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Dipyridamole enhances interleukin-1β-stimulated nitric oxide production by cultured rat vascular smooth muscle cells

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Abstract

We examined whether dipyridamole affected interleukin-1β-stimulated nitric oxide (NO) production by cultured rat vascular smooth muscle cells. Interleukin-1β stimulated the production of nitrite and nitrate, stable metabolites of NO, in a dose- and time-dependent manner in vascular smooth muscle cells. Dipyridamole (1–100 μM) enhanced interleukin-1β-induced nitrite production in a dose- and time-dependent manner. The mRNA expression of inducible NO synthase was up-regulated by dipyridamole (0.3–10 μM) treatment in a dose-dependent manner. Both 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-bromo-cGMP) and dibutyryl adenosine 3′,5′-cyclic monophosphate (db-cAMP) enhanced the nitrite production in the presence of interleukin-1β. Dipyridamole up-regulated the effect of both 8-bromo-cGMP and db-cAMP on the interleukin-1β-induced nitrite production. Dipyridamole increased the intracellular cAMP content in the presence of interleukin-1β (10 ng/ml), but did not affect the intracellular cGMP content. 8*R**,9*S**,11*S**-(−)-9-hydroxy-9-*n*-hexyloxy-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-triazadibenzo-(*a*,*g*)-cycloocta(*c*,*d*,*e*)-trinden-1-one (KT 5720), a selective inhibitor of cAMP-dependent protein kinase, abolished the enhancement of interleukin-1β-induced nitrite production by dipyridamole, whereas 8*R**,9*S**,11*S**-(−)-9-methoxy-carbamyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-trizadibenzo-(*a*,*g*)-cyclo-octa-(*c*,*d*,*e*)-trinden-1-one (KT 5823), an inhibitor of cGMP-dependent protein kinase, did not attenuate the enhancement. Furthermore, Rolipram and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro-20-1724), cAMP-specific phosphodiesterase type IV inhibitors, augmented the interleukin-1β-induced nitrite production. We concluded that dipyridamole enhanced the interleukin-1β-induced NO production via an increase in intracellular cAMP content in cultured rat vascular smooth muscle cells.

Keywords: Dipyridamole; Nitric oxide (NO); Smooth muscle cells, vascular; Interleukin-1β; Cyclic nucleotide

1. Introduction

Nitric oxide (NO) is a messenger molecule with diverse actions including vascular homeostasis, neurotransmission, and antimicrobial defense (Moncada et al., 1991; Lowenstein and Snyder, 1992). Endothelium-derived NO activates guanylyl cyclase in vascular smooth muscle cells to generate intracellular cyclic 3′,5′-guanosine monophosphate (cGMP), which stimulates cGMP-dependent protein kinase with subsequent muscle relaxation. Vascular smooth muscle cells themselves produce NO in response to cytokines such as interleukin-1β, tumor necrotic factor, interferon-γ and lipopolysaccharide through an induction of inducible type of NO synthase (Koide et al., 1993). Using immunohistochemical methods, production of these cytokines has been detected in atherosclerotic lesions (Moyer et al., 1991; Barath et al., 1990), implicating some role of these cytokines in forming atherosclerosis in vessels. However, the in vivo roles of cytokine-induced NO have not been clearly understood yet.

Several factors of hormones and nucleotides have been found to modulate cytokine-induced NO production. Norepinephrine suppressed lipopolysaccharide-induced NO synthase induction (Feinstein et al., 1993). Conversely, angiotensin II inhibited the inducible NO

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synthase mRNA expression and protein production via an activation of protein kinase C (Nakayama et al., 1994). With regard to cyclic nucleotides, cyclic 3′,5′-adenosine monophosphate (cAMP) is known to have a potency to stimulate inducible NO synthase mRNA expression and NO production (Kunz et al., 1994; Muhl et al., 1994; Sowa and Przewlocki, 1994). Recently, we found that both atrial natriuretic peptide and cGMP enhanced interleukin-1β-induced NO production in cultured vascular smooth muscle cells possibly through an activation of cAMP-dependent protein kinase (Iimura et al., submitted).

