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ALTERATION OF CYCLIC GMP METABOLISM BY CD-349, A NOVEL CALCIUM ANTAGONIST, AND BY SODIUM NITROPRUSSIDE IN BOVINE INTRAPULMONARY ARTERY AND VEIN

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Abstract—The effects of 2-nitratopropyl 3-nitratopropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (CD-349) and sodium nitroprusside (NP) on cyclic GMP (cGMP) metabolism in bovine intrapulmonary artery (BPA) and vein (BPV) were examined. CD-349 inhibited cGMP phosphodiesterase (PDE) activity in BPA and BPV. In the latter, about 40% of the cGMP PDE activity was Ca^{2+} dependent. The inhibition of cGMP PDE activity by CD-349 also depended on Ca^{2+}. The inhibitory effect of CD-349 was more potent than that of nicardipine or nifedipine. The conversion of cGMP from GTP in the homogenates of BPA and BPV was stimulated by NP in a concentration-dependent manner. The NP-induced cGMP formation was stimulated further by CD-349. This effect of CD-349 depended on Ca^{2+} in the BPV but not in the BPA. The NP-induced elevation of cGMP levels in the tissue preparations of BPA and BPV was also potentiated by CD-349. These results suggest that CD-349 inhibited Ca^{2+}-dependent cGMP PDE activity and that the levels of cGMP were elevated in vascular smooth muscle, particularly when guanylate cyclase was activated.

Diamond and co-workers [1, 2] observed that the potent vasodilator, nitroglycerin, elevates cyclic GMP (cGMP)† levels in vascular and non-vascular smooth muscles. Subsequently, other investigators also reported that various smooth muscle relaxants activate soluble guanylate cyclase and elevate cGMP levels in tissue [3, 4]. A good correlation between vasodilation and cGMP accumulation elicited by nitrogen oxide-containing vasodilators [5–9] or muscarinic receptors agonists, mediated through endothelium-derived relaxing factor (EDRF) [9–13], or atrial natriuretic factor (atriopeptin II) [8, 9, 14], has been reported. In addition to guanylate cyclase stimulants, cGMP phosphodiesterase (PDE) inhibitors also probably dilate smooth muscle since they inhibit cGMP hydrolysis and elevate levels of tissue cGMP. Indeed, cGMP PDE inhibitors solely dilate non-vascular [15] and vascular [16] smooth muscles. The cGMP PDE inhibitor has a marked potentiating action on relaxant responses of nitrogen oxide-containing vasodilators [16, 17] and endothelium-dependent vasodilators [10, 18].

A newly synthesized 1,4-dihydropyridine derivative, CD-349, has a high affinity for the Ca^{2+} channel [19] and dilates canine vascular smooth muscle *in vivo* [20]. CD-349 also inhibits Ca^{2+}-dependent cyclic nucleotide PDE activity in the vessels [21, 22]. In the present study, we examined the effects of CD-349 on cGMP metabolism, in relation to the guanylate cyclase stimulant, sodium nitroprusside (NP), and compared the responsiveness of bovine intrapulmonary artery (BPA) and vein (BPV) to this compound.

METHODS

Materials. Bovine lungs obtained from the local slaughterhouse were immersed in an ice-cold buffered salt solution containing (mM): NaCl, 145; KCl, 4; $MgCl_2$, 1; $CaCl_2$, 2; Tris/HCl (pH 7.5), 20; and glucose, 10. They were then transported to our laboratory.

CD-349 and nicardipine were synthesized in the Taisho Research Laboratories. Nifedipine (Adalat) was purchased from the Bayer Yakuhin Co. Ltd (Osaka, Japan); cGMP, GTP, guanosine, NP, ethyleneglycol-bis-(β-aminoethyl ether) *N,N'*-tetraacetic acid (EGTA) and snake venom (*Crotalus atrox*) were purchased from the Sigma Chemical Co. (St Louis, MO). [^{3}H]cGMP (33.9 Ci/mmol) was purchased from New England Nuclear (Boston, MA). All other chemicals were of reagent grade or of higher quality.

CD-349, nicardipine and nifedipine were dissolved in 100% dimethyl sulfoxide at a concentration of 15 mM and stored at −20°. Dilutions were prepared just before use. Buffered salt solution and NP were freshly prepared. Reagent grade water used to prepare the solutions was obtained using a Milli Q Water System (Millipore Corp., Bedford, MA).

