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Original Article

Increase of Norepinephrine-Induced Endothelium-Dependent Relaxation of Pulmonary Artery in Rats after Chronic Exposure to Cold

Osamu KASHIMURA

Department of Physical Education, Honan College, Nagano

Abstract

The present study was designed to determine whether norepinephrine (NE) mediate endothelium-dependent relaxations in arteries of the pulmonary vasculature of cold-acclimated rats. Twenty male Sprague-Dawley rats comprising two groups (Cold-acclimated for 12 weeks at 6℃, CA; Warm-acclimated for 12 weeks at 24℃, WA) were used. After anesthesia, the pulmonary artery (4 mm long) was isolated. Pulmonary artery with and without endothelium were suspended for isometric force measurements in a buffered salt solution. The dose-response relations for the vascular responses to the isolated pulmonary artery to norepinephrine (NE), phenylephrine (PE) and acetylcholine (Ach) were determined and compared in the CA group and the WA group. In the CA group, the vascular sensitivities to NE and PE-induced contraction in the pulmonary artery was significantly lowered than that in the WA group. NE and PE-induced contractions were significantly greater in endothelium-denuded compared with endothelium-intact arteries. These differences of contraction responses to NE and PE between arteries with without endothelium were significantly greater in the CA group than in the WA group. There was no significant difference between the pulmonary arterial response to Ach in the CA group and that in the WA group. Our data suggest that chronic exposure to cold show decreased NE and PE-induced contraction responses in isolated pulmonary arteries and may decrease NE-induced contraction responses due to enhancing NE-induced endothelium derived relaxing factor release via up-regulating endothelial α_1-adrenoceptors.

Key words: endothelium derived relaxing factor, cold-acclimation, α_1-adrenoceptors, phenylephrine

Introduction

Endothelial cells are important local regulators of vascular function and the relaxation of rabbit thoracic aorta by acetylcholine (Ach) required an intact endothelium[1]. A substance termed endothelium derived relaxing factor (EDRF) was shown to be produced by the endothelium in response to Ach and to be released and transferred to the vascular smooth muscle. Also, EDRF released from the pulmonary endothelial cell can be transferred to vascular smooth muscle, where it causes relaxation. It is possible that the normal low vascular resistance of the pulmonary circulation is dependent on the presence of an EDRF[2].

It was reported that the pulmonary hypertension with cold exposure was elicited by an increase in pulmonary blood flow and catecholamines[3,4,5]. Chronic exposure to cold, cold-acclimation, can reduce the vascular reactivity to norepinephrine (NE) in smooth muscle of aorta isolated from rats and rabbits[6,7,8]. However, whether the pulmonary vascular responses to NE could be altered by cold-acclimation was unclear. Endothelial cell-mediated vasodilation in response to marked increases in blood flow has been reported in canine coronary and femoral arteries in situ[9,10]. Therefore, increase of blood flow during chronic exposure to cold may change endothelial function and increase the sensitivity of stimulated EDRF release. It was reported that adrenergic agents may stimulate EDRF release via endothelium-dependent α-adrenoreceptors[11]. If pulmonary artery in cold-acclimated rats was less sensitive to adrenergic stimulations by norepinephrine (NE) and phenylephrine (PE) than that of warm-acclimated rats, such reduction in responsiveness was dependent on the presence of the endothelial cell layer or not should be determined.

To verify the possible involvement of endothelial cells in the alteration of adrenergic stimulation-induced vasoconstriction after chronic exposure to cold, we compared the pulmonary vascular responses to NE and PE in endothelium-intact or

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Reprint Requests to: Osamu KASHIMURA,
72 Nakayama, Tatsuno, Nagano 399-0424, Japan
TEL: +81(266)41-4411
FAX: +81(266)41-4420

denuded vessel segments.

