ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb；21 (2)：131－138
http；//www. simm. ac. cn； www. chinainfo. gov. cn/periodical

Prostacyclin-induced relaxations of small porcine pulmonary arteries are enhanced by the basal release of endothelium-derived nitric oxide through an effect on cyclic GMP-inhibited-cyclic AMP phosphodiesterase[1]

Thomas M ZELLERS[2], Yi-Qun WU, Jennifer McCORMICK, Paul M VANHOUTTE[3]
(*Department of Pediatrics, Division of Cardiology University of Texas Southwestern Medical Center at Dallas 5323 Harry Hines Boulevard, Dallas, TX 75235*；[3]*IRIS 6 Place de Pleiades, 92415 Courbevoie, France*)

KEY WORDS pulmonary artery；vascular endothelium；nitric acid；epoprostenol；cyclic GMP；cyclic AMP；phosphoric diester hydrolases

ABSTRACT

AIM：To study the interactions between prostacyclin and endothelium-derived nitric oxide in porcine pulmonary arteries. METHODS：Rings of 5th order of porcine pulmonary arteries were studied *in vitro* for the measurement of tension and the content in cyclic nucleotides. RESULTS：Prostacyclin, given exogenously, caused endothelium-potentiated relaxations (inhibition of phenylephrine contraction) that were inhibited by the inhibitors of the *L*-arginine nitric oxide pathway, oxyhemoglobin and N^{ω}-nitro-*L*-arginine. These inhibitors did not affect the tension in rings without endothelium. Cyclic GMP-concentrations were not increased above basal concentrations in the presence of prostacyclin. Increases were seen with acetylcholine and sodium nitroprusside. Prostacyclin-stimulated cyclic AMP concentrations did not reach statistical significance compared to controls. The addition of 8-bromo-cyclic GMP to prostacyclin, however, increased the cyclic AMP content. The nitric oxide synthase inhibitor, nitro-*L*-arginine (NLA), reduced the prostacyclin-stimulated cyclic AMP content to basal level. Inhibition of cyclic GMP-inhibited cyclic AMP phosphodiesterase by 8-bromo-cyclic GMP or amrinone (a specific inhibitor of this enzyme) potentiated the prostacyclin-induced relaxations in rings without endothelium to a magnitude similar to that observed in rings with endothelium. CONCLUSION：These data suggest that the augmentation by the endothelium of the prostacyclin-induced relaxation of porcine pulmonary arteries is secondary to the inhibition of cyclic GMP-inhibited cyclic AMP phosphodiesterase by basally released endothelium-derived nitric oxide.

INTRODUCTION

The endothelium synthesizes and releases relaxing and contracting factors that are responsible for vascular tone under normal and pathologic conditions[1－4]. Prostacyclin and endothelium-derived nitric oxide (EDNO) are synthesized and released by pulmonary endothelial cells；both factors cause vasodilatation[5,6] and inhibit platelet aggregation[7,8]. Prostacyclin mediates these actions through the accumulation of cyclic AMP while nitric oxide mediates vasodilation through the accumulation of cyclic GMP[9－11]. In porcine systemic vessels, prostacyclin stimulates the release of EDNO, the quantity of which varies with the type of blood vessel[12]. Shimokawa *et al*[12] concluded that the vasodilator effects of endothelium-derived relaxing factor (EDRF)/nitric oxide and prostacyclin are synergistic in the porcine coronary artery. They interpreted their data to suggest that the synergism is secondary to stimulated release of EDRF/nitric oxide by the prostanoid. Another synergism exists between agonists that stimulate cyclic AMP and those that stimulate cyclic GMP[13]. A cyclic GMP-inhibited cyclic AMP phosphodiesterase (cGI-PDE) was first isolated and characterized in bovine cardiac muscle[13]. The enzyme is also present in platelets[14] and vascular smooth muscle[15,16]. Inhibition of this enzyme results in elevated platelet cyclic AMP concentrations and is responsible for the synergistic antiaggregatory actions of sodium nitroprusside and prostaglandin E_1[14], and likely EDRF/nitric oxide and prostacyclin[8], on platelets.

[1] Project supported by grant 91G-083 from the American Heart Association, Texas Affiliate and grants HL 31183 and HL 31547 from the National Institutes of Health.
[2] Correspondence to Dr Thomas M ZELLERS.
Phn 214-640-2333. Fax 214-640-6154.
E-mail tzellers@childmed. dallas. tx. us
Received 1999-04-23 Accepted 1999-10-15

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb；21 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Dilatation of vascular smooth muscle also results from the inhibition of the enzyme[16,17]. The cardiotonic drugs, amrinone and milrinone, inhibit cGI-PDE[15,18], increasing cardiac myocyte cyclic AMP concentrations[13] and causing peripheral vasodilatation[19-21], presumably secondary to an increase in cyclic AMP concentration in the vascular smooth muscle. Inhibition of the enzyme by nitrovasodilators appears responsible for the enhanced relaxations to β-adrenergic agonists[16]. Indeed, the presence of the endothelium enhances relaxations of pulmonary arterial smooth muscle to the β-adrenergic agonist, isoproterenol[4].