Enzyme preparations. Bovine intrapulmonary vessels were dissected from the parenchyma and placed in ice-cold Krebs–Ringer bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 4.7;

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† Abbreviations: cGMP, cyclic GMP; EDRF, endothelium-derived relaxing factor; PDE, phosphodiesterase; CD-349, 2-nitratopropyl 3-nitrapropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate; NP, sodium nitroprusside; BPA, bovine intrapulmonary artery; BPV, bovine intrapulmonary vein; and EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N,N'*-tetraacetic acid.

$CaCl_2$, 1.5; $NaHCO_3$, 25; $MgSO_4$, 1.1; KH_2PO_4, 1.2; and glucose, 5.6. After removal of connecting tissues, the strips were weighed, minced with scissors, and homogenized in a Physcotron homogenizer (Niti-on Medical and Physical Instruments Mfg. Co. Ltd, Funabashi, Japan) in 5 vol. of ice-cold buffer. The homogenate prepared with 20 mM Tris/HCl (pH 7.7) containing 0.25 M sucrose, and the supernatant fraction of the homogenate, prepared with 40 mM Tris/HCl (pH 7.5) containing 0.25 M sucrose, 5 mM 2-mercaptoethanol and 5 mM $MgCl_2$, obtained by ultracentrifugation at 100,000 *g* for 60 min, were used to measure the accumulation of cGMP generated from GTP and cGMP PDE activity respectively. The responsiveness of BPA and BPV to CD-349 or NP was measured in vessels obtained from the same lung.

Protein concentration was determined by the method of Lowry *et al.* [23] using bovine serum albumin as the standard.

Procedure for cGMP PDE assay. Cyclic GMP PDE activity was assayed by the method of Beavo *et al.* [24], with slight modification [21]. The standard reaction mixture contained 30 mM Tris/HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 100 mM NaCl, 1 μM cGMP (including 150,000 dpm [^{3}H]cGMP), 50 μg bovine serum albumin and enzyme preparation (280–300 μg and 160–190 μg protein for BPA and BPV respectively) in a total volume of 0.5 ml. The reaction mixture was incubated at 37° for 2 min with protection from the light. The reaction was halted by placing the tubes for 2 min in a boiling water bath. Subsequently, 10 μl of snake venom (*C. atrox*, 5 mg/ml) was added to the reaction mixture and the preparation was incubated at 37° for an additional 30 min. After the incubation, the reaction mixture was diluted to 1 ml with a 0.5 mM aqueous solution of guanosine. The entire mixture was applied to a column (0.6 × 2 cm) of Dowex 1 × 8 (chloride form). The column was eluted with 8 ml of 0.1 M Tris/HCl (pH 7.5), and the radioactivity in a 1-ml aliquot was counted with 10 ml of Aquasol-2 scintillator (New England Nuclear) by a liquid scintillation spectrometer (Tri-Carb model 4430, Packard Instrument Co. Inc., Downers Grove, IL).

Accumulation of cGMP generated from GTP in intrapulmonary vessel homogenates. The standard reaction mixture contained 50 mM Tris/HCl (pH 7.5), 0.1 mM GTP, 4 mM $MgCl_2$ and 0.1 ml of homogenate (1.2 to 1.4 mg and 0.7 to 1.0 mg protein for BPA and BPV respectively) in a total volume of 0.5 ml. After incubating the reaction mixture at 37° for 2 (BPA) or 5(BPV) min, with protection from the light, the reaction was terminated by placing the tubes in a boiling bath for 2 min. Following centrifugation at 3000 rpm for 10 min, appropriate supernatant aliquots were directly assayed by radioimmunoassay for cGMP. The method for radioimmunoassay of cGMP is described below.

Procedure for determination of cGMP contents in tissues. Bovine intrapulmonary vessels were prepared as described above. Krebs–Ringer bicarbonate solution saturated with 95% O_2–5% CO_2 was used throughout the experiment. Cleaned vessels were cut into strips (5–8 mm wide, 10–15 mm long, approximately 100 mg weight) and put into glass test tubes containing 2.0 ml of Krebs–Ringer bicarbonate solution. After the addition of drugs, incubation was carried out for 5 or 20 min at 37°. At the end of incubation, each strip was wiped with filter paper, clamped with a stainless-steel clamp that had been precooled in liquid nitrogen, and plunged into liquid nitrogen. Frozen strips were stored at −85° until the measurement of cGMP.

