

Moderate and Deep Hypothermia Produces Hyporesponsiveness to Phenylephrine in Isolated Rat Aorta

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Background: Moderate and severe hypothermia with cardiopulmonary bypass during aortic surgery can cause some complications such as endothelial cell dysfunction or coagulation disorders. This study found out the difference of vascular reactivity by phenylephrine in moderate and severe hypothermia. **Methods:** Preserved aortic endothelium by excised rat thoracic aorta was sectioned, and then down the temperature rapidly to 25°C by 15 minutes at 38°C and then the vascular tension was measured. The vascular tension was also measured in rewarming at 25°C for temperatures up to 38°C. To investigate the mechanism of the changes in vascular tension on hypothermia, NG-nitro-L-arginine methyl ester (L-NAME) and indomethacin administered 30 minutes before the phenylephrine administration. And to find out the hypothermic effect can persist after rewarming, endothelium intact vessel and endothelium denuded vessel exposed to hypothermia. The bradykinin dose-response curve was obtained for ascertainment whether endothelium-dependent hyperpolarization factor involves decreasing the phenylephrine vascular reactivity on hypothermia. **Results:** Fifteen minutes of the moderate hypothermia blocked the maximum contractile response of phenylephrine about 95%. The vasorelaxation induced by hypothermia was significantly reduced with L-NAME and indomethacin administration together. There was a significant decreasing in phenylephrine susceptibility and maximum contractility after 2 hours rewarming from moderate and severe hypothermia in the endothelium intact vessel compared with contrast group. **Conclusion:** The vasoplegic syndrome after cardiac surgery might be caused by hypothermia when considering the vascular reactivity to phenylephrine was decreased in the endothelium-dependent mechanism.

Key words: 1. Hypothermia
2. Nitric oxide
3. Epoprostenol
4. Endothelium-dependent hyperpolarization factor
5. Phenylephrine

INTRODUCTION

The interaction between vasopressor agents and hypothermia has attracted considerable research interest because of the frequent observations of hyporesponsiveness to vaso-

pressors during or after hypothermic cardiopulmonary bypass (CPB) [1,2]. It has been demonstrated that hypothermia potentiates vasopressor responsiveness in cutaneous vessels but decreases such responsiveness in deep vessels, such as the human middle cerebral artery [3], rabbit carotid artery [4],

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human coronary artery [5], and the rat pulmonary artery and thoracic aorta [6]. Extensive studies with deep vessels have focused on the influence of endothelial nitric oxide (NO) or prostacyclin (PGI₂) on the hyporesponsiveness to vasopressor during or after hypothermia [7-9]. Although this hypothermic effect was mainly reported in *in vitro* isolated vessels, there are inconsistencies in the role of the endothelium in the hyporesponsiveness to phenylephrine (Phe) during hypothermia. Despite the fact that increasing evidence shows that endothelium-derived hyperpolarizing factor (EDHF) participates in the control of the vasomotor tone, it is unclear whether EDHF is associated with hyporesponsiveness to vasopressor during or after hypothermia.

The optimal body temperature during aortic surgery with circulatory arrest has been widely debated. It has been demonstrated that moderate hypothermia (30°C) has suboptimal organ protection during an hour of hypothermic circulatory arrest. In contrast, Allibhai et al. [10] recently demonstrated that moderate hypothermic circulatory arrest (25°C) with selective cerebral perfusion compared with deep hypothermic circulatory arrest (18°C) is associated with fewer changes in cerebrospinal fluid proteins in a piglet model of CPB. However, whether the degree of hyporesponsiveness to vasopressor after moderate hypothermia (25°C) is less than that of deep hypothermia (18°C) has not been investigated.

Vasoplegic syndrome is a common complication of hypothermic CPB, appearing with an incidence of 5% to 25% [11]. It is characterized by significant hypotension, high or normal cardiac output, and low systemic vascular resistance. Further, whether hypothermia itself can be an important causative factor of the vasoplegic syndrome is still unclear.

Thus, the purpose of the present study is to improve our understanding of vascular responses to α_1 adrenoceptor agonist and Phe during or after hypothermia and rewarming. The primary objective of this study was to investigate the influence of endothelial NO, PGI₂, or EDHF on hyporesponsiveness to Phe during hypothermia or after hypothermia and rewarming. Second, this study was designed to determine whether moderate (25°C) and deep hypothermia (18°C) influence the physiological responses to Phe and compare the degree of hyporesponsiveness to Phe after the completion of rewarming.

METHODS

1) Preparation of aortic rings for tension measurement

All the experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of Catholic University of Daegu School of Medicine. Male Sprague-Dawley rats, weighing 250 to 350 g, were anesthetized with an intramuscular administration of ketamine (80 mg/kg). The descending thoracic aorta was dissected free and immersed in a cold modified Krebs-Ringer bicarbonate (KRB) solution with the following composition (mM): 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.4 CaCl₂, 25 NaHCO₃, 11.1 glucose, and 0.016 ethylenediaminetetraacetic acid. After removing the connective tissue, the aorta was cut into ring segments 5 mm in length, with care taken not to damage the endothelium. In some of the aortic rings, the endothelium was removed prior to mounting the vessels in the organ bath. The removal of the endothelium was accomplished by gently rubbing the lumen of the vessel with a roughened steel rod.