In patients with cardiac coronary ischemia, dipyridamole has been used for the prevention of coronary artery restenosis after percutaneous transluminal coronary angioplasty or coronary bypass operation, sometimes in combination with acetylsalicylic acid (Schwartz et al., 1988; White et al., 1987). Dipyridamole is used as an anti-platelet drug mostly in those patients with coronary diseases, and has vasodilatative potency as well (Bult et al., 1991). In terms of NO, dipyridamole has augmented both vasodilatation in the thoracic aorta and anti-aggregating activity in platelets induced by exogenous NO (Bult et al., 1991). This enhancement of NO action by dipyridamole may be due to the function of dipyridamole as an inhibitor of cGMP-specific phosphodiesterase. Dipyridamole is known to act not only as a cGMP-phosphodiesterase inhibitor but also as a cAMP-specific phosphodiesterase inhibitor in aorta (Saeki and Saito, 1993; Olivera and Lopez-Novoa, 1992; Lugnier et al., 1986; Schoeffter et al., 1987). Thus, it is possible that phosphodiesterase inhibitors including dipyridamole would affect cytokine-induced NO production through an increase in intracellular cyclic nucleotides content. Here, we examined whether dipyridamole could affect interleukin-1β-induced NO production.

2. Materials and methods

2.1. Cell preparation

Vascular smooth muscle cells were harvested from rat aortas by modifications of the methods described before in our laboratory (Ikeda et al., 1991). In brief, male Sprague-Dawley rats (150–200 g) were killed by cervical dislocation. The aorta was excised and placed in a Petri dish containing phosphate-buffered saline. Fat and adventitia were removed with fine forceps, and the cleaned aorta was incubated in Dulbecco's modification of Eagle's medium (DMEM, ICN Biomedicals CA, USA) containing 1.0 mg/ml collagenase (type I) and 1.0 mg/ml bovine albumin. After incubation at 37°C for 60 min, the intima was gently removed with a scalpel, after which the media was cut into smaller pieces (1 mm^2) and digested with the above enzyme solution at 37°C for 120 min. The tissue suspension was centrifuged and the cell pellet was resuspended in DMEM supplemented with 10% fetal bovine serum (ICN Biomedicals, Osaka, Japan), 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technology Inc., USA) and seeded in primary culture. Cells grown to confluence were detached by a 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 (Boehringer Mannheim), which selectively recognized muscle forms of actin but did not react with endothelial cells or fibroblasts. The typical growth experiment was performed with vascular smooth muscle cells at passage levels of 5–10. Cells were plated at $1-2 \times 10^4$ cells/ml in 24-well dishes (Falcon), in DMEM, supplemented as described above and allowed to grow subconfluently for 48–72 h. They were then made 'quiescent' by a 24 h incubation in serum-free DMEM.

2.2. Experimental procedures

After being rinsed with phosphate buffered saline at pH 7.35, confluent cells were incubated for 24 h with serum-free DMEM containing 10 ng/ml human recombinant interleukin-1β (Genzyme, MA) with an exception of the study of cytokine dose dependency. Agents like dipyridamole, rolipram, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724), 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-bromo-cGMP), dibutyryl adenosine 3′,5′-cyclic monophosphate (db-cAMP) were added to the incubation medium concomitantly with interleukin-1β with the exceptions indicated above.

2.3. Determination of nitrite and nitrate

Nitrite and nitrate were measured by the method of Green et al. (1982). Briefly, nitrate was reduced to nitrite using Cd-Cu reduction column (Tokyo Kasei Kogyo, Tokyo, Japan). Assay samples were mixed with an equal volume of the Griess reagent (0.1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 3% H_3PO_4) and incubated to yield a chromophore. The absorbance at 540 nm was measured, and nitrite concentration was determined using a curve calibrated from sodium nitrite standards.