Materials and Methods

Animals

This study was conducted in conformity with the policies and procedures detailed in the "Guide for the Care and Use of Laboratory Animals" (Declaration of Helsinki)[12]. A total of 20 male Sprague-Dawley rats aged 5 weeks was used. The control group (10 rats) was kept for 12 weeks in a room maintained at about 24 ℃ (warm-acclimated group, WA) for 10 weeks while the cold-acclimated group at 5 ℃ (cold-acclimated group, CA) in a cold room for the same period of time. The rats were housed in groups of three per cage in an environmentally controlled room. Both groups were maintained with a 12-hours light/dark cycle and fed a standard rat chow and water ad libitum.

Reagents

All chemicals for the preparation of physiological saline solution (PSS) were purchased from Sigma Chemical (St. Louis, MO). In addition, the following pharmacological agents were used: L-norepinephrine bitartrate (NE), L-phenylephrine (PE) and Ach (Sigma Chemical; St. Louis, MO). Vasoactive compounds were dissolved in double-distilled water and were added to the baths in volumes of 100 μ L generate accumulative dose-response curves as described by Van Rossun[13]. Concentrations of drugs are expressed as final concentrations (molar) in the organ chambers.

Preparation of tissue

At the end of a 12-week period of continuous exposure to warm and cold, all rats were anesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), and were then killed by exsanguination through the carotid artery. Pulmonary artery from the diaphragm to the arch was prepared as quickly as possible (<10 min), and then placed in an isolated tissue bath containing PSS of the following composition (expressed in millimoles per liter): 119 NaCl, 4.7 KCl, 1.17 $MgSO_4 \cdot H_2O$, 22.6 $NaHCO_3$, 1.18 KH_2PO_4, 5.5 D-glucose, and 3.2 $CaCl_2 \cdot H_2O$. The pulmonary artery was rapidly dissected free and placed in PSS of the same composition at 4 ℃. Adherent fat and connective tissue were removed, and paired 4-mm arteries were cut from each segment using two surgical blades. One artery of each pair was denuded of endothelium by gently rubbing the intimal surface with roughened steel rob[14].

The arteries were suspended between two stainless steel hooks inserted into the lumen and placed into water jacketed baths. During the equilibration, the PSS in the isolated bath was constantly aerated with bubbling gas mixtures of 5% CO_2, 21% O_2 and 74% N_2, and the temperature was held constant at 37 ℃. The arteries were maintained at an extracellular pH of 7.4. The volume of PSS in the isolated bath was 10 mL. The arteries were then attached to a force transducer (LVS-A, Kyowa, Tokyo). The resting tension of artery was set at the beginning of each experiment at 500 mg by mechanically elevating the transducers and thereby imposing strain on the strips. Resting passive tension was maintained at 500 mg for at least 60 min. The artery was assessed by maximal membrane depolarization with KCl in the following manner: KCl was added to each bath to bring the concentration to 80 mM. Contraction was allowed to proceed for 30 min, at which time a stable maximum had been achieved. After the addition of fresh PSS buffer three times, the artery was readjusted to the 500 mg resting tension. The artery was then contracted with 10^{-5} M from 10^{-8} M PE and after reaching a maximal contraction, 10^{-5} from 10^{-8} M Ach were added and vasorelaxation measured.

After the rings were freshened with PSS buffer three times, rings were re-equilibrated to the 500 mg resting tension, and reconstricted with 10^{-6} M from 10^{-9} M NE. The responses to 10^{-5} M from 10^{-8} M Ach were measured. After the NE and PE concentration-response curves were completed, the maximal contraction after addition of 80 mM KCl was determined.

Organic weight

After the rats were killed under anesthesia, the heart and interscapular brown fat were removed, and the atria were separated from the ventricles, leaving the isolated ventricular myocardium, and specifically, the left ventricular wall with the septum (LV+S)[15]. The ventricles and interscapular brown fat were weighed as rapidly as possible in a tension balance.

Data analysis

All values are expressed as means ± SD while responses to NE and PE are expressed as the percentage changes in the contraction to 80 mM KCl. Ach relaxations in the remaining arteries were expressed as percentage relaxation from the maximal contraction to PE. Statistical evaluation of the data was performed using Student's t test for paired or unpaired analysis. When more than two means were compared, analysis of variance was performed. Differences were considered to be statistically significant when $p<0.05$.

