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Original Article

Responsiveness of Isolated Thoracic Aorta to Norepinephrine and Acetylcholine in Cold-Acclimated Rats

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Abstract

We investigated the responses of thoracic aortae to adrenergic contraction and endothelium-dependent relaxation following chronic exposure to cold in rats. Two groups (CA, cold-acclimated for 12 weeks at 5 ℃; WA, warm-acclimated for 12 weeks at 24 ℃) of 10 male Sprague-Dawley rats were used. After anesthesia, the thoracic aortae (4 mm long) were isolated and the vascular tension was measured with a force transducer. The dose-response relations for aortic responses to norepinephrine (NE), phenylephrine (PE) and acetylcholine (Ach) were determined and compared between the CA and the WA groups. In the CA rats, the thoracic aortae became more sensitive to Ach-induced vasorelaxation. The vascular sensitivities to NE- or PE-induced contraction in the thoracic aortae were lowered. Chronic exposure to cold decreased NE- and PE-induced vasoconstrictive responses and increased Ach-induced vasorelaxative response of the isolated thoracic aortae, which were suggested to be due to enhanced release of NE-induced endothelium-derived relaxing factor by up-regulating endothelial α_1-adrenoceptors.

Key words: Adrenergic vasoconstriction, Endothelium-derived relaxing factor, Phenylephrine

Introduction

Chronic exposure to cold has been reported to cause an elevation in metabolic activity, levels of catecholamines, systemic arterial pressure and heart rate as well as an increase in the weight of heart in rats[1-3]. These rats are characterized as having a large increase in activity in their sympathetic nervous system[4].

Furchgott and Zawadzki[5] demonstrated that the vascular responses to acetylcholine (Ach) are strongly dependent on the presence of the endothelial cell layer. Their findings suggest the existence of a mediator, known as endothelium-derived relaxing factor (EDRF), passing from the endothelial cells to vascular smooth muscle. Previous studies suggest a pattern of increased EDRF in vessels exposed to chronically elevated blood flow[6]. Also, it was reported that shear stress due to elevated blood flow on endothelial cells modulates adrenergic vasoconstriction by augmenting release of EDRF[7]. The increases in both blood pressure and heart rate of rats exposed chronically to cold suggest that these responses may be associated with the increased blood flow observed to occur during exposure to cold and to be responsible for shear stress on endothelial cells. The present study was speculated that, in cold-acclimated rats the acceleration of metabolism due to chronic exposure to cold would decrease adrenergic vasoconstriction and increase endothelium-dependent vasodilation mediated by enhanced release of EDRF in response to an increase in shear stress. No reports indicate whether Ach-induced vasorelaxation response would be altered by chronic exposure to cold in rats.

The present study was designed to determine the vascular responses of isolated thoracic aortae to norepinephrine (NE) and Ach in cold-acclimated rats.

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Materials and Methods

Animals

This study was conducted with a total 20 rats comprising two groups of male Sprague-Dawley rats at age of 5 weeks. Half of the rats acted as control group was kept for 12 weeks in a room maintained at about 24 ℃ (warm-acclimated group, WA) and the cold-acclimated group at 5 ℃ (cold-acclimated group, CA) in another cold room for the same period of time. Both groups were maintained with a 12-hour light/dark cycle and given food and water ad libitum.

Drugs

The following pharmacological agents were used: L-norepinephrine bitartrate (NE), L-phenylephrine hydrochloride (PE) and acetylcholine (Ach). Vasoactive compounds were dissolved in double-distilled water and were added to the baths in volumes of 100 μL to generate accumulative dose-response curves as described by Van Rossun[8]. Concentrations of drugs are expressed as final (molar) concentrations in the organ chambers.

Preparation of tissue

At the end of a 12 week period of exposure to warm and cold, all rats were killed with sodium pentobarbital (50 mg kg^{-1}, i.p.), thoracic aortae from the diaphragm to the arch were prepared as quickly as possible (<10 min), and placed in an isolated tissue bath containing physiological saline solution (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 H_2PO_4, 5.5 D-Glucose, 3.2 $CaCl_2 \cdot H_2O$. The aortae were rapidly dissected free and placed in PSS at 4 ℃. Adherent fat and connective tissue were removed. Aortae (4mm long) were cut from each segment using two surgical blades. Care was taken to avoid inadvertent damage to the endothelium.

The aorta was 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 was held at a temperature of 37 ℃. The volume of PSS in the isolated bath was 10 ml. The aorta was maintained at an extracellular pH of 7.4, and aorta was attached to a force transducer (LVS-A, Kyowa, Tokyo).

