Role of the endothelium in the response to cholinceptor stimulation of rabbit ear and femoral arteries during cooling

L. Monge, A.L. García-Villalón, J.J. Montoya, J.L. García, N. Fernández, B. Gómez & ¹G. Diéguez

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, 28029 Madrid, Spain

1 The role of the endothelium in the effects of cooling on the response to cholinceptor stimulation of the rabbit central ear (cutaneous) and femoral (non-cutaneous) arteries was studied using 2 mm long cylindrical segments.

2 Concentration-response curves for acetylcholine (10^{-9} – 10^{-5} M), methacholine (10^{-9} – 10^{-5} M) and sodium nitroprusside (10^{-9} – 10^{-4} M) were isometrically recorded in arteries under conditions, with and without endothelium or following pretreatment with the nitric oxide-synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-6} – 3×10^{-4} M) at 37°C and at 24°C (cooling).

3 Ear and femoral arteries showed endothelium-dependent relaxation to acetylcholine and methacholine at 37°C and 24°C. The extent of relaxation of the control ear arteries, but not of the control femoral arteries, to acetylcholine and methacholine increased during cooling.

4 L-NAME (10^{-6} – 3×10^{-4} M) reduced in a concentration-dependent way the response of ear arteries to acetylcholine at both 37°C and 24°C, this reduction being more potent at 37°C. L-Arginine (10^{-5} – 10^{-3} M) reversed in a concentration-dependent manner the inhibitor effects of 10^{-5} M L-NAME at both temperatures.

5 Sodium nitroprusside caused a concentration-dependent relaxation in both arteries that was endothelium-independent. However, the extent of relaxation to this nitrovasodilator in ear and femoral arteries was lower at 24°C.

6 These results suggest that cooling augments the reactivity of cutaneous (ear) arteries, but not that of non-cutaneous (femoral) arteries to cholinceptor stimulation by endothelium-mediated mechanisms. Cooling could therefore facilitate the stimulated release of endothelial nitric oxide in cutaneous vessels.

Keywords: Skin arteries; temperature; nitric oxide; acetylcholine; sodium nitroprusside

Introduction

It appears that cooling affects cutaneous vascular responses (Vanhoutte, 1980) and also it is widely accepted that the endothelium plays a major role in the regulation of vascular tone by releasing relaxing and contracting factors (Furchgott & Vanhoutte, 1989). The endothelium can produce nitric oxide, the precursor of which is L-arginine (Palmer *et al.*, 1987; 1988; Ignarro *et al.*, 1987). The role of the endothelium in the effects of cooling on vascular reactivity has been studied very little (Karaki & Nagase, 1987; Bodelsson *et al.*, 1989) and it could be of interest, especially in cutaneous blood vessels which have a thermoregulatory function.

In our laboratory we have recently observed that in the rabbit ear artery, a cutaneous blood vessel, the response to endothelin-1 (Monge *et al.*, 1991) and to adrenergic activation (García-Villalón *et al.*, 1992) decreases during cooling by endothelium-dependent mechanisms, probably by increasing the availability of endothelial nitric oxide. To investigate further the endothelial mechanisms underlying the effects of cooling on the response of cutaneous blood vessels, the present study was designed to examine the response of the rabbit central ear artery to acetylcholine, methacholine and sodium nitroprusside in isolated arterial segments under isometric conditions at 37°C and during cooling (24°C). The experiments were performed in ear arteries with and without the endothelium or treated with an inhibitor of nitric oxide synthesis, and compared to those obtained in rabbit femoral (non-cutaneous) arteries. Cholinceptor stimulation produces vascular relaxation by releasing endothelial nitric oxide (Aisaka *et al.*, 1989; Furchgott & Vanhoutte, 1989; Vallance *et*

al., 1989; Moore *et al.*, 1990; Rees *et al.*, 1990; Gardiner *et al.*, 1991) and sodium nitroprusside relaxes vascular smooth muscle by generating nitric oxide (Ignarro & Kadowitz, 1985). The ear artery was selected because this artery is easily accessible and used as a model of cutaneous blood vessels (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988).