2.4. Northern blot analysis

Total RNA was isolated using the phenol-acid precipitation procedure (Chomczynski and Sacchi, 1987). In brief, vascular smooth muscle cells were grown to

confluence on 100-mm dishes and lysed using Isogen (Nippon Gene, Tokyo, Japan), which contained phenol and guanidine isothiocyanate. The lysate was extracted with chloroform/isopropanol, washed with 75% ethanol, and dissolved in 20 μl of diethylpyrocarbonate (DEPC)-treated water. The RNA was quantitated by ultraviolet absorbance at 260 nm. Total RNA (30 μg) was fractionated on 1% denaturing agarose-formaldehyde gels and capillary blotted onto nylon membranes (Hybond N, Amersham, Brucks, UK) in 20 × standard saline citrate (contains 0.15 M NaCl and 0.0015 M sodium citrate, pH 7.0) overnight. Filters were prehybridized for 30 min at 68°C before hybridization using Quick-Hyb (Stratagene, La Jolla, CA, USA). Filters were then hybridized for 1 h at 68°C in the same solution with 10^6 cpm/ml of [α-^{32}P]dCTP random primer-labeled inducible NO synthase probes from mice (Koide et al. 1993). Filters were washed twice for 10 min at room temperature in 2 × standard saline citrate and 0.1% sodium dodecyl sulfate followed by a 10 min wash at 45°C in 0.1 × standard saline citrate and 0.1% sodium dodecyl sulfate. The hybridized filters were then exposed to Kodak XAR film overnight at −70°C with one intensifying screen. Autoradiography was performed at −70°C and quantified by densitometric scanning (Immunomedica Image Analyzer TIF-64). The filter membranes were also hybridized with [γ-^{32}P]ATP-labeled rat glyceraldehyde-3-phosphate dehydrogenase oligonucleotide probe.

2.5. Determination of intracellular-cyclic GMP and intracellular-cyclic AMP and protein content

We measured these cyclic nucleotides by enzyme immuno-assay (Iimura et al., 1995). Briefly, medium was aspirated and cells were immediately immersed in 0.2 ml of 0.1 N HCl to stop the reaction. Cells were then collected into glass tubes and boiled for 3 min. After 15 min of centrifugation at 6500 rpm, the supernatant was decanted and 50 μl of 50 mM sodium acetate were added for the assay of cyclic nucleotides. The pellet was used for protein measurements. Cyclic GMP and cAMP content of the supernatant were assayed by enzyme immuno-assay kits (Amersham, Bucks, UK). The protein content was measured by a protein assay kit (BCA protein assay kit, Pierce, IL, USA).

2.6. Reagents

Human recombinant interleukin-1β was purchased from Genzyme (Cambridge, MA, USA). Dipyridamole (Boehringer Ingelheim, Tokyo, Japan) was dissolved in pure methanol to provide stock solution of 50 mM; final concentration of methanol was 0.1% in culture medium including the experiment of dipyridamole dose dependency. Rolipram (Meiji Seika, Tokyo, Japan) and Ro 20-1724 (Miomol, PA, USA) were dissolved in pure ethanol to provide stock solution of 100 mM. The final concentration of ethanol was made to 0.1% in the culture medium. 8*R**,9*S**,11*S**-(−)-9-hydroxy-9-*n*-hexyloxy-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-triazadibenzo-(*a*,*g*)-cycloocta(*c*,*d*,*e*)-trinden-1-one (KT 5720) and 8*R**,9*S**,11*S**-(−)-9-methoxy-carbamyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-trizadibenzo-(*a*,*g*)-cyclo-octa-(*c*,*d*,*e*)-trinden-1-one (KT 5823) were purchased from Kyowa Medicals (Tokyo, Japan). Other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA), with exceptions of those specially described. A mouse macrophage inducible NO synthase cDNA fragment (nucleotides 1621-2653) prepared by reverse transcription-polymerase chain reaction (Koide et al., 1993) was kindly donated by Dr. Y. Kawahara (Kobe University, Kobe, Japan).

2.7. Statistical analysis

The results are expressed as means ± S.E.M. Statistical determination was performed by Student's *t*-test for unpaired observations. The differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Interleukin-1β-induced nitrite and nitrate production

Interleukin-1β stimulated nitrite and nitrate production by vascular smooth muscle cells in a dose- and time-dependent manner with a submaximum dose of 10 ng/ml (Fig. 1A,B). As shown in Fig. 1A, the nitrate production was linearly correlated with nitrite production ($r = 0.99$, $P < 0.01$). Thus, in the following experiments, we measured nitrite content instead of measuring both nitrate and nitrite. L-N^G-Monomethyl arginine (L-NMMA; 1 mM), an inhibitor of NO synthase, inhibited approximately 90% of nitrite production induced by 10 ng/ml interleukin-1β (data not shown). In subsequent experiments, we incubated vascular smooth muscle cells with 10 ng/ml interleukin-1β for 24 h.