The resting tension of the strips were set at the beginning of each experiment at 1000 mg by elevating the transducers mechanically and thereby imposing strain on the strips. The resting passive tension was maintained at 1000 mg for at least 60 min. The aorta 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; Once a stable maximum had been achieved, resting tension was readjusted to 1000 mg with addition of fresh PSS buffer three times. The aorta was then constricted with PE of concentrations from 10^{-8} to 10^{-5} M. When the maximal contraction wa reached, concentrations of 10^{-8} to 10^{-5} M Ach were added sequentially and the vasorelaxation was measured.

After the addition of fresh PSS buffer three times, the aorta was re-equilibrated to the 1000 mg resting tension, and recontracted with NE of concentrations from 10^{-9} to 10^{-6} M. The response to 10^{-8} to 10^{-5} M Ach was then measured. After the NE and PE concentration-response curves were completed, the maximal contraction after addition of 80 mM KCl was determined.

Organic weights

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 ventricular myocardium isolated specifically. The ventricles and interscapular brown fat were weighed as rapidly as possible in a tension balance.

Data analysis

Contractions to NE and PE are expressed as the percentage contraction to 80mM KCl. The relaxation to Ach is expressed as the percentage of the contracted muscle tension in response to PE.

Statistical analysis

Statistical evaluation of the difference between the WA and CA groups for the areas under the curves was conducted using unpaired Student's t-test. Differences were considered to be statistically significant at $p<0.05$.

Results

Group characteristics

The body weight was significantly lighter in the CA rats compared to the WA rats (Table 1). However, the weights of interscapular brown fat to body weight and of the ventricular wall to body weight were significantly greater in the CA rats compared to the WA rats (Table 1). This finding indicated that the present protocol had effects of chronic exposure to cold.

Dose-response relationships

There were significant relations in tension generated by smooth muscles of the aortae from the CA rats when depolarized with 80 mM KCl compared with that of the WA rats (Fig. 1).

Table 1 **Effects of chronic exposure to cold on body weight, ventricular wall weight and interscapular brown adipose tissue weight of rats.**

	WA (n=10)	CA (n=10)
Body weight (g)	562.5 ± 6.5	515.6 ± 6.9*
TV (mg)	1250.89 ± 3.83	1263.27 ± 8.60
IBAT (mg)	347.57 ± 22.22	586.65 ± 49.65*
TV (mg/100 g body wt)	222.38 ± 0.68	245.01 ± 1.69*
IBAT (mg/100g body wt)	61.79 ± 3.95	113.78 ± 9.63*

Data presented as means ± SD. * significantly different from the WA group. WA, warm acclimated group; CA, cold acclimated group; TV, total ventricular wall weight; IBAT, interscapular brown adipose tissue weight

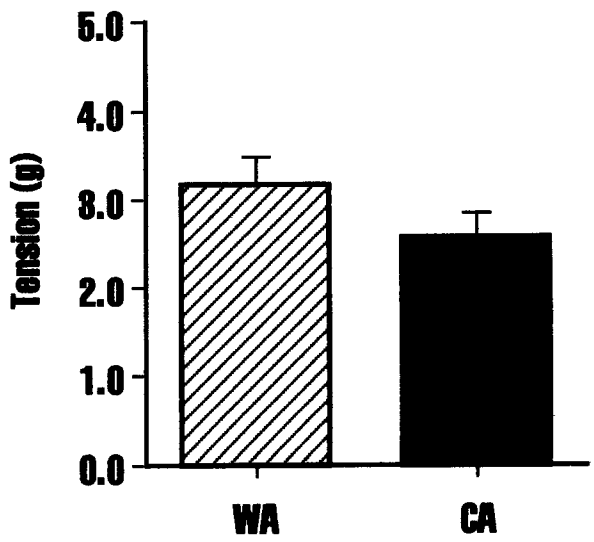

Fig.1 **Contraction in responses to 80 mM KCl in thoracic aortae from warm-acclimated (WA) and cold-acclimated (CA) rats. Contraction in responses to 80 mM KCl showed significantly lesser in thoracic aortae of CA rats than in those of WA rats ($p<0.05$).**