2) Isometric tension experiments

The aortic rings were vertically suspended between two steel hooks in an organ chamber filled with 10 mL of modified KRB solution gassed with 95% O₂ and 5% CO₂. The temperature of the organ bath was controlled with a refrigerated bath circulator (RBC-10; Jeio Tech, Seoul, Korea). One of the hooks was anchored, and the other was connected to a strain gauge (FT-03; Grass Instrument, Quincy, MA, USA) in order to measure isometric tension. The rings were stretched at 10-minute intervals in increments of 0.5 g to reach optimal tension. Optimal tension was defined as the minimum level of stretch required to achieve the largest contractile response to 60-mM KCl and was determined in the preliminary experiment to be 2.0 g for the size of aortic rings used in these experiments. After the rings had been stretched to their optimal resting tension, the contractile response to 60-mM KCl was measured. After washing out the KCl from the organ bath and returning the isometric tension to the prestimulation value, we checked for endothelial integrity. The presence of the endothelium in the endothelium-intact (non-rubbed) rings and the adequacy of endothelium removal

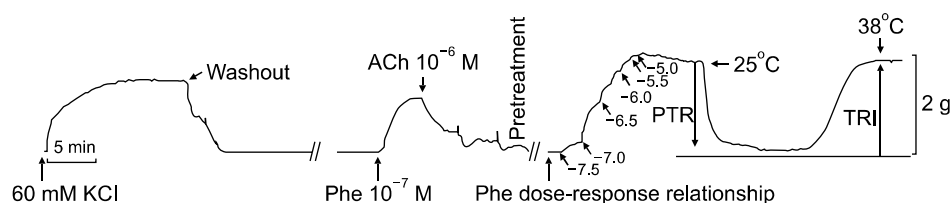


Fig. 1. This picture shows the first series of experimental protocol with endothelium-intact (E+) rat aortic rings. After obtaining of phenylephrine (Phe, 10^{-9} - 10^{-5} M)-dose response relationships at 38°C , the temperature of organ baths was maintained at 25°C for 15 minutes (hypothermia) and then increased to 38°C (rewarming). Percent tension reduction (PTR) by hypothermia and tension recovery index (TRI) by rewarming in endothelium-intact rings were compared with rings pretreated with NG-nitro-L-arginine methyl ester or indomethacin.

in the endothelium-denuded (rubbed) rings were tested functionally by recording the response to acetylcholine (ACh, 10^{-6} M) in the aortic rings precontracted with Phe (10^{-7} M). Endothelium-intact rings were verified by $>60\%$ relaxation, whereas denudation was verified by $<5\%$ relaxation in response to ACh.

The first experiment was designed to investigate the direct effects of moderate hypothermia (25°C). Endothelium-intact rings were suspended, and Phe concentration-response studies were performed at 38°C . When the contractile response at the highest concentration of the Phe (10^{-5} M) was sustained, the temperature of the organ baths was maintained at 25°C for 15 minutes and then increased to 38°C . The degree of vasorelaxation response induced by hypothermia was expressed as percentage tension reduction (PTR), which refers to a maximal vasorelaxing percentage change from the contractile response of Phe (10^{-5} M). The degree of tension recovery after rapid rewarming showed the tension recovery index (TRI), which refers to the maximal vasocontracting percentage change from the maximal vasorelaxation by hypothermia. To analyze the role of endothelial nitric oxide or PGI_2 on hypothermia-induced PTR, the NO synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) or PGI_2 inhibitor, indomethacin (10^{-5} M) was added to the bath 30 minutes before checking the Phe concentration response at 38°C . Then, we checked the effect of L-NAME or indomethacin on hypothermia-induced PTR and rewarming-induced TRI.

The second experiment was designed to determine whether moderate (25°C) and deep hypothermia (18°C) influence the physiological responses to Phe and to compare the degree of hyporesponsiveness to Phe after the completion of rewarming. After checking endothelial integrity, the temperature of the

experimental ring was maintained at 18°C for 1 hour and then increased gradually. A 2-hour rewarming protocol included 15 minutes of rewarming to 25°C , 15 minutes of rewarming to 30°C , and 90 minutes of rewarming to 38°C . To determine the degree of hyporesponsiveness to Phe during moderate hypothermia, the same experiment was performed at 25°C .

The third experiment was designed to investigate the influence of EDHF on the hypothermic effect of the physiological response to Phe. After completion of the 2-hour rewarming protocol, endothelium-intact and endothelium-denuded rat aortic rings were precontracted with Phe (3×10^{-7} M). When the tension was sustained, we checked the dose-response relationships to bradykinin (3×10^{-10} to 10^{-5} M), which is known to produce endothelium-dependent vasorelaxation. To determine the influence of EDHF on the hyporesponsiveness to Phe after exposure to deep hypothermia (18°C), some rings were pretreated with L-NAME and indomethacin for 30 minutes before checking the bradykinin dose-response relationships.

3) Drugs and solutions

The following drugs showed the highest purity commercially available: Phe HCl, ACh, L-NAME, indomethacin (Sigma Chemical, St. Louis, MO, USA). All of the other drugs were dissolved and diluted in distilled water. All drug concentrations are expressed as the final molar concentration in the organ bath.

4) Data analysis

The logarithm of the drug concentration (Log EC_{50}) eliciting 50% of the maximal contractile response was calculated using a non-linear regression analysis by fitting the concen-

Table 1. Log EC₅₀ and E_{max} of phenylephrine concentration-response relationships at 38°C, the degree of hypothermia (25°C)-induced PTR and rewarming (38°C)-induced TRI

	N	Log EC ₅₀	E _{max} (% of 60 mM KCl)	PTR (%)	TRI (%)
Control	22	-7.18±0.04	131.30±5.56	95.05±1.50	102.10±2.41
L-NAME (10 ⁻⁴ M)	8	-7.64±0.05 ^{a)}	157.07±4.71 ^{a)}	83.44±5.65	97.61±4.10
Indomethacin (10 ⁻⁵ M)	7	-7.40±0.11	147.80±16.05	84.13±6.09	101.61±2.10
L-NAME+indomethacin	6	-7.51±0.11 ^{a)}	190.30±19.87 ^{a)}	75.84±6.49 ^{a)}	96.85±3.82

The data are shown as the mean±standard error of the mean. Log EC₅₀ indicate the concentration eliciting 50% of the maximal contractile response. E_{max} means the maximum contraction in response to phenylephrine. PTR means the degree of hypothermia (25°C)-induced PTR. TRI indicates rewarming (38°C)-induced TRI.

Log EC₅₀, logarithm of the drug concentration; E_{max}, maximum response; PTR, percent tension reduction; TRI, tension recovery index; L-NAME, NG-nitro-L-arginine methyl ester.

^{a)}p<0.05 vs. rings with control.

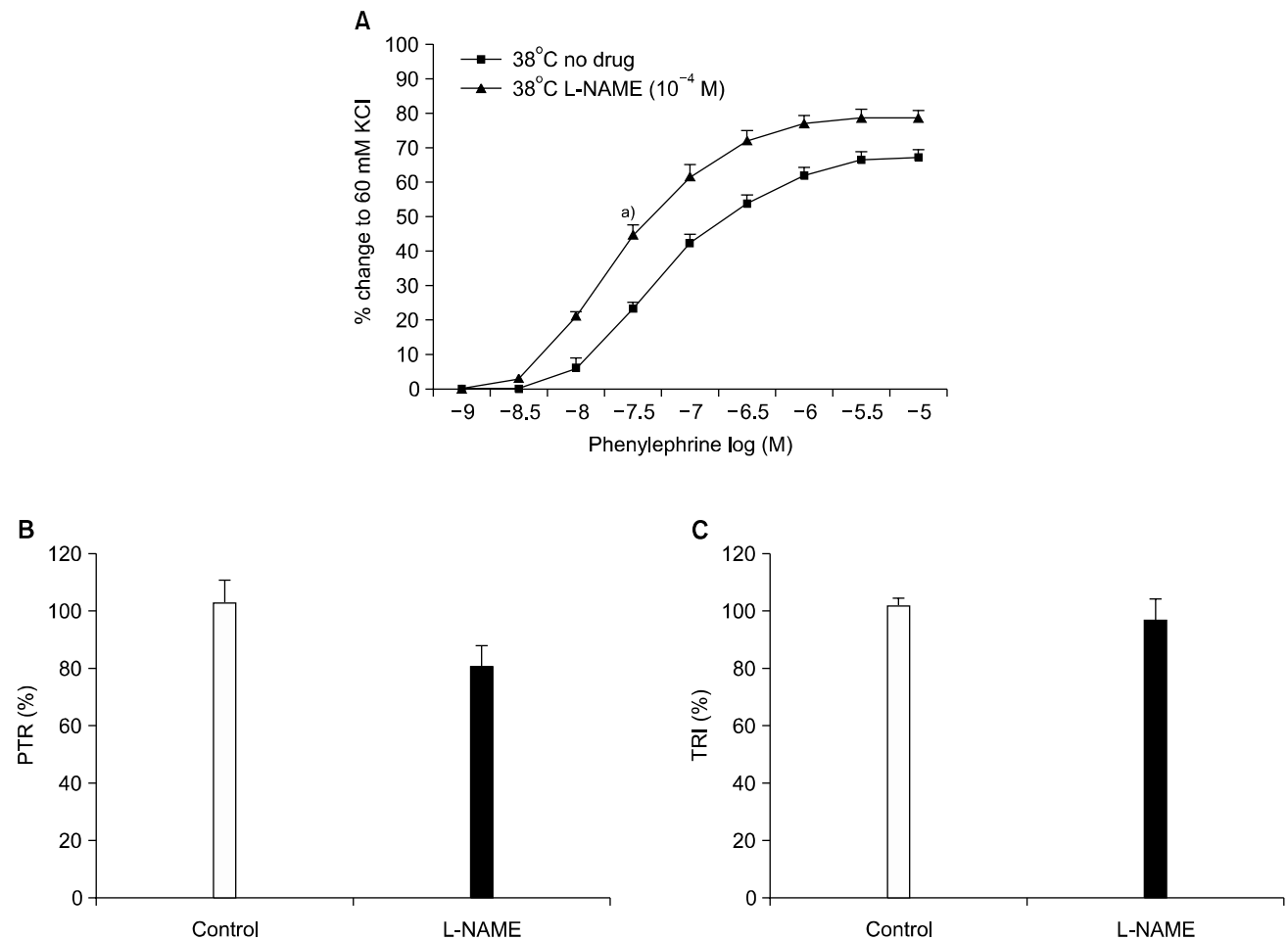


Fig. 2. (A) Nitric oxide synthase inhibitor (NG-nitro-L-arginine methyl ester, L-NAME, 10⁻⁴ M) caused ($p < 0.05$) a leftward shift in phenylephrine dose response relationships in endothelium-intact rat thoracic aortic rings ($n=8$). (B) L-NAME slightly attenuated ($p > 0.05$) hypothermia-induced percent tension reduction (PTR) (C) whereas L-NAME had no effect ($p > 0.05$) on rewarming-induced tension recovery index (TRI). ^{a)}p<0.05 compared with control.

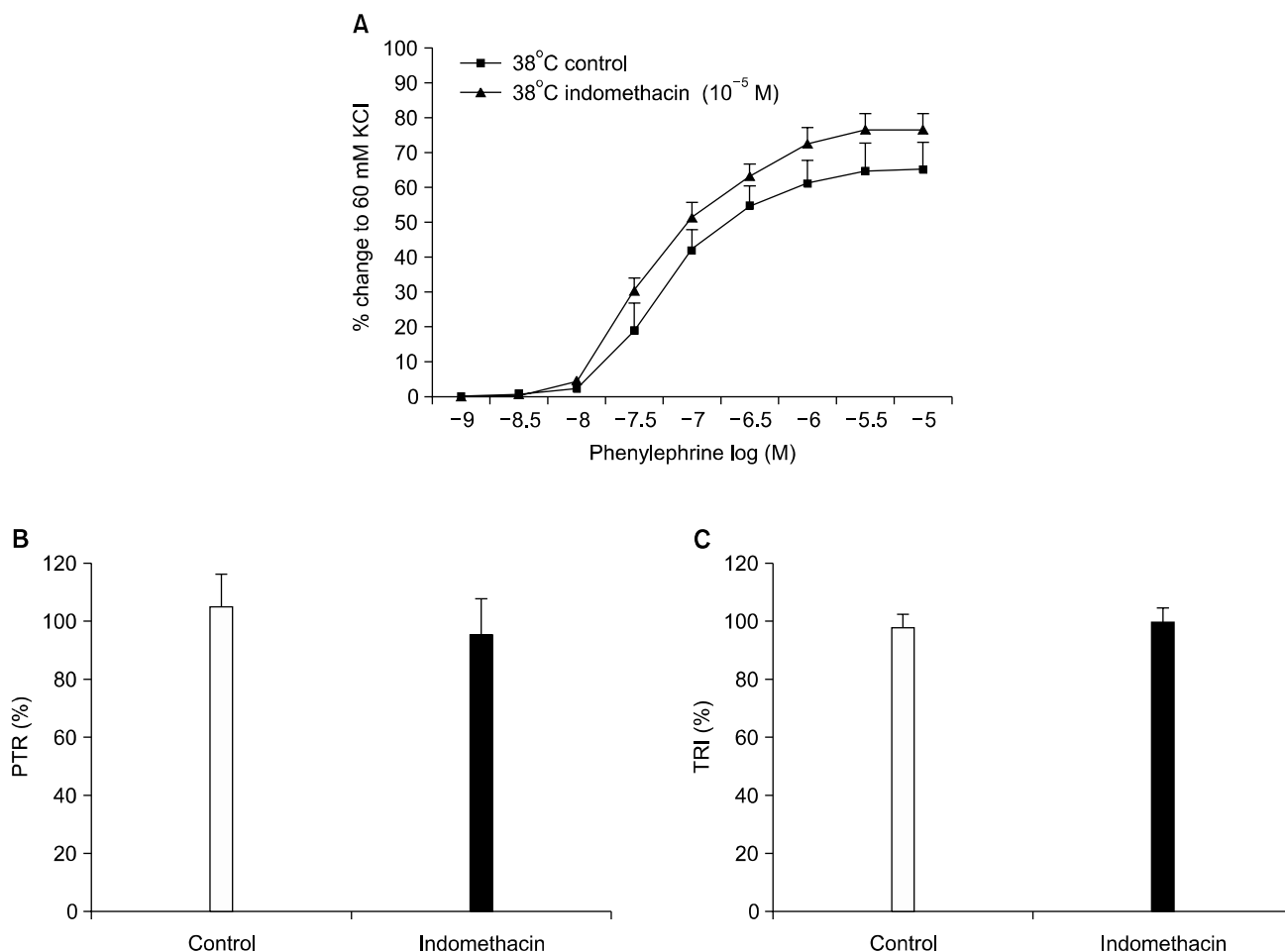


Fig. 3. (A) Indomethacin (10^{-5} M) had no effect ($p > 0.05$) on phenylephrine dose response relationships in endothelium-intact rat thoracic aortic rings ($n=7$). (B) Indomethacin had no effect ($p > 0.05$) on hypothermia-induced percent tension reduction (PTR) (C) whereas Indomethacin had no effect ($p > 0.05$) on rewarming-induced tension recovery index (TRI).

tration-response relationships for Phe to a sigmoidal curve using commercially available software (Prism ver. 4.0; Graph Pad Software, San Diego, CA, USA). The maximum response (E_{max}) was considered to be the maximal amplitude of the response reached in the concentration-response curves of Phe. Statistical analysis for the comparison of $\log EC_{50}$, E_{max} , and the degree of PTR and TRI was conducted using the one-way analysis of variance test followed by Fisher's least significant difference. The differences were considered to be statistically significant at p -values < 0.05 . All data are expressed as mean \pm standard error of the mean. The contractile responses to Phe are expressed as the percentage of the contractile response at 60 mM KCl. N refers to the number of rats used in each protocol.

RESULTS

1) Effect of NO synthase inhibition or PGI₂ inhibition on hypothermia-induced PTR or rewarming-induced TRI

The original tracing of the first series of experiments is shown in Fig. 1. As summarized in Table 1, moderate hypothermia (25°C) produced approximately 95% of the PTR in the control rings. L-NAME (10^{-4} M), but not indomethacin (10^{-5} M), significantly increased ($p < 0.05$) the sensitivity and the maximal response to Phe, as compared to the control rings at 38°C (Figs. 2A, 3A). L-NAME or indomethacin had no significant effect on hypothermia-induced PTR (Figs. 2B, 3B). However, combined pretreatment with L-NAME and in-

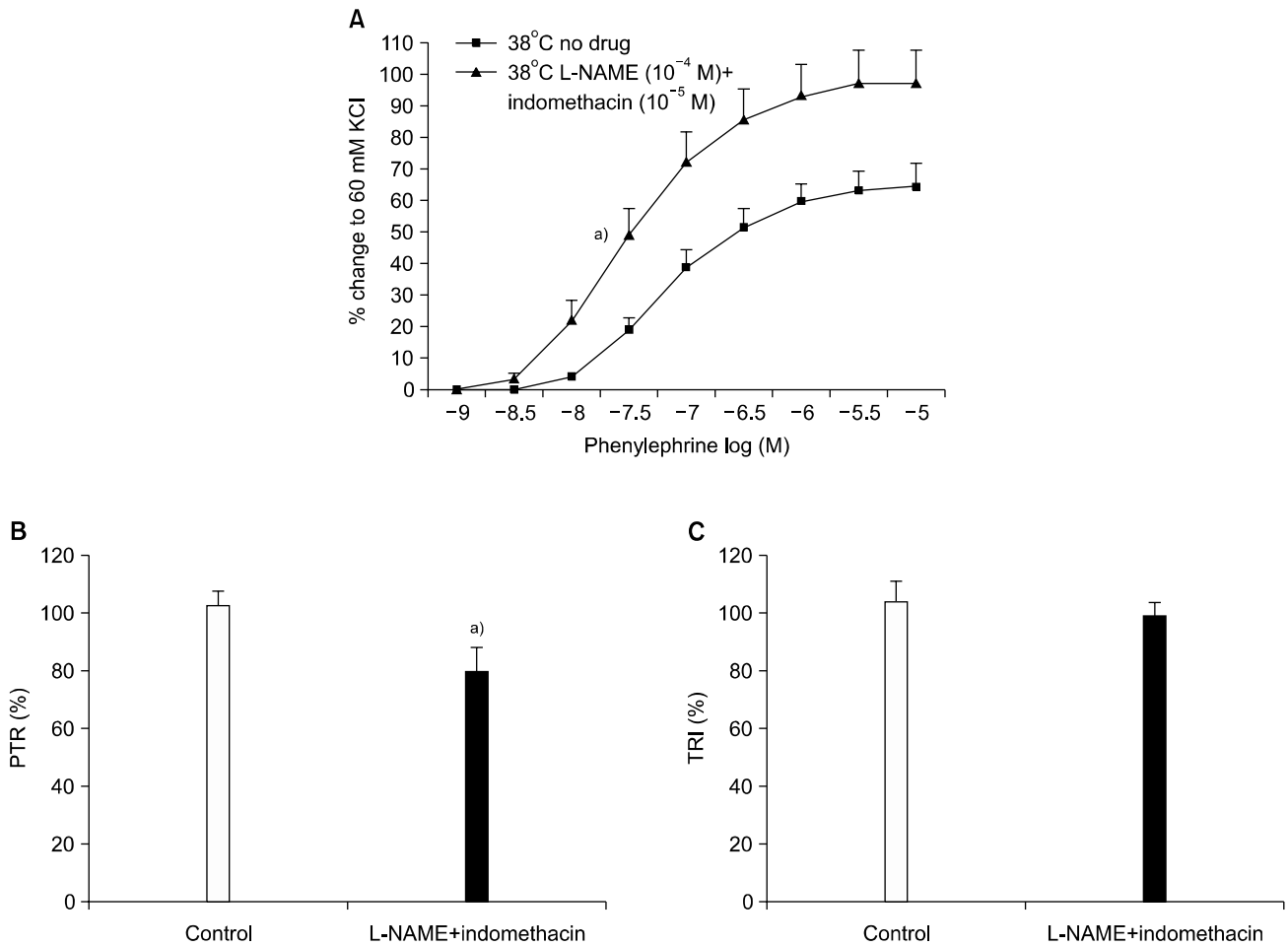


Fig. 4. (A) Combined pretreatment with nitric oxide synthase inhibitor (NG-nitro-L-arginine methyl ester, L-NAME, 10^{-4} M) and indomethacin (10^{-5} M) caused ($p < 0.05$) a leftward shift in phenylephrine dose response relationships in endothelium-intact rat thoracic aortic rings ($n=8$). (B) Combined pretreatment with L-NAME and indomethacin significantly attenuated ($p < 0.05$) hypothermia-induced percent tension reduction (PTR) (C) whereas these drugs had no effect ($p > 0.05$) on rewarming-induced tension recovery index (TRI). ^{a)} $p < 0.05$ compared with control.

domethacin significantly attenuated ($p < 0.05$) hypothermia-induced PTR (Fig. 4B). In contrast, in the comparison of rewarming-induced TRI, there were no differences between pretreated rings and control rings (Figs. 2C, 3C, 4C).

2) Effect of deep hypothermia (18°C) and rewarming on Phe dose-response relationships

The original tracing of the second series of experiments is shown in Fig. 5. In endothelium-intact rings, rings pretreated with deep hypothermia (18°C) and 2-hour rewarming significantly decreased ($p < 0.05$) the sensitivity and maximal response to Phe compared with the control rings at 38°C (Table 2, Fig. 6). However, these hypothermic effects were not ob-

served in the case of endothelium-denuded rings. In a comparison of the effect of the rewarming time, the Phe dose-response relationships of 2-hour rewarming were not different from those of 1-hour rewarming (data not shown).

3) Effect of moderate hypothermia (25°C) on Phe dose-response relationships

In endothelium-intact rings, rings pretreated with moderate hypothermia (25°C) and 2-hour rewarming significantly decreased ($p < 0.05$) the sensitivity and maximal response to Phe as compared to the control rings at 38°C (Table 2, Fig. 7). However, these hypothermic effects were not observed in the case of endothelium-denuded rings. Phe dose-response re-

