Phosphodiesterase 5 inhibition restores impaired ACh relaxation in hypertensive conduit pulmonary arteries

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Oka, Masahiko. Phosphodiesterase 5 inhibition restores impaired ACh relaxation in hypertensive conduit pulmonary arteries. Am J Physiol Lung Cell Mol Physiol 280: L432–L435, 2001.—Responses to acetylcholine (ACh) and sodium nitroprusside (SNP) were compared in large (LPA) and small pulmonary artery (SPA) rings from normoxic and chronically hypoxic (CH) rats. In addition, the effects of a selective phosphodiesterase (PDE) 5 inhibitor, E-4021, on ACh-induced relaxation were evaluated. Chronic hypoxia markedly decreased both ACh- and SNP-induced relaxations in LPA but not in SPA rings. Pretreatment with E-4021 caused a much greater leftward shift of the concentration-response curve for ACh in hypoxic than in normoxic LPA rings, eliminating the difference in response to ACh between these two vessels. These results suggest that cGMP-dependent relaxation is impaired in the proximal but not in the distal pulmonary artery of CH rats and that increased PDE5 activity could be a mechanism responsible for this impaired responsiveness.

chronic hypoxia; endothelium-dependent relaxation; guanosine 3',5'-cyclic monophosphate; sodium nitroprusside

IT HAS BEEN REPORTED THAT endothelium-dependent relaxation is impaired in conduit pulmonary arteries (PAs) isolated from chronically hypoxic (CH) animals (10, 13, 14, 17, 19). However, most studies using isolated perfused lung or in vivo studies of animals with pulmonary hypertension have demonstrated normal or increased responsiveness to endothelium-dependent vasodilators (13, 14, 16). Similarly in humans, whereas endothelium-dependent relaxation has been reported to be impaired in isolated large PA preparations from patients with pulmonary hypertension associated with chronic obstructive pulmonary disease (3), an in vivo study has demonstrated a maintained vasodilator response to acetylcholine (ACh) in the pulmonary circulation of patients with this disease (1). The reason for these discrepant results has not been satisfactorily explained. Furthermore, a number of studies have shown that in addition to impairment of endothelium-dependent relaxation, responses to endothelium-independent nitric oxide (NO)-donor agents such as sodium nitroprusside (SNP) are also reduced in conduit PAs isolated from CH rats (10, 17, 19).

One major factor that mediates endothelium-dependent relaxation is endothelium-derived NO. NO causes vascular smooth muscle cell relaxation by increasing the intracellular concentration of cGMP via activation of soluble guanylate cyclase (sGC) and subsequent activation of protein kinase G (4). The increased intracellular cGMP is rapidly inactivated to GMP by phosphodiesterase (PDE). Thus if the activity of PDE is increased, then the effect of NO should be reduced. The PDE isoenzymes have been subdivided into at least seven distinct families (PDE1–7). The PDE5 family is termed cGMP specific because these isoenzymes specifically hydrolyze cGMP. They are reported to be distributed abundantly in lung tissue (15), and it has been demonstrated recently that chronic hypoxia increases PDE5 activity in the proximal but not in the distal PAs of the rat (9).

We therefore hypothesized that cGMP-dependent relaxation would be impaired in the proximal but not in the distal PAs and that increased PDE5 activity would be responsible for this impairment. The different responsiveness between proximal and distal PAs could account for discrepant findings in isolated conduit PA rings and perfused lungs where small-vessel effects could predominate. To address these issues, responses to ACh and SNP were compared in the proximal and distal PAs from normal and CH rats. The effects of the selective PDE5 inhibitor E-4021 (sodium 1-[6-chloro-4-(3,4-methylenedioxybenzyl)aminoquinazolin-2-yl]piperdine-4-carboxylate sesquihydrate) (6, 18) on ACh-induced relaxation were also evaluated. We used E-4021 in this study because this compound has been shown to be highly selective and potent compared with previously used PDE5 inhibitors such as zaprinast. For instance, the reported IC50 value of E-4021 for PDE5 from porcine aorta is 0.0039 μM vs. 0.51 μM for zaprinast, whereas its IC50 value for the next most sensitive PDE isozyme (PDE2) is 8.7 μM (18). In addition, our group has demonstrated that E-4021 (1 μM) augmented the response to a cGMP-dependent vasodilator SNP but not to the cGMP-independent (cAMP-
METHODS

All protocols and surgical procedures were approved by the Institutional Animal Use Committee of Juntendo University School of Medicine.