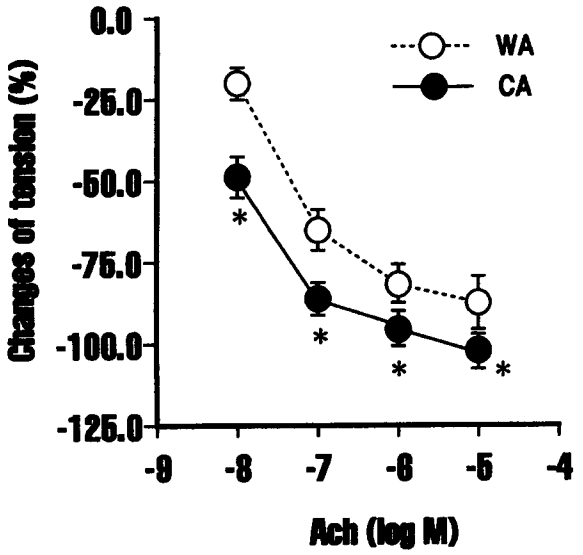

Fig.2 Percentage change of relaxation in responses to acetylcholine (Ach) in thoracic aortae from warm-acclimated (WA) and cold-acclimated (CA) rats. Data are percentage changes of maximal contraction to norepinephrine (10^{-5} M) (means ± SD). * indicates Ach concentrations at which thoracic aortae of CA showed greater relaxation response than did those of WA ($p<0.05$ vs. WA).

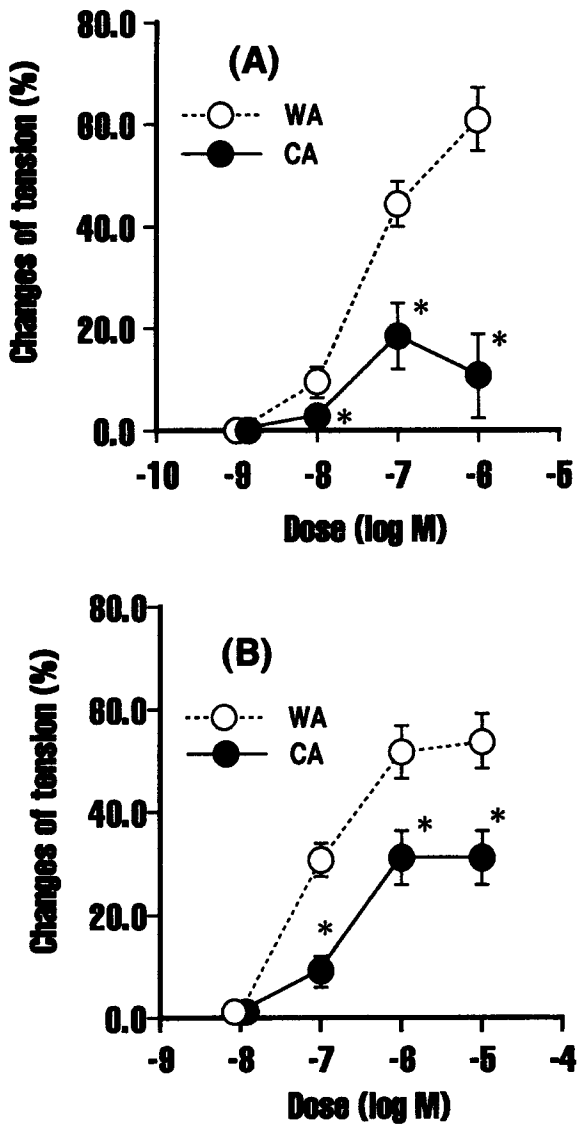

Fig.3 Percentage changes of contraction in responses to norepinephrine (A) and phenylephrine (B) in thoracic aortae from warm-acclimated (WA) and cold-acclimated (CA) rats. Data are percentage changes of the contraction in responses to 80 mM KCl (means ± SD). * indicates NE and PE concentrations at which the thoracic aortae of CA rats showed lesser constriction responses than did those of WA rats ($p<0.05$ vs. WA).

The aortic vascular smooth muscle reactivity to KCl was reduced significantly in the CA rats.

Ach evoked concentration-dependent relaxations in both groups. The relaxations induced by Ach were greater in the CA rats than in the WA rats at concentrations of 10^{-8} to 10^{-5} M (Fig. 2). The results showed that the CA rats had higher sensitivity of Ach-induced vasorelaxation in the thoracic aortae than did the WA rats.

The thoracic aortic contraction in response to NE (10^{-9}-10^{-6} M) was significantly lesser in the CA rats than in the WA rats at concentrations of 10^{-8} to 10^{-6} M, but not at 10^{-9} M (Fig. 3, upper panel (A)). The thoracic aortic contraction in response to PE was significantly lesser in the CA rats than in the WA rats at concentrations of 10^{-7} to 10^{-5} M, but not at 10^{-8} M (Fig. 3, lower panel (B)). The dose-response curves of NE- and PE-induced vasoconstriction in the thoracic aortae were shifted to the right by chronic exposure to cold. Also, chronic exposure to cold significantly altered the maximal contractive force.

