Cooling and response to adrenoceptor agonists of rabbit ear and femoral artery: role of the endothelium

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

2 Concentration-response curves for noradrenaline (10^{-9} – 3×10^{-4} M), phenylephrine (α_1 -adrenoceptor agonist, 10^{-9} – 3×10^{-4} M) and B-HT 920 (α_2 -adrenoceptor agonist, 10^{-7} – 10^{-3} M) were recorded isometrically in arteries with and without endothelium at 37°C and at 24°C (cooling). To analyze further the endothelial mechanisms in the responses to adrenoceptor stimulation during cooling, the effects of the adrenoceptor agonists on ear arteries in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-5} M) were also determined.

3 In every condition tested, the three adrenoceptor agonists produced a concentration-dependent arterial contraction and the order of potency in ear and femoral arteries was noradrenaline $\geq$ phenylephrine $>$ B-HT 920. The response of ear and femoral arteries to phenylephrine or B-HT 920 was blocked by prazosin (10^{-6} M). Yohimbine (10^{-6} M) decreased slightly the response of ear arteries and increased that of femoral arteries to B-HT 920.

4 The sensitivity of both ear and femoral arteries to the three adrenoceptor agonists was significantly lower at 24°C than at 37°C.

5 In ear arteries, endothelium removal or treatment with L-NAME did not influence the response at 37°C, but did increase it during cooling to adrenoceptor stimulation. In femoral arteries, endothelium removal increased the sensitivity to noradrenaline and, especially, to B-HT 920 at 37°C, but did not affect the response at 24°C.

6 The results suggest that: (a) rabbit ear and femoral arteries are equipped mainly with α_1 -adrenoceptors; (b) at 37°C, the contraction of the ear artery to adrenoceptor agonists is mostly endothelium-independent, and in the femoral artery the contraction to α_2 -adrenoceptor activation is endothelium-dependent; (c) cooling inhibits the contraction to adrenoceptor agonists in both ear and femoral arteries: in the ear artery probably by increasing the availability of endothelial nitric oxide, but in the femoral artery by depressing the sensitivity of α -adrenoceptors in the smooth musculature.

7 The results suggest that the endothelium may modulate the adrenoceptor response of cutaneous arteries during changes in temperature.

Keywords: Skin arteries; temperature; nitric oxide; noradrenaline; phenylephrine; B-HT 920; adrenoceptors

Introduction

The endothelium appears to play a major role in the regulation of vascular tone by releasing relaxing and contracting factors (Furchgott & Vanhoutte, 1989). As vascular relaxants, the endothelium can produce nitric oxide from L-arginine (Palmer *et al.*, 1987; 1988), which acts via the stimulation of the soluble guanylate cyclase in the vascular smooth muscle (Rapoport & Murad, 1983). The synthesis of endothelial nitric oxide can be inhibited by arginine analogues such as N^G-nitro-L-arginine methyl ester (L-NAME) (Moore *et al.*, 1990; Rees *et al.*, 1990).

At present very little is known about the role of the endothelium in the effects of temperature on vascular reactivity (Karaki & Nagase, 1987; Bodelsson *et al.*, 1989). This could be of relevance in blood vessels such as cutaneous ones that have thermoregulatory functions. Experimental evidence shows that cutaneous vessels of several species, including man, are equipped with a mixed population of postjunctional α_1 - and α_2 -adrenoceptors, frequently with a predominance of the α_2 -adrenoceptor subtypes (Vanhoutte & Flavahan, 1986; Borbujo *et al.*, 1989; Harker *et al.*, 1991),

and that cooling augments the constrictor response of these vessels to adrenergic activation by an increased α_2 -adrenoceptor responsiveness (Flavahan *et al.*, 1985) or by an increased responsiveness of both α_1 - and α_2 -adrenoceptors (Gómez *et al.*, 1991). The role of the endothelium in these cooling-induced effects has not been studied.