The present experiments were designed to determine whether or not, in isolated porcine pulmonary arteries, the potentiation by the presence of the endothelium of the response to prostacyclin is secondary to the basal or the stimulated release of nitric oxide, and if it depends upon a cyclic GMP-inhibited cyclic AMP phosphodiesterase.

MATERIALS AND METHODS

Drugs The following drugs were used in the experiments: acetylcholine chloride, amrinone, bradykinin, 8-bromo-cyclic GMP, hemoglobin (bovine), indomethacin, methylene blue, N^{ω}-nitro-*L*-arginine (NLA), potassium chloride and prostacyclin (all from Sigma Chemical Co, St. Louis, Mo). The drugs were made fresh daily using distilled water. Indomethacin was first dissolved in $NaCO_2$ (10 μmol·L^{-1}) and then distilled water. NLA was first dissolved in HCl (1 mol/L；100 μL) and then 10 mL of distilled water. Prostacyclin was first dissolved in $NaHCO_3$ and then NaOH (100－200 μL) was added to bring the pH of the solution to 9.0. The drugs were kept on ice during the experiments.

Bovine hemoglobin (type 1) was obtained from Sigma Chemical Co. It contains a mixture of oxyhemoglobin and methemoglobin. The oxyhemoglobin solution was prepared by adding 600 mg of bovine hemoglobin to 10 mL of distilled water containing 70 mg of sodium dithionite ($Na_2S_2O_4$). The sodium dithionite was then removed by dialyzing the solution against 15 liters of water, containing 0.001 % edetic acid, which was maintained at room temperature and bubbled with nitrogen. Following two hours of dialysis, the percent of oxyhemoglobin was determined spectrophotometrically[23].

Animal research was performed using an Institutional Review Board approved protocol and was carried out according to guidelines set forth by the National Institutes of Health for animal research.

Organ chamber experiments Lungs were obtained from mature pigs (n = 40, three months old) following ketamine (300 mg/kg intramuscularly) and pentobarbital (12.5 mg/kg intravenously) anesthesia and euthanasia, or fresh from a local slaughterhouse. The lungs were immersed in cold modified Krebs-Ringers bicarbonate solution (control solution) of the following composition (in mmol·L^{-1})：NaCl 118；KCl 4.7；$CaCl_2$ 2.5；$MgSO_4$ 1.2；KH_2PO_4；$NaHCO_3$ 25；edetic acid-Ca 0.026；glucose 11.1 and transported to the laboratory. The small pulmonary arteries (5th order, 2－3 mm diameter) were dissected free from the parenchyma and loose connective tissue was removed taking care not to touch and damage the endoluminal surface. In some rings, the endothelium was removed by passing a thin stainless steel wire (0.25 mm) into the lumen and gently rolling the vessels back and forth on tissue soaked with control solution. This method has proven effective in removing the endothelium without damaging the underlying vascular smooth muscle[4,22]. The cleaned vessels were cut into rings and placed in organ chambers filled with control solution (25 mL) that was maintained at constant temperature (37 ℃) and pH (7.4) and bubbled with a 95 % O_2－5 % CO_2 gas mixture. The rings were suspended between a fixed stirrup (within the organ chamber) and a force transducer (grass UTC3, Grass Instruments, Quincy, MA). Isometric tension was recorded (Gould 8000S, Gould Electronics, Cleveland, OH). The rings were stretched gradually to the optimal point on the length-tension curve as determined by a maximal contraction to either phenylephrine (50 μmol·L^{-1}) or histamine (3 μmol·L^{-1}). The vessels were washed and allowed to equilibrate for 30 minutes in the presence of indomethacin (10 μmol·L^{-1}；an inhibitor of cyclooxygenase). Experimental protocols were then performed. Rings with and without endothelium were studied in parallel. The integrity of the endothelium was verified by the response to bradykinin (300 nmol·L^{-1}).

Endothelium-dependent responses Following the equilibration period, rings, with and without endothelium, were contracted with a submaximal dose of phenylephrine (1 μmol·L^{-1} to 50 μmol·L^{-1}) or KCl (20 mmol·L^{-1}). These concentrations cause a contraction that approximates 50 % (ED_{50}) of the maximal contraction to 10 mmol·L^{-1} norepinephrine (ED_{50} =