The cGMP content was determined by radioimmunoassay as described previously [25–27]. Immediately after measuring the weight, the frozen strips were homogenized with 1.0 ml of ice-cold 6% trichloroacetic acid using a Physcotron homogenizer. The homogenate was centrifuged at 3000 rpm for 15 min at 4°. After transfer of the resulting supernatant fraction to another test tube, the pellet was rehomogenized. The same procedure was repeated. The supernatant fractions were combined, and 6.0 ml of water-saturated diethyl ether was added to the mixture, which was then mixed vigorously using a Physcotron homogenizer. The ether and aqueous phase were separated by centrifugation at 3000 rpm for 5 min at 4°, and the ether layer was removed by aspiration. This extraction procedure was repeated three times. The remaining aqueous solution was evaporated to dryness under reduced pressure with a centrifugal evaporator (Speed Vac SVC-200H, Savant Instruments Inc., Hicksville, NY), and the remnants were dissolved with 0.5 ml water. Cyclic GMP was measured with a commercially available radioimmunoassay kit (Yamasa Shoyu Co., Choshi, Japan). Following succinylation of cGMP in 0.1-ml aliquots and a standard solution using succinic anhydride in dioxane and triethylamine, the samples were incubated with [^{125}I]succinylated cGMP tyrosine methyl ester and mouse anti-cGMP monoclonal antibody for 20–24 hr at 4°. To separate free and bound antigen, 0.5 ml of dextran-coated charcoal was added, mixed, allowed to stand for 10 min in an ice-bath, and then centrifuged at 3000 rpm for 5 min at 4°. The radioactivity in a 0.5-ml aliquot of the resulting supernatant fraction was counted using a Gamma counter (ARC-300, Aloka Co., Tokyo, Japan). The recovery of added [^{3}H]cGMP from the tissue or homogenate exceeded 90%.

Data analysis. The concentration of the agent necessary to produce 50% inhibition of control (IC_{50}) was calculated from computerized nonlinear least squares fits of the data points. The approach used was the Marquardt–Levenberg curve-fitting procedure of the RS/1 program (BBN Research Systems, Cambridge, MA) running on a VAX-8600/VMS system. Statistical analysis of the values of cGMP was carried out using ANOVA.

RESULTS

Effect of Ca^{2+} on the inhibition of cGMP PDE activity by CD-349 in BPA and BPV. CD-349 inhibited cGMP PDE activity in the presence of 10 μM Ca^{2+} or 0.2 mM EGTA, dose-dependently, in BPA and BPV (Figs. 1 and 2). EGTA (0.2 mM) decreased basal activity of cGMP PDE by about 15 and 40% in BPA and BPV respectively. Values for cGMP PDE activity in the presence of 10 μM Ca^{2+} and in the presence of 0.2 mM EGTA were

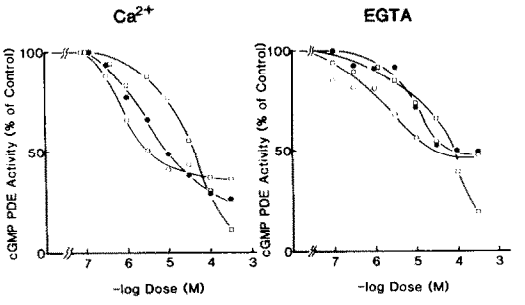

Fig. 1. Effect of CD-349 on cGMP PDE activity of bovine intrapulmonary artery in the presence and absence of Ca^{2+}. Assay of cGMP PDE activity was carried out as described in Methods, in the presence of 10 μM Ca^{2+} (left panel) or 0.2 mM EGTA (right panel). Cyclic GMP PDE activity without inhibitor was taken as 100%, and results are the average of three separate experiments of duplicate determinations. Control cGMP PDE activities in the presence of 10 μM Ca^{2+} and 0.2 mM EGTA were 702.1 ± 87.6 and 607.0 ± 18.7 pmol/mg protein/min respectively. Key: CD-349 (○), nicardipine (●), and nifedipine (□).

respectively. In BPA, however, Ca^{2-} had no significant effect on the inhibition of cGMP PDE activity by CD-349 (Fig. 1). The IC_{50} values of CD-349, nicardipine and nifedipine in the presence of 10 μM Ca^{2+} were 7.9×10^{-6}, 3.3×10^{-5} and 9.1×10^{-5} M, respectively, and in the presence of 0.2 mM EGTA, 1.6×10^{-5}, 6.1×10^{-5} and 1.0×10^{-4} M respectively.

