Effect of mild hypothermia on the vascular actions of phenylephrine in rat aortic rings

F. Lagneau*, P. Kirstetter², C. Bernard³ and J. Marty¹

¹Department of Anaesthesia, Hôpital Beaujon, Clichy, INSERM U-408, Hôpital Bichat, Paris, France.
²Polyclinique de Savoie, Annemasse, Paris, France. ³Department of Anaesthesia, Hôpital Lariboisière, Paris, France

*Corresponding author: Service d’Anesthésie–Réanimation, Hôpital Beaujon, 100 Bd du Général Leclerc, F-92100 Clichy, France

Intraoperative mild hypothermia is common. We have investigated the effects of mild hypothermia (34 vs 38°C) on phenylephrine- (10⁻⁸ to 10⁻⁵ mol litre⁻¹) induced contractions of rat aortic rings mounted for isometric tension recordings. A marked decrease in Emax (maximal tension) (P<0.05) and significant increase in EC₅₀ (phenylephrine concentration producing 50% of maximal tension) were observed at the lower temperature in endothelium intact rings, but there was no effect of temperature when the endothelium had been removed. The decreased contraction with hypothermia in the endothelium intact vessels was restored to 84% by administration of the nitric oxide synthase inhibitor L-NNA and a small additional amount of tone was restored in the presence of the cyclooxygenase inhibitor, indomethacin. We conclude that mild hypothermia markedly decreased phenylephrine-induced rat aortic contraction in vitro by endothelium dependent mechanisms, largely related to increased nitric oxide production or action.

Br J Anaesth 1999; 82: 938–40

Keywords: sympathetic nervous system, phenylephrine; temperature, effects; complications, hypothermia; pharmacology, nitric oxide; rat

Accepted for publication: January 20, 1999

Mild intraoperative hypothermia may modify the pharmacological properties of phenylephrine, which is used commonly during this period.¹ Direct vascular effects of drugs are difficult to investigate in vivo. In this study, we have assessed mild hypothermia-induced changes in the vascular pharmacodynamic proprieties of phenylephrine in vitro.

Methods and results

Male Sprague–Dauley rats (Ifacredo, France) were used according to European legislation involving animals. Aortic reactivity was analysed in aortic rings in organ chambers using a standardized protocol, as reported previously in our laboratory.² Briefly, animals were anaesthetized with pentobarbital 50 mg kg⁻¹ i.p. The thoracic aorta was removed and placed in a modified HEPES buffer aerated with a mixture of 95% oxygen–5% carbon dioxide. Aortae were divided into 3.5–4.0-mm ring segments. In some rings, endothelium was removed mechanically by gently rubbing the intimal surface with a stainless steel rod. The rings were suspended between two L-shaped stainless hooks and placed in a 10-ml water-jacketed organ chamber containing a solution composed of (mmol litre⁻¹): NaCl 118.2, KCl 4.7, NaHCO₃ 25.0, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, glucose 11.1 and HEPES 5. Organ chambers were aerated continuously with 95% oxygen–5% carbon dioxide and pH was maintained at 7.35–7.4. Organ bath temperature was set at either 34 or 38°C, and controlled strictly during the experiment by thermal probes (Physitemp BAT-10, Bioblock Scientific, France). Isometric tension was measured using a force displacement transducer (UF1 Pioden Controls, Paris, France) connected to the upper hook. Tension was recorded using a data acquisition system (MacLab System, AD Instruments Pty Ltd, Australia). A 90-min period was used to stabilize resting tension at 0.5 g, with systematic replacement of bath solution every 15 min.

Vascular response to phenylephrine was tested using increasing concentrations of phenephrine from 10⁻⁸ to 10⁻⁵ mol litre⁻¹ (L-Phenylephrine, Sigma Chemical Co., St Louis, MO, USA). The integrity of the endothelium was assessed by testing relaxation produced by addition of acetylcholine 10⁻⁶ mol litre⁻¹ on phenylephrine 10⁻⁵ mol litre⁻¹ precontracted rings. The endothelium was considered to be intact when acetylcholine elicited a minimum of 30% vasodilatation. The endothelium was considered to be removed when acetylcholine failed to produce vasodilatation. The role of
endothelium was assessed by comparing phenylephrine-induced contractions in the presence (E+) or absence (E–) of endothelium. Specific roles of nitric oxide and prostanoid endothelial pathways were assessed by comparing phenylephrine-induced contraction of intact rings in the presence or absence of N-nitro-L-arginine 10−5 mol litre−1 (L-NNA, Sigma Chemical Co., St Louis, MO, USA) for nitric oxide, indomethacin 10−5 mol litre−1 (Sigma Chemical Co.,) for prostanoids, and both L-NNA and indomethacin together. Media relaxation was tested using increasing concentrations of a direct nitric oxide donor (sodium nitroprusside (SNP), Nitriate, L’arguenon, France) from 10−8 to 10−6 mol litre−1. Mean (SEM) variations in tension were expressed as gram per milligram of dry weight (g mg−1) for contraction, and as percentage decrease in phenylephrine-induced maximal tension for relaxation.

