Effects of Norepinephrine on Isolated Rabbit Aorta at Different Temperatures

Huei-Yann Tsai[1,2], Chiou-Ling Chu[1], Wen-Tsong Hsieh[1],
Yuh-Fung Chen[1], Cheng-Chun Lee[3]

[1]Department of Pharmacology and Institute of Medical Sciences, China Medical College; [2]Department of Pharmacy and [3]Department of Medical Research, China Medical College Hospital, Taichung, Taiwan, R.O.C.

The change in body temperature is one of the most important factors in physiological functions. Early studies have suggested that the cutaneous arteries of the rabbit are influenced by temperature changes. Low temperature stimulates the release of nitric oxide from the endothelium and prevents a cold-induced vasospasm by relaxation of the vessels. However, the influence of temperature changes on deep vessels is not clear. The preparation of isolated rat aortic strips was used in this study. The temperatures in the organ bath were changed, using a thermometer as a guide. From our results, the isolated rabbit aorta relaxed when the organ bath temperature was lowered from 37°C to 20°C. The tension of the vascular smooth muscle increased when the temperature was increased from 20°C to 37°C. Norepinephrine-induced contraction in the isolated rabbit aorta was influenced by different temperatures. The optimal temperature for the isolated rabbit aorta was 35°C to 37°C in the *in vitro* experiment. **(Mid Taiwan J Med 1999 : 4 : 57-61)**

Key words

different temperatures, norepinephrine, vascular smooth muscle

INTRODUCTION

In *in vitro* studies, the temperature is always carefully controlled at 37°C, which is the normal human body temperature. However, some scholars have suggested that the temperature should be set at 34°C [1,2] or 35°C [3] because the *in vitro* studies did not consider the impact of the effects of temperature upon the blood vessels. Early in the 1980s, Janssens et al. [4] and Shepherd et al. [5] observed that the cutaneous arteries reacted with a change in temperature. In 1991, Monge et al. [6] started discussing the differences between femoral arteries and cutaneous ear arteries at a lower temperature, i.e., 24°C, and found that this was related to the endothelium. Later on, they discovered that vasodilation of the cutaneous arteries was due to the production of nitric oxide (NO) at low temperatures. However, no one has discussed why vasocontraction and vasodilation occurred under continuous temperature changes and what the impact of temperatures on norepinephrine (NE) was. Therefore, the main goal of this study was to explore the effects of norepinephrine on isolated rabbit aortas under different temperatures.

Received : August 18, 1998. Revised : October 29, 1998.
Accepted : November 23, 1998.

Address reprint requests to : Huei-yann Tsai, Department of Pharmacology and Institute of Medical Sciences, China Medical College, No 91, Hsueh-Shih Road, Taichung, Taiwan, R.O.C.

MATERIALS AND METHODS

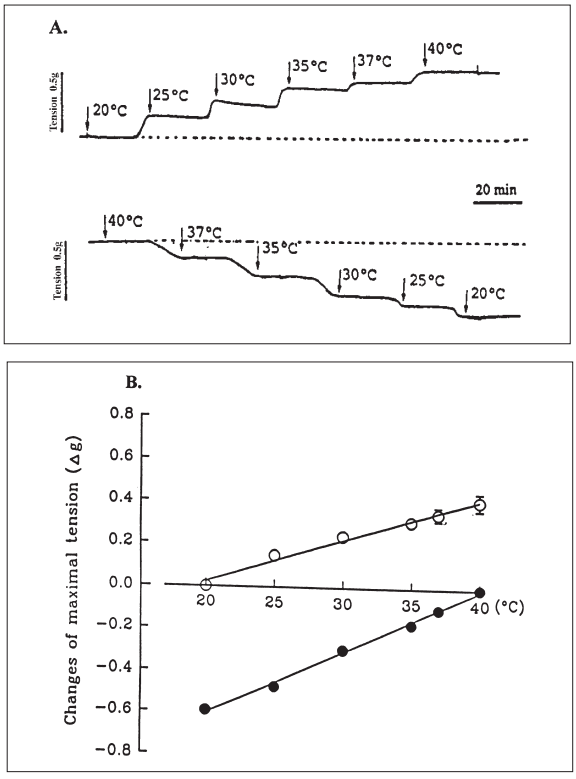

Fig.1 Effect of norepinephrine (NE, 10^{-7}M) in different temperatures on the isolated rabbit aorta. A: A typical pattern of NE-induced vasocontraction in different temperatures. B: The averaged response of NE-induced vasocontraction in different temperatures (n=8). O : Temperature from 20°C to 40°C; ● : Temperature from 40°C to 20°C.