Effects of CD-349 and NP on the accumulation of cGMP generated from GTP in the homogenates of BPA and BPV. To determine the effects of CD-349 on cGMP metabolism, tissue homogenate was used as an enzyme source since it contains enzymes generating and degrading cGMP. In BPA, 10 μM CD-349 only slightly increased the accumulation of cGMP at 1–2 min after the start of incubation, whereas the guanylate cyclase stimulating vasodilator, NP (100 μM), markedly elevated the accumulation of cGMP 1–2 min after incubation (Fig. 3, upper). The cGMP levels elevated by NP rapidly decreased and returned to the control level within 30 min (Fig. 3, upper). Ten micromolar CD-349 had no effect on the time–course of cGMP accumulation produced by NP in BPA. In BPV, however, 10 μM CD-349 slightly but clearly

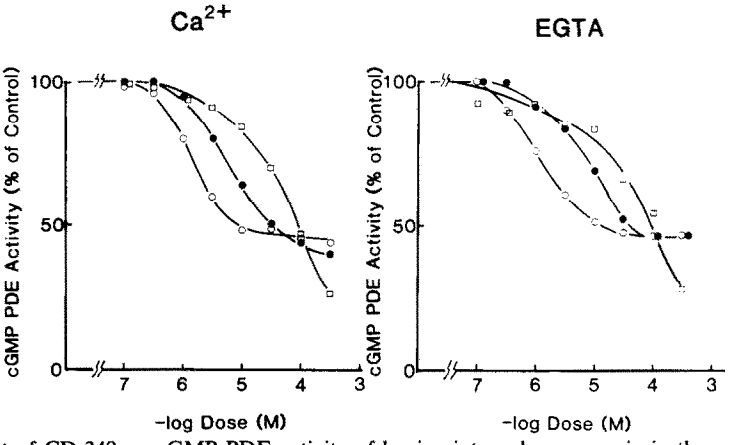

Fig. 2. Effect of CD-349 on cGMP PDE activity of bovine intrapulmonary vein in the presence and absence of Ca^{2+}. Assay of cGMP PDE activity was carried out as described in Methods, in the presence of 10 μM Ca^{2+} (left panel) or 0.2 mM EGTA (right panel). Cyclic GMP PDE activity without inhibitor was taken as 100%, and results are the average of three separate experiments of duplicate determinations. Control cGMP PDE activities in the presence of 10 μM Ca^{2+} and 0.2 mM EGTA were 361.0 ± 85.8 and 216.1 ± 54.0 pmol/mg protein/min respectively. Key: CD-349 (○), nicardipine (●), and nifedipine (□).

702.1 ± 87.6 and 607.0 ± 18.7 pmol/mg protein/min in the BPA, and 361.0 ± 85.8 and 216.1 ± 54.0 pmol/mg protein/min in the BPV respectively. The inhibitory effects of CD-349 on cGMP PDE activity in the BPV were affected markedly by the presence of Ca^{2+} (Fig. 2). In the presence of 10 μM Ca^{2+}, CD-349 showed an inhibitory effect on cGMP PDE activity that was 10 times more potent than that in the presence of 0.2 mM EGTA. A similar effect of Ca^{2+} on the inhibition of cGMP PDE activity was observed with nicardipine but not with nifedipine (Fig. 2). The IC_{50} values of CD-349, nicardipine and nifedipine in the presence of 10 μM Ca^{2+} were 3.3×10^{-6}, 9.5×10^{-6} and 4.1×10^{-5} M respectively. When free Ca^{2+} was removed by 0.2 mM EGTA, the values were 3.3×10^{-5}, 6.4×10^{-5} and 6.4×10^{-5} M increased the accumulation of cGMP after 2–5 min of incubation (Fig. 3, lower). NP elevated the cGMP levels in BPV as well as in BPA. CD-349 potentiated the elevation of cGMP levels produced by NP about 2.1 times at 5 min. The point of maximal increase in NP-induced cGMP levels was moved to 15 min, from 5 min, after the incubation by addition of CD-349, with a 4.0 times increase of cGMP (Fig. 3, lower).

Figures 4 and 5 show the concentration–response relationship of NP and CD-349 on the accumulation of cGMP generated from GTP. Above 100 and 10 μM, NP produced a concentration-dependent increase in cGMP accumulation in BPA and BPV respectively (Fig. 4). The sensitivity to NP-induced stimulation of guanylate cyclase activity in BPV was higher than in BPA. CD-349 (10 μM) remarkably

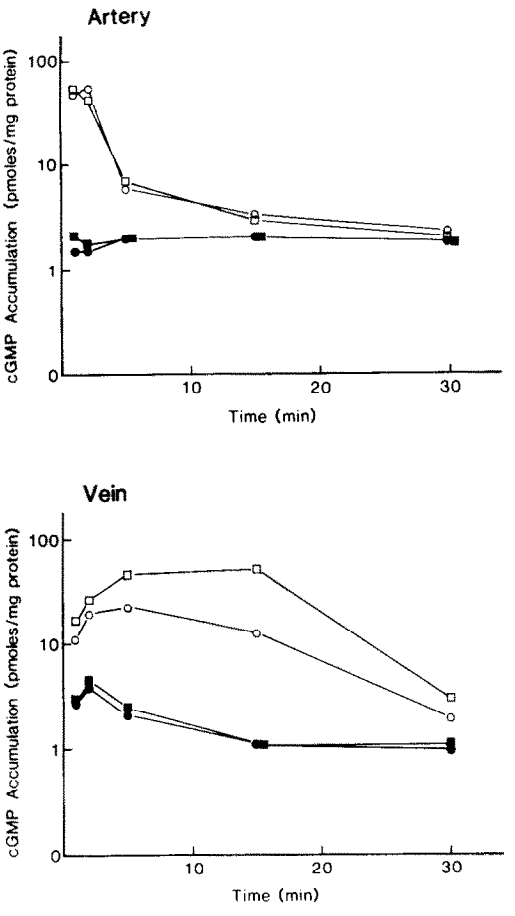

Fig. 3. Effects of CD-349, NP, and NP plus CD-349 on the time-course of accumulation of cGMP generated from GTP in bovine intrapulmonary arterial and venous homogenate. Accumulation of cGMP generated from GTP was measured as described in Methods, using arterial (upper panel) and venous (lower panel) homogenate. Each point is the mean value of duplicate determinations. Key: control (●), 10 μM CD-349 (■), 100 μM NP (○), and 100 μM NP + 10 μM CD-349 (□).

potentiated the accumulation of cGMP generated from GTP produced by NP in both BPA and BPV.

CD-349 did not stimulate the accumulation of cGMP generated from GTP in BPA homogenate at concentrations between 0.3 and 30 μM; at 10 and 30 μM, it slightly stimulated the accumulation in BPV homogenate (Fig. 5). However, when guanylate cyclase activity was stimulated by 100 μM NP, CD-349 further increased, in a concentration-dependent manner, the accumulation of cGMP generated from GTP in BPA and BPV at concentrations over 10 and 3.0 μM respectively. This potentiating effect of CD-349 was observed more significantly in BPV than in BPA. BPV showed a higher sensitivity to NP than BPA, and CD-349 also had a larger potentiating effect on BPV than on BPA for accumulation of cGMP.

Effect of Ca^{2+} *on the accumulation of cGMP generated from GTP induced by CD-349 and NP.* Since Ca^{2+} inhibits NP-induced activation of guanylate cyclase activity in the presence of Mg^{2+} [28], the Ca^{2+}-free assay mixture was used to measure cGMP synthesis. The accumulation of cGMP generated from GTP in the control was not affected by Ca^{2+} in either BPA or BPV (Fig. 6, left). However, the NP-induced stimulation of cGMP accumulation was attenuated significantly by 100 μM Ca^{2+} and augmented significantly by 2 mM EGTA in both BPA and BPV (Fig. 6, right). Ten micromolar CD-349 increased cGMP accumulation in BPV but not in BPA (Fig. 6, left). CD-349 augmented the stimulation of cGMP accumulation produced by NP in BPA and BPV. When free Ca^{2+} was removed by EGTA, the augmentation by CD-349 was not observed in BPV. In BPA, the effect of CD-349 on NP-induced stimulation of cGMP accumulation was observed in the presence of EGTA, but the increasing ratio of cGMP accumulation was significantly smaller compared with that obtained in the presence of free Ca^{2+} (Fig. 6, right).