The present experiments were designed to study the effects of moderate cooling on the response of cutaneous and non-cutaneous arteries to α -adrenoceptor activation with analysis of the role of the endothelium in these effects. To achieve this, central ear and femoral arteries from rabbits were mounted in an organ bath at 37°C and 24°C (cooling), and the effects of α -adrenoceptor activation were recorded isometrically in intact arteries and in arteries without endothelium or treated with an inhibitor of nitric oxide synthesis. The central ear artery was selected because this vessel is easily accessible and used as a model of cutaneous blood vessels (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988).

Methods

Seventeen male New Zealand White rabbits, weighing 2–2.5 kg, were killed by intraperitoneal injection of sodium

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pentobarbitone, 100 mg kg^{-1} . Central ear and femoral arteries were dissected free and cut into cylindrical segments 2 mm in length. Each segment was prepared for isometric tension recording in a 6-ml organ bath containing modified Krebs-Henseleit solution of the following composition (millimolar): NaCl 115, KCl 4.6, KH_2PO_4 1.2, MgSO_4 1.2, CaCl_2 2.5, NaHCO_3 25 and glucose 11.1. The solution was equilibrated with 95% O_2 and 5% CO_2 to give a pH of 7.3–7.4. Briefly, the method consists of passing two fine, stainless steel pins, 150 μm in diameter, through the lumen of the vascular segment. One pin is fixed to the organ bath wall while the other is connected to a strain gauge for isometric tension 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 before any drug was added.

Part of the ear and femoral arteries were kept in the bath at a temperature of 37°C throughout the experiment, and the others were kept at 24°C (cooling). In some of the arterial segments, at 37°C or 24°C , the endothelium was removed prior to mounting the vessels in the organ bath. The removal of the endothelium was accomplished by gently rubbing the lumen of the vessel with a roughened steel rod. The presence of the endothelium in non-rubbed arteries as well as the adequacy of endothelium removal in rubbed arteries was functionally tested by recording the response to acetylcholine (10^{-6} M) in arteries precontracted with 5-hydroxytryptamine (10^{-6} M), and at the end of the experiments by morphological observation after 'en face' silver staining. In non-rubbed, intact arteries acetylcholine produced relaxation and more than 60–70% of the luminal surface was covered by endothelium. In rubbed arteries acetylcholine did not cause any response and less than 5% of the luminal surface was covered by endothelium.

Cumulative concentration-response curves were determined in central ear and femoral arteries with and without endothelium for noradrenaline (10^{-9} – $3 \times 10^{-4} \text{ M}$), the specific α_1 -adrenoceptor agonist, phenylephrine (10^{-9} – $3 \times 10^{-4} \text{ M}$) and the α_2 -adrenoceptor agonist, B-HT 920 (10^{-7} – 10^{-3} M) at 37°C and at 24°C . Propranolol (10^{-6} M) was added to the bath 10 min before adding the agonists to block a possible β -adrenoceptor effect of the drugs. To test the specificity of B-HT 920 for α_2 -adrenoceptors and phenylephrine for α_1 -adrenoceptors, experiments with these drugs were also done in presence of the α_1 -adrenoceptor antagonist, prazosin (10^{-6} M) or the α_2 -adrenoceptor antagonist, yohimbine (10^{-6} M), at 37°C and at 24°C .

In order to analyze the role of endothelial nitric oxide in the vascular response, concentration-response curves to each adrenoceptor agonist were obtained in ear arteries in the presence of N^G -nitro-L-arginine methyl ester (L-NAME, 10^{-5} M), a nitric oxide-synthase inhibitor (Moore *et al.*, 1990; Rees *et al.*, 1990) at 37°C and 24°C . This drug was added to the bath 30 min before beginning the experiments. These types of experiments were not performed in femoral arteries because in these vessels endothelial removal did not affect the response to the α -adrenoceptor agonists used.

Concentrations of the α -adrenoceptor drugs causing 50% of the maximal response (EC_{50}) were calculated from each individual concentration-response curve. The geometric mean of EC_{50} and its 95% confidence interval were obtained for each group of experiments. At 37°C and 24°C the logarithms of the EC_{50} obtained in vascular segments with or without endothelium, and in the presence of L-NAME or the α -adrenoceptor antagonists were compared statistically. The data expressed as means $\pm$ s.e.mean, were evaluated by Student's *t* test, considering as significant a probability value of less than 0.05.

Drugs used were: (–)-noradrenaline bitartrate (Sigma), (–)-phenylephrine hydrochloride (Sigma), B-HT 920 (5-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5]-dazepin dihydrochloride, Boehringer Ingelheim), L-NAME (N^G -nitro-L-arginine methyl ester hydrochloride, Sigma), prazosin (1-[4-amino-6,7-dimethoxy-2-quinazolinyl]-4-[furan-2-yl]-piperazine hydrochloride, Sigma) and yohimbine hydrochloride (Sigma).

Results

Ear arteries

Noradrenaline The effects of noradrenaline on ear arteries with and without endothelium obtained at 37°C and 24°C are summarized in Figure 1. At 37°C , noradrenaline (10^{-9} – $3 \times 10^{-4} \text{ M}$) produced a concentration-dependent contraction of intact ear arteries ($\text{EC}_{50} = 1.3 \times 10^{-7} \text{ M}$, 95% confidence interval = 7×10^{-8} – $1.6 \times 10^{-7} \text{ M}$), and after endothelium removal the sensitivity was not significantly ($P > 0.05$) affected and the maximal contraction was decreased ($P < 0.001$) to noradrenaline.

Figure 1 Contractile response of rabbit central ear (a,b) and femoral (c,d) arteries to noradrenaline at 37°C and 24°C in segments with (○) and without (●) endothelium. Values are means with s.e.mean shown by vertical bars. Statistically significant difference * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *n* = number of animals.

During cooling (24°C), the sensitivity ($EC_{50} = 5.6 \times 10^{-7}$ M, 95% confidence interval = 3.4×10^{-7} M), but not the maximal response, of intact ear arteries to noradrenaline was lower (4 times, $P < 0.001$) than at 37°C. At 24°C, ear arteries without endothelium showed an increased sensitivity (4 times, $P < 0.001$) and lower maximal contraction with respect to intact arteries to noradrenaline.

At 37°C ear arteries treated with L-NAME (10^{-5} M) exhibited a comparable sensitivity ($P < 0.05$) and a lower maximal contraction ($P < 0.05$) than non-treated arteries, whereas at 24°C treatment with L-NAME induced a parallel leftward shift (2.5 times, $P < 0.01$) of the concentration-response curve to noradrenaline (Table 1).

Phenylephrine The effects of phenylephrine on ear arteries with and without endothelium at 37°C and 24°C are summarized in Figure 2. At 37°C, phenylephrine (10^{-9} – 3×10^{-4} M) induced a concentration-dependent contraction of intact arteries ($EC_{50} = 1.7 \times 10^{-7}$ M, 95% confidence interval = 1.2×10^{-7} – 2.4×10^{-7} M), and after endothelium removal the sensitivity was comparable ($P > 0.05$) and the maximal contraction was significantly reduced ($P < 0.001$) to this α_1 -adrenoceptor agonist.

During cooling the sensitivity ($EC_{50} = 3.4 \times 10^{-7}$ M, 95% confidence interval = 2.2×10^{-7} – 5.1×10^{-7} M), but not the maximal contraction, of intact arteries to phenylephrine was reduced about 2 times ($P < 0.001$) as compared to that obtained at 37°C. At 24°C, in arteries without endothelium the concentration-response curves for phenylephrine ($EC_{50} = 1.0 \times 10^{-7}$ M, 95% confidence interval = 7.5×10^{-8} – 1.4×10^{-7} M) were shifted to the left in a parallel way about 3.4 times ($P < 0.001$) in comparison to intact arteries.

Treatment with L-NAME (10^{-5} M) did not significantly affect the response of ear arteries at 37°C, and tended to induce a parallel leftward shift of the response at 24°C to phenylephrine (Table 1) although this was not significant.

Prazosin (10^{-6} M) induced a parallel rightward shift of the concentration-response curve to phenylephrine in intact arteries at 37°C and at 24°C. The displacement at 37°C was about 82 times ($P < 0.001$), and at 24°C was about 132 times ($P < 0.001$). Yohimbine (10^{-6} M) did not significantly modify the response of intact ear arteries to phenylephrine at either temperature.

B-HT 920 The effects of B-HT 920 on ear arteries with and without endothelium at 37°C and at 24°C are summarized in Figure 3. At 37°C this substance contracted the intact ear arteries in a concentration-dependent manner ($EC_{50} = 2.1 \times 10^{-6}$ M, 95% confidence interval = 1.4×10^{-6} –

2.7×10^{-6} M) and the response was not significantly affected by endothelium removal.

During cooling a rightward shift ($EC_{50} = 5.7 \times 10^{-5}$ M, 95% confidence interval = 3.3×10^{-5} – 7.1×10^{-5} M; 27 times, $P < 0.001$) was found in the response of the intact ear arteries to B-HT 920 with respect to that obtained at 37°C. At 24°C, arteries without endothelium showed a response to B-HT 920 that was shifted to the left in a parallel way (5 times, $P < 0.001$) in comparison to that recorded in intact arteries.

Yohimbine (10^{-6} M) produced a rightward shift of the response at 37°C (3 times, $P < 0.001$), but it did not significantly affect the response at 24°C ($P > 0.05$) of intact arteries to B-HT 920. Prazosin (10^{-6} M) also shifted to the right in a parallel way the response of intact ear arteries at 37°C (124 times, $P < 0.001$), and it markedly reduced the response at 24°C to B-HT 920.

Treatment with L-NAME (10^{-5} M) did not modify significantly the concentration-response curve at 37°C, but it did produce a parallel leftward shift (3.5 times, $P < 0.001$) of the response at 24°C to B-HT 920 (Table 1).

Femoral arteries

Noradrenaline (10^{-9} – 3×10^{-4} M) produced a concentration-dependent contraction of intact femoral arteries at 37°C, and the sensitivity ($EC_{50} = 3.1 \times 10^{-8}$ M, 95% confidence interval = 1.8×10^{-8} – 4.4×10^{-8} M) and maximal contraction (5.5 ± 0.27 g) exhibited by these arteries were significantly ($P < 0.001$) higher than with intact ear arteries ($EC_{50} = 1.3 \times 10^{-7}$ M, 95% confidence interval = 7×10^{-8} – 1.6×10^{-7} M; maximal contraction = 4.1 ± 0.13 g) (Figure 1). At 37°C the response of femoral arteries without endothelium to noradrenaline was shifted to the left (2.4 times, $P < 0.05$) as compared with the intact arteries.

During cooling, the sensitivity ($EC_{50} = 2.5 \times 10^{-7}$ M, 95% confidence interval = 1.4×10^{-7} – 4.6×10^{-7} M), but not the maximal response, of the intact femoral arteries was significantly lower (8 times, $P < 0.001$) than at 37°C. At 24°C, endothelium removal did not affect significantly the arterial response to noradrenaline with respect to intact arteries (Figure 1).

Phenylephrine (10^{-9} – 3×10^{-4} M) caused a concentration-dependent contraction of intact femoral arteries, the sensitivity being similar ($EC_{50} = 2.4 \times 10^{-7}$ M, 95% confidence interval = 1.2×10^{-7} – 4.8×10^{-7} M vs. 1.7×10^{-7} M, 95% confidence interval = 1.2×10^{-7} – 2.4×10^{-7} M, $P > 0.05$) and the maximal response being higher (5.5 ± 0.15 vs. 3.9 ± 0.02 g, $P < 0.001$) than in ear arteries at 37°C (Figure 2).

Table 1 EC_{50} values with 95% confidence intervals and maximal contraction for the adrenoceptor agonists used in rabbit central ear arteries in absence (control) and in the presence of N^G -nitro-L-arginine methyl ester (L-NAME, 10^{-5} M) at 37°C and at 24°C

	EC_{50} (M)		Maximal contraction (g)	
	37°C	24°C	37°C	24°C
Noradrenaline				
Control	1.3×10^{-7} (7.0×10^{-8} – 1.6×10^{-7})	5.6×10^{-7} (3.4×10^{-7} – 7.0×10^{-7})	4.1 ± 0.13 (n = 13)	4.3 ± 0.11 (n = 12)
With L-NAME	1.6×10^{-7} (1.0×10^{-7} – 2.3×10^{-7})	2.3×10^{-7} ** (1.3×10^{-7} – 3.9×10^{-7})	$3.0 \pm 0.56^*$ (n = 6)	3.8 ± 0.33 (n = 6)
Phenylephrine				
Control	1.7×10^{-7} (1.2×10^{-7} – 2.4×10^{-7})	3.4×10^{-7} (2.2×10^{-7} – 5.1×10^{-7})	3.9 ± 0.09 (n = 16)	4.1 ± 0.15 (n = 17)
With L-NAME	1.1×10^{-7} (6.3×10^{-8} – 1.8×10^{-7})	1.7×10^{-7} (1.0×10^{-7} – 3.5×10^{-6})	3.8 ± 0.42 (n = 8)	4.2 ± 0.32 (n = 8)
B-HT 920				
Control	2.1×10^{-6} (1.4×10^{-6} – 2.7×10^{-6})	5.7×10^{-5} (3.3×10^{-5} – 7.1×10^{-5})	3.2 ± 0.35 (n = 8)	2.1 ± 0.18 (n = 14)
With L-NAME	1.1×10^{-6} (5.8×10^{-7} – 1.8×10^{-6})	1.6×10^{-5} *** (9.1×10^{-6} – 2.1×10^{-5})	3.7 ± 0.43 (n = 5)	3.0 ± 0.20 *** (n = 10)

Statistically different with respect to its control at the same temperature. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. n = number of animals.

Figure 2 Contractile response of rabbit central ear (a,b) and femoral (c,d) arteries to phenylephrine at 37°C and 24°C in segments with (O) and without (●) endothelium. Values are means with s.e.mean shown by vertical bars. Statistically significant difference * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n = number of animals.

During cooling, the sensitivity ($EC_{50} = 1.5 \times 10^{-6}$ M, 95% confidence interval = 9.6×10^{-7} – 2.3×10^{-6} M), but not the maximal response, of intact femoral arteries to phenylephrine was lower (6 times, $P < 0.001$) than at 37°C (Figure 2).

At 37°C and 24°C endothelium removal did not significantly influence the response of femoral arteries with respect to intact arteries to phenylephrine.

Prazosin (10^{-6} M) induced a parallel rightward shift of the response of femoral arteries to phenylephrine at 37°C (207 times, $P < 0.001$) and at 24°C (80 times, $P < 0.001$). Yohimbine (10^{-6} M) also produced a small parallel rightward shift of the response of these arteries to phenylephrine at 37°C and 24°C, these displacements being only about 2 times ($P < 0.05$) in both cases.

B-HT 920 (10^{-7} – 10^{-3} M) contracted intact femoral arteries in a concentration-dependent manner, the sensitivity ($EC_{50} = 6.8 \times 10^{-5}$ M, 95% confidence interval = 4.7×10^{-5} – 9×10^{-5} M vs. 2.1×10^{-6} M, 95% confidence interval = 1.4×10^{-6} – 2.7×10^{-6} M, $P < 0.001$) and maximal response (2.1 ± 0.43 vs. 3.2 ± 0.35 g, $P < 0.05$) being significantly lower than

in intact ear arteries at 37°C (Figure 3).

During cooling the response of intact femoral arteries to B-HT 920 was markedly depressed, and this response was very small so that reliable values of EC_{50} for these responses could not be obtained (Figure 3).

At 37°C femoral arteries without endothelium showed a response to B-HT 920 ($EC_{50} = 2.3 \times 10^{-5}$ M, 95% confidence interval = 1.1×10^{-5} – 3.6×10^{-5} M) that was significantly shifted to the left (2 times, $P < 0.05$) in comparison with intact arteries. At 24°C the response to this agonist was very small and similar in endothelium-denuded and intact arteries (Figure 3).

Yohimbine (10^{-6} M) produced, paradoxically, a parallel leftward shift (2 times, $P < 0.05$) of the response of femoral arteries to B-HT at 37°C. The response to B-HT 920 in the presence of yohimbine (10^{-6} M) at 24°C was very small and difficult to evaluate. Prazosin (10^{-6} M) abolished completely the response of intact arteries to this agonist at 37°C and at 24°C.

Discussion

In the present work we have studied the effects of moderate cooling on the response of the ear (cutaneous) artery of the rabbit to α -adrenoceptor stimulation, paying special attention to the influence of the endothelium in these effects. The ear artery was used because this superficial vessel has been used as a model of cutaneous blood vessels (Patton & Wallace,

Figure 3 Contractile response of rabbit central ear (a,b) and femoral (c,d) arteries to B-HT 920 at 37°C and 24°C in segments with (O) and without (●) endothelium. Values are means with s.e.mean shown by vertical bars. Statistically significant difference * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n = number of animals.

1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988). Cooling to 24°C was chosen because it represents a modest tissue cooling that is likely to occur in skin surface exposed to ambient temperature under certain circumstance and comparable data are available in the literature for blood vessels. The results in this cutaneous artery are compared with those obtained in the femoral artery of the rabbit, which is a deep, non-cutaneous vessel.

Our results indicate that at 37°C the α -adrenoceptor agonists used produce contraction in rabbit ear and femoral arteries by stimulating postjunctional α -adrenoceptors located probably on the smooth muscle cells. In both types of arteries the sensitivity to noradrenaline and to the α_1 -adrenoceptor agonist phenylephrine was similar, but the sensitivity to the α_2 -adrenoceptor agonist, B-HT 920, was much lower than to noradrenaline or phenylephrine, this being more evident for femoral arteries. The response of both types of arteries to phenylephrine was blocked, as expected, by prazosin but not by yohimbine, indicating that this adrenoceptor agonist indeed acts on these arteries by activating α_1 -adrenoceptors. The α_1 -adrenoceptor antagonist prazosin, paradoxically, also blocked in a competitive manner the response to B-HT 920 in both femoral and ear arteries, whereas the α_2 -adrenoceptor antagonist, yohimbine, produced a very small blockade of the response in ear arteries, or increased the sensitivity of femoral arteries to B-HT 920. This suggests that at the concentrations producing arterial contraction in these arteries, B-HT 920 produces contraction by acting mainly on α_1 -adrenoceptors in ear and femoral arteries. Therefore, our results suggest that in the ear and femoral arteries of the rabbit α_1 -adrenoceptors predominate over α_2 -adrenoceptors to mediate vasoconstriction, probably by a higher concentration and/or sensitivity of the α_1 -adrenoceptor subtype. The α_2 -adrenoceptor subtype would have a relatively small importance for inducing constriction, especially in femoral arteries. Our data for the ear artery agree with those found in this artery by others (Harker & Vanhoutte, 1988). The ear artery, a cutaneous thermoregulatory vessel, seems to differ from other cutaneous vessels in which a predominance of the α_2 -adrenoceptor over the α_1 -adrenoceptor subtype has frequently been observed (Vanhoutte & Flavahan, 1986; Borbujo *et al.*, 1989; Harker *et al.*, 1991). The observations in the femoral artery correlate with those found in deep, non-cutaneous vessels (Vanhoutte & Flavahan, 1986).

At 37°C the sensitivity of the ear artery to the three adrenoceptor agonists used was not affected by removing the endothelium, thus suggesting that the adrenergic contraction of this artery is mostly caused by activation of the α -adrenoceptors located in the smooth musculature. The maximal contraction to noradrenaline and phenylephrine was found to be significantly reduced and tended to be lower to B-HT 920 in the ear arterial segments without endothelium. This feature could be related to some damage of the smooth muscle of these particular arteries when rubbed for removing the endothelium. This procedure, however, did not apparently affect the sensitivity of ear arteries to the adrenoceptor agonists used. In the femoral artery at 37°C, endothelium removal did not affect the effects by phenylephrine, but increased the response to noradrenaline and, especially, to B-HT 920. This suggests that α_2 -adrenoceptors are also present in the endothelial cells of the femoral artery, and the activation of these particular receptors would induce the release of relaxant factors to oppose the adrenergic vasoconstriction mediated by activation of α -adrenoceptors of smooth musculature. This correlates with the paradoxical facilitