DOI: 10.4149/BLL_2013_117

Bratisl Lek Listy 2013; 114 (10)
556 – 560

EXPERIMENTAL STUDY

Cilostazol-induced relaxation of calf cardiac vein and coronary artery during cooling

Nurullahoglu-Atalik KE[1], Nurullahoglu ZU[2], Kilic M[1], Aribas A[3]

Department of Pharmacology, Faculty of Meram Medicine, University of Necmettin Erbakan University, Konya, Turkey. **esraatalik@hotmail.com**

Abstract: *Objective:* At present very little is known about the role of endothelial nitric oxide (NO) in the effects of temperature on vascular reactivity. The aim of the present study is to evaluate the influence of cooling (to 28 °C) on the vasodilatation induced by cilostazol(10^{-9}–$3x10^{-4}$M) on carbachol (10^{-6})-precontracted calf cardiac vein and coronary artery and the role of NO in these effects.
Materials and methods: Ring preparations of great cardiac vein and the anterior interventricular branch of left coronary artery were used.
Results: Cilostazol produced concentration-dependent relaxation of calf cardiac vein and coronary artery rings precontracted with carbachol. During cooling, the pIC_{50} values, but not the maximal responses to cilostazol were significantly lower than at 37 °C in both preparations. Cooling to 28 °C in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) did not modify the effect of temperature both in cardiac vein and coronary artery. These results demonstrate for the first time that cooling-induced changes of cilostazol in calf cardiac vein and coronary artery are independent of NO *(Tab. 2, Fig. 3, Ref. 32)*. Full Text in PDF *www.elis.sk.*
Key words: cardiac vein, cilostazol, cooling, coronary artery, nitric oxide.

Cooling has been shown to induce significant changes in non-cutaneous vascular smooth muscle responses to various drugs (1, 2). Most of the previous studies examining the effect of cooling on smooth muscle responses have focused on the effects of contractile agents and information about vasodilators is rather limited.

Cilostazol is a phosphodiesterase III inhibitor and is used in diabetic patients as an antiplatelet and antithrombotic drug (3, 4). It has been found that phosphodiesterase III inhibitors including cilostazol cause vasodilation leading to a concomitant reduction in arterial pressure (5, 6). Cyclic nucleotide phosphodiesterases catalyze the degradation of cAMP and cGMP (7). An increase in levels of cAMP or cGMP in smooth muscle cells was regulated by activation of adenylyl cyclase or guanylyl cyclase and inhibition of phosphodiesterases (8). The endothelium plays an important role in regulating vascular tone, through the release of a variety of vasoactive substances. Endothelium-derived relaxing factor, identified as nitric oxide (NO) or as a nitric oxide compound, is one of the most important (9). Cilostazol is suggested to relax smooth muscle cells by elevating cAMP.

[1]Department of Pharmacology, Faculty of Meram Medicine, University of Necmettin Erbakan University, Konya, Turkey, [2]Department of Biology, Faculty of Art and Science, Marmara University, Istanbul, Turkey, and [3]Department of Cardiology, Faculty of Meram Medicine, University of Necmettin Erbakan University, Konya, Turkey

Address for correspondence: K. Esra Atalik, Department of Pharmacology, Faculty of Meram Medicine, Necmettin Erbakan University, Konya, 42080, Turkey.
Fax: +90.3322237124

Acknowledgements: We are grateful to Abdi Ibrahim Drug Industry for kindly providing the cilostazol for the study. We thank Mehmet KÖSEN for assistance during the experiments.

Thus, despite current research to determine the effects of cooling on vascular reactivity of different animal species, studies with vasodilator agents remain incomplete and to our knowledge, there are no studies that analyze the effects of cooling on the cilostazol-dependent relaxations.

The purpose of this study was to determine the effects of cooling (28 ºC) on vascular smooth muscle responses to cilostazol in calf cardiac vein and coronary artery, analyzing the role of NO in these effects.

Materials and methods

Tissue preparations

Calf hearts were obtained from a slaughterhouse and immediately placed in Krebs-Henseleit solution. Segments of the great cardiac vein and anterior interventricular branch of left coronary artery were removed and cut into rings 2.5 mm in length. Care was taken not to damage the endothelium. Each ring was mounted in 25 ml organ baths containing Krebs-Henseleit Solution (KHS), aerated with 95 % O_2 and 5 % CO_2. KHS was composed of (mM): NaCl 119, KCl 4.70, $MgSO_4$ 1.50, KH_2PO_4 1.20, $CaCl_2$ 2.50, $NaHCO_3$ 25, Glucose 11.