3.2. The effects of dipyridamole on interleukin-1β-induced nitrite production

As shown in Fig. 2A, addition of dipyridamole enhanced interleukin-1β-stimulated nitrite generation in a dose-dependent fashion. Only a 3-h coincubation with 10 μM dipyridamole significantly increased the nitrite content accumulated in culture medium in the presence of interleukin-1β (Fig. 2B). Dipyridamole had no effects on the level of nitrite without cytokine, at least up to 100 μM (data not shown).

3.3. The effects of dipyridamole on mRNA expression of inducible NO synthase

As shown in Fig. 3, addition of dipyridamole strongly up-regulated mRNA expression of inducible NO synthase in the presence of interleukin-1β in a dose-dependent manner. Incubation with 10 μM dipyridamole for 24 h increased the mRNA expression by approximately 6-fold compared with the control condition without dipyridamole.

3.4. Cyclic nucleotide-enhanced nitrite production and dipyridamole

Both 8-bromo-cGMP and db-cAMP increased interleukin-1β-induced nitrite production (Table 1). Interleukin-1β-induced nitrite production was increased by 2.1-fold and 7.0-fold with 1 mM 8-bromo-cGMP and 100 μM db-cAMP respectively. Coincubation with dipyridamole (10 μM) augmented the effects of 8-bromo-cGMP and db-cAMP on the nitrite production.

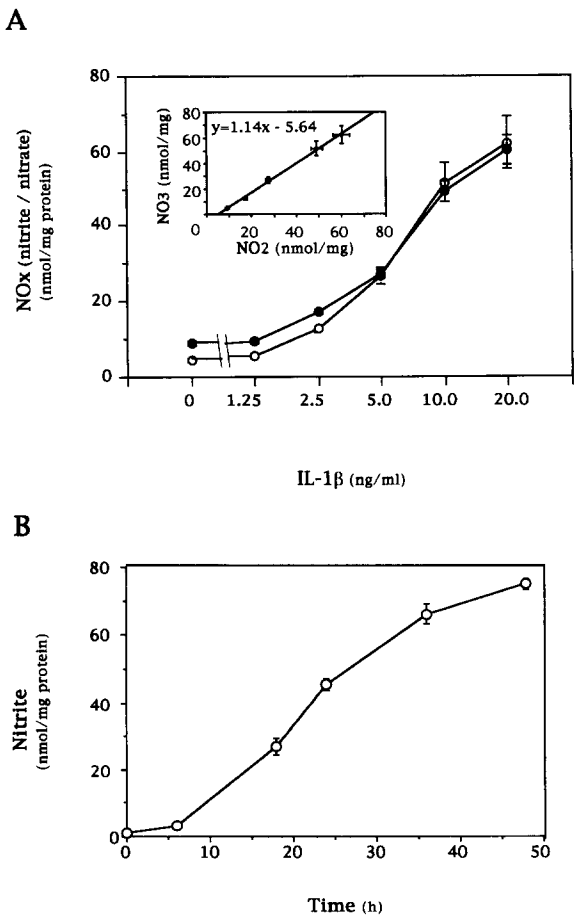

Fig. 1. Dose- and time-response curves of interleukin-1β-induced nitrite and nitrate production. (A) Vascular smooth muscle cells were stimulated with various concentrations of interleukin-1β for 24 h. Nitrite (closed circle) and nitrate (open circle) released into medium were measured. Inset: the correlation between nitrite and nitrate. (B) Vascular smooth muscle cells were stimulated with 10 ng/ml interleukin-1β for various times as indicated and nitrite production was measured. Each point and bar represents mean ± S.E.M. ($n = 8$).