The preparation of aortic strips was similar to that originally described by Furchgott and Zawadzki [7]. Male New Zealand white rabbits (n=8) weighing 2-3 kg were killed by a blow on the head and exsanguinated. The thoracic aorta, which was isolated from the animal, was carefully cleaned of surrounding fat and connective tissue and cut into rectangular strips (15 mm in length, 2 mm in width). Preparations were mounted vertically in 20-ml organ baths containing 10 ml oxygenated (95:5 O_2/CO_2) Krebs Ringer solution (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, $KH_2PO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 2.5 and glucose 11, pH 7.3-7.4 at 37°C. The tissue was allowed to equilibrate for 90 min under a resting tension of 2 g prior to experimentation. The isometric tension was recorded with a force-displacement transducer (Gould Instrument Systems, U.S.A.) connected to a Gould 2600s polygraph (Gould Instrument Systems, U.S.A.).

The reagents, NaCl, $NaHCO_3$, KCl, $KH_2PO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$ and glucose, were purchased from Merck KgaA Darmstadt, Germany. NE was purchased from Sigma Chemical Co., U.S.A.

Results of the experiment were expressed as mean ± S.E. The data were analyzed by Student's *t*-test. A $p < 0.05$ was defined as statistically significant.

RESULTS

When the temperature in the organ bath was increased gradually from 20°C to 25°C, the contraction effect of the vascular smooth muscle increased, and this effect became optimal when the temperature reached 40°C. When the temperature was decreased from 40°C to 20°C, the effect on vasocontraction was decreased, and became minimal at 20°C. The effect of vasocontraction of the vascular smooth muscles was temperature dependent. A better effect was seen when the temperature was kept at 37 ± 0.5°C because the vascular smooth muscles may possibly be damaged at 40°C despite the stronger effect of vasocontraction at this temperature (Fig. 1). At different temperatures, such as 20, 25, 30, 35, and 40°C, the effect of NE on vasocontraction was the strongest when the temperature reached 37°C. When NE was added in the second and the third trials, the effect was even more profound than in the previous trial (Fig. 2).

DISCUSSION

The initial phasic contraction induced by NE in smooth muscle results from intracellular Ca^{2+} release following an increase in the turnover of phosphatidylinositol and the production of inositol 1,4,5-triphosphate [8]. This contractile process is related to the depletion of Ca^{2+} stores sensitive to NE and is

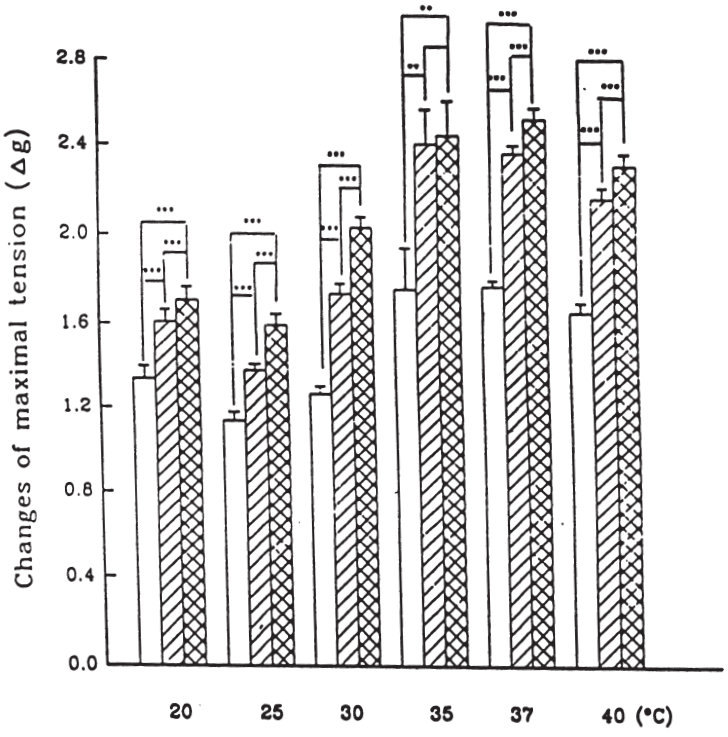

Fig.2 Effect of norepinephrine (NE, 10^{-7}M) and temperatures on tension of isolated rabbit aorta (n=8). ▨: the first time of NE administration, ▧: the second time of NE administration, ▭: the third time of NE administration. $^{**}p< 0.01$ and $^{***}p< 0.001$ as compared with NE, respectively.

linked to Ca^{2+} entry through voltage-operated Ca^{2+} channels and α-adrenoceptors [9]. As for the aorta, the effect of vasocontraction was weaker when the temperature was lower than 37°C. Different agents produced different effects. For example, caffeine could induce a stronger vasocon-traction effect when the temperature was changed from 37°C to 22°C [10]. However, in 1993, Booth et al. [11] observed the effect of glyceryltrinitrate (GTN) on rabbit aortas, and found that GTN produced less NO when the temperature was decreased from 37°C to 27°C. The choice of temperature was, therefore, a major factor in this experiment. The temperature dependence of the transient increase in free intracellular Ca^{2+} to bradykinin and Ca^{2+}/Mg^{2+} entry through a Ca^{2+} store-operated Ca^{2+} entry pathway was suggested by Paltauf-Doburzynska and Graier [12]. Moritoki et al. [1,2] used 34°C because 37°C is not applicable in some parts of the experiment to produce NO. Therefore, in this experiment, by changing the temperature, whether increasing the temperature from 20°C to 37°C, or decreasing the temperature, the temperature in the 35°C to 37°C range showed a better result in the rabbit aorta experiment. Analyzing the effect of vasocontraction, 35°C to 37°C was the most suitable temperature in the rabbit aorta experiment. When the temperature gradually changed, the vasocon-traction also gradually changed. NE (10^{-7} M) showed the best effect for contraction in rabbit aorta at 35°C to 37°C.

REFERENCES

1. Moritoki H, Miyano H, Takeuchi S, et al. Endothelin-3-induced relaxation of rat thoracic aorta: a role for nitric oxide formation. *Br J Pharmacol* 1994;108:1125-30.
2. Moritoki H, Hisayama T, Takeuchi S, et al. Relaxation of rat thoracic aorta induced by the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid, possibly through nitric oxide formation. *Br J Pharmacol* 1994;111:655-62.
3. Kenny BA, Fraser S, Kilpatrick AT, et al. Selective

antagonism of calcium channel activators by fluspirilene. *Br J Pharmacol* 1990;100:211-6.
4. Janssens WJ, Verbeuren TJ, Vanhoutte PM. Effect of moderate cooling on adrenergic neuroeffector interaction in canine cutaneous veins. *Blood Vessels* 1981;18:281-95.
5. Shephered JT, Rusch NJ, Vanhoutte PM. Effect of cold on the blood vessel wall. *General Pharmacol* 1983;14:61-4.
6. Monge L, Garcia-Villalon AL, Montoya JJ, et al. Response of rabbit ear artery to endothelin-1 during cooling. *Br J Pharmacol* 1991;104:609-12.
7. Funchgott RF, Zawadzki JV. The oligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288:373-6.
8. Bray KM, Weston AH, Duty S, et al. Differences between the effects of cromakalim and nifedipine on agonist-induced responses in rabbit aorta. *Br J Pharmacol* 1991;102:337-44.
9. Noguera MA, D'Ocon MP. Evidence that depletion of internal calcium stores sensitive to noradrenaline elicits a contractile response dependent on extracellular calcium in aorta. *Br J Pharmacol* 1993;110:861-7.
10. Sato K, Ozaki H, Karaki H. Multiple effects of caffeine on contraction and cytosolic free Ca^{2+} levels in vascular smooth muscle of rat aorta. *Naunyn-Schmiedeberg's Arch Pharmacol* 1988; 338:443-8.
11. Booth BP, Brien JF, Marks G, et al. Effects of temperature on glyceryl trinitrate induced relaxation of rabbit aorta. *Can J Physiol Pharmacol* 1993;71:629-32.
12. Paltauf-Dobuzynska J, Graier WF. Temperature dependence of agonist-stimulated Ca^{2+} signaling in cultured endothelial cells. *Cell Calcium* 1997; 21:43-51.

不同溫度下Norepinephrine 對於家兔主動脈血管收縮之影響

蔡輝彥[1,2] 朱秋玲[1] 謝文聰[1] 陳玉芳[1] 李正淳[3]

中國醫藥學院 藥理學科暨醫學研究所[1]

中國醫藥學院附設醫院 藥劑部[2] 醫學研究部[3]

體溫的變化是影響生物體生理功能的因素之一，早期有學者提出溫度對於兔子體表動脈血管(cutaneous arteries)具影響，並發現低溫會引起內皮（endothelium）產生一氧化氮（nitric oxide, NO）舒張血管，以防止血管因低溫過度攣縮(cold-induced cutaneous vasospasm)，對於深層血管（deep vessels）例如主動脈血管（aorta）的溫度連續變化的影響研究報告尚少。本實驗結果顯現，當存在血管的溫度低於37°C時，其收縮力較弱；隨著溫度增加，收縮力增加；反之，隨溫度降低，血管收縮力亦降低，呈現溫度依存性（temperature-dependence）。並且觀察到norepinephrine 在不同溫度時收縮力亦不同，37°C時血管收縮力最大，而20°C 時收縮力最小。由以上實驗顯示，在家兔的離體主動脈血管實驗中，器官槽中的溫度選擇以35°至37°C反應能力最佳。（ **中台灣醫學科學雜誌 1999；4：57-61** ）

關鍵詞

不同溫度，norepinephrine， 血管平滑肌

聯絡作者：蔡輝彥

地　　址：台中市學士路91號

中國醫藥學院 藥理學科暨醫學研究所

收文日期：8/18/1998　　修改日期：10/29/1998

接受日期：11/23/1998