

# Moderate hypothermia attenuates $\alpha_1$ -adrenoceptor-mediated contraction in human varicose spermatic vein: The role of nitric oxide (Short communication)

KE Nurullahoglu-Atalik<sup>1</sup>, A Cenker<sup>2</sup>

<sup>1</sup>Department of Pharmacology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

<sup>2</sup>Department of Urology, Medline Hospital, Konya, Turkey

Received: February 15, 2016

Accepted: September 5, 2016

The effects of moderate hypothermia (28 °C) on the response of human varicose spermatic vein to  $\alpha_1$ -adrenoceptor agonist phenylephrine and the role of endothelial nitric oxide (NO) in these effects were studied. Concentration–response curves for phenylephrine ( $10^{-9}$  to  $3 \times 10^{-4}$  M) were recorded in rings with and without endothelium at 37 and 28 °C. To further analyze the role of NO, in the response to phenylephrine during hypothermia, the effects of this agonist in the presence of N<sup>G</sup>-nitro-L-arginine methyl ester ( $10^{-4}$  M) were also determined. Under every condition tested, phenylephrine produced a marked, concentration-dependent contraction. Sensitivity of intact veins to the agonist was consistently lower at 28 °C than at 37 °C. There was no significant difference in phenylephrine response at 28 and 37 °C in vessels without endothelium but at 28 °C veins without endothelium showed a higher sensitivity than intact veins to phenylephrine. The sensitivity of veins with and without endothelium to nitroprusside ( $10^{-9}$  to  $3 \times 10^{-3}$  M) was significantly decreased during hypothermia, and endothelium removal did not affect the relaxation to this nitrovasodilator. These results suggest that moderate hypothermia decreases the sensitivity of human varicose spermatic vein to phenylephrine probably by increasing the availability of endothelial NO.

**Keywords:** endothelium, hypothermia, NO, phenylephrine, spermatic vein, temperature

## Introduction

Temperature can influence endothelial and vascular smooth muscle cell function and alter the reactivity of the vessels. Moderate hypothermia, cooling to 25–31 °C, has been shown to have a variable influence on the vascular sensitivity to various drugs and endogenous substances in different parts of the vascular system of animals (5). We have previously studied the thermal responses of smooth muscle in various vessels and observed that the sensitivity of different agents were temperature dependent (2, 11). Thus, despite current research to determine the effects of temperature on vascular reactivity of different animal species, studies with human tissues remain incomplete. A varicocele is a dilation of the pampiniform plexus of the spermatic cord; this network of veins is dependent on the spermatic vein. Varicoceles develop as a result of venous reflux in the presence of increased venous pressure or defective venous valves (14). In non-cutaneous vessels, very little was known about the role played by the endothelium in vascular reactivity during moderate hypothermia. Human varicose spermatic vein is an easily accessible smooth muscle preparation but the studies on this vessel are

---

Corresponding author: K Esra Nurullahoglu-Atalik, PhD

Department of Pharmacology, Meram Faculty of Medicine, Necmettin Erbakan University, Konya 42080, Turkey

Phone: +90 332 2237956; Fax: +90 332 2237952; E-mail: [esraatalik@hotmail.com](mailto:esraatalik@hotmail.com)

limited and the effects of cooling on this non-cutaneous vessel have not been reported so far. The present study is the first demonstration of functional changes in the vascular smooth muscle of human spermatic varicose vein during moderate hypothermia.

Endothelium plays a major role in the regulation of vascular tone by releasing relaxing and contracting factors (6). Among the various mediators released by the endothelium, nitric oxide (NO) is of major importance. It has vasodilator activity and its local level is controlled by biosynthesis from the inactive precursor L-arginine. As vascular relaxants, the endothelium can produce NO from L-arginine (9).

In addition to agonist-mediated constriction, limited evidence suggests that vascular smooth muscle responsiveness to NO and related factors can be influenced by temperature (10–12). Recently, we studied the effects of temperature on the contractile response of smooth muscle in various species and vessels, and observed that the contractile responses were temperature dependent but the endothelium seems to have no role in the temperature-induced responses (13–15).