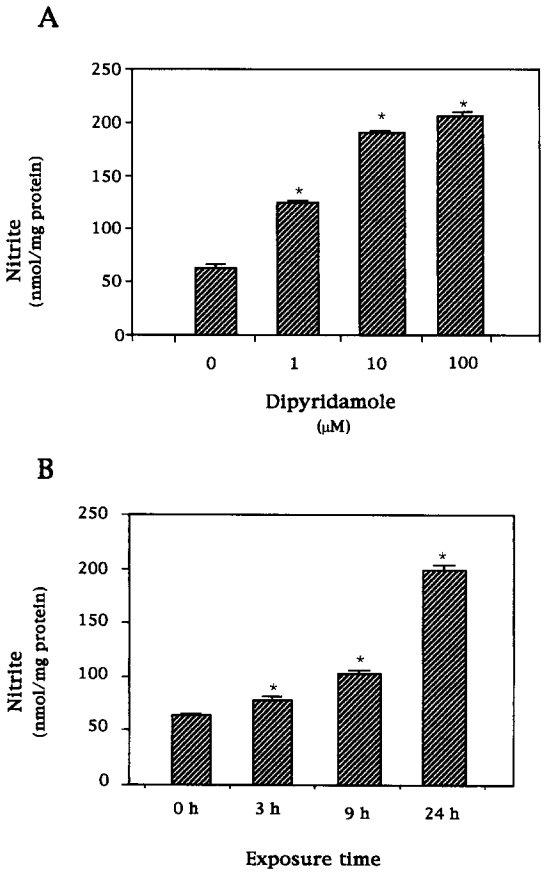

Fig. 2. Effects of dipyridamole on interleukin-1β-induced nitrite production. (A) Dose dependency of dipyridamole-enhanced nitrite production. Vascular smooth muscle cells were incubated with various concentrations of dipyridamole in the presence of interleukin-1β (10 ng/ml) for 24 h. Each bar represents mean ± S.E.M. ($n = 8$). $^*P < 0.01$ vs. dipyridamole 0 μM. (B) Time dependency of dipyridamole-enhanced nitrite production. Vascular smooth muscle cells were incubated with dipyridamole (10 μM) for the time indicated in the presence of interleukin-1β (10 ng/ml). After incubation with dipyridamole, the medium was changed to a newly prepared medium of interleukin-1β (10 ng/ml) without dipyridamole and further incubated until 24 h. Each bar represents mean ± S.E.M. ($n = 8$). $^*P < 0.01$ vs. incubation 0 h.

3.5. Changes of intracellular cyclic nucleotides content by dipyridamole

In this study, we measured intracellular cyclic nucleotides concentrations with or without dipyridamole. As shown in Fig. 4, the intracellular cAMP content was significantly increased in the presence of 10 μM dipyridamole. On the other hand, cGMP content was not affected by the addition of dipyridamole.

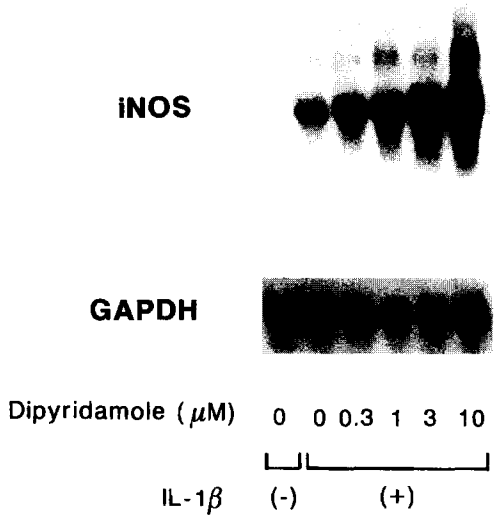

Fig. 3. Effects of dipyridamole on interleukin-1β-stimulated inducible NO synthase mRNA expression. VSMC were stimulated with 10 ng/ml interleukin-1β for 24 h. Inducible NO synthase mRNA (upper panel) and GAPDH and mRNA (lower panel) by Northern blot analysis are shown. Lane 1: interleukin-1β (−); 2: interleukin-1β 10 ng/ml; 3–6: interleukin-1β 10 ng/ml + dipyridamole. The figure is a representative of three independent experiments, which gave a similar result.