Discussion

After 12 weeks of cold exposure there were significant increases in the ratios of ventricular wall, and brown fat to body weight. These changes are similar to those reported earlier for longer periods of cold exposure[9]. The increase in the weights of both ventricle and brown fat with cold acclimation can be mimicked by chronic administration of isoproterenol[9]. These changes during cold exposure may be a result of increased secretion of catecholamines induced by exposure to cold. Also, the increase in heat production[1], blood pressure[3] and heart rate[3] of rats exposed chronically to cold suggest that these responses may be associated with the increased secretion of norepinephrine observed during exposure to cold. The ventricular hypertrophy evidenced by the increased weight of the ventricular wall in the CA rats may be due to the higher metabolism, particularly to increased ventricular work rate secondary to chronic high blood flow. The stimulus produced by exposure to cold that adaptation of the thoracic aortae could be physical (e.g. increased blood flow and mechanical compression) and/or chemical (e.g. circulating metabolites, catecholamines, and other vasoactive substances)[10]. Therefore, the thoracic aortae in the systemic circulation were studied.

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[11]. Also, KCl is easily mobilized by membrane depolarization through voltage-dependent Ca^{2+} channels[12]. The contraction of the vascular smooth muscle from the CA rats to 80 mM KCl was diminished when compared with the WA rats. This probably indicated diminished movement of Ca^{2+} from cytoplasm into the contractile elements. The cause of this reduction is unknown but the decrease in availability of Ca^{2+} channels or alterations in

membrane charge may result in reduced intracellular Ca^{2+} mobility.

The response of the vascular strips to Ach was demonstrated previously to be dependent on the presence of the endothelial cell layer[3,13]. Their findings suggested the existence of a mediator derived from the endothelial cells and induced the relaxation of vascular smooth muscle. Its characteristics are similar to those of nitric oxide (NO)[14]. Therefore, we conducted this study to characterize the Ach-induced endothelium-dependent vasorelaxative response in the CA rats. The response of Ach-stimulated EDRF release in the thoracic aortae may cause more sensitive in the CA rats than in WA rats. There are several possible explanations for the greater Ach-stimulated EDRF release in the thoracic aortae. The CA rats may have increased the density of muscarinic receptors on the endothelial cell membrane, which in turn would enhance the induction of Ach-stimulated EDRF release. We speculated that chronically elevated blood flow because of higher metabolic activity, enhances the agonist-stimulated EDRF release in the CA rats[1]. It might also be one possible underlying mechanism to explain elevations in shear stress caused by increases in blood flow through an artery trigger release of EDRF from endothelium[15]. However, it is not possible at this time to establish whether the EDRF release is altered in the CA rats. More research is needed for such determination.

The objective of these experiments was to test the responsiveness of cold-adapted rats to NE and PE agents. Our results demonstrated that the vascular sensitivities to NE- and PE-induced contractions in the thoracic aortae were lower in the 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 presser response[16,17]. 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[18,19]. In addition, there is abundant evidence of enhanced β-adrenergic responsiveness of aortic vascular smooth muscle following acclimation of rats to cold[18-20]. The increase in β-adrenergic responsiveness in CA rats is possibily to facilitate an increase in metabolic rate to offset the increased heat loss induced by cold exposure[21]. 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 the CA rabbits when compared to similar vessels from WA controls[19]. The differences in results may reflect differences in species of animals used, length of cold acclimation and different vessels studied.

In addition to the systemic adrenosympathetic adaptation to chronic exposure to cold, the possibility that alteration of local modulation in vascular tone by chronic exposure to cold 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 seems to vary with different vessels of different species[22,23]. In the present study, cold acclimation could reduced the vasoconstrictive responses to 10^{-8}-10^{-6} M of NE and 10^{-7}-10^{-5} M of PE. In the experiments of NE and PE (a specific α_1-agonist) -induced responses of the studied aortae of the CA rats, it was observed that chronic exposure to cold might enhance α_1-receptor mediated EDRF release from endothelial cells. The present results suggest that increased shear stress in aortae of the CA rats inhibits adrenergic neurogenic vasoconstriction by augmenting release of endothelial cell vasodilators.

In conclusion, chronic exposure to cold decreased NE- and PE-induced vasoconstrictive responses and increased Ach-induced vasorelaxative response of the isolated thoracic aortae, which were suggested to be due to enhanced release of NE-induced endothelium-derived relaxing factor by up-regulating endothelial α_1-adrenoceptors.

Acknowledgments

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