1.0－1.5 grams of tension). Increasing cumulative concentrations of prostacyclin (100 fmol·L^{-1} to 1 μmol·L^{-1}) or acetylcholine (1 pmol·L^{-1} to 1 μmol·L^{-1}) were added to organ chambers containing rings with and without endothelium, to determine the effect of the endothelium on the agonist-induced relaxations. All of these experiments were performed in the presence of indomethacin (10 μmol·L^{-1}). Vehicle controls for prostacyclin, using $NaHCO_3$ and NaOH, were performed. The pH of the bath following vehicle and prostacyclin addition was measured. To determine the contribution of nitric oxide to these relaxations, the following antagonists were added to the organ chamber (5－30 min) prior to the addition of prostacyclin or acetylcholine: oxyhemoglobin (10 μmol·L^{-1}; a scavenger of EDRF/nitric oxide); methylene blue (10 μmol·L^{-1}; a nonspecific inhibitor of soluble guanylate cyclase); N^{ω}-nitro-*L*-arginine (NLA, 10 μmol·L^{-1}; a stereoselective antagonist of nitric oxide synthases). Concentrations of cyclic GMP and cyclic AMP in the vascular smooth muscle were measured by radioimmunoassay to determine if increased nitric oxide release and the subsequent accumulation of cyclic GMP and AMP were responsible for the endothelium-potentiated relaxations observed to prostacyclin.

Cyclic GMP-inhibited cyclic AMP phosphodiesterase To determine the contribution of nitric oxide, with subsequent accumulation of cyclic GMP in the vascular smooth muscle, leading to inhibition of cGI-PDE, to the prostacyclin-induced relaxation, amrinone (10 μmol·L^{-1}, a specific cGI-PDE inhibitor[20]) and 8-bromo-cyclic GMP (10 μmol·L^{-1}), the soluble cyclic GMP analog, were added to the organ chambers containing rings without endothelium. Cumulative increases in the concentration of the prostacyclin were then added to the organ baths, using rings with and without endothelium from the same lungs as parallel controls. All of these experiments were performed in the presence of indomethacin (10 μmol·L^{-1}).

Cyclic nucleotides Rings with and without endothelium were placed in test tubes containing 2 ml of control solution. The rings were allowed to equilibrate for 30 minutes at 37 ℃ and then changed to solution containing isobutylmethylxanthine (IBMX, 100 μmol·L^{-1}), a phosphodiesterase inhibitor. The rings were exposed for two minutes to agonists. The rings were then removed from the tube, frozen in liquid nitrogen and the reaction was stopped by the addition of 21 % trichloroacetic acid to a final concentration of 7 % of the latter. The rings were then stored at －20 ℃ until the radioimmunoassay was performed. The rings were thawed, homogenized and centrifuged at 1300 g for 15 minutes at 4 ℃. The supernatant was then extracted under water-saturated ether and dried at 40 ℃. Cyclic GMP or cyclic AMP were quantified by radioimmunoassay following acetylation (New England Nuclear). Protein analysis was performed by a modification of the Lowry method. The cyclic GMP content is expressed as pmol/g protein, and the cyclic AMP content is expressed as nmol/g protein.

Statistics Results are expressed as means ± SEM. Values from rings with and without endothelium were compared using Student's *t*-test for paired or unpaired observations. When multiple means were compared, an analysis of variance (ANOVA) was employed. For the isolated ring-tension experiments, "*n*" equals the number of animals and rings used for each experiment. For cyclic nucleotide experiments, "*n*" equals the number of animals used for each experiment; two rings from contiguous sites were used for each drug exposure and the values were averaged. Values were considered statistically different when P was less than 0.05.

RESULTS

Endothelium-dependent relaxations In rings contracted with phenylephrine, concentrations of prostacyclin (0.1 pmol to 1 μmol·L^{-1}) caused concentration-dependent relaxations in rings, with and without endothelium, that were larger in rings with endothelium (n = 11; Fig 1). The endothelium-dependent potentiation was abolished by oxyhemoglobin (10 μmol·L^{-1}; n = 6) and NLA (10 μmol·L^{-1}; n = 7). (Fig 1) The small relaxations to prostacyclin observed in control rings without endothelium were not altered when exposed to these inhibitors of the *L*-arginine-nitric oxide pathway. Vehicle controls using $NaHCO_3$ and NaOH did not alter the tension of the contracted rings (n = 8 paired rings; Fig 2) nor did it alter the pH of the solution more than 0.04 units (7.40 *vs* 7.44; n = 12). Acetylcholine (1 pmol to 1 μmol·L^{-1}) caused endothelium-dependent relaxations (n = 6) that were abolished by oxyhemoglobin (n = 3), or methylene blue (10 mmol·L^{-1}; n = 3), and reduced by NLA (n = 6; $P<0.05$; Fig 1).