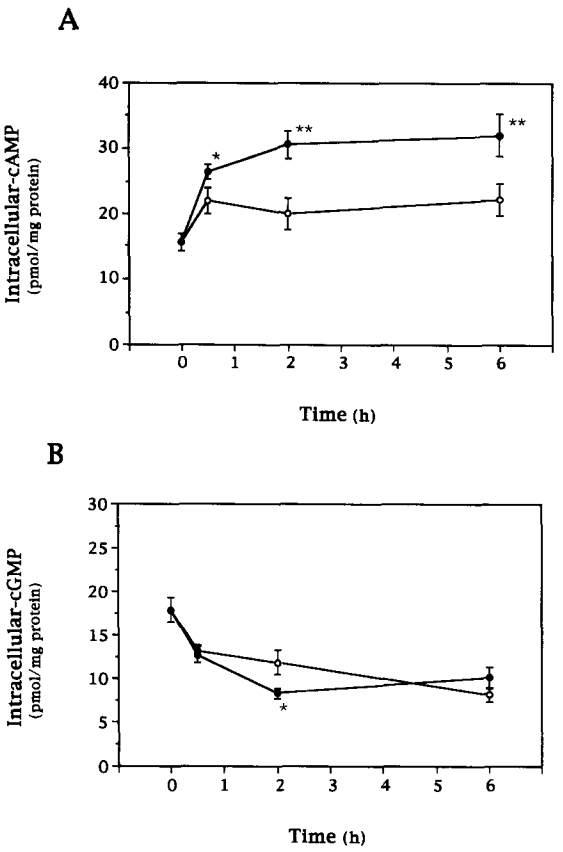

Fig. 4. Changes of intracellular cyclic nucleotides content by dipyridamole. Vascular smooth muscle cells were incubated with (closed circle) or without (open circle) dipyridamole (10 μM) in the presence of 10 ng/ml interleukin-1β. Intracellular cAMP (A) and intracellular cGMP(B) were assayed by enzyme-immunoassay. Each point and bar represents mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the condition without dipyridamole.

3.6. Dipyridamole and protein kinase inhibitors

In the next step, to determine the role of protein kinase in the augmentation of nitrite production by dipyridamole, two protein kinase inhibitors were added to the medium concomitantly with dipyridamole (Fig. 5). KT 5720, a selective inhibitor of cAMP-dependent protein kinase, abolished the enhancement of nitrite production by dipyridamole, while KT 5823, a selective inhibitor of cGMP-dependent protein kinase, did not attenuate the effect.

3.7. The effects of phosphodiesterase inhibitors on interleukin-1β-induced nitrite production

As shown in Fig. 6, Rolipram and Ro-20-1724 dose dependently enhanced interleukin-1β-stimulated ni-

Table 1
The effects of cyclic nucleotides and dipyridamole on the IL-1β-stimulated nitrite production

		Nitrite (nmol/mg protein)		
		IL-1β (−)	IL-1β 10 ng/ml	
			Dipyridamole (−)	Dipyridamole 10 μM
Control		16.2 ± 2.2	49.9 ± 3.8	271.6 ± 13.7
Db-cAMP	10 μM	23.7 ± 3.2	97.3 ± 4.9 [a]	322.7 ± 10.5 [a]
	100 μM	19.0 ± 1.7	349.4 ± 11.5 [a]	385.1 ± 3.9 [a]
8-Bromo-cGMP	100 μM	18.0 ± 1.8	67.7 ± 2.3 [a]	294.1 ± 11.3
	1 mM	16.6 ± 2.0	104.8 ± 4.7 [a]	322.3 ± 11.5 [b]

Cyclic nucleotides and/or dipyridamole were simultaneously added to the medium with IL-1β. The nitrite content released into medium during 24 h was measured. Results are means ± S.E.M. ($n = 8$). [a] $P < 0.01$, [b] $P < 0.05$ versus respected control values.

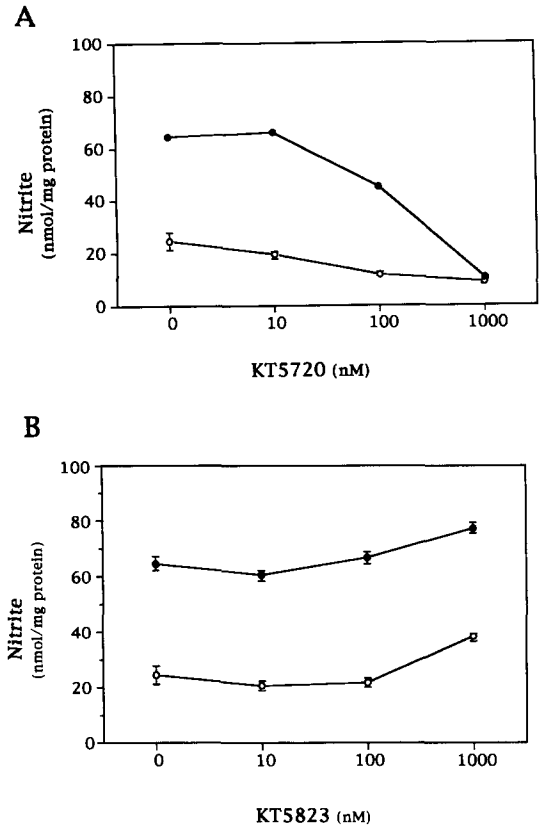

Fig. 5. Effects of protein kinase inhibitors on interleukin-1β-induced nitrite production. (A) KT 5720 was added to culture medium concomitantly with interleukin-1β (10 ng/ml) and incubated for 24 h. (B) KT 5823 was added to culture medium concomitantly with interleukin-1β (10 ng/ml) and incubated for 24 h. Control (open circle), 10 μM dipyridamole (closed circle). Each point and bar represents mean $\pm$ S.E.M. ($n = 8$).

trite generation. These agents had no effects on the level of nitrite without cytokine, at least up to 100 μM (data not shown).

4. Discussion

4.1. Dipyridamole and NO production

There have been several investigations on the interaction of clinically used drugs with nitric oxide production. Nifedipine, a calcium channel antagonist, enhances lipopolysaccharide-induced nitric oxide production in a macrophage cell line (Szabo et al., 1993). In vascular smooth muscle cells, colchicine, a drug used for the hyperuricacidemia, inhibits cytokine-induced cGMP production through depolymerization of the microtubule system (Marczin et al., 1993). Dipyridamole potentiates the vasodilatative action of NO by suppressing the degradation of cGMP in aorta (Bult et al., 1991). However, to our knowledge, there has been no report on the interaction of dipyridamole with NO production. In healthy volunteers receiving 400 mg dipyridamole daily during 3 days, the serum concentration is estimated to be approximately 2.0–4.5 μM (Chevolet et al. 1981). Dipyridamole concentrations used in our experiments are comparable to the in vivo concentration. Dipyridamole did not induce the nitrite production without cytokines. Moreover, there was no morphological change of cells treated with dipyridamole, at least up to 100 μM for 24 h. In contrast, even 1 μM dipyridamole, when coincubated for 24 h,

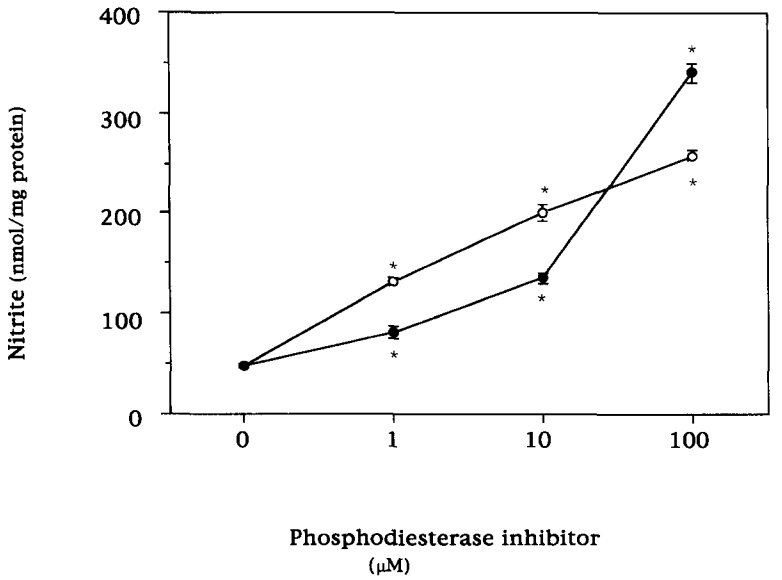

Fig. 6. Effects of phosphodiesterase inhibitors on interleukin-1β-induced nitrite production. Vascular smooth muscle cells were incubated with Rolipram (open circle) or Ro-20-1724 (closed circle) in the presence of 10 ng/ml interleukin-1β for 24 h. Each point and bar represents mean $\pm$ S.E.M. ($n = 8$). $^*P < 0.01$ vs. the condition without inhibitors.

was potent enough to enhance interleukin-1β-induced nitrite production.

4.2. NO and cyclic nucleotides

Among many factors that regulate inducible NO synthase induction, cAMP is very potent in a variety of cells including vascular smooth muscle cells (Koide et al., 1993; Imai et al., 1993), rat renal mesangial cells (Kunz et al., 1994; Muhl et al., 1994) and rat peritoneal macrophages (Sowa et al., 1994). In cultured rat vascular smooth muscle cells, we found that cGMP-generating agents including ANP could enhance the interleukin-1β-induced NO production through activation of protein kinase A (Iimura et al., submitted). In the present study, both db-cAMP and 8-bromo-cGMP upregulated the interleukin-1β-induced nitrite production that was augmented by the addition of dipyridamole. *However, the effect of 8-bromo-cGMP is rather weak compared to that of db-cAMP. A 100-fold higher concentration of cGMP analogue (1 mM) is required to obtain effects comparable to 10 μM of cAMP analogue (Table 1). Therefore, we can't rule out that a 100-fold excess of cGMP may cause non-physiological activation of cAMP-dependent protein kinase.*

4.3. Dipyridamole and cyclic nucleotides

Dipyridamole is known to inhibit some types of phosphodiesterase in vitro (Saeki and Saito, 1993; Schoeffter et al., 1987; Lugnier et al., 1986). An investigation using separated phosphodiesterase isozymes from pig aortic smooth muscle homogenates has revealed that dipyridamole is relatively specific to phosphodiesterase isozymes II, IV, V (values of IC_{50} for each enzyme are 3.6 μM, 0.52 μM, 6.4 μM, respectively) (Saeki and Saito, 1993). Types II and IV isozymes of phosphodiesterase are relatively specific to cAMP, while type V is to cGMP. In another study, using isolated rat aorta, dipyridamole increased both cyclic-nucleotides (Lugnier et al., 1986). Thus, it seemed possible that cAMP and cGMP was increased by dipyridamole and responsible for the enhancement of NO production. Nonetheless, in the present study, an increase only in cAMP content was recognized in the presence of dipyridamole. It may be due to a difference in activity of cGMP-specific phosphodiesterase and result in the change of intracellular cGMP content, although it is not yet tested.

These findings imply that an increase in cAMP concentration would be responsible for the enhancement of nitrite production by dipyridamole. To confirm this, we used two protein kinase inhibitors (Kase et al., 1987). KT 5720 is extremely selective for protein kinase A (K_i values for protein kinase C, protein kinase A, protein kinase G, myosin light chain kinase are > 2000 nM, 56 nM, > 2000 nM, > 2000 nM, respectively). KT 5823 is rather specific for protein kinase G (K_i values for protein kinase C, protein kinase A, protein kinase G, myosine light chain kinase are 4000 nM, > 10000 nM, 234 nM, > 10000 nM, respectively). KT 5720 totally abolished the enhancement of nitrite production by dipyridamole, while KT 5823 did not affect it.

The enhancement of nitrite production by two inhibitors of type IV phosphodiesterase strongly suggested that the inhibition of type IV phosphodiesterase would be responsible for the action of dipyridamole to a large extent, although we could not completely exclude the role of other type phosphodiesterases in the action of dipyridamole. In addition, dipyridamole is a pleiotrophic drug. Attenuation of adenosine uptake into cells by dipyridamole increases the extracellular concentration of adenosine and augments the cellular action of adenosine (Marangos et al., 1985; Chevolet et al., 1981). Adenosine enhances the interleukin-1β-induced NO production in our cells (data not shown). Thus, we can't preclude the role of dipyridamole as an adenosine potentiator in NO production.

In summary, dipyridamole enhanced the interleukin-1β-stimulated NO production in rat vascular smooth muscle cells possibly through protein kinase A activation, which was assigned to an increase in intracellular cAMP concentration. It seems possible that NO production is increased in the patients taking dipyridamole. Further investigations are underway in our laboratory to elucidate the in vivo roles of dipyridamole in relation to NO production.

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