In contrast to the effect of moderate hypothermia on cutaneous vessels, the influence on non-cutaneous vessels especially human vessels is unclear because of limited information and disparate results. Therefore, the purpose of the present study was to determine the effects of moderate hypothermia on phenylephrine-induced responses of human spermatic varicose vein, a non-cutaneous vein, paying special attention to the role of the endothelial NO in these effects.

## Materials and Methods

### *Tissue preparations*

Human spermatic vein was obtained from patients undergoing varicocelelectomy. All the patients had symptomatic varicocele and were suffering from pain and discomfort from swelling. None of our patients had undergone an operation due to infertility. Men with a history of hydrocele and/or absence of any testicular part for any reason, any musculoskeletal disease, or deformity were excluded from the study. The ethics committee of Necmettin Erbakan University Meram Faculty of Medicine granted approval to use the spermatic vein tissue (2015/149). Spermatic veins were placed in 4 °C Krebs–Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, MgSO<sub>4</sub> 1.50, KH<sub>2</sub>PO<sub>4</sub> 1.20, CaCl<sub>2</sub> 2.50, NaHCO<sub>3</sub> 25, glucose 11) and transferred immediately into the laboratory. The veins were cleaned and cut into 3–4 mm rings. The rings were mounted in 15 ml organ baths which were maintained at 37 °C and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The resting tension was adjusted to 0.5 g. Changes in the isometric tension of the rings were recorded using a four-channel force–displacement transducer (BIOPAC MP36, Santa Barbara, CA, USA) connected through amplifiers to a ITBS08 Integrated Tissue Bath System (Commat, Ankara, Turkey).

### *Experimental procedure*

Adequate care was taken to insert the hooks without damaging the endothelium. After the stabilization period, isometric contraction was induced by 5-HT (10<sup>−6</sup> M) and ACh (10<sup>−6</sup> M) was added to verify the integrity of the endothelium. The vascular endothelium was considered complete when the aortic rings showed a relaxation of ≥50%, and non-functional if relaxation was ≤10%. Only one concentration–response curve was generated with phenylephrine in each tissue.

Initially, cumulative concentration–response curves were determined in human spermatic veins with and without endothelium for phenylephrine (10<sup>−9</sup> to 3 × 10<sup>−4</sup> M) at 37 °C.

Another set of experiments was designed to study the effect of moderate hypothermia on the phenylephrine-induced contraction of human spermatic vein rings; 28 °C was considered to be “moderate cooling” temperature according to the previous literature (2). After regaining basal conditions, the temperature was changed from 37 to 28 °C. Temperature changes were rapidly achieved and maintained for 30 min before and during the construction of the concentration–response curve for phenylephrine. Then, cumulative concentration–response curves were determined in the tissues with and without endothelium at 28 °C. To analyze the role of endothelial NO in the vascular response, concentration–response curves to phenylephrine were obtained in intact vein rings in the presence of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME, 10<sup>-4</sup> M), an NO synthase inhibitor, at 37 and 28 °C. This drug was added to the bath 20 min before applying phenylephrine. Furthermore, the relaxant action of sodium nitroprusside (10<sup>-9</sup> to 3 × 10<sup>-3</sup> M) in preparations precontracted by 5-HT (10<sup>-6</sup> M) was investigated at 37 and 28 °C.

### Drugs

The following compounds were used: phenylephrine, acetylcholine chloride, N<sup>G</sup>-nitro-L-arginine methyl ester hydrochloride and sodium nitroprusside. All drugs were obtained from Sigma (St. Louis, MO, USA) and dissolved in distilled water.