Methods

Thirty-eight New Zealand White rabbits of either sex, weighing 2–2.5 kg, were killed by i.p. injection of sodium pentobarbitone (Sigma, 100 mg kg⁻¹). Central ear and femoral arteries were dissected free, cut into 2 mm-cylindrical segments, and prepared for isometric tension recording in a 6 ml organ bath containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 115, KCl 4.6, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, glucose 11.1. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Two fine, stainless steel pins, 150 µm in diameter, were introduced through the lumen of the vascular segment. One pin was fixed to the organ bath wall while the other was connected to a strain gauge for isometric recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments, Inc.), a Statham Microscale Accessory UL5 (Statham Instruments, Inc.) and a Beckman Type RS Recorder (model R-411, Beckman Instruments, Inc.). A resting tension of 0.5 g was applied to the tissue and the segments were allowed to equilibrate for 60–90 min.

¹ Author for correspondence.

First, responses of the vascular segments under resting conditions to KCl (50 mM) were recorded in ear and femoral arteries with and without endothelium at both temperatures to test functional ability; and the solution was renewed by repeated wash-outs. Then, the relaxant responses to acetylcholine, methacholine and sodium nitroprusside were determined in the arteries precontracted with 5-hydroxytryptamine. One group of arteries were precontracted with 10^{-7} or 10^{-6} M 5-hydroxytryptamine, and with these doses the arteries developed an active tone that was lower in ear arteries than in femoral arteries, and in both types of arteries it was lower at 24°C than at 37°C. As these differences in active tone could interfere with the results, another group of ear arteries at 24°C were precontracted with higher concentrations of 5-hydroxytryptamine (10^{-5} – 3×10^{-5} M) in order to achieve a similar tone to that obtained at 37°C with lower concentrations of this amine (10^{-7} or 10^{-6} M).

In the presence of the 5-hydroxytryptamine-induced tone, cumulative concentration-response curves for acetylcholine (10^{-9} – 10^{-4} M), methacholine (10^{-9} – 10^{-4} M) and sodium nitroprusside (10^{-9} – 10^{-4} M) were obtained in ear and femoral arteries with (intact) and without endothelium at 37°C and 24°C. Also, the effects of acetylcholine were recorded in precontracted ear arteries in the presence of different concentrations of N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-6} – 3×10^{-4} M), an inhibitor of nitric oxide synthase (Moore *et al.*, 1990; Rees *et al.*, 1990). The action of an intermediate concentration of L-NAME (10^{-5} M) on the acetylcholine-induced response was tested in the presence of different concentrations of L-arginine (10^{-5} – 10^{-3} M) at 37°C and 24°C.

Acetylcholine and methacholine were used as cholinergic agonists, and sodium nitroprusside as an exogenous donor of nitric oxide, and all of them were dissolved in physiological saline solution. Removal of the endothelium was accomplished by gently rubbing the lumen of the vessel with a roughened steel rod. The presence of the endothelium and the adequacy of endothelial removal was tested morphologically at the end of the experiments after silver staining (Caplan *et al.*, 1974).

L-NAME and L-arginine, dissolved in distilled water, were added to the bath 20–30 min before recording the response to acetylcholine. The effect of L-NAME and L-arginine on the relaxant response to acetylcholine was studied only in ear arteries since cooling affected the response of these arteries, but not that of femoral arteries.

Data analysis

The relaxation produced by the vasodilator drugs was expressed as a percentage of the active tone achieved with 5-hydroxytryptamine in each artery, and the concentration of drugs causing 50% of maximum effect (EC₅₀) was calculated from each individual concentration-response curve. The geometric mean of the EC₅₀ and its 95% confidence interval were calculated for each group of experiments. The logarithms of

the EC₅₀ at every condition were compared statistically and the data were evaluated by Student's *t* test. To compare the inhibitory effects of L-NAME at 37°C and at 24°C, a two-way analysis of variance followed by the Dunnett's test was applied to the data of the relaxation of ear arteries to acetylcholine at both temperatures in the presence of different concentrations of L-NAME. In each case, $P < 0.05$ was considered statistically significant.

Drugs

Materials used were: 5-hydroxytryptamine creatinine sulphate, acetylcholine chloride, methacholine chloride, sodium nitroprusside, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) and L-arginine (free base), all from Sigma.