Changes in isometric tension were recorded by a force-displacement transducer (BIOPAC MP36, Santa Barbara, California, USA) connected through amplifiers to a ITBS08 Integrated Tissue Bath System (Commat, Ankara, Turkey). The tissues were allowed to equilibrate for 60 mins under a resting tension of 1 g with repeated washing every 15 min.

Experimental design

The endothelial cell integrity was determined in each ring before all experiments. Relaxation responses to acetylcholine (ACh, 10-6

M) in rings pre-constricted with 5-hydroxytryptamine (5-HT, 10^{-6} M) were used to test endothelial cell integrity. Preparations which were relaxed by >70 % of the 5-HT-induced tone after addition of ACh were considered to have undamaged endothelium. Thereafter, experimental procedures were performed as describe below.

After the stabilization period, cardiac vein and coronary artery preparations were contracted with 10^{-6} M carbachol. After the contraction had reached steady state, cilostazol was added to the organ bath cumulatively (10^{-9}–$3x10^{-4}$M) at 37 °C. The maximal carbachol contraction was used as a standard by which subsequent responses of the tissue could be expressed (as a percentage of this contraction).

After the first concentration-response curve was completed, preparations were washed and allowed to reestablish resting tension. After the contractile responses to carbachol, the temperature was changed from 37 to 28 °C (cooling). Cooling was rapidly achieved and preparations were allowed to equilibrate at this temperature for 30 mins before a second concentration-response curve was determined for cilostazol. Cilostazol was prepared daily and added to the organ bath cumulatively.

The influence of NO on relaxations to cilostazol was specifically addressed by pre-treating the rings with the nitric oxide synthase inhibitor NG nitro-L-arginine methlyl esther (L-NAME, 10^{-4} M) (10). Again, after the contractile response to carbachol, the temperature was changed from 37 to 28 °C. The tissues were allowed to equilibrate at 28 °C for 30 mins. L-NAME was added to the organ bath 20 mins before concentration-response curves were obtained. Endothelium was not denuded because only the role of endothelial NO was examined in this study.

In another series of experiments, the relaxant effect of ACh was investigated in preparations precontracted with 5-HT at 37 and 28 °C. In a different series of the study, the action of sodium nitroprusside (10^{-9}–$3x10^{-4}$ M) in preparations pre-contracted by carbachol (10^{-6} M) was investigated at 37 and 28 °C.

Tab. 1. pIC_{50} values for cilostazol in calf cardiac vein and coronary artery at 37, 28 °C and in the presence of L-NAME (10-6 M). Each value is derived from six experiments. Data are means ± S.E.M.

	pIC50	
	Cardiac vein	Coronary artery
37 °C	5.9±0.3	7.0±0.3
37 °C – L-NAME	4.4±0.2*	5.8±0.4*
28 °C	6.4±0.3**	8.0±0.4**
28 °C – L-NAME	5.8±0.3***	6.5±0.2***

*,**p<0.05 compared to pIC50 values obtained at 37 °C
*** p<0.05 compared to pIC50 values obtained at 28 °C

Tab. 2. Maximum responses (Emax) for cilostazol at 37 °C, 28 °C and in the presence of L-NAME (10-6 M) . Each value is derived from six experiments.

	Emax(%)	
	Cardiac vein	Coronary artery
37 °C	100±0.0[a]	100±0.0
37 °C – L-NAME	89.0±3.0	90.0±4.0
28 °C	81.3±9.0	93.3±6.0
28 °C – L-NAME	82.0±2.0	95.6±4.3

[a] Data are means ± S.E.M.

Statistical analysis

Relaxation responses to cilostazol were expressed as percentages of the carbachol (10^{-6} M) induced contraction. Concentrations of cilostazol causing 50 % of the maximal response (IC_{50}) were calculated from each individual concentration-response curve. Maximal responses (Emax) and pIC_{50} (-log IC_{50}) values for curves obtained before and during cooling were compared by using Student's t test. Statistical significance was set at $p<0.05$.

Drugs

Carbachol chloride, NG nitro-L-arginine methlyl ester, acetylcholine chloride (all dissolved in distilled water) and cilostazol (dissolved in dimethyl sulphoxide; DMSO. The concentration of DMSO in the tissue bath has always been kept below 0.4%) were used. Carbachol and ACh were obtained from Sigma (St. Louis, MO, USA). Cilostazol was kindly provided by Abdi İbrahim Drug Industry (Istanbul, Turkey).