### Statistical analysis

Concentrations of phenylephrine causing 50% of the maximal response (EC<sub>50</sub>) were calculated from each individual concentration–response curve. The maximum effect values were calculated as the percentage of the maximum response of the tissue to phenylephrine at 37, 28, and 28 °C in the presence of L-NAME, respectively. All data were expressed as mean ± SE of the mean. Statistical analysis was performed using two-way ANOVA and unpaired Student's *t*-test where appropriate. All statistical analyses were performed using the SPSS for Windows version 13.0 software program (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant when *p* < 0.05.

## Results

Phenylephrine produced a marked, concentration-dependent contraction of the vein rings in all cases. The response of veins with endothelium differed at 37 and 28 °C (Fig. 1). The sensitivity of intact veins to phenylephrine was 6.00 ± 0.20 at 37 °C and 5.00 ± 0.15 during hypothermia. Hypothermia decreased the sensitivity (10 times, *p* < 0.05) and maximal contraction was similar at both temperatures. Veins without endothelium showed a response that was similar at 37 °C (pD<sub>2</sub> = 6.22 ± 0.16) and 28 °C (pD<sub>2</sub> = 6.15 ± 0.30). At 37 °C, the sensitivity of vessels to phenylephrine was similar in veins with and without endothelium. However, at 28 °C in veins without endothelium, the concentration–response curves were shifted to the left (*p* < 0.05) in comparison to intact veins. Endothelium removal did not significantly affect the maximal contraction to phenylephrine at either temperature. In intact veins, treatment with L-NAME (10<sup>-4</sup> M) attenuated the effects of moderate hypothermia on the response to phenylephrine (pD<sub>2</sub> = 5.74 ± 0.13, *p* < 0.05).

During hypothermia, a rightward shift (*p* < 0.05) was found in response to sodium nitroprusside with respect to that obtained at 37 °C (Fig. 2) and endothelium removal did not affect the relaxation evoked by this nitrovasodilator either at 37 or 28 °C (data not given).

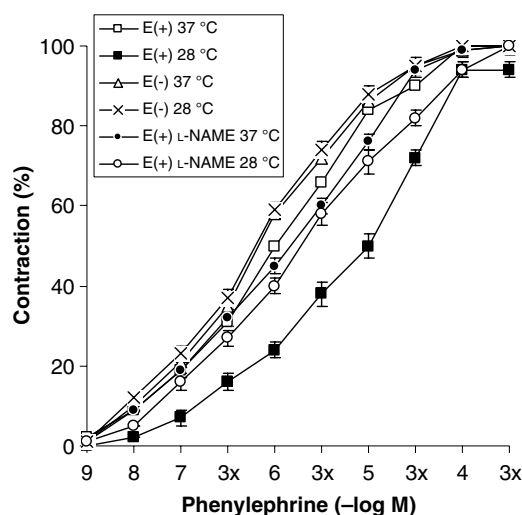


Fig. 1. Contractile response of human spermatic vein to phenylephrine at 37 and 28 °C in rings with and without endothelium and in the presence of L-NAME ( $10^{-4}$  M) during moderate hypothermia. Each point is the mean  $\pm$  SEM of eight experiments

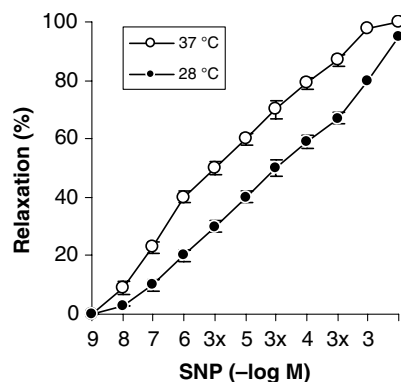


Fig. 2. Relaxation response of human varicose spermatic vein to sodium nitroprusside. Responses obtained at 37 °C (control) and 28 °C in veins with endothelium. Each point is the mean  $\pm$  SEM of eight experiments

## Discussion

We studied the effects of moderate hypothermia on  $\alpha_1$ -adrenergic receptor agonist phenylephrine-induced contractions of human spermatic varicose vein, paying special attention to the role of NO in these effects. The human spermatic vein is a non-cutaneous vessel and this *in vitro* study is the first demonstration of moderate hypothermia-induced changes on  $\alpha_1$ -adrenergic receptor-induced contractile responses of this vessel. In our previous study (unpublished data), we observed no response with  $\alpha_2$ -adrenergic receptor agonists in human varicose spermatic vein.

In this study, phenylephrine caused concentration-dependent contractions of human spermatic varicose vein with endothelium at 37 °C. As we know, phenylephrine, an  $\alpha_1$ -adrenoceptor agonist, can cause contractions both via an influx of extracellular  $\text{Ca}^{2+}$  and the intracellular release of  $\text{Ca}^{2+}$ . At 37 °C, the sensitivity of the spermatic vein to phenylephrine was not affected significantly by removing the endothelium, thus suggesting that the  $\alpha_1$ -adrenergic contraction of this vein is mostly caused by activation of  $\alpha_1$ -adrenoceptors located in the smooth musculature. The main finding of this study was

that moderate hypothermia significantly decreased the sensitivity to phenylephrine. The reduction of the adrenergic contraction by moderate hypothermia found in this vein is in accordance with that described in deep, non-cutaneous blood vessels (15). Furthermore, it has been reported that contrary to the contractile responses in cutaneous vessels, the aorta and pulmonary artery dilate when exposed to hypothermia (7).

In the vasculature, NO is physiologically important for maintaining vascular homeostasis and it keeps the vessels dilated. Although efforts are currently being made to understand the regulation, production, and function of endothelial NO, its role in the effects of cooling on vascular reactivity has been little studied. Limited data suggest that changing temperature may also alter the ability of the endothelium to generate or release NO (6). In this study, there was no significant difference in response to phenylephrine at 28 and 37 °C in vessels without endothelium. In our study, endothelium removal reversed the inhibitory effect of moderate hypothermia on the  $\alpha_1$ -adrenoceptor response. This suggests that the inhibitory effect of moderate hypothermia on the adrenergic constriction is mediated by an endothelium-dependent mechanism. Treatment with L-NAME also increased the sensitivity to phenylephrine at 28 °C, suggesting that the production of NO in the endothelium can be increased under moderate hypothermia. Thus, the response of the spermatic vein to moderate hypothermia in this study is in agreement with that of the cutaneous vessels of different species (9, 17). The reason for this discrepancy may be related to differences between arteries and veins, skin vascular regions, and perhaps species.

Some recent studies showed an excessive release of NO in the dilated spermatic vein (13, 16), but no study investigated the role of NO in vascular reactivity of this vein. Although the vessel used in this study was non-cutaneous, the results contrasted our previous findings (1) that NO had no role in the cooling-induced responses of different non-cutaneous vessels. However, we have no explanation for this feature. Perhaps, the effects of NO during moderate hypothermia may be spermatic varicose vein specific. Further studies must be performed for this postulate. The basic mechanisms underlying moderate hypothermia-induced reactions of smooth muscle in blood vessels and other conduits of the body are regionally different and adjusted to serve the functional requirements of the organism when exposed to moderate hypothermia (3). It has also been reported that changes in temperature might affect the production of NO in a different way depending on vascular beds (10).

In this study, we also observed that the relaxation of the human spermatic varicose vein to sodium nitroprusside, a compound that relaxes vascular smooth muscle in a similar way to NO (4), decreased during moderate hypothermia. Similarly, it has been shown that the relaxation of the rabbit ear artery to nitroprusside was decreased during moderate hypothermia (9). These results support our observation. The decreased relaxation to nitroprusside observed during cooling could indicate that increased availability of endogenous NO downregulates sensitivity to exogenous NO (8).

The limitation of this study is that the study preparation was selected from only varicocele patients. The vessels we used were obtained from patients with symptomatic varicocele. We did not evaluate the fertility of these patients, whose major complaint was pain and discomfort from swelling. This study did not have a chance of making a control group.

In conclusion, the present results suggest that moderate hypothermia decreases the sensitivity of human spermatic varicose vein, a non-cutaneous vein, to phenylephrine probably by increasing the availability of endothelial NO.

## REFERENCES

1. Atalik KE, Şahin AS, Kiliç M, Doğan N: Role of the endothelium on the response to adrenoceptor agonists of rabbit aorta during cooling. *Fundam. Clin. Pharmacol.* 14, 25–30 (2000)
2. Atalik KE, Kiliç M, Doğan N: Role of the nitric oxide on diazoxide-induced relaxation of the calf cardiac vein and coronary artery during cooling. *Fundam. Clin. Pharmacol.* 23, 271–277 (2009)
3. Fernández N, Monge L, García-Villalón AL, García JL, Gómez B, Diéguez G: Cooling effects on nitric oxide production by rabbit ear and femoral arteries during cholinergic stimulation. *Br. J. Pharmacol.* 113, 550–554 (1994)
4. Ignarro LJ, Kadowitz PJ: The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu. Rev. Pharmacol. Toxicol.* 25, 171–191 (1985)
5. Jantschak F, Popp AM, Hofmann RA, Villalón CM, Centurión D, Pertz HH: Postjunctional  $\alpha_{2c}$ -adrenoceptors mediate vasoconstriction in rat tail artery: influence of precontraction and temperature on vasoreactivity. *Naunyn Schmiedebergs Arch. Pharmacol.* 382, 487–497 (2010)
6. Karaki H, Nagase H: Low temperature augments the endothelium dependent relaxation in isolated rat aorta. *Eur. J. Pharmacol.* 142, 129–132 (1987)
7. Lubbe AS: Heat shock attenuates endothelium-dependent vasodilation in skeletal muscle microcirculation. *Shock* 2, 179–184 (1994)
8. Moncada S, Rees DD, Schulz R, Palmer RM: Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc. Natl. Acad. Sci. U S A* 88, 2166–2170 (1991)
9. Monge L, García-Villalón AL, Montoya JJ, García JL, Fernández N, Gómez B, Diéguez G: Role of the endothelium in the response to cholinceptor stimulation of rabbit ear and femoral arteries during cooling. *Br. J. Pharmacol.* 109, 61–67 (1993)
10. Monge L, García-Villalón AL, Montoya JJ, García JL, Gómez B, Diéguez G: Response of rabbit ear artery to endothelin-1 during cooling. *Br. J. Pharmacol.* 104, 609–612 (1991)
11. Nurullahoglu Atalik KE: Role of calcium on contractile response of calf cardiac vein during cooling. *J. Smooth Muscle Res.* 47, 135–142 (2011)
12. Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327(6122), 524–526 (1987)
13. Romeo C, Ientile R, Santoro G, Impellizzeri P, Turiaco N, Impalà P, Cifalà S, Cutroneo G, Trimarchi F, Gentile F: Nitric oxide production is increased in the spermatic veins of adolescents with left idiopathic varicocele. *J. Pediatr. Surg.* 36, 389–393 (2001)
14. Stahl P, Schlegel PN: Standardization and documentation of varicocele evaluation. *Curr. Opin. Urol.* 21, 500–505 (2011)
15. Yamazaki F, Sone R, Zhao K, Alvarez GE, Kosiba WA, Johnson JM: Rate dependency and role of nitric oxide in the vascular response to direct cooling in human skin. *J. Appl. Physiol.* 100, 42–50 (2006)
16. Yildiz O, Gul H, Ozgok Y, Onguru O, Kilciler M, Aydin A, Isimer A, Harmankaya AC: Increased vasoconstrictor reactivity and decreased endothelial function in high grade varicocele; functional and morphological study. *Urol. Res.* 31, 323–328 (2003)
17. Zerpä H, Berhane Y, Elliott J, Bailey SR: The effect of cooling on the contractility of equine digital small lamellar arteries: modulating role of the endothelium. *Exp. Physiol.* 95, 1033–1042 (2010)