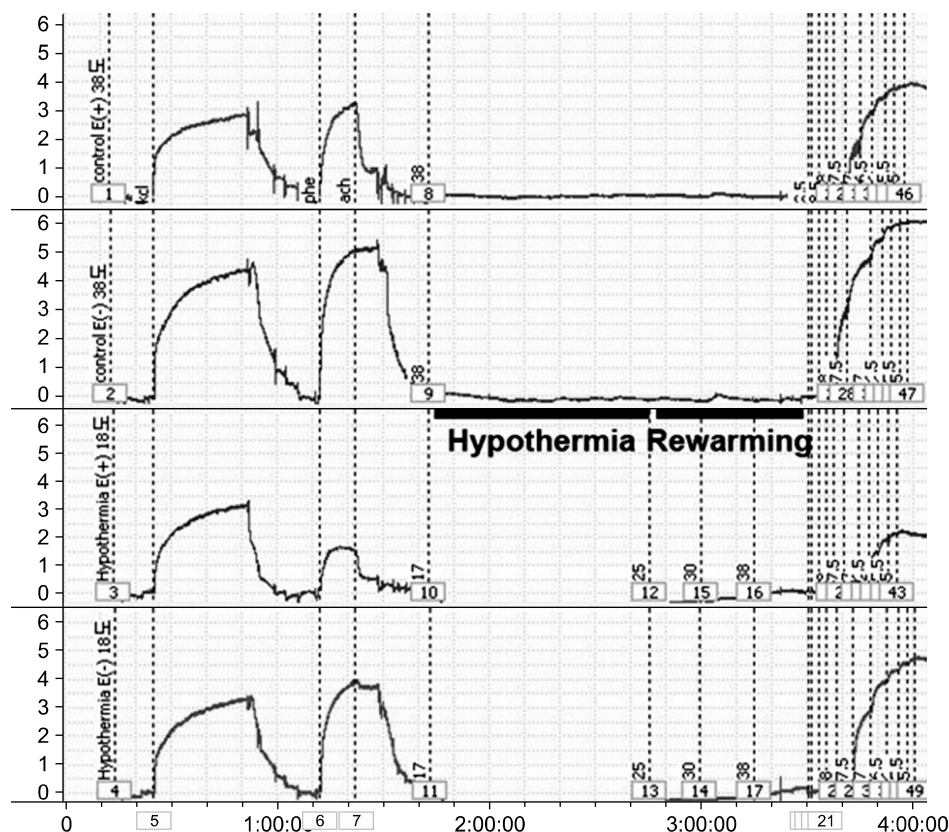


Fig. 5. This picture shows the second series of experimental protocol with endothelium intact (E+) and denuded (E-) rat aortic rings. After checking endothelial integrity, a pair of ring was exposed to hypothermia period (25°C or 18°C) for 1 hour and rewarming period to 38°C (1 hour or 2 hours).

Table 2. Log EC₅₀ and E_{max} of phenylephrine concentration-response relationships in isolated endothelium-intact and -denuded aortic rings pre-exposed at 38°C, 25°C, and 18°C

	N	Log EC ₅₀	E _{max} (% of 60 mM KCl)
38°C endothelium-intact rings	24	-7.07±0.05	115.40±5.22
38°C endothelium-denuded rings	24	-7.93±0.05	186.53±9.89
25°C endothelium-intact rings	7	-6.86±0.12 ^{a)}	83.84±3.72 ^{a)}
25°C endothelium-denuded rings	7	-8.07±0.18	202.55±9.53
18°C endothelium-intact rings	7	-6.77±0.21 ^{a)}	83.40±7.77 ^{a)}
18°C endothelium-denuded rings	7	-7.81±0.25	186.00±11.16

The data are shown as the mean±standard error of the mean. Log EC₅₀ indicate the concentration eliciting 50% of the maximal contractile response. E_{max} means the maximum contraction in response to phenylephrine.

Log EC₅₀, logarithm of the drug concentration; E_{max}, maximum response.

^{a)}p<0.05 vs. endothelium-intact rings of 38°C.

relationships in endothelium-intact rings at 25°C were not different from those observed at 18°C.

4) Effect of deep hypothermia (18°C) on bradykinin dose-response relationships

In endothelium-intact rings, bradykinin produced dose-dependent vasorelaxation in the non-pretreated control rings at

38°C (Table 3, Fig. 8). Rings pretreated with deep hypothermia (18°C) had a similar bradykinin dose-response relationship as the control rings at 38°C. Maximal relaxation to bradykinin in rings pretreated with L-NAME and indomethacin at 18°C significantly decreased (p<0.05) as compared to that in non-pretreated rings. However, there were no differences between the effects at 18°C and those at 38°C.

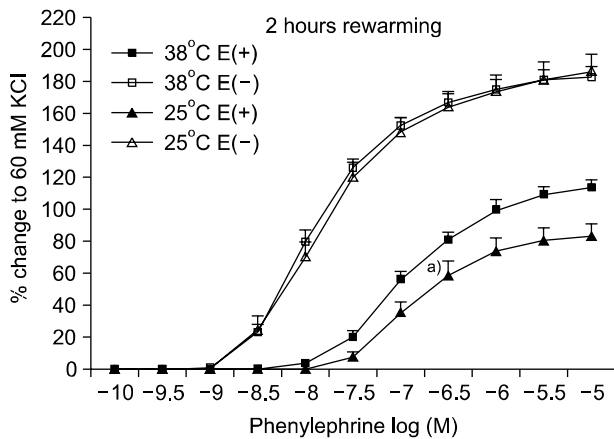


Fig. 6. One hour hypothermia at 18°C and 2 hours rewarming attenuated (^a $p < 0.05$) phenylephrine dose response relationships in endothelium-intact rings whereas these hypothermic effects did not appear at endothelium-denuded rings ($n=7$).

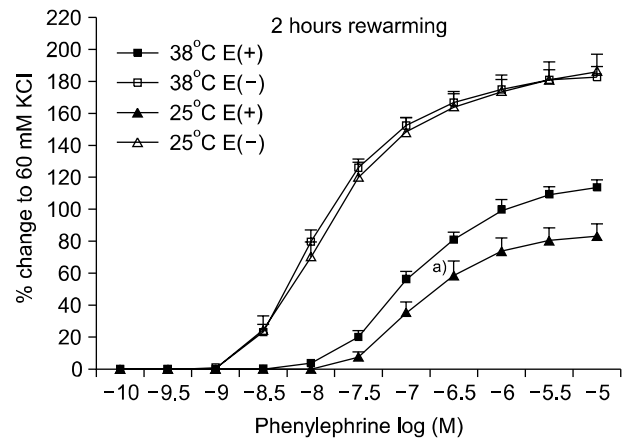


Fig. 7. One hour hypothermia at 25°C and 2 hours rewarming attenuated (^a $p < 0.05$) phenylephrine dose response relationships in endothelium-intact rings whereas these hypothermic effects did not appear at endothelium-denuded rings ($n=7$).