Results

Cardiac vein

In calf cardiac vein, carbachol (10^{-6} M) produced reproducible contractions. After the maximal contractile response to carbachol, cooling to 28 °C did not change this contraction. Maximal response (g) to carbachol was found 0.65±0.04 and 0.62±0.03, at 37 and 28 °C, respectively. Cilostazol (10^{-9}-$3x10^{-4}$ M) produced concentration-dependent relaxation of calf cardiac vein preparations pre-contracted with carbachol at both 37 and 28 °C (cooling).

Figure 1 shows the results in cardiac vein at 37, 28 °C and also in the presence of L-NAME. Compared with 37 °C, the pIC_{50} value of cilostazol was significantly higher ($p<0.05$) at 28 °C (Tab. 1). Furthermore, E_{max} value was not significantly different ($p>0.05$) (Tab. 2).

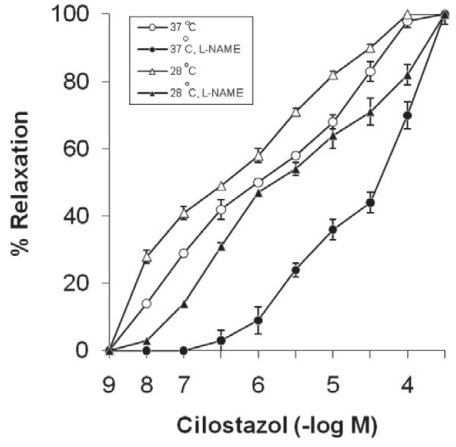

Fig. 1. Concentration-response curves for cilostazol at 37 and 28 °C and in the presence of N^G nitro-L-arginine methyl esther (L-NAME, 10^{-6} M), at 37 and 28 °C, in calf cardiac vein. The preparations were pre-constricted with carbachol (10^{-6} M). Each point is the mean ± SEM of six experiments.

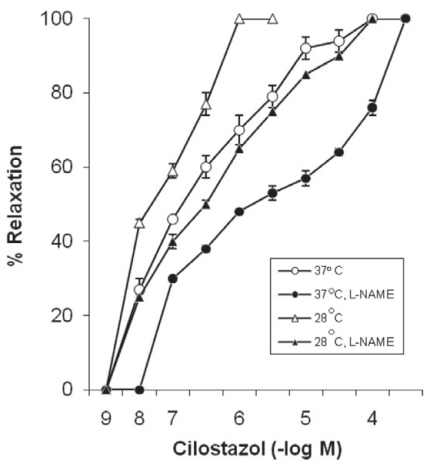

Fig. 2. Concentration-response curves for cilostazol at 37 and 28 °C and in the presence of N^G nitro-L-arginine methyl ester (L-NAME, 10^{-6} M), at 37 and 28 °C, in calf coronary artery. The preparations were pre-constricted with carbachol (10^{-6} M). Each point is the mean ± SEM of six experiments.

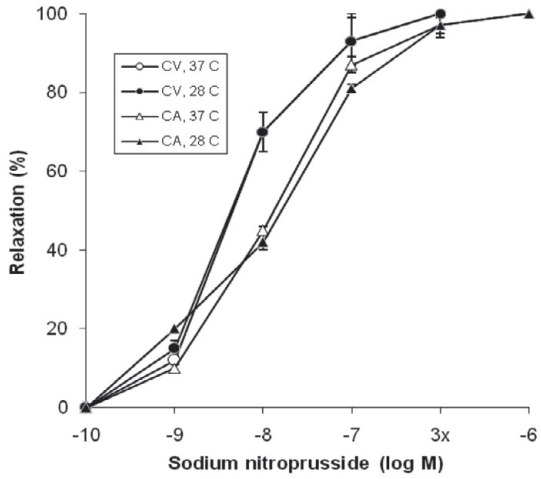

Fig. 3. Responses of calf cardiac vein (CV) and coronary artery (CA) to sodium nitroprusside at 37 and 28 °C. Each point is the mean ± SEM of six experiments.

Pre-incubation with L-NAME significantly decreased the pIC_{50} values of cilostazol at both temperatures ($p<0.05$) (Tab. 1). Furthermore, the sensitivity to ACh was not significantly different between the two temperatures studied (pD_2=6.56±0.05 at 37 °C and 6.58±0.06 at 28 °C, $p>0.05$).

Coronary artery

Figure 2 shows the effects of cilostazol (10^{-9}–$3x10^{-4}$ M) on calf coronary artery at 37 and 28 °C and also in the presence of L-NAME. Carbachol (10^{-6} M) produced contraction in calf coronary artery. Responses to carbachol were reproducible. After the maximal contractile response to carbachol, cooling to 28 °C did not change this contraction. Maximal response (g) to carbachol was found 0.92±0.04 and 0.90±0.12, at 37 and 28 °C, respectively. Cumulative addition of cilostazol (10^{-9}-$3x10^{-4}$ M) produced concentration-dependent relaxation of calf coronary arteries pre-contracted with carbachol at both 37 and 28 °C (cooling). During cooling the pIC_{50} value (Tab. 1) of the coronary artery was significantly higher ($p<0.05$) than at 37 °C. Furthermore, E_{max} value was not significantly different ($p>0.05$) (Tab. 2).

The sensitivity to ACh was not significantly different between the two temperatures studied (pD_2=6.81±0.08 at 37 °C and 6.85±0.04 at 28 °C, $p>0.05$).

Effect of sodium nitroprusside

Sodium nitroprusside-induced concentration-dependent relaxation in cardiac vein and coronary artery and the relaxation to this agent was not influenced by cooling in both preparations (Fig. 3).

Discussion

In the present work, we studied the effects of cooling on cilostazol-induced vasodilatation of calf cardiac vein and coronary artery, paying special attention to the role of NO in these responses. The calf cardiac vein and coronary artery are easily accesible smooth muscle preparations and there is limited information (11–13) about the effect of temperature in these vessels.

The temperature utilized in this study; 28 ºC, for cooling was considered to be 'moderate cooling' temperature according to our previous studies (14,15).

Nitric oxide is a major factor in the cardiovascular system. Its multiple roles include regulation of vasomotor tone and cell adhesion to the endothelium, inhibition of platelet aggregation and vascular smooth muscle cell proliferation (16). This suggests that NO plays a crucial role in the prevention of cardiovascular damage as seen in hypertension, atherosclerosis and other diseases (17). Our results indicate that at 37 ºC, carbachol-induced reproducible contractions in cardiac vein and coronary artery. At this temperature, cilostazol-induced concentration-dependent vasodilatation in both preparations pre-contracted with carbachol. As we know, cilostazol is a selective inhibitor of phosphodiesterase III (3). By increasing intracellular cAMP, in vascular smooth muscle cells are thought to be the following: (i) stimulation of cAMP-dependent protein kinase, which activates a sarcolemmal calcium pump, (ii) stimulation of Na+/K+-ATPase, which results in hyperpolarization and removal of intracellular sodium and calcium, (iii) augmentation of dephosphorylation of the myosin light chain cilostazol activates protein kinase A (PKA), which activates eNOS (18, 19). Nakamura et al (6) reported that cilostazol induced relaxation of the phenylephrine-precontracted rat thoracic aorta in a concentration-dependent manner. In our study, compared with the control responses at 37 ºC, treatment with L-NAME, a NO synthase inhibitor decreased the sensitivity, but not maximal relaxation to cilostazol in both vessels. Our results are in aggrement with another reports (20, 21) showing that cilostazol has elicited a vasodilation of rabbit spinal arterioles regardless of the endothelium function. Birk et al (22) also demonstrated that cilostazol produced a vasodilation of guinea-pig basiler arteries which was

not dependent on the NO pathway. On the other hand, Nakamura et al (6) have reported that the vasodilative action of cilostazol is partially endothelium-dependent, and NO production is increased with cilostazol in the rat thoracic aorta. Itis also reported that (23) urinary excretion of nitrites, a stable metabolite of NO, and basal production of NO of the aortic ring obtained from cilostazol-treated rats were greater than in control animals. These data suggest that cilostazol-induced vasodilation is dependent on NO.

In our study, compared with the control responses at 37 ºC, cooling increased the sensitivity, but not maximal relaxation to cilostazol in both vessels. No previous data on the effects of cilostazol of calf cardiac vein and coronary artery during cooling have been published. On the other hand, there are limited and conflicting reports about the effects of cooling due to using vasodilator agents and tissues. For example, Fernandez et al (24) reported that cooling increased the sensitivity of the relaxation of the central ear artery, a cutaneous vessel, but it did not affect the response of the femoral artery, a noncutaneous vessel, to histamine. It has also been shown that the relaxation to cholinergic stimulation of ear artery, but not of femoral artery, from rabbit was increased during cooling (25). Tiritilli (26) reported that cooling decreased nicorandil, an ATP-sensitive K+ channel-opener-induced relaxation in umbilical arteries. Furthermore, Suzuki et al (27) supported that when temperature was decreased, human pulmonary arteries presented a dilatation which has not been observed in pulmonary veins; during cooling the pulmonary veins constrict while pulmonary arteries dilate. In this study, we used cardiac vein and coronary artery preparations of calf, and observed the same responses in both vessels.

Limited data suggest that changing temperature may also alter the ability of the endothelium to generate or release NO (28–30). In this study, we also investigated the role of NO in cooling induced responses to cilostazol. At 28 °C, inhibition of NO synthesis decreased the sensitivity to cilostazol in both vessels, thus suggesting that during cooling the response to cilostazol may be modulated by NO. With our data we cannot suggest which mechanisms underlie this decreased sensitivity to cilostazol during cooling because the sensitivity was also decreased similarly at 37 °C, in the presence of L-NAME. In a previous study, we observed cooling-induced decreased sensitivity to diazoxide, a K_{ATP} opening drug in calf cardiac vein and coronary artery; the same tissues with this study (15). However, Monge et al (25) reported that changes in temperature might affect the production of NO in a different way depending on vascular beds. Moreover, it is also reported that the increased sensitivity of the relaxation in ear arteries (cutaneous vessels), but not in femoral arteries (deep vessels), to histamine found during cooling seems to be independent of the release of NO (24). The discrepancy between this study (24) and ours may be related to the different relaxant agent used. However, no studies have analyzed the role of NO on cilostazol-induced relaxation during cooling of cardiac vein or coronary artery. In our study, the relaxation to ACh was also studied in preparations pre-contracted with 5-HT at 37 and 28 °C. The sensitivity to ACh was not significantly different between two temperatures studied. Furthermore, L-NAME had no effect on ACh-induced relaxations during cooling. This finding is in line with the findings of others in non-cutaneous vessels such as femoral arteries (23, 31). In our study, the effects of cooling to sodium nitroprusside, a nitrous compound believed to relax vascular smooth muscle cells in a similar way to NO (32) were also studied, and it was found that the relaxations to this substance were not influenced by cooling and it can therefore be assumed that the ability of the smooth muscle cells to relax to nitrous compounds remains essentially unaltered at lowered temperatures both in cardiac vein and coronary artery of calf.

In conclusion, the present results suggest that in calf cardiac vein and coronary artery cooling increased the sensitivity to cilostazol independently on NO. Further studies must be performed to clarify the mechanism of cooling-induced increase in the sensitivity of cilostazol.

References

1. Mustafa S, Thulesius O. Cooling is a potent vasodilator of deep vessels in the rat. Can J Physiol Pharmacol 2001; 79: 899–904.

2. Mustafa S, Thulesius O. Cooling-induced carotid artery dilatation: an experimental study in isolated vessels. Stroke 2002; 33: 256–260.

3. Kambayashi J, Liu Y, Sun B, Shakur Y, Yoshitake M, Czerwiec F. Cilostazol as a unique antithrombotic agent. Curr Pharm Des 2003; 9: 2289–2302.

4. Polson JB, Strada SJ. Cyclic nucleotide phosphodiesterases and vascular smooth muscle. Annu Rev Pharmacol Toxicol 1996; 36: 403–427.

5. Mori K, Takeuchi S, Moritoki H et al. Endothelium-dependent relaxation of rat thoracic aorta by amrinone-induced nitric oxide release. Eur Heart J 1996; 17: 308–316.

6. Nakamura T, Houchi H, Minami A et al. Endothelium-dependent relaxation by cilostazol, a phosphodiesteras III inhibitor, on rat thoracic aorta. Life Sci 2001; 69: 1709–1715.

7. Beavo JA. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol Rev 1995; 75: 725–748.

8. Busch CJ, Graveline AR, Jiramongkolchai K, Sanchez LS, Bloch KD. Phosphodiesterase 3A expression is modulated by nitric oxide in rat pulmonary artery smooth muscle cells. J Physiol Pharmacol 2010; 61: 663–669.

9. Palmer RM. Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524–526.

10. Rees DD, Schulz R, Hudson HF, Palmer RMJ, Moncada S. Identification of some novel inhibitors of the vascular nitric oxide synthase in vivo and in vitro, in: Moncada S., Higgs E.A. (Eds), Nitric oxide from L-arginine: a bioregulatory system, Elsevier, Amsterdam, 1990: 485–487.