Results

Group characteristics

The body weight was significantly lighter in CA (515.6 ± 6.98 g) compared to WA (562.5 ± 6.50 g) ($p<0.05$), but the weights of interscapular brown fat was significantly greater in CA (113.78 ± 19.63 mg/100 g body weight) than in WA (61.79 ± 3.95 mg/100 g body weight) ($p<0.05$). The weights of the right ventricular wall was significantly greater in CA (47.22 ± 2.67 mg/100 g body weight) than in WA (42.40 ± 2.35 mg/100 g body weight) ($p<0.05$), and left ventricular wall including the septum was significantly greater in CA (197.78 ± 10.99 mg/100 g body weight) than in WA (179.98 ± 9.2 mg/100 g body weight) ($p<0.05$).

Responses to NE, PE and Ach

The smooth muscles of the pulmonary arteries were contracted with 80 mM KCl. The contractions in response to KCl was lesser in CA than in WA (Fig. 1, $p<0.05$). Pulmonary artery contraction in response to NE (Fig. 2) was significantly lesser in CA than in WA at concentrations of 10^{-8} to 10^{-6} M ($p<0.05$), but not at 10^{-9} M. PE evoked concentration-dependent increase in the tension of the pulmonary arteries in both groups (Fig. 3), and the responses to PE in CA were significantly less than WA at concentrations of 10^{-7} to 10^{-5} M ($p<0.05$), but not at 10^{-8} M. NE-induced contraction was significantly greater in endothelium-denuded compared with endothelium-intact arteries at concentrations of 10^{-8}-10^{-6} M (Fig.4, $p<0.05$), but not at 10^{-9} M. PE-induced contraction was significantly greater in endothelium-denuded compared with endothelium-intact rings

at concentrations of 10^{-8}-10^{-5} M (Fig.5, $p<0.05$). These differences of contraction in responses to NE and PE between arteries with and without endothelium were clearly greater in CA than in WA. Ach evoked concentration-dependent relaxations for the pulmonary arteries preconstricted with NE and PE in both groups. No significant differences between CA and WA were observed.

Discussion

Organic adaptation to chronic cold exposure

After 12 weeks of cold exposure there were significant increases in the ratios of left ventricular wall and interscapular brown adipose tissue to body weight. These changes are similar to

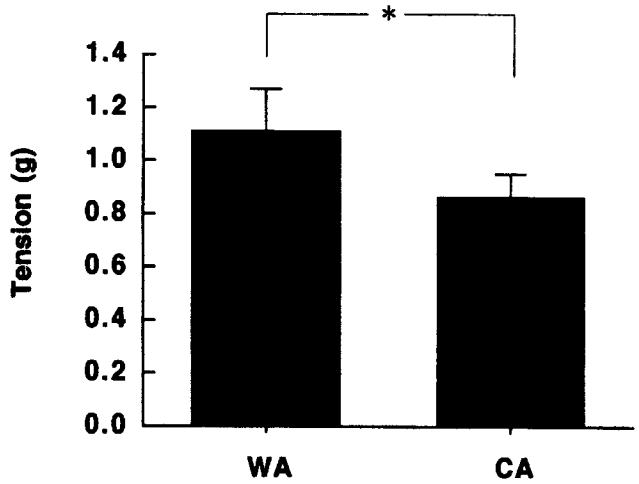

Fig. 1 Contractions of isolated pulmonary arteries in WA rats and CA rats in 80 mM KCl. Results are presented as means ± SD.* statistically significant difference between the two groups ($p<0.05$).
WA, warm acclimated group; CA, cold acclimated group