Cyclic GMP content Rings with and without

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb；21 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

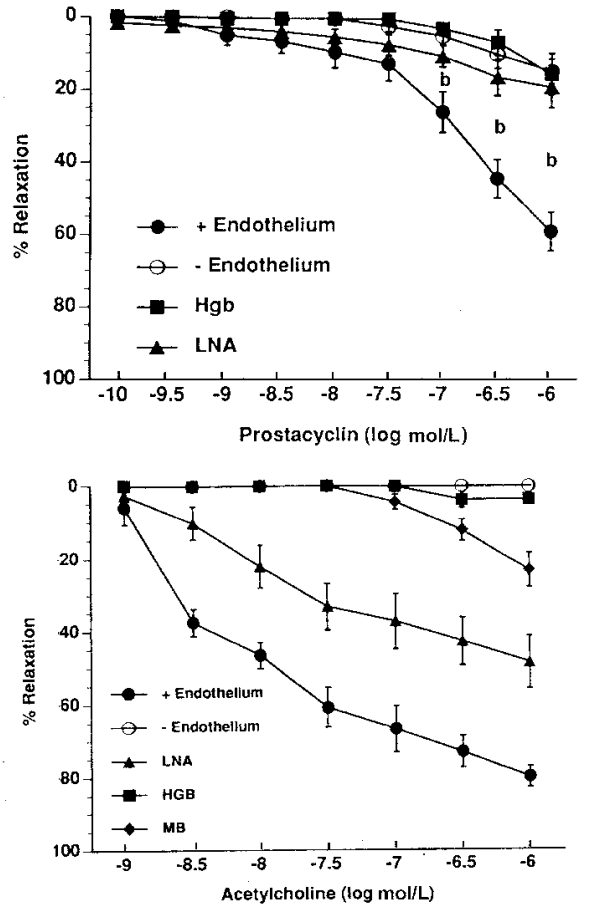

Fig 1. Cumulative concentration-response curves to prostacyclin (Above) in isolated porcine pulmonary artery rings with (+；closed symbols) and without (−；open symbols) endothelium ($n = 11$) in the presence of hemoglobin (Hgb, 10 μmol·L^{-1}；$n = 6$) and N^{ω}-nitro-*L*-arginine (NLA, 10 μmol·L^{-1}；$n = 7$). $^{b}P < 0.05$ between rings with and without endothelium and rings treated with Hgb and NLA. Cumulative concentration-response curve to acetylcholine (bottom) in isolated rings with and without endothelium ($n = 6$) and in the presence of hemoglobin ($n = 3$), N^{ω}-nitro-*L*-arginine ($n = 6$) and methylene blue ($n = 3$). Data are expressed as means ± SEM；% relaxation is the percent change in tension, relative to the phenylephrine contraction, induced by the agonist tested. Indomethacin (10 μmol·L^{-1}) was present in all experiments. Relaxations in rings with endothelium were different ($^{b}P < 0.05$) from those rings without endothelium (−7.5 to −6 log mol·L^{-1} acetylcholine), those treated with Hgb and MB (−7.5 to −6 log mol·L^{-1} acetylcholine), and those treated with NLA (−7 to −6 log mol·L^{-1} acetylcholine).

endothelium ($n = 10 - 12$) were studied under basal conditions (IBMX only) and during stimulation with acetylcholine, sodium nitroprusside and prostacyclin (IBMX plus agonist). Rings with endothelium accumulated more cyclic GMP compared to rings without endothelium (117.2 ± 24.5 *vs* 11.05 ± 3.4 pmol/g protein), under basal conditions, indicating the presence of basal release of nitric oxide. The cyclic GMP content was increased over basal production in the presence of acetylcholine (1 μmol·L^{-1}；rings with endothelium；339 ± 91 pmol/g protein) and sodium nitroprusside (10 μmol·L^{-1}；rings with (227.3 ± 44.5 pmol/g protein) and without endothelium (96.8 ± 50.3 pmol/g protein *vs* basal levels without endothelium = 11.05 ± 3.4 pmol/g protein)；$P < 0.05$ for all comparisons). However, no significant increase over basal production was seen with exposure to prostacyclin (1 μmol·L^{-1}) in rings with endothelium (160.2 ± 40 pmol/g protein). (Fig 3).

Cyclic AMP content Rings with and without endothelium ($n = 5 - 6$) were studied under basal conditions and during stimulation with prostacyclin. The cyclic AMP content was increased by prostacyclin (1 μmol·L^{-1}) compared to basal levels, but this did not reach statistical significance (3.99 ± 0.97 *vs* 1.86 ± 0.52 nmol/g protein；$P = 0.07$). The addition of the cyclic GMP analog, 8-bromo-cyclic GMP (10 μmol·L^{-1}), to prostacyclin increased the cyclic AMP levels (4.14 ± 0.53 nmol/g protein, $P = 0.019$ compared to basal levels). The increase in cyclic AMP caused by prostacyclin was reduced when NLA (10 μmol·L^{-1}), was added (1.93 ± 0.24 pmol/mg protein, $P = 0.005$). The addition of 8-bromo-cyclic GMP to rings in which nitric oxide production was inhibited by NLA restored the cyclic AMP levels toward the prostacyclin-induced levels (3.22 ± 0.35 nmol/g protein). Results with 8-bromo-cyclic GMP alone were similar (3.26 ± 0.61 nmol/g protein). (Fig 4)

