulation of adenosine production at the cell surface. J Biol Chem 1989;264:18986–18995.
[9] Pearson JD, Gordon JL. Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. Nature 1979;281:384–386.
[10] Headrick JP, Berne RM. Endothelium-dependent and -independent relaxations to adenosine in guinea pig aorta. Am J Physiol 1990;259:H62–H67.
[11] Cristalli G, Vittori S, Thompson RD, et al. Inhibition of platelet aggregation by adenosine receptor antagonists. Naunyn-Schmiedebergs Arch Pharmacol 1994;349:644–650.
[12] Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. J Appl Physiol 1994;76:5–13.
[13] Jackson EK. Adenosine: a physiological brake on renin release. Annu Rev Pharmacol Toxicol 1991;31:1–35.
[14] Murray RD, Churchill PC. Effects of adenosine receptor agonists in the isolated perfused rat kidney. Am J Physiol 1984;247:H343–H348.
[15] Dubey RK, Gillespie DG, Osaka K, Suzuki F, Jackson EK. Adenosine inhibits growth of rat aortic smooth muscle cells. Hypertension 1996;27:786–793.
[16] Dalzierl HH, Westfall DP. Receptors for adenine nucleotides and nucleosides: subclassification, distribution, and molecular characterization. Pharmacol Rev 1994;46:449–466.
[17] Fredholm BB, Abbracchio MP, Burnstock G, et al. Nomenclature and classification of purinoceptors. Pharmacol Rev 1994;46:143–156.
[18] Van Calker D, Müller M, Hamprecht B. Adenosine regulates, via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurochem 1979;33:999–1005.
[19] Linden J, Tucker AL, Lynch KR. Molecular cloning of adenosine A_1 and A_2 receptors. Trends Pharmacol Sci 1991;12:326–328.
[20] Zhou QY, Li C, Olah ME, Johnson RA, Stiles GL, Givelli O. Molecular cloning and characterization of an adenosine receptor: the A_3 adenosine receptor. Proc Natl Acad Sci USA 1992;89:7432–7436.
[21] Anand-Srivastava MB, Franks DJ, Cantin M, Genest J. Presence of 'Ra' and 'P' site receptors for adenylate cyclase in cultured vascular smooth muscle cells. Biochem Biophys Res Commun 1982;108:213–219.
[22] Jonzon B, Nilsson J, Fredholm BB. Adenosine receptor-mediated changes in cyclic AMP production and DNA synthesis in cultured arterial smooth muscle cells. J Cell Physiol 1985;124:451–456.
[23] Silver PJ, Walus K, DiSalvo J. Adenosine-mediated relaxation and activation of cyclic AMP-dependent protein kinase in coronary arterial smooth muscle. J Pharmacol Exp Ther 1984;228:342–347.
[24] Vials A, Burnstock G. A_2-purinoceptor-mediated relaxation in the guinea-pig coronary vasculature: a role for nitric oxide. Br J Pharmacol 1993;109:424–429.
[25] Steinhorn RH, Morin FC, Van Wylen DG, Gugino SF, Giese E, Russell JA. Endothelium-dependent relaxations to adenosine in juvenile rabbit pulmonary arteries and veins. Am J Physiol 1994;266:H2001–H2006.
[26] Ikeda U, Ikeda M, Oohara T, et al. Interleukin 6 stimulates growth of vascular smooth muscle cells in a PDGF-dependent manner. Am J Physiol 1991;260:H1713–H1717.
[27] Knowles RG, Noncada S. Nitric oxide synthase in mammals. Biochem J 1994;298:249–258.
[28] Green LC, Wagner DA, Glogowski J, Skiper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. Anal Biochem 1982;126:131–138.
[29] Koide M, Kawahara Y, Nakayama I, Tsuda T, Yokoyama M. Cyclic AMP-elevating agents induce an inducible type of nitric oxide synthase in cultured vascular smooth muscle cells. J Biol Chem 1993;268:24959–24966.
[30] Imai T, Hirata Y, Kanno K, Marumo F. Induction of nitric oxide synthase by cyclic AMP in rat vascular smooth muscle cells. J Clin Invest 1994;93:543–549.
[31] Hirokawa K, O'Shaughnessy K, Moore K, Ramrakha P, Wilkins MR. Induction of nitric oxide synthase in cultured vascular smooth muscle cells: the role of cyclic AMP. Br J Pharmacol 1994;112:396–402.
[32] Jarvis MF, Rainer R, Hutchison AJ, Do UH, Williams M. [^{3}H]CGS21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. J Pharmacol Exp Ther 1989;251:888–893.
[33] Gallo-Rodriguez C, Ji X, Melman N, et al. Structure–activity relationships of N^6-benzyladenosine-5′-uronamides as A_3-selective adenosine agonists. J Med Chem 1994;37:636–646.
[34] Strickler J, Jacobson KA, Liang BT. Direct preconditioning of cultured chick ventricular myocytes: Novel functions of cardiac adenosine A_{2a} and A_3 receptors. J Clin Invest 1996;98:1773–1779.
[35] Nonaka H, Mori A, Ichimura M, et al. Binding of [^{3}H]KF17837S, a selective adenosine A_2 receptor antagonist, to rat brain membranes. Mol Pharmacol 1994;46:817–822.
[36] Gurden MF, Coates J, Ellis F, et al. Functional characterization of three adenosine receptor types. Br J Pharmacol 1993;109:693–698.
[37] Oddis CV, Simmons RL, Hattler BG, Finkel MS. cAMP enhances inducible nitric oxide synthase mRNA stability in cardiac myocytes. Am J Physiol 1995;269:H2044–H2050.
[38] Joly GA, Schini VB, Vanhoutte PM. Balloon injury and interleukin-1β induce nitric oxide synthase activity in rat carotid arteries. Circ Res 1992;71:331–338.
[39] Buttery LD, Springall DR, Chester AH, et al. Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. Lab Invest 1996;75:77–85.
[40] Lloyd-Jones D, Bloch KD. The vascular biology of nitric oxide and its role in atherogenesis. Annu Rev Med 1996;47:365–375.
[41] Yan Z, Yokota T, Zhang W, Hansson GK. Expression of inducible nitric oxide synthase inhibits platelet adhesion and restores blood flow in the injured artery. Circ Res 1996;79:38–44.
[42] Hamon M, Vallet B, Bauters C, et al. Long-term oral administration of L-arginine reduces intimal thickening and enhances neoendothelium-dependent acetylcholine-induced relaxation after arterial injury. Circulation 1994;90:1357–1362.