Results

Effects of potassium chloride

Potassium chloride (50 mM) contracted both ear and femoral arteries and the response in both types of arteries was significantly lower at 24°C than at 37°C. At 37°C the contraction of ear arteries with and without endothelium was not significantly different, whereas at 24°C the ear arteries without endothelium showed a 40% higher contraction than those with endothelium. In femoral arteries at 37°C and 24°C no significant differences were observed between intact and endothelium-free arterial segments (Table 1).

Tone induced by 5-hydroxytryptamine

When 10^{-7} M or 10^{-6} M 5-hydroxytryptamine was applied to the arteries to achieve active tone, the contraction of ear arteries was significantly lower than that of femoral arteries, and in both types of arteries the contraction at 24°C was significantly lower than at 37°C. Endothelium removal did not significantly modify the magnitude of contraction developed by both ear and femoral arteries at 24°C and 37°C to these doses of 5-hydroxytryptamine (10^{-6} or 10^{-7} M) respectively (Table 1). In another group of ear arteries (12 segments) higher concentrations of 5-hydroxytryptamine (10^{-5} – 3×10^{-5} M) induced a contraction at 24°C (2.0 ± 0.14 g) that was similar to that developed by these arteries (13 segments) at 37°C with lower concentrations of this amine (10^{-6} M) (2.2 ± 0.17 g) ($P > 0.05$).

Effects of cholinergic stimulation

At 37°C, acetylcholine (10^{-9} – 10^{-4} M) produced concentration-dependent relaxations of the intact ear (30 segments) and femoral (10 segments) arteries precontracted with 5-hydroxytryptamine, and the maximal relaxation was about

Table 1 Contractile response (g) elicited by KCl (50 mM) or 5-hydroxytryptamine (5-HT, 10^{-6} or 10^{-7} M respectively) in rabbit central ear and femoral arteries at 37°C and 24°C

	Central ear artery		Femoral artery	
	37°C	24°C	37°C	24°C
KCl				
Control	1.95 ± 0.11	$0.73 \pm 0.07^*$	3.10 ± 0.27	$2.03 \pm 0.13^*$
Without endothelium	2.08 ± 0.16	$1.02 \pm 0.10^{*†}$	2.78 ± 0.29	$1.72 \pm 0.13^*$
5-HT				
Control	2.12 ± 0.19	$1.65 \pm 0.15^*$	2.46 ± 0.18	$2.02 \pm 0.16^*$
Without endothelium	2.09 ± 0.15	$1.70 \pm 0.13^*$	2.45 ± 0.17	$1.97 \pm 0.18^*$

Values are means $\pm$ s.e.mean from 22–27 animals. *Statistically significant differences ($P < 0.01$) between 37°C and 24°C. †Statistically significant differences ($P < 0.05$) between arteries with (control) and without endothelium at 24°C.

90–100% of the achieved active tone. Methacholine (10^{-9} – 10^{-4} M) also induced concentration-dependent relaxations of these arteries (7 ear and 6 femoral arterial segments), and the response to this drug was comparable to that induced by acetylcholine in the corresponding arteries. After removal of the endothelium, ear and femoral arteries exhibited absence of, or a very little relaxation to acetylcholine (23 ear and 10 femoral arterial segments) and methacholine (7 ear and 6 femoral arterial segments) (Figures 1 and 2, and Table 2).

At 24°C precontracted, intact ear and femoral arteries also

exhibited a concentration-dependent relaxation to acetylcholine (28 ear and 10 femoral arterial segments) and methacholine (7 ear and 5 femoral arterial segments), and the maximal relaxation to these agents was about 90–100% of the active tone. However, the extent of relaxation and sensitivity of intact ear arteries, but not of intact femoral arteries, to both acetylcholine and methacholine at 24°C was significantly higher than at 37°C. The increase in the relaxation of ear arteries induced with acetylcholine at 24°C was observed both when the arteries had a lower tone at 24°C (16

Figure 1 Relaxation of ear arteries with (○, ●) and without (□, ■) endothelium to (a) acetylcholine, (b) methacholine and (c) sodium nitroprusside obtained at 37°C (○, □) or 24°C (●, ■). Relaxation is expressed as a percentage of the active tone achieved with 5-hydroxytryptamine. Values are means with s.e.mean. *Statistically significant difference ($P < 0.01$) between arteries with endothelium at 37°C and 24°C. n = number of animals.