Effects of CD-349 and NP on tissue cGMP levels. Figure 7 shows the effects of CD-349, NP, and NP plus CD-349 on cGMP levels in BPA and BPV

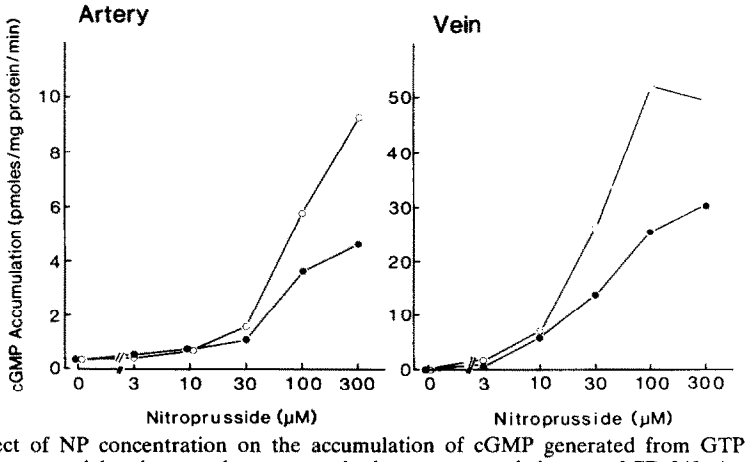

Fig. 4. Effect of NP concentration on the accumulation of cGMP generated from GTP in bovine intrapulmonary arterial and venous homogenate in the presence and absence of CD-349. Accumulation of cGMP generated from GTP was measured as described in Methods, using arterial (left panel) and venous (right panel) homogenate. Data are from one experiment, representative of several. Each point is the mean of triplicate determinations. Key: control (●), and 10 μM CD-349 (○).

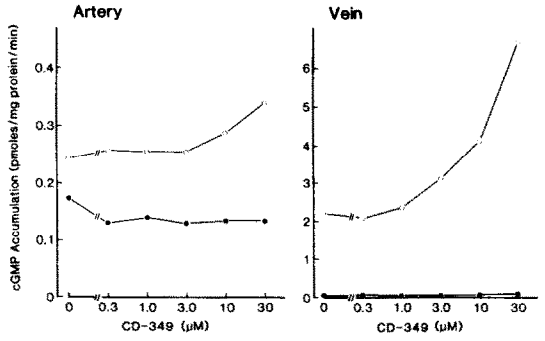

Fig. 5. Effect of CD-349 concentration on the accumulation of cGMP generated from GTP in bovine intrapulmonary arterial and venous homogenate in the presence and absence of NP. Accumulation of cGMP generated from GTP was measured as described in Methods, using arterial (left panel) and venous (right panel) homogenate. Data are from one experiment, representative of several. Each point is the mean of quadruplicate determinations. Key: control (●), and 100 μM NP (○).

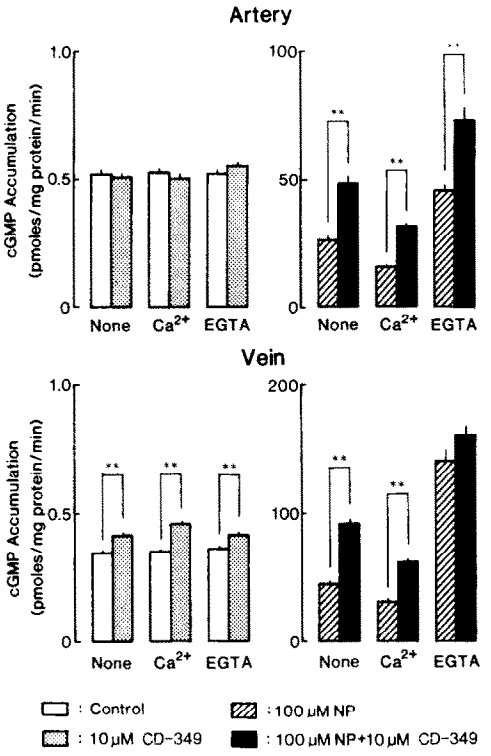

Fig. 6. Effects of CD-349, NP, and NP plus CD-349 on the accumulation of cGMP generated from GTP in the presence and absence of Ca^{2+} in bovine intrapulmonary arterial and venous homogenate. Accumulation of cGMP generated from GTP was measured as described in Methods, using arterial (upper) and venous (lower) homogenate. None, Ca^{2+} and EGTA refer to the standard assay condition, plus 100 μM Ca^{2+} and plus 2 mM EGTA respectively. Columns are mean values of quintuplicate determinations and vertical lines on each column indicate SE. Key: (**)$P < 0.01$.

tissues. CD-349 tended to elevate cGMP levels slightly but not significantly. On the other hand, NP (100 μM) markedly elevated tissue cGMP levels; further stimulation occurred with the addition of 10 μM CD-349, in both BPA and BPV after 20 min and 5 min of incubation respectively.

DISCUSSION

We obtained evidence that CD-349 inhibits Ca^{2+}-dependent cGMP PDE activity in both BPA and BPV. CD-349 augmented the NP-induced accumulation of cGMP generated from GTP in homogenates of the vessels. Stimulation of NP-induced elevation of cGMP levels by CD-349 in blood vessel tissues was also observed.

Concerning the mechanism of vasodilation mediated by cyclic nucleotide, many investigators have proposed that cGMP is a candidate of vasodilative "second messenger" since the nitro-containing vasodilator, EDRF, and atrial natriuretic factor elevate cGMP levels in vascular smooth muscle, with subsequent vasodilation [5–14]. CD-349 inhibited cGMP PDE activity of BPA and BPV in the presence and absence of free Ca^{2+}. Basal cGMP PDE activity was attenuated significantly by EGTA in BPV but not in BPA. Removal of free Ca^{2+} by 0.2 mM EGTA weakened the inhibitory potency of CD-349 on cGMP PDE activity in BPV by about one-tenth, but not in BPA. These results suggest that Ca^{2+}-dependent cGMP PDE is contained abundantly in BPV, and CD-349 inhibits the enzyme, Ca^{2+} dependently. Similar results have also been observed in the porcine coronary artery [21].

Ignarro *et al.* [29] reported that organic nitrate (R—ONO_2) reacts with intracellular —SH groups to form NO_2^- which subsequently converts to NO and to unstable intermediates *S*-nitrosothiols, which activate soluble guanylate cyclase activity. Activation of guanylate cyclase by CD-349 could be considered since it has two —ONO_2 groups in its molecule. CD-349 stimulated cGMP accumulation, especially in BPV, but the effect was not so potent as NP in the homogenate of the vessels. CD-349 dose-dependently potentiated and maintained the stimulated accumulation of cGMP produced by NP. These results may explain why CD-349 predominantly acts as a cyclic nucleotide PDE inhibitor.

Since CD-349 selectively inhibits Ca^{2+}-dependent cyclic nucleotide PDE activity [21, 22], in the present study we focused on the effect of CD-349 on the accumulation of cGMP generated from GTP in relation to Ca^{2+}. Cyclic GMP accumulation was activated more strongly by NP in the absence of Ca^{2+} than in its presence in homogenates of the pulmonary vessels. This may be due to the presence of Mg^{2+} in the reaction mixture because Ca^{2+} is an inhibitor of guanylate cyclase in the presence of Mg^{2+} [28]. The disappearance or attenuation of the potentiating effect of CD-349 on the NP-induced accumulation of cGMP in the presence of EGTA also suggests that CD-349 exerts its action predominantly via inhibition of Ca^{2+}-dependent cyclic nucleotide PDE.

Despite a similar basal level of cGMP accumulation in BPA and BPV, sensitivities of the vessels

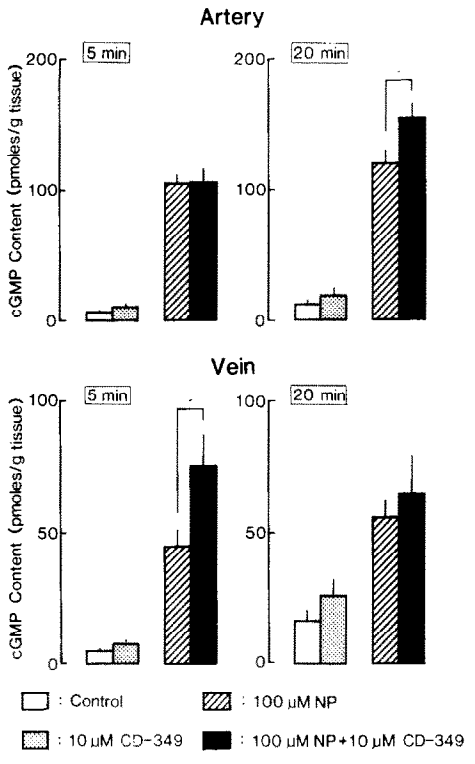

Fig. 7. Effects of CD-349, NP, and NP plus CD-349 on cGMP contents in bovine intrapulmonary artery and vein. Cyclic GMP contents in artery (upper) and vein (lower) were measured as described in Methods, at 5 min (left) and 20 min (right). The columns are mean values from eleven tissues. Vertical lines on each column indicate SE. Key: (*)$P < 0.05$.

to NP in cGMP generation differed, BPV being more sensitive than BPA to NP. Edwards *et al.* [7] reported that nitrogen oxide-containing vasodilators, including NP, relax BPA and BPV, but that these vasodilators are more potent in venous than arterial vessels as relaxants or elevators of cGMP levels. They also reported that activation of soluble guanylate cyclase activity was stimulated by organic nitrates more effectively in BPV than in BPA, whereas basal enzymatic activities were similar in BPA and BPV. The present results obtained from the homogenate preparations are in agreement with those obtained by Edwards *et al.* [7]. Moreover, the potentiation of the stimulatory effect of NP by CD-349 in the accumulation of cGMP in BPA differed from that in BPV. The effect of CD-349 was exerted at a lower concentration in BPV than in BPA. The high sensitivity of CD-349 to BPV also suggests that CD-349 inhibited Ca^{2+}-dependent cGMP PDE activity and increased cGMP accumulation in BPV more than in BPA.

The physiological significance of PDE inhibition in CD-349-induced vasodilation has not been confirmed. In the present study, CD-349 inhibited Ca^{2+}-dependent cGMP PDE activity in BPA and BPV. CD-349 also specifically binds to rat myocardial and brain membrane fractions and porcine coronary arterial membrane fractions, with a high affinity [19]. The binding site was the same as that of 1,4-dihydropyridines, such as nifedipine, nicardipine and nitrendipine. CD-349 inhibits KCl, 5-hydroxytryptophan and prostaglandin $F_{2\alpha}$-induced contractions of canine basilar artery [20]. Thus, CD-349 probably binds to the Ca^{2+} channel, prevents Ca^{2+} influx into the vascular smooth muscle cells, and dilates vascular smooth muscle. CD-349 exerts effects on smooth muscle contraction and on Ca^{2+} channel blockade at concentrations several orders of magnitude lower than their IC_{50} values for the inhibition of PDE activity [19–21]. Several investigators have reported that Ca^{2+} antagonists may accumulate in isolated vascular smooth muscle cells [30–32] and are concentrated about 500-fold, intracellularly [33, 34]. We also observed this same accumulation of CD-349 in BPA and BPV*. These observations suggest that the concentrations of CD-349 in the target organ likely reach 10^{-6}–10^{-5} M levels, under physiological conditions, since the clinically available plasma concentration of Ca^{2+} antagonists is at the 10^{-7} M level [35, 36]. Thus, the inhibition of Ca^{2+}-dependent PDE activity by CD-349 may be involved in the vasodilative action of CD-349.

Our interpretation of the results is that CD-349 inhibits Ca^{2+}-dependent cGMP PDE activity with high selectivity and that the inhibition may prevent cGMP hydrolysis. Moreover, the effects of CD-349 on cGMP accumulation or cGMP levels were marked when guanylate cyclase activity was stimulated by a nitrogen oxide-containing vasodilator. Responsiveness of the tissues to CD-349 was always higher in BPV than in BPA, perhaps because of a different ratio of Ca^2-dependent PDE to total PDE in BPA and BPV. In either case, the Ca^{2+} dependency of blood vessel cyclic nucleotide PDE was large compared with that of the heart [21]. From these results, cGMP may well play a role in the vasodilation by CD-349 by inhibiting Ca^{2+}-dependent PDE activity, at least to some extent.

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