Curve-fitting was performed using a non-linear regression analysis (SPSS software package) to obtain the variables of the Hill equation:

\[ E(C) = E_0 + ((Emax \times C^N)/(C^N + EC_{50}^N)) \]

where \( E(C) = \) tension induced by the drug at concentration \( C \), \( E_0 = \) resting tension, \( Emax = \) maximum tension in response to the drug, \( EC_{50} = \) concentration that produces 50% of (Emax–E0) and \( N = \) steepness of the curve. Comparisons between groups were made by comparing Emax and EC50 using ANOVA. The statistical package was Statview SE (Abacus Concept, Inc, Berkley, CA, USA). \( P < 0.05 \) was considered statistically significant.

At 34°C, when endothelium was present (n=12), Emax markedly and significantly decreased compared with at 38°C (n=15), and EC50 increased significantly (Fig. 1). In contrast, when the endothelium was removed, there was no difference between temperatures of 34°C (n=24) and 38°C for both Emax and EC50. At 38°C, there was no significant difference in Emax or EC50 values with or without indomethacin, L-NNA, or the combination. However, Emax was significantly increased and EC50 significantly decreased by indomethacin (n=10), L-NNA (n=12), or both (n=16) at 34°C (Fig. 1). When indomethacin and L-NNA were both added to the bath at 34°C, the resulting Emax and EC50 values were not significantly different from those in the control group at 38°C. SNP-induced endothelium independent vasorelaxation was not different at 34°C compared with at 38°C, for both Emax and EC50.

**Comment**

The main finding of the study was that mild hypothermia significantly decreased the pressor response of intact aortic vessels to the α-agonist, phenylephrine. We demonstrated that the media environment was not involved in this alteration as there was no significant difference in pressor responses at 34 and 38°C in vessels without endothelium. Therefore, we have shown that the endothelium was responsible mainly for this change in phenylephrine-induced contraction at 34°C. Endothelium-derived vasodilators (nitric oxide and prostanoids) may be involved as pretreatment with L-NNA, indomethacin, or both, partially restored normal reactivity to phenylephrine at 34°C compared with at 38°C.

The mechanisms by which nitric oxide and prostanoids are involved in this altered response to phenylephrine at 34°C are still unclear. Increased sensitivity of smooth muscle cells to nitric oxide can be excluded in part as there was no significant difference in the response to SNP at 34 and 38°C. An increase in endothelium-dependent nitric oxide production is possible as endothelium-dependent relaxation, which occurs with administration of phenylephrine, is mediated via \( \alpha_2 \)-adrenergic receptors. An increase in phenylephrine affinity for such receptors during mild hypothermia has been reported and could then increase nitric oxide production. Moreover, endothelial nitric oxide production depends on constitutive nitric oxide synthase enzyme activity which depends on cytosolic calcium concentration. Marked hypothermia increases free calcium concentration by changing cytoskeleton properties, enzyme activities, Na–Ca exchanger function and acid–base metabolism. Hypothermia-induced decrease in nitric oxide clearance may also be involved. Indeed, a decrease in the production of oxygen-derived free radicals, which are known to be nitric oxide scavengers, is suspected during mild hypothermia causing an increase in nitric oxide availability in organ baths.

In conclusion, mild hypothermia markedly decreased phenylephrine-induced rat aortic contraction in vitro by endothelium-dependent mechanisms. Endothelium-depend-
ent vasodilators are probably involved, in particular an increase in nitric oxide production or the action of nitric oxide in response to phenylephrine when temperature moderately decreases, as often occurs during anaesthesia.

Acknowledgements

We thank D. Rouelle for technical assistance and A. Tedgui for useful comments. Supported by Contrat INSERM-CNAMTS 1992.

References