Cyclic GMP-inhibited cyclic AMP phosphodiesterase Rings with endothelium exhibited endothelium-potentiated relaxations to prostacyclin under control conditions ($n = 10$). Rings without endothelium, in the presence of amrinone (10 μmol·L^{-1}；$n = 10$) and 8-bromo-cyclic GMP (10 μmol·L^{-1}；$n = 10$), exhibited relaxations to prostacyclin that were statistically increased compared to control rings without endothelium. They were similar to those observed in control rings with endothelium. (Fig 5)

DISCUSSION

Prostacyclin is an endothelium-derived vasodilator

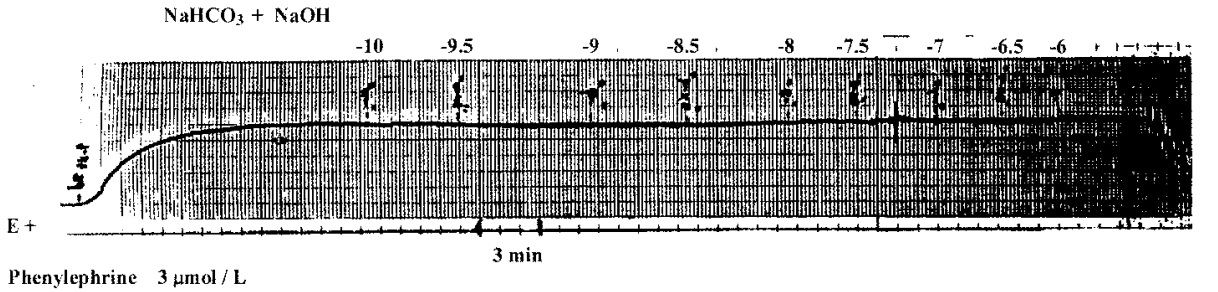

Fig 2. Representative cumulative dose-response curve for $NaHCO_3$ and NaOH against a phenylephrine contraction in an isolated porcine pulmonary artery ring. There is no change in tension with increasing concentrations of the buffers used.

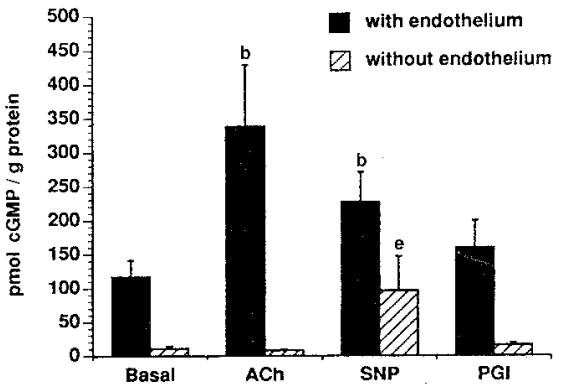

Fig 3. Changes in cyclic GMP content under basal conditions (in the presence of isobutylmethylxanthine, 100 μmol·L^{-1}), and in the presence of acetylcholine (ACh, 1 μmol·L^{-1}), sodium nitroprusside (SNP, 10 μmol·L^{-1}) and prostacyclin (PGI_2, 1 μmol·L^{-1}). Rings with and without endothelium were tested. Data are expressed as means ± SEM (n = 10 − 12). $^bP<0.05$ compared to basal with endothelium and $^eP<0.05$ compared to basal without endothelium. Indomethacin (10 μmol·L^{-1}) was present in all experiments.

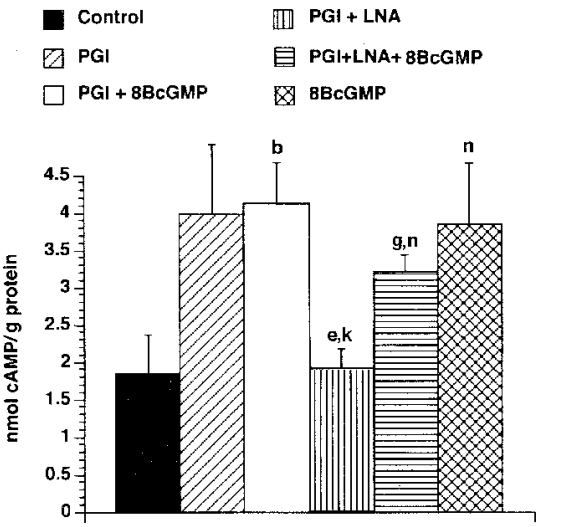

Fig 4. Changes in cyclic AMP concentrations under basal conditions (in the presence of isobutylmethylxanthine 100 μmol·L^{-1}), and in the presence of prostacyclin (PGI_2, 1 μmol·L^{-1}), prostacyclin plus 8-bromo-cyclic GMP (10 μmol·L^{-1}) and prostacyclin plus NLA (10 μmol·L^{-1}). Rings with and without endothelium were tested. Data are expressed as means ± SEM (n = 5 − 6). $^bP<0.05$ compared to basal (with endothelium). $^eP<0.05$ compared to PGI_2. $^gP>0.05$ compared to control. $^kP<0.05$ compared to PGI_2 and 8-BcGMP. $^nP<0.05$ compared to PGI_2 + NLA. Indomethacin (10 μmol·L^{-1}) was present in all experiments.

prostaglandin that dilates several vascular beds and inhibits its platelet aggregation[1,8,9,24]. This process occurs through the activation of adenylate cyclase with subsequent formation and accumulation of cyclic AMP[6]. In 1980, Furchgott and Zawadzki[2] demonstrated the existence of EDRF subsequently identified as EDNO or a nitric oxide-liberating nitrosothiol[24-28]. It causes relaxation of vascular smooth muscle and inhibits platelet aggregation by activating soluble guanylate cyclase with the subsequent accumulation of cyclic GMP[2,10,11,24,27]. Several agonists and stimuli can cause the concomitant release of nitric oxide and prostacyclin; these include bradykinin, the calcium ionophore A23187, thrombin, flow and shear stress[4-6,29,30]. In porcine coronary vessels, prostacyclin and EDNO appear synergistic in causing relaxation of vascular smooth muscle, with prostacyclin stimulating the release of EDNO at least *in vitro*[7-12].

In the present studies, prostacyclin-induced relaxations were potentiated in the porcine pulmonary artery

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb；21 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

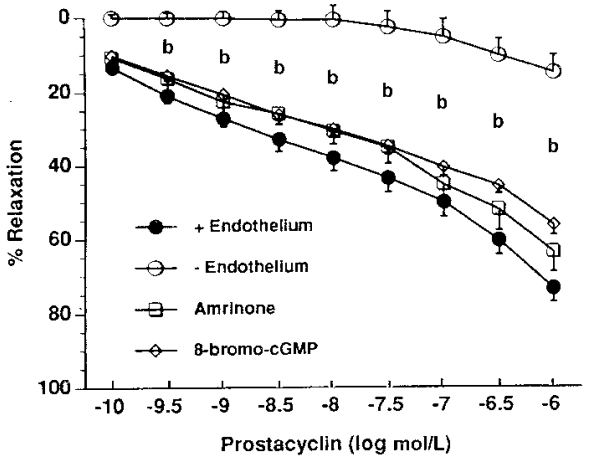

Fig 5. Cumulative concentration-response curves to prostacyclin in isolated rings of porcine pulmonary artery in rings with (+; closed symbols) and without endothelium (−; open symbols). The effects of 8-bromo-cGMP (10 μmol·L^{-1}) and amrinone (10 μmol·L^{-1}) were tested in rings without endothelium. Dta are expressed as means ± SEM (n = 10). Indomethacin (10 μmol·L^{-1}) was present in all experiments. ^{b} $P<0.05$ between control rings with endothelium, as well as amrinone and 8-bromo-cGMP treated rings without endothelium compared to control rings without endothelium.

by the presence of an intact endothelium. These observations could be explained by prostacyclin stimulating the release of nitric oxide from the pulmonary arterial endothelium. This would be concordant with the observations that oxyhemoglobin, a scavenger of EDRF/nitric oxide[23], and N^{ω}-nitro-L-arginine, a stereospecific inhibitor of nitric oxide synthase[31], both inhibited the endothelium-potentiated relaxations induced by prostacyclin. Confirmation that the inhibition of the L-arginine nitric oxide pathways occurs in isolated porcine pulmonary artery rings with oxyhemoglobin and N^{ω}-nitro-L-arginine, is provided by the antagonists' effects upon the acetylcholine-induced endothelium-dependent relaxation[2]. Methylene blue, which nonspecifically inhibits soluble guanylate cyclase, the target of nitric oxide, also inhibited the acetylcholine-induced relaxation, further strengthening the evidence that this factor is released from the rings studied[4,11,23]. However, although the cyclic GMP content measured in isolated rings of pulmonary artery is increased above basal by acetylcholine and sodium nitroprusside, this is not the case with prostacyclin. These data indicate that in the porcine pulmonary artery, prostacyclin does not stimulate the release of a significant amount of additional nitric oxide above the basal levels, but potentiates the relaxation by another mechanism.