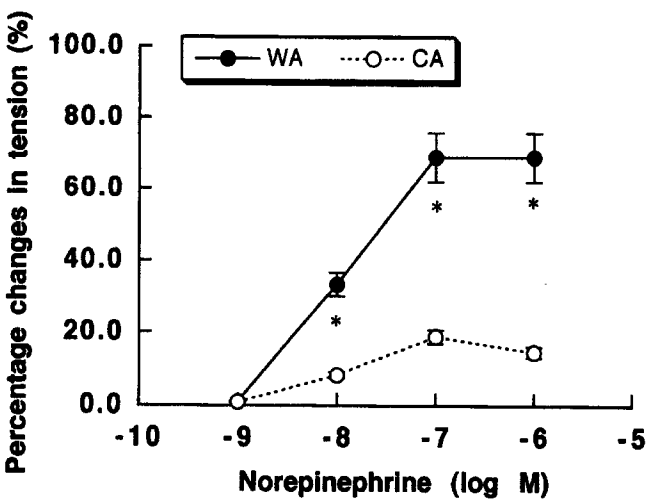

Fig. 2 Concentration-effect of the responses to norepinephrine (NE, 10^{-9}-10^{-6} M) in isolated endothelium-intact pulmonary arteries in WA and CA rats. Results are expressed as a percentage of response to 80 mM KCl and are presented as means ± SD. * indicates NE concentrations at which pulmonary arteries of CA had less constrictive responses than WA at concentrations of 10^{-8} to 10^{-6} M ($p<0.05$ vs WA value).
WA, warm acclimated group; CA, cold acclimated group

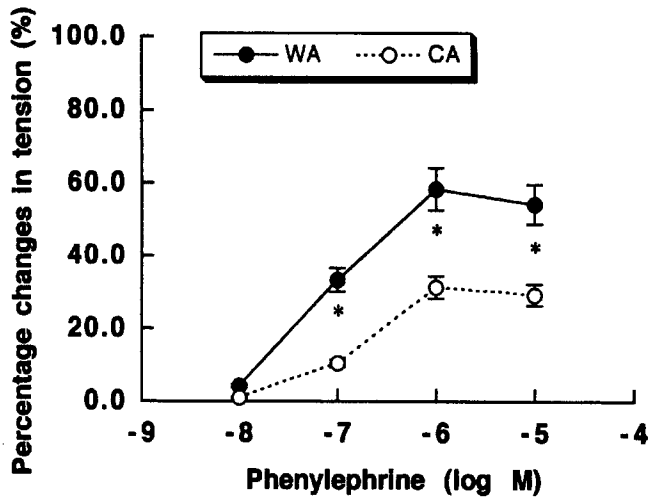

Fig. 3 Concentration-effect of the responses to phenylephrine (PE, 10^{-8}-10^{-5} M) in isolated endothelium-intact pulmonary arteries in WA and CA rats. Results are expressed as a percentage of response to 80 mM KCl and are presented as means ± SD. * indicates PE concentrations at which pulmonary arteries of CA had less constrictive responses than WA at concentrations of 10^{-7} to 10^{-5} M ($p<0.05$ vs WA value).
WA, warm acclimated group; CA, cold acclimated group