11. Atalik KE, Şahin AS, Dogan N. Effects of cooling on contractions of calf coronary artery: Role of the endothelium. Asia Pac J Pharmacol 2001; 15: 33–36.

12. Bodelsson M, Arneklo-Nobin B, Chester AH, Tadjkarimi S, Törnebrandt K, Yacoub M. Differential effect of hypothermia on the vascular tone and reactivity of the human coronary artery and graft vessels. J Cardiavasc Surg 1991; 32: 288–294.

13. Dahdah NS, Russo P, Wagerle LC. Phosphorylation in coronary artery cold-induced contraction in the newborn lamb. Cryobiology 2001; 42: 40–48.

14. Atalik KE, Kilic M, Nurullahoglu ZU, Dogan N. Effects of cooling and warming on 5-hydroxytryptamine- and acetylcholine-induced contractions of human umbilical vessels: role of nitric oxide. Fundam Clin Pharmacol 2008; 22: 37–44.

15. Atalik KE, Kilic M, Dogan N. Role of the nitric oxide on diazoxide-induced relaxation of the calf cardiac vein and coronary artery during cooling. Fundam Clin Pharmacol 2001; 23: 271–277.

16. Andrew JP, Mayer B. Enzymatic function of nitric oxide synthase. Cardiovasc Res 1999; 43: 521–523.

17. Kodja G, Harrison D. Interaction between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. Cardiovasc Res 1999; 43: 562–571.

18. Harris MB, Blackstone MA, Sood SG et al. Acute activation and phosphorylation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. Am J Physiol Heart Circ Physiol 2004; 287: 560–566.

19. Honerjager P. Pharmacology of positive inotropic phosphodiesterase III inhibitors. Eur Heart J 1989; 10: 25–31.

20. Nakamura K, Ikomi F, Ohhashi T. Cilostazol, an inhibitor of type 3 phosphodiesterase, produces endothelium-independent vasodilation in pressurized rabbit cerebral penetrating arterioles. J Vasc Res 2006; 43: 86–94.

21. Yashiro Y, Ohhashi T. Effects of cilostazol, a selective cyclic AMP phosphodiesterase inhibitor on isolated rabbit spinal arterioles. Jpn J Physiol 2002; 52: 471–477.

22. Birk S, Edvinsson L, Olesen J, Kruuse C. Analysis of the effects of phosphodiesterase type 3 and 4 inhibitors in cerebral arteries. Eur J Pharmacol 2004; 489: 93–100.

23. Weintraub WS. The vascular effects of cilostazol. Adv Vasc Biol 2006; 22: 56–60.

24. Fernandez N, Garcia-Villalon AL, Borbujo J et al. Cooing effects on the histaminergic response of rabbit ear and femoral arteries: role of the endothelium. Acta Physiol Scand 1994; 151: 441–451.

25. Monge L, Garcia-Villalon AL, Montoya JJ et al. Role of the endothelium in the cholinergic response of rabbit ear and femoral arteries during cooling. Br J Pharmacol 1993; 109: 61–67.

26. Tiritilli A. Guanylate cyclase and not ATP-dependent K^+ channels seems temperature-dependent in smooth muscle relaxation of human umbilical arteries. Eur J Pharmacol 2000; 406: 79–84.

27. Suzuki S, Sugita M, Ono S, Song C, Kondo T, Fujimura S. Difference in the effects of low temperatures on the tension of human pulmonary artery and vein ring segments. Respiration 2000; 67: 189–193.

28. Bodelsson M, Arneklo-Nobin B, Törnebrandt K. Cooling augments contractile response to 5-hydroxytryptamine via an endothelium-dependent mechanism. Blood Vessels 1989; 26: 347–355.

29. Feelisch M, Noack EA. Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. Eur J Pharmacol 1987; 139: 19–30.

30. Karaki H, Nagase H. Low temperature augments the endothelium-dependent relaxation in isolated rat aorta. Eur J Pharmacol 1987; 142: 129–132.

31. Garcia-Villalon AL, Fernandez N, Monge L, Garcia Jl, Gomez B, Dieguez G. Role of nitric oxide and potassium channels in the cholinergic relaxation of rabbit ear and femoral arteries: effects of cooling. J Vasc Res 1995; 32: 387–397.

32. Ignarro LJ, Kadowitz PJ. The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. Ann Rev Pharmacol 1985, 25: 171–191.

Received March 28, 2012.
Accepted July 20, 2013.