Animals. Experiments were performed with two groups of adult male Sprague-Dawley rats (250–320 g). The normoxic pulmonary-normotensive (N) group was kept at normal sea-level atmospheric pressure. The chronically hypoxic pulmonary-hypertensive (CH) group was exposed to a simulated high altitude of 5,500 m (barometric pressure, 380 mmHg) for 1 wk in a hypobaric chamber flushed continuously with room air to prevent accumulation of CO₂, NH₃, and water vapor. The hypobaric pressure was maintained 24 h/day except when the chamber was opened for 10–15 min every 2 days to remove rats or to clean the cages and replenish food and water. All rats were exposed to a 12:12-h light-dark cycle and allowed free access to standard rat food and water.

Right ventricular hypertrophy. To demonstrate the presence of pulmonary hypertension in the CH rats, the hearts were dissected and an index of right ventricular hypertrophy was calculated as the ratio of the wet weight of the free wall of the right ventricle (RV) over the wet weight of the left ventricle wall plus septum (LV + S).

Isolated PA rings. The left first branch of the extralobar large pulmonary artery (LPA) and fifth-order small pulmonary artery (SPA) rings were prepared as described previously (2, 13) with minor modifications. Briefly, after rats had been anesthetized with 30 mg of intraperitoneal pentobarbital sodium, the chest was opened and heparin sulfate (100 IU) was injected into the right ventricle. The rats were then exsanguinated, and the heart and lungs were removed en bloc. The left branch of the extralobar LPA, as well as the distal fifth-order SPA (200–300 µm ID), was isolated using small iris scissors under observation with a dissecting microscope. Adventitial tissue was removed, and PA rings were cut and placed in a physiologic salt solution (PSS) containing (in mM) 116.3 NaCl, 5.4 KCl, 0.83 MgSO₄, 19.0 NaHCO₃, 1.04 NaH₂PO₄, 1.8 CaCl₂·H₂O and 5.5 d-glucose (Earle's balanced salt solution, Sigma). Care was taken to avoid damage to the endothelium. LPA rings were placed on 11-mil steel wires attached to a transducer and suspended in baths containing 10 ml of PSS at 37°C. SPA rings were suspended in PSS using two strands of 0.002-mm tungsten wire threaded through the vessel lumen, fastening the free ends with screws positioned on either side of round Teflon disks and attaching the disks to a hanger. After the hanger assembly had been transferred to the transducer apparatus, the hanger was removed and the free ends of the tungsten wire were attached to the transducer. Resting passive force was adjusted to a previously determined optimum tension (determined by maximum response to 80 mM KCl; 400 mg for SPA and 750 mg for LPA from N rats, and 750 mg for SPA and 1,500 mg for LPA from CH rats). Rings were gassed with 21% O₂, 5% CO₂, and 74% N₂, and allowed to equilibrate for 60 min.

Experimental protocol. After equilibration and readjustment of the resting force, all rings were depolarized with 80 mM KCl for 30 min. Vasodilator responses to ACh (10 µM) and SNP (1 µM) were assessed in phenylephrine (1 µM)-contracted vessel rings. In a separate series of experiments, the effects of a PDE5 inhibitor (E-4021, 0.1 µM) on ACh-induced relaxation were evaluated in LPA rings from N and CH rats. After exposure to 80 mM KCl, the dose-response curves for ACh were determined in phenylephrine-contracted vessel rings with and without pretreatment of E-4021. E-4021 or its vehicle was given in the bath 15 min before addition of phenylephrine. We used phenylephrine because it is well established as a preconstriction agent in vessel ring studies (13, 17). All subsequent values for vasodilation are expressed as the percentage relaxation of the precontracted force.

Drugs. All reagents were obtained from Sigma (St. Louis, MO), except for E-4021 (provided by Eisai Pharmaceutical, Tokyo, Japan). E-4021 was dissolved in dimethyl sulfoxide and diluted with saline.

Statistics. Data are expressed as means ± SE. Statistical analysis was done by analysis of variance (ANOVA) with Scheffé's post hoc test for multiple comparisons or repeated-measures ANOVA. Differences were considered significant at P < 0.05.

RESULTS

The presence of pulmonary hypertension in the CH rats was reflected in an increased ratio of RV to LV + S weight, which averaged 0.43 ± 0.02 (n = 6) vs. 0.26 ± 0.01 (n = 5) in N rats (P < 0.05). There was no difference in LV + S weight between N and CH rats. The maximum contraction in response to KCl (80 mM) tended to be greater in LPA than in SPA rings from both N and CH rats, but the difference did not reach statistical significance (810 ± 70 and 460 ± 70 mg in LPA and SPA rings, respectively, from N rats and 680 ± 96 and 500 ± 130 mg in LPA and SPA rings, respectively, from CH rats). The contractile response to phenylephrine (1 µM) in LPA rings was significantly greater than in SPA rings from both
groups of rats (480 ± 26 and 120 ± 16 mg in LPA and SPA rings, respectively, from N rats, \( P < 0.05 \); 530 ± 46 and 100 ± 19 mg in LPA and SPA rings, respectively, from CH rats, \( P < 0.05 \)). Figure 1 shows the relaxant responses to ACh (A) and SNP (B) in LPA and SPA rings from N and CH rats. Responses to both ACh and SNP were markedly impaired by chronic hypoxia in LPA but not in SPA rings.

In the next set of experiments, the effects of the selective PDE5 inhibitor E-4021 on the dose response to ACh-induced relaxation in LPA rings from N (\( n = 6 \)) and CH (\( n = 6 \)) rats were examined. Although ACh caused concentration-dependent relaxation of LPA rings from both groups of rats, the relaxation was markedly attenuated in vessels from CH rats (Fig. 2). Neither E-4021 (0.1 \( \mu M \)) nor vehicle had any effect on either basal tension or the contractile response to phenylephrine in LPA rings from both groups of rats (data not shown). Pretreatment with E-4021 caused a much greater leftward shift of the concentration-response curve for LPA rings from CH rats than that from N rats. There was no statistical difference in the concentration-response curves between vessels from N rats and those from CH rats after addition of E-4021 (Fig. 2).

**DISCUSSION**

This study has demonstrated that endothelium-dependent (ACh-induced) relaxation is markedly impaired by chronic hypoxia in rings from the proximal PA of CH rats, whereas it is not reduced in rings from the distal PA. This finding suggests that the responsiveness of hypertensive PA rings to endothelium-dependent vasodilators may differ depending on the part of the PA from which the rings are taken; i.e., impaired responsiveness to endothelium-dependent vasodilators may occur only in the proximal portion of the PA. This may explain the previous contradictory findings that the responsiveness to endothelium-dependent vasodilators is impaired in conduit PAs isolated from CH rats (10, 13, 17, 19) but not in the intact hypertensive pulmonary circulation (13, 16).

The mechanism of the impaired responsiveness to endothelium-dependent vasodilators in hypertensive conduit PAs is not fully understood. One possibility is decreased NO activity in endothelial cells after exposure to chronic hypoxia. This seems unlikely, however, because numerous studies (10, 17, 19), including this one, have found that impaired responsiveness is not restricted to endothelium-dependent vasodilators but also includes NO donor agents such as SNP. In addition, other studies have shown that endothelial NO synthase mRNA and protein (7, 20) and NO production (5, 11) are increased in the hypertensive lungs and PAs of CH rats. These findings suggest that the problem exists downstream from NO production.

Another possible cause of the blunted relaxation of hypertensive conduit PAs is a reduction of sGC activity through which NO increases the intracellular level of cGMP. Although sGC activity was not assessed in this investigation, a recent study has demonstrated that sGC mRNA, protein, and enzyme activity are upregulated during the development of hypoxia-induced pulmonary hypertension in rats (8). Therefore, this possibility also seems unlikely.

Intracellular cGMP levels are regulated not only by GC activity but also by PDE activity. PDE5, which specifically hydrolyzes cGMP and is abundant in lung tissue (15), is thought to have a major role in inactivating cGMP in the pulmonary vasculature. Recently, MacLean et al. (9) have reported that PDE5 activity is increased in first-branch and intrapulmonary (0.2–2 mm ID) arteries but not in resistance arteries (100–300 \( \mu m \) ID) removed from rats with pulmonary hypertension induced by chronic hypoxia. This may explain the impaired responsiveness to cGMP-dependent vasodilators such as ACh and SNP in conduit, but not in resistance, PAs from CH rats. Indeed, the present finding that the impaired ACh-induced relaxation in LPA from CH rats was fully restored by a selective PDE5 inhibitor strongly supports this possibility.

In summary, the results of this study indicate that cGMP-dependent relaxation is impaired in the proximal, but not in the distal, PAs from rats with pulmonary hypertension induced by chronic hypoxia, probably through increased PDE5 activity. In isolated perfused lungs and in vivo studies, responses to stimuli reflect the summation of effects on the longitudinal series of different types of vessels, and small-vessel effects predominate. Thus our finding of different responsiveness between small and large PAs could account for divergent findings in isolated vessels and perfused lungs. It should be borne in mind that responsiveness to vasoactive substances may differ between proximal conduit arteries and the more distal resistance arteries, which determine the pulmonary arterial pressure. Large vessels dilate and accommodate stroke volume and recoil during diastole to maintain...
peripheral flow, whereas smaller vessels are the main site of resistance to flow.

REFERENCES