Table 3. Log EC₅₀ and E_{max} of bradykinin concentration-response relationships in isolated endothelium-intact aortic rings at 38°C and 18°C

	N	Log EC ₅₀	E _{max} (% of 60 mM KCl)
38°C non-pretreated	9	-9.15±0.49	-75.86±4.20
38°C L-NAME (10 ⁻⁴ M)+indomethacin (10 ⁻⁵ M)	9	-8.32±1.41	-32.09±6.68 ^{a)}
18°C non-pretreated	9	-9.15±0.30	-76.72±5.75
18°C L-NAME (10 ⁻⁴ M)+indomethacin (10 ⁻⁵ M)	9	-8.65±0.23	-30.49±4.74 ^{b)}

The data are shown as the mean±standard error of the mean. Log EC₅₀ indicate the concentration eliciting 50% of the maximal contractile response. E_{max} means the maximum relaxation in response to bradykinin.

Log EC₅₀, logarithm of the drug concentration; E_{max}, maximum response; L-NAME, NG-nitro-L-arginine methyl ester.

^{a)} $p < 0.05$ vs. non-pretreated rings of 38°C. ^{b)} $p < 0.05$ vs. non-pretreated rings at 18°C.

DISCUSSION

The first of these *in vitro* experiments using the rat thoracic aorta revealed that lowering the temperature of the organ bath from 38°C to 25°C produced profound vasorelaxation in endothelium-intact rings precontracted with Phe (10⁻⁵ M), suggesting that moderate hypothermia attenuated α_1 -adrenoceptor-mediated contraction. These hypothermic effects were attenuated by the presence of L-NAME, an inhibitor of NO synthase, and indomethacin, an inhibitor of PGI₂, suggesting that the direct effect of moderate hypothermia might be associated with the production of NO and PGI₂ in the endothelium. Evora et al. [12] demonstrated that the endothelium was sensitive to temperature variations and that PGI₂ and NO-dependent pathways might be involved in endothelium-depend-

ent vasorelaxation to hypothermia. In our previous studies [13], we found the enhancement of cyclic guanosine monophosphate (cGMP) in the radioimmunoassay of the endothelium-intact rings at 25°C, suggesting that moderate hypothermia stimulated the release of endothelial NO, which activated the guanylate cyclase-cGMP pathway in vascular smooth muscle cells and consequently relaxed vascular tone. In addition, we confirmed the enhancement of cyclic adenosine monophosphate (cAMP) only in endothelium-intact rings at 25°C. Endothelial PGI₂ was prostanoid produced by the cyclooxygenase pathway, and the activation of the G protein-coupled PGI₂ receptors relaxed vascular smooth muscle cells by increasing the cellular concentration of cAMP [14]. Taken together, these findings suggest that the underlying mechanism for the endothelial-dependent component of hypo-

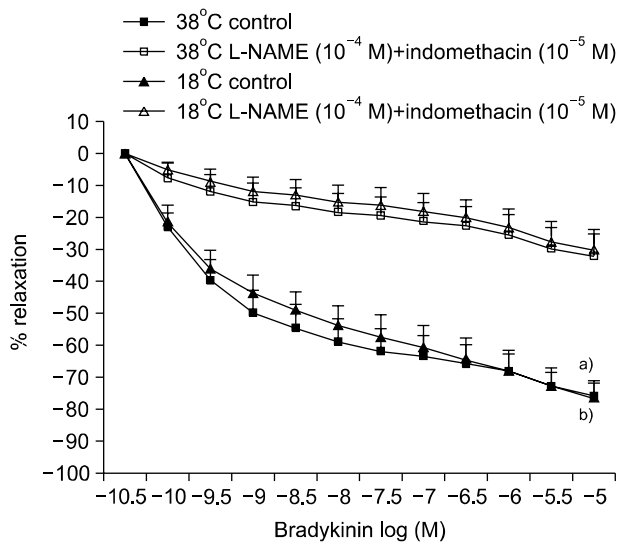


Fig. 8. The effect of L-NAME (10^{-4} M) and indomethacin (10^{-5} M) on bradykinin-dose response relationships in the endothelium-intact or denuded rings with or without pretreatment with hypothermia (18°C) and 1 hour rewarming ($n=9$). ^{a)} $p < 0.05$ vs. non-pretreated rings of 38°C . ^{b)} $p < 0.05$ vs. non-pretreated rings at 18°C .

thermia-induced vasorelaxation involves both the NO-cGMP and the PGI_2 -cAMP pathways in the rat aorta.

The first series of experiments in the present study shows that a short period of moderate hypothermia (25°C) produces a profound vasorelaxation. Several studies also demonstrated that lowering the temperature of the organ bath decreases vascular tension [7,8,12,13]. The hypothermic effect of profound vasorelaxation is a similar finding to severe hypotension at the initial phase of hypothermic CPB. Although it is generally accepted that hemodilution and non-pulsatile flow are associated with a sudden drop in blood pressure when CPB is applied, the present study shows that hypothermia itself can play a major role in producing a profound hypotension in the initial phase of hypothermic CPB.