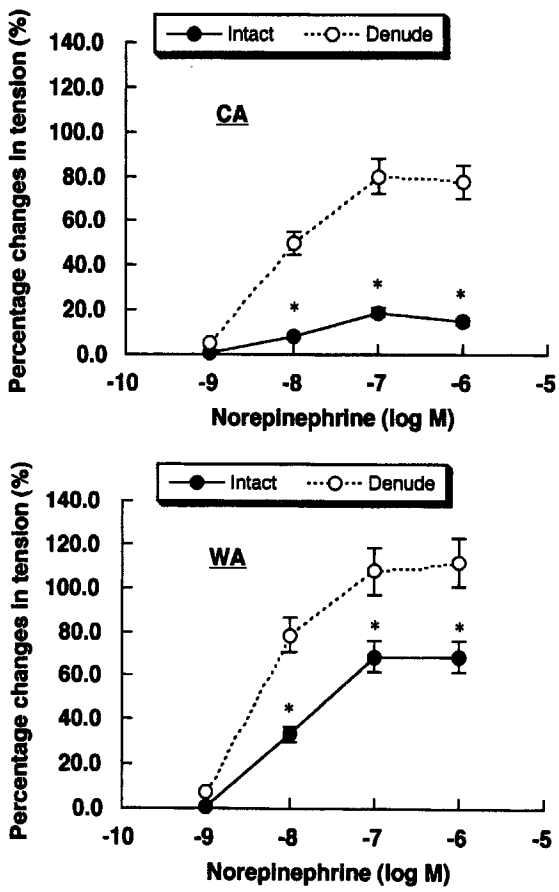

Fig. 4 Concentration-effect of the responses to norepinephrine (NE, 10^{-9}-10^{-6} M) in isolated pulmonary arteries with intact or denude endothelium in WA (bottom) and CA (top) rats. Results are expressed as a percentage of response to 80 mM KCl and are presented as means ± SD. *statistically significant difference from corresponding value with intact endothelium ($p<0.05$).
WA, warm acclimated group; CA, cold acclimated group

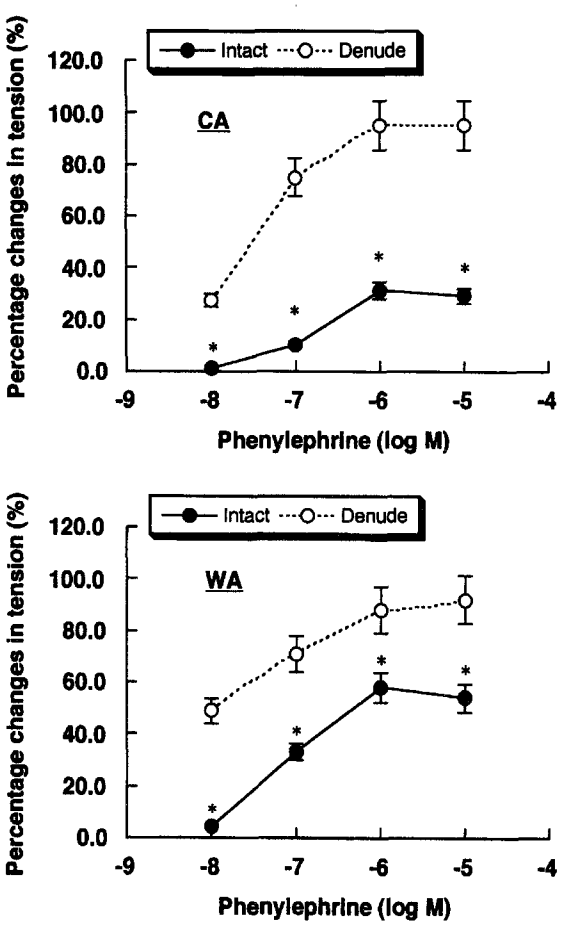

Fig. 5 Concentration-effect of the responses to phenylephrine (PE, 10^{-8}-10^{-5} M) in isolated pulmonary arteries with intact or denude endothelium in WA (bottom) and CA (top) rats. Results are expressed as a percentage of response to 80 mM KCl and are presented as means ± SD. *statistically significant difference from corresponding value with intact endothelium ($p<0.05$).
WA, warm acclimated group; CA, cold acclimated group

those reported earlier for longer periods of cold exposure[16]. These changes during cold exposure may be a result of increased secretion of catecholamines induced by exposure to cold. The ventricular hypertrophies may be induced by higher rates of metabolism, in particular chronically increased blood flow to systemic and pulmonary circulations. There was a marked increase in pulmonary arterial pressures and pulmonary circulatory resistance at cold exposure[5] through increase of shear stress in pulmonary artery.

NE and PE-induced responses

The objective of this experiment was to test the responsiveness of cold-acclimated rats to NE and PE. If pulmonary artery in CA rats was less sensitive to adrenergic stimulations by NE and PE than that of WA rats, we would like to investigate whether their reduced responsiveness was dependent on the presence of the endothelial cell layer or not. Our results demonstrated that the vascular sensitivity to NE and PE-induced contractions in the pulmonary arteries were lowered in CA rats than in WA rats. These reduction may be due to decrease in either number or sensitivity of α_1-adrenoceptors in these vessels. Previous studies have suggested that prolonged infusion of NE or long-term physical activity, repetitive increase in NE, can inhibit pressure response[17,18]. It was showed that this suggestion is supported by the differences in active tension developed in response to α_1-adrenergic stimulation by low doses of PE, NE and NE in the presence of propranolol[6,7]. However, blockade of the β-adrenergic receptors in these vessels by propranolol resulted in a greater sensitivity to α-adrenergic receptor-mediated constriction by NE in cold-acclimated rabbits when compared to similar vessels from warm-acclimated controls[7].