Cyclic GMP reduces the hydrolysis of cyclic AMP through inhibition of cyclic AMP phosphodiesterases[13]. These enzymes have been found in cardiac muscle, platelets and vascular smooth muscle[13-18]. In the rat aorta, cyclic GMP, even in low concentrations, increases the content of cyclic AMP[16]. Inhibition of the cyclic GMP-inhibited cyclic AMP phosphodiesterases causes relaxation of vascular smooth muscle. This conclusion has been reached when the selective inhibitors of the enzyme, milrinone and amrinone were given to rat aortic and guinea pig pulmonary artery rings *in vitro*[15-17,20]. *In vivo*, peripheral vasodilatation has also been observed with those drugs in humans[19,21]. Additionally, agonists that stimulate cyclic GMP accumulation act synergistically to enhance β-adrenergic agonist-induced relaxation of isolated rat aortic rings as well as to enhance the antiaggregatory action of prostaglandin E1 and prostacyclin on platelets[8,14,16,32]. In the present study, the endothelium potentiated the relaxations to prostacyclin, a cyclic AMP-mediated agonist. The basal release of nitric oxide and the resulting accumulation of cyclic GMP could potentiate the relaxations to prostacyclin by inhibiting the cGI-PDE. This is supported by the documented accumulation of cyclic GMP under basal conditions, which is not ($P>0.05$) changed by exposure to prostacyclin, but which is accompanied by enhanced relaxations. Inhibition of basal release of nitric oxide, using hemoglobin and NLA, also inhibited the potentiation of the relaxation to prostacyclin. When simulating the presence of basally released nitric oxide in rings without endothelium by using 8-bromo-cyclic GMP, prostacyclin-induced relaxations were enhanced to the level observed in rings without endothelium. In addition, amrinone, the specific inhibitor of cGI-PDE, similarly enhanced the prostacyclin-induced relaxations in rings without endothelium, mimicking relaxations observed in control rings. These observations strongly suggest that indeed the basal release of nitric oxide results in inhibition of cGI-PDE. The measurements of cyclic AMP levels offers further evidence that this is the case. The cyclic AMP-concentration is increased by the addition of 8-bromo-cyclic GMP

to prostacyclin. Inhibition of the cGI-PDE, with subsequent increase in cyclic AMP levels, would be expected with 8-bromo-cyclic GMP if this mechanism were active in the studied preparation. The addition of the nitric oxide inhibitor, NLA, decreased the cyclic AMP content toward basal levels. The most likely explanation for this observation is that the reduction in basal nitric oxide-induced cyclic GMP accumulation prevents the inhibition of cGI-PDE and the subsequent accumulation of cyclic AMP. A similar phenomenon has been observed in cerebral vessels and labeled cyclic nucleotide crosstalk[33].

In conclusion, these present data confirm that the endothelium potentiates the relaxation to prostacyclin in the porcine pulmonary artery. This potentiation can be attributed to the basal release of nitric oxide, which stimulates the accumulation of cyclic GMP on the vascular smooth muscle cells. Cyclic GMP, in turn, inhibits the cGI-PDE in these cells, inhibiting the hydrolysis of cyclic AMP and increasing its concentration. This, is turn, enhances relaxation of the vascular smooth muscle by the cyclic nucleotides. Physiologically, the potentiated relaxations by the basal release of nitric oxide may represent an important synergy to perpetuate vasodilatation *in vivo*.

REFERENCES

1 Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 1976；263：663－5.
2 Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980；288：373－6.
3 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, *et al*. Endothelin：A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988；332：411－5.
4 Zellers TM, Vanhoutte PM. Heterogeneity of endothelium-dependent and independent responses in porcine pulmonary arteries. Pulm Pharmacol 1989；2：201－8.
5 Chand N, Altura BM. Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells：role in lung vascular disease. Science 1981；213：1376－9.
6 Gordon JL, Martin W. Stimulation of endothelial prostacyclin production plays a role in endothelium-dependent relaxation of the pig aorta. Br J Pharmacol 1983；80：179－86.
7 Azuma H, Ishikawa M, Sekizaki S. Endothelium-dependent inhibition of platelet aggregation. Br J Pharmacol 1986；88：411－5.
8 Radomski MW, Palmer RM J, Moncada S. The antiaggregatory properties of the vascular endothelium：interactions between prostacyclin and nitric oxide. Br J Pharmacol 1987；92：639－46.
9 Gorman RR, Bunting S, Miller OV. Modulation of human platelet adenylate cyclase by prostaglandin (PGX). Prostaglandins 1977；13：377－88.
10 Furlong B, Henderson AH, Lewis MJ, Smith JA. Endothelium-derived relaxing factor inhibits *in vitro* platelet aggregation. Br J Pharmacol 1987；90：687－92.
11 Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxations in rat thoracic aorta may be mediated through cGMP. Circ Res 1983；52：352－7.
12 Shimokawa H, Flavahan NA, Lorenz RR, Vanhoutte PM. Prostacyclin releases endothelium-derived relaxing factor and potentiates its action in coronary arteries of the pig. Br J Pharmacol 1988；95：1197－203.
13 Harrison SA, Reifsynder DH, Gallis B, Cadd GC, Beavo JA. Isolation and characterization of bovine cardiac muscle cGMP-inhibited phosphodiesterase：a receptor for new cardiotonic drugs. J Pharmacol Exp Ther 1986；29：506－14.
14 Maurice DH, Haslam RJ. Molecular basis of the synergistic inhibition of platelet function by nitrovasodilators and activators of adenylate cyclase：inhibition of cyclic AMP breakdown by cyclic GMP. J Pharmacol Exp Ther 1990；37：671－81.
15 Lindgren S, Rascon A, Andersson KE, Manganiello V, Degerman E. Selective inhibition of cGMP-inhibited and cGMP-noninhibited cyclic nucleotide phosphodiesterases and relaxation of rat aorta. Biochem Pharmacol 1991；42：545－52.
16 Maurice DH, Haslam RJ. Nitroprusside enhances isoprenaline-induced increases in cAMP in rat aortic smooth muscle. Eur J Pharmacol 1990；191：471－5.
17 Rossing TH, Drazen JM. Effects of milrinone on contractile responses of guinea pig trachea, lung parenchyma and pulmonary artery. J Pharmacol Exp Ther 1986；238：874－9.
18 Silver PJ, Lepore RE, O'Connor B, Lemp BM, Hamel LT, Bentley RG, *et al*. Inhibition of the low Km cyclic AMP phosphodiesterase and activation of the cyclic AMP system in vascular smooth muscle by milrinone. J Pharmacol Exp Ther 1988；247：34－42.
19 Cody RJ, Muller FB, Kubo SH, Rutman H, Leonard D. Identification of the direct vasodilator effect of milrinone with an isolated limb preparation in patients with congestive heart failure. Circulation 1986；73：124－9.
20 Kauffman RF, Schenck KW, Utterback BG, Crowe VG, Cohen ML. *In vitro* vascular relaxation by new inotropic agents：relationship to phosphodiesterase inhibition and cyclic nucleotides. J Pharmacol Exp Ther 1987；242：864－72.
21 Ludmer PL, Wright RF, Arnold JMO, Ganz P, Braunwald E, Colucci WS. Separation of the direct myocardial and vasodilator actions of milrinone administered by an intracoronary infusion technique. Circ 1986；73：130－7.
22 Zellers TM, Vanhoutte PM. Endothelium-dependent relax-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb；21 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

ations of piglet pulmonary arteries augment with maturation. Pediatr Res 1991；30：176－80.
23 Martin W，Villani GM，Jothianandin D，Furchgott RF. Selective blockade of endothelium-dependent and glyceryl-trinitrate induced relaxations by hemoglobin and methylene blue in the rabbit aorta. J Pharmacol Exp Ther 1984；232：708－16.
24 Moncada S，Palmer RMJ，Higgs EA. Relationship between prostacyclin and nitric oxide in the thrombotic process. Thromb Res 1990；Suppl XI：3－13.
25 Palmer RMJ，Ferrige AG，Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987；327：524－6.
26 Palmer RMJ，Ashton D，Moncada S. Vascular endothelial cells synthesize nitric oxide from *l*-arginine. Nature 1988；333：664－6.
27 Moncada S，Radomski MW，Palmer RMJ. Endothelium-derived relaxing factor：identification as nitric oxide and role in the control of vascular tone and platelet function. Biochem Pharmacol 1988；37：2495－501.
28 Myers PR，Minor RL Jr，Guerra R Jr，Bates JN，Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles S-nitrosocysteine than nitric oxide. Nature 1990；345：161－3.
29 Rubanyi G，Romero JC，Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986；250：H1145－9.
30 Zellers TM，Shimokawa H，Yunginger J，Vanhoutte PM. Heterogeneity of endothelium-dependent and endothelium-independent responses to aggregating platelets in porcine pulmonary arteries. Circ Res 1991；68：1437－45.
31 Moore PK，Al-Swayeh OA，Chong NWS，Evans RA，Gibson A. *L*-N^G-nitro-arginine (*L*-NOARG)，a novel *L*-arginine reversible inhibitor of endothelium-dependent vasodilatation in vitro. Br J Pharmacol 1990；99：408－12.
32 Nishikawa M，Kanamori M，Hidaka H. Inhibition of platelet aggregation and stimulation of guanylate cyclase by an antianginal agent molsidomine and its metabolites. J Pharmacol Exp Ther 1982；220：183－90.
33 Pelligrino DA，Wang Q. Cyclic nucleotide crosstalk and the regulation of cerebral vasodilation. Prog Neurobiol 1998；56：1－18.

环 GMP 抑制的环 AMP 磷酸二酯酶产生的内皮源性 NO 的基础释放促进前列环素引起的小猪肺动脉舒张[1]

Thomas M ZELLERS[2]，Yi-Qun WU，Jennifer McCORMICK，Paul M VANHOUTTE[3]

关键词 肺动脉；血管内皮；硝酸；依前列醇；环鸟苷一磷酸；环腺苷一磷酸；磷酸二酯水解酶

（责任编辑 李 颖）