Figure 2 Relaxation of femoral arteries with (○, ●) and without (□, ■) endothelium to (a) acetylcholine, (b) methacholine and (c) sodium nitroprusside obtained at 37°C (○, □) or 24°C (●, ■). Relaxation is expressed as a percentage of the active tone achieved with 5-hydroxytryptamine. Values are means with s.e.mean. *Statistically significant difference ($P < 0.05$; ** $P < 0.01$) between arteries with endothelium at 37°C and 24°C. n = number of animals.

Table 2 EC₅₀ (M) values for the relaxation to acetylcholine and methacholine in rabbit central ear and femoral arteries precontracted with 5-hydroxytryptamine, at 37°C and 24°C

	Central ear artery		Femoral artery	
	37°C	24°C	37°C	24°C
Acetylcholine	1.4 × 10 ⁻⁷ (1.0 × 10 ⁻⁷ –2.0 × 10 ⁻⁷)	5.5 × 10 ^{-8***} (4.0 × 10 ⁻⁸ –7.6 × 10 ⁻⁷)	9.9 × 10 ⁻⁸ (6.3 × 10 ⁻⁸ –1.6 × 10 ⁻⁷)	1.2 × 10 ⁻⁷ (7.9 × 10 ⁻⁸ –1.7 × 10 ⁻⁷)
Methacholine	1.8 × 10 ⁻⁷ (1.2 × 10 ⁻⁷ –2.5 × 10 ⁻⁷)	7.7 × 10 ^{-8*} (5.6 × 10 ⁻⁸ –1.1 × 10 ⁻⁷)	2.1 × 10 ⁻⁷ (1.5 × 10 ⁻⁷ –3.1 × 10 ⁻⁷)	2.4 × 10 ⁻⁷ (1.6 × 10 ⁻⁷ –3.7 × 10 ⁻⁷)

Values are geometric means and, in parentheses, 95% confidence intervals from 6–10 animals. Statistically significant differences (**P* < 0.01; ***P* < 0.001) between 37°C and 24°C.

segments) than at 37°C (17 segments), and when the concentration of 5-hydroxytryptamine was increased to ensure that the preparations had comparable tone at 24°C (12 segments) and at 37°C (13 segments). In both cases, the increase in the degree of relaxation at 24°C was of similar magnitude and the experiments on ear arteries with acetylcholine at each temperature were therefore pooled. In terms of EC₅₀ values, the sensitivity of intact ear arteries to acetylcholine at 24°C was 2.5 times higher (*P* < 0.001) than at 37°C, and the sensitivity to methacholine at 24°C was 2.3 times higher (*P* < 0.01) than at 37°C. On the contrary, in intact femoral arteries, the sensitivity to acetylcholine as well as to methacholine was not significantly different at 24°C and 37°C (*P* > 0.05). In both ear and femoral arteries without endothelium the relaxation to acetylcholine (17 ear and 10 femoral arterial segments) or methacholine (6 ear and 5 femoral arterial segments) was abolished or greatly reduced during cooling as occurred at 37°C (Figures 1 and 2, Table 2).

L-NAME (10⁻⁶–3 × 10⁻⁴ M) reduced in a concentration-dependent way the relaxation of ear arteries to acetylcholine, but these inhibitory effects of L-NAME were significantly more potent at 37°C than at 24°C (Figure 3). At both temperatures, 10⁻⁶ M or 3 × 10⁻⁶ M L-NAME did not affect significantly the relaxation of ear arteries to acetylcholine. At 37°C, 10⁻⁵ M L-NAME increased the EC₅₀ values (*P* < 0.01) and also decreased the maximal relaxation (*P* < 0.01) to acetylcholine; these blocking effects of 10⁻⁵ M L-NAME were not significantly increased with the higher concentrations (3 × 10⁻⁵ and 10⁻⁴ M) of this substance. At 24°C, 10⁻⁵ M L-NAME increased the EC₅₀ values (*P* < 0.01) without affecting significantly the maximal relaxation to acetylcholine; the higher concentrations of L-NAME (3 × 10⁻⁵ and 10⁻⁴ M) did reduce the maximal relaxation to acetylcholine, but did not change significantly the EC₅₀ values with regard to those obtained with 10⁻⁵ M L-NAME. By comparing the blocking effects of L-NAME on the acetylcholine-induced relaxation at 37°C and 24°C, we found that 10⁻⁵ M L-NAME was less effective in blocking the relaxation to acetylcholine at 24°C than at 37°C: the increment in the EC₅₀ values and the reduction in the maximal relaxation induced with 10⁻⁵ M L-NAME on the response to acetylcholine were significantly (*P* < 0.003) lower at 24°C than at 37°C.