In addition to the systemic adrenosympathetic adaptation to chronic exposure to cold, the possibility that alteration of local modulation in vascular tone by cold acclimation should not be ruled out. Previous studies have suggested that adrenergic agents may stimulate EDRF release via endothelium-dependent α_1 or α_2 -adrenoreceptors, which may vary with different vessels of different animals[11,19]. In the present study, cold acclimation could reduce the vasoconstrictive responses to 10^{-8}-10^{-6} M of NE and 10^{-7}-10^{-5} M of PE. In blood vessels, removal of the endothelium enhanced both the sensitivity and the magnitude of the contractile responses of the smooth muscle to adrenergic stimulation[20]. Furthermore, the differences of vasoconstrictive responses to NE and PE between rings with and without endothelium were greater in cold-acclimated rats than in warm-acclimated rats. The present study suggests that the presence of the endothelium exerts a protective role at least partially by decreasing the contractile responses induced directly at the smooth muscle level by adrenergic stimulations in rat pulmonary artery. Moreover, the results confirmed the occurrence of endothelial-dependent relaxations through α_1-adrenergic stimulation. Therefore, it is possible that chronic exposure to cold may, at least in part, decrease NE-induced vasoconstriction by enhancing NE-induced EDRF release via up-regulating endothelial α_1-adrenoceptors. The results may then postulate taht increases in shear stress due to pulmonary hypertension in endothelium of cold-acclimated rats inhibit adrenergic neurogenic vasoconstriction by augmenting release of endothelial cell vasodilators.

The contraction of the pulmonary vascular smooth muscle from cold-acclimated rats in response to 80 mM KCl was diminished when compared with WA. From several studies it appears that two Ca^{2+} fractions are involved in smooth muscle activation. In canine coronary artery, depolarization induced by 30-90 mM KCl-physiological salt solution produced KCl concentration dependent increase in cytoplasmic Ca^{2+} concentration and force[21]. Moreover, KCl is easily mobilized by membrane depolarization through voltage-dependent Ca^{2+} channels[22]. However, the causes of this reduction is unknown.

Ach-induced responses

The response of vascular strips to Ach was reported to be strongly dependent on the presence of the endothelial cell layer[1,23]. Their findings suggested the existence of a mediator passing from endothelial cells to induce the relaxation of vascular smooth muscle. The relaxations of Ach-stimulated EDRF release were similar in the pulmonary arteries from CA rats and WA rats. It was suggested that reduced NE-induced responses in vessels from CA rats were related to selective changes in α_1-receptor signal transduction-second messenger mechanisms. It might be one possible underlying mechanism to explain elevations in shear stress caused by increases in blood flow through an artery triggers release of EDRF from endothelium[9]. However, whether the changes of EDRF release are direct causes in the case of cold-

acclimated rats remains to be investigated. It is important to recognize limitations of our study. We only examined responses to vasoactive substances in our isolated vessels. Furthermore, the shear stress-induced release of nitric oxide (via increased flow) may also contribute to chronic cold-induced vasodilation. Other critical roles of release of nitric oxide from endothelium have also been proposed, including modulation of myocardial metabolism[24] and structural remodeling of the arterial wall during chronic adaptations to flow-induced changes in shear stress[25]. Our experimental preparation, unfortunately, does not permit evaluation of this possibility.

In conclusion, we found that the isolated pulmonary arteries from CA rats show decreased NE-induced vasoconstrictive response, and that NE agonist-stimulated EDRF release via endothelial α_1-adrenergic receptors may, at least in part, be involved in this diminished response.

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