Recently, Chung et al. [13] suggested that the hyporesponsiveness to Phe during hypothermia is produced by endothelium-dependent mechanisms. Because their study was performed in the state of hypothermia, their results cannot explain the phenomenon of hyporesponsiveness to Phe after the completion of rewarming. Therefore, the present study was designed to focus on the hyporesponsiveness to Phe after the completion of rewarming during cardiac or aortic surgery

with hypothermic CPB. In the second series of our experiments, Phe dose-response relationships in endothelium-intact rings pre-exposed with deep hypothermia (18°C) shifted to the right, indicating that there is hyporesponsiveness to Phe. These findings did not appear in endothelium-denuded rings, suggesting that hyporesponsiveness to Phe is produced by endothelium-dependent mechanisms. We believe that these endothelium-dependent mechanisms involve NO-cGMP pathways and PGI_2 -cAMP pathways, as mentioned above. We also found this hyporesponsiveness to Phe in endothelium-intact rings pre-exposed to moderate hypothermia (25°C). Therefore, the present study strongly suggests that the hyporesponsiveness to Phe can be caused by the endothelium-dependent mechanisms in spite of the completion of 2 hours of rewarming after moderate or deep hypothermia. Interestingly, the degree of hyporesponsiveness to Phe in the endothelium-intact rings pre-exposed to moderate hypothermia (25°C) was not different from that in the case of deep hypothermia (18°C). A similar finding in a previous study was found under conditions of a hypothermic state [13]. Chung et al. [13] demonstrated that the degree of hyporesponsiveness to Phe at 30°C is similar to that at 25°C . Consequently, the hyporesponsiveness to Phe is endothelium-dependent but may not be temperature-dependent at temperatures below 30°C . We believe that a certain temperature sensor in the endothelium might act as a temperature on-off switch and stimulate the endothelium when there is a certain degree of change in temperature.

In the present study, rewarming-induced TRI was almost 100% in the first experiment, suggesting that the effect of the short period of hypothermia is a reversible process. However, the hypothermic effect in the second experiment of our study was not reversible in spite of the completion of 2 hours of rewarming. This discrepancy may be associated with the duration of hypothermia. The first experiment involved 15 minutes of hypothermia at 25°C , whereas the second experiment involved 1 hour of hypothermia at 18°C and 25°C . Because 1 hour of hypothermia at 18°C and 25°C is frequently used in aortic surgery, we believe that hyporesponsiveness to Phe after the completion of the 2-hour rewarming is a clinically relevant observation.

In the third experiment of the present study, we tested a hypothesis related to whether EDHF relaxation is involved in

the hypothermia-induced vasorelaxation or hyporesponsiveness to Phe. In general, EDHF relaxation induced by agonists such as bradykinin has been assessed in the condition of an NO synthase inhibitor (L-NAME or L-NG-nitro-L-arginine), an NO scavenger (oxyhemoglobin), and a cyclooxygenase inhibitor (indomethacin) [15]. The EDHF in bradykinin-induced vasorelaxation is composed of non-NO pathways and non-PGI₂ pathways. In the present study, the effects of L-NAME and indomethacin on bradykinin dose-response relationships at 18°C were not different from those of the same condition at 38°C, suggesting that the component of EDHF in bradykinin-induced vasorelaxation of the endothelium-intact rings with pre-exposed deep hypothermia (18°C) may not be involved in hypothermia-induced vasorelaxation or hyporesponsiveness to Phe.

There are some limitations of this *in vitro* study. In the present study, the underlying mechanisms for the vascular smooth muscle component of hypothermia-induced vasorelaxation in the first experiment of our study need to be clarified. We thought that the endothelium-independent component of hypothermia-induced vasorelaxation might be associated with the direct effect of moderate hypothermia on the vascular smooth muscle cells via an inhibitory effect of hypothermia on intracellular enzymes or various Ca²⁺ channels such as voltage-gated Ca²⁺ channels or store-operated Ca²⁺ channels [16]. Furthermore, the bath application of a relatively specific α_1 -adrenoceptor agonist, Phe, certainly does not mimic the release of norepinephrine, adenosine triphosphate, and vasoactive peptides at specialized sympathetic neuroeffector junctions [17]. Even with these limitations, however, our findings provide the new insight that the therapeutic effect of α -adrenoceptor agonists, such as Phe or norepinephrine, can be attenuated during or after hypothermic CPB in cardiac surgery. To control systemic hypotension with these drugs during or after hypothermic CPB, the anesthesiologist and the surgeon should keep in mind the occurrence of hyporesponsiveness to these vasopressors. In addition, this study also provides the basic data to elucidate the underlying mechanisms of low systemic vascular resistance or vasoplegic syndrome in cardiac or aortic surgery with hypothermic CPB [18,19]. Cutaneous vessels start to vasodilate in the rewarming period. The present study shows that deep vessels such as

rat thoracic aortae are also easy to vasodilate during hypothermia or even after the rewarming period. Therefore, we conclude that the rewarming or after-rewarming period is vulnerable to the production of severe hypotension or low systemic vascular resistance because both types of vessels are prone to vasodilation. Although vasoplegic syndromes are linked to the NO-cGMP pathway [19], the mechanisms responsible for these syndromes have not yet been fully understood. We believe that the hypothermic condition itself is one of the important factors responsible for vasoplegic syndromes in cardiac or aortic surgery.

In conclusion, moderate and deep hypothermia stimulates the endothelium-dependent component of α_1 -adrenoceptor-mediated contraction, and this effect depends on the NO-cGMP and PGI₂-cAMP pathways. EDHF-mediated relaxation is not involved in the hyporesponsiveness to Phe. Hyporesponsiveness to Phe after the completion of rewarming during cardiac or aortic surgery is endothelium-dependent but temperature-independent; it is observed at temperatures below 30°C. Further, the degree of hyporesponsiveness to Phe after moderate hypothermia (25°C) is not different from that in the case of deep hypothermia (18°C).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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