The addition of L-arginine (10⁻⁵–10⁻³ M) to the ear arteries previously treated with 10⁻⁵ M L-NAME reversed in a concentration-dependent manner the inhibitory effect of L-NAME on the relaxation to acetylcholine, and the reversal of this inhibition by L-arginine was not significantly different at 37°C and 24°C (Figure 4).

Effect of sodium nitroprusside

At 37°C sodium nitroprusside (10⁻⁹–10⁻⁴ M) produced a comparable concentration-dependent relaxation of precontracted ear (Figure 1) and femoral (Figure 2) arteries, and in both cases the maximal relaxant effect was 100% of the achieved tone. The sensitivity to sodium nitroprusside was not significantly different in ear arteries with (30 segments) and without (26 segments) endothelium, or in femoral

Figure 3 Relaxation of ear arteries from 5 rabbits to acetylcholine obtained at 37°C (a) or 24°C (b), under control conditions (○, ●) and in the presence of N^G-nitro L-arginine methyl ester at concentrations of 10⁻⁶ M (■), 3 × 10⁻⁶ M (□), 10⁻⁵ M (▲), 3 × 10⁻⁵ M (△), 10⁻⁴ M (◆) or 3 × 10⁻⁴ M (◇). Relaxation is expressed as a percentage of the active tone achieved with 5-hydroxytryptamine. Values are means with s.e. mean. Statistically significant difference (**P* < 0.05, ***P* < 0.01) from control. In parentheses, number of segments.

arteries with (30 segments) and without (18 segments) endothelium.

During cooling, sodium nitroprusside also induced a concentration-dependent relaxation of ear and femoral arteries, the maximal relaxation being about 100% of the active tone. However, the sensitivity of the intact ear (30 segments) and femoral (31 segments) arteries to sodium nitroprusside, in terms of the EC₅₀ values, was significantly lower at 24°C than at 37°C (Figure 1 and 2, Table 3). In intact ear arteries the sensitivity during cooling was 4.4 times lower than at 37°C, and in intact femoral arteries it was 6 times lower at 24°C

than at 37°C. As occurred at 37°C, the sensitivity to sodium nitroprusside at 24°C was not significantly different in ear and femoral arteries with (30 ear and 31 femoral arterial segments) and without (26 ear and 20 femoral arterial segments) endothelium.

Discussion

In the present work we have studied the effects of moderate cooling (24°C) on the response of central ear and femoral

arteries from the rabbit to cholinergic stimulation. The ear artery is a cutaneous vessel that is likely to be exposed *in vivo* to the range of temperatures studied in the present *in vitro* work, and the results in this artery were compared with those obtained in the femoral artery, a deep blood vessel.

At 37°C, both ear and femoral arteries showed a similar relaxation to acetylcholine and methacholine that was endothelium-dependent as occurs in many vascular preparations (Furchgott & Vanhoutte, 1989). The relaxation of ear arteries to acetylcholine was also reduced in a concentration-dependent manner by L-NAME, and this reduction was reversed in a concentration-dependent way by L-arginine. These findings agree with the idea that the vascular relaxation to cholinergic stimulation is mediated, at least in part, by endothelial nitric oxide (Aisaka *et al.*, 1989; Furchgott & Vanhoutte, 1989; Vallance *et al.*, 1989; Moore *et al.*, 1990; Rees *et al.*, 1990; Gardiner *et al.*, 1991).

During cooling, both ear and femoral arteries also exhibited endothelium-dependent relaxation to acetylcholine and methacholine. This relaxation of ear arteries to acetylcholine was concentration-dependently reduced by L-NAME, and this reduction was reversed by L-arginine. Thus endothelial nitric oxide could be also involved in the relaxation of ear arteries to cholinergic stimulation during cooling, as occurs at 37°C. However, the effects of cooling on the response to acetylcholine and methacholine differed between ear and femoral arteries. In ear arteries, cooling increased, whereas in femoral arteries it did not affect the relaxation to cholinergic stimulation. The increased relaxation of ear arteries to acetylcholine at 24°C was found when these arteries had a lower or similar active tone at 24°C than at 37°C, thus indicating that the facilitatory effects of cooling on the relaxation of ear arteries to cholinergic stimulation are not dependent on the level of active tone, but on specific action of cold on these particular arteries. In addition, our results show that cooling decreased the ability of L-NAME to block the acetylcholine-induced relaxation of ear arteries.

Sodium nitroprusside has been used as a donor of nitric oxide and produces vascular relaxation in a similar way to nitric oxide by stimulating the guanylate cyclase of vascular smooth musculature (Rapoport & Murad, 1983; Ignarro & Kadowitz, 1985). On the other hand, evidence that the endothelium modulates the effect of nitrovasodilators is controversial and ranges from findings of potentiation (Shirasaki & Su, 1985; Gardiner *et al.*, 1991; Moncada *et al.*, 1991; Garcia *et al.*, 1992) to attenuation (White *et al.*, 1986) and no response whatsoever (Furchgott *et al.*, 1991; Rapoport & Murad, 1983). In our experiments we found that sodium nitroprusside induced endothelium-independent relaxation of ear and femoral arteries, and that this relaxation was reduced in both types of arteries during cooling as compared to 37°C. The decreased relaxation to sodium nitroprusside clearly contrasts with the increased relaxation to cholinergic stimulation in ear arteries during cooling.

Although an explanation for these differences is at present not available, some considerations can be made on the basis that both sodium nitroprusside and stimulation of the endothelium with cholinergic agonists lead to formation of nitric oxide and a reduced sensitivity of arterial smooth

Figure 4 Relaxation of ear arteries from 4 rabbits to acetylcholine obtained at 37°C (a) or 24°C (b), under control conditions (○, ●) and in the presence of N^G-nitro L-arginine methyl ester (L-NAME) 10⁻⁵ M alone (▲), and in the presence of L-NAME 10⁻⁵ M plus L-arginine at concentrations of 10⁻⁵ M (■), 3 × 10⁻⁵ M (□), 10⁻⁴ M (△), or 10⁻³ M (◆). Relaxation is expressed as a percentage of the active tone achieved with 5-hydroxytryptamine. Values are means with s.e. mean. Statistically significant difference (**P* < 0.05, ***P* < 0.01) from L-NAME alone. In parentheses, number of segments.

Table 3 EC₅₀ (M) values for the relaxation to sodium nitroprusside in rabbit central ear and femoral arteries precontracted with 5-hydroxytryptamine, at 37°C and 24°C

	Central ear artery		Femoral artery	
	37°C	24°C	37°C	24°C
Control	1.8 × 10 ⁻⁷ (1 × 10 ⁻⁷ –3 × 10 ⁻⁷)	7.7 × 10 ⁻⁷ * (5.3 × 10 ⁻⁷ –1.1 × 10 ⁻⁶)	5.3 × 10 ⁻⁷ (2.6 × 10 ⁻⁷ –1.1 × 10 ⁻⁶)	3.2 × 10 ⁻⁶ * (1.6 × 10 ⁻⁶ –6.6 × 10 ⁻⁶)
Without endothelium	1.2 × 10 ⁻⁷ (8 × 10 ⁻⁸ –1.9 × 10 ⁻⁷)	4.8 × 10 ⁻⁷ * (3.2 × 10 ⁻⁷ –7.2 × 10 ⁻⁷)	2.9 × 10 ⁻⁷ (1.7 × 10 ⁻⁷ –4.9 × 10 ⁻⁷)	8.8 × 10 ⁻⁷ * (3.7 × 10 ⁻⁷ –2.1 × 10 ⁻⁶)

Values are geometric means and, in parentheses, 95% confidence intervals from 13–14 animals. Statistically significant differences (**P* < 0.01) between 37°C and 24°C.

musculature to nitric oxide could occur during cooling. The possibility that an increased stability of nitric oxide and a higher rate of its reaction with -SH groups to form S-nitrosothiols accounts for the facilitatory effects of cooling on cholinergic-induced relaxation in ear arteries can also reasonably be excluded, because if that were the case, these facilitatory effects of cooling should also be present in femoral arteries, as well as in both types of arteries, when exposed to sodium nitroprusside. Thus, a more probable explanation is that an increased release of endothelial nitric oxide might occur in ear arteries, but not in femoral arteries when stimulated with acetylcholine or methacholine during cooling. This might be because cooling increases the interaction of the cholinergic agonists with the muscarinic receptors or the signal transduction mechanisms in the endothelial cells, or to cooling directly stimulating the production of nitric oxide in ear artery, a feature that could be specific for cutaneous arteries.

The reduced relaxation of both ear and femoral arteries to sodium nitroprusside might be related not only to a decreased sensitivity of arterial smooth musculature but also to a decreased formation of nitric oxide from sodium nitroprusside during cooling. The process that leads to nitrocompounds providing nitric oxide in the organ bath might be altered at a low temperature. It has been found that the ability of sodium nitroprusside to release nitric oxide and to form S-nitrosothiols varies with the medium conditions (Ignarro *et al.*, 1981).

L-NAME was able to block the relaxation of ear arteries to acetylcholine in a concentration-dependent way at both temperatures. However, higher concentrations of L-NAME were needed at 24°C than at 37°C to block the relaxation to acetylcholine. Also, the concentration of L-NAME that caused the maximal blockade to acetylcholine at 37°C, was less effective at 24°C than at 37°C in reducing the relaxation of ear arteries to cholinergic stimulation. These results with L-NAME suggest that the biochemical reaction involved in the conversion of L-arginine to nitric oxide in the endothelium of ear arteries is less sensitive to inhibition during cooling. With our data we cannot suggest which mechanisms underlie this lower sensitivity to inhibition by L-NAME during cooling, but our results support the hypothesis that the formation of endothelial nitric oxide in stimulated ear arteries increases during cooling.

We also found that cooling reduced the contraction to potassium chloride in ear and femoral arteries, a feature that is known to occur in cutaneous and non-cutaneous blood vessels (Vanhoutte, 1980). In our experiments we observed that endothelium removal did not affect the contraction of either ear or femoral arteries to potassium chloride at 37°C. However, at 24°C the ear arteries without endothelium showed a higher contraction than the arteries with endothelium to potassium chloride, a phenomenon that did not occur in the femoral arteries. This suggests that during cooling the endothelium also exerts an inhibitory effect on the contractile response of cutaneous arteries to stimuli considered as direct activators of smooth musculature. We have recently observed that the endothelium inhibits the contraction of underlying smooth musculature of cutaneous arteries to endothelin-1 (Monge *et al.*, 1991) and to adrenergic stimulation (García-Villalón *et al.*, 1992) at low temperature, probably by an increased availability of endothelial nitric oxide. These two studies (Monge *et al.*, 1991; García-Villalón *et al.*, 1992) and the present results suggest that cooling could non-specifically facilitate the release of nitric oxide from the endothelium of cutaneous vessels when it is activated by different types of vasoactive stimuli.

In summary, the present results suggest that cooling facilitates the stimulated release of nitric oxide from the endothelium in cutaneous, but not in deep vessels. If the results in the ear artery could be extended to other cutaneous vessels, the endothelium function may be of relevance to thermoregulatory function of the cutaneous circulation, as well as to the pathophysiology of some pathological situations. The endothelium is thought to be of some importance in the pathophysiology of Raynaud's phenomenon (Yang *et al.*, 1990), which is in part characterized by intense cold-induced cutaneous vasospasm (Harper & Le Roy, 1987). From our results we can speculate that during cooling the increased endothelium function would limit or avoid cutaneous vasospasm, and, in consequence, a damage of the cutaneous vascular endothelium could lead to uncover or increase the cold-induced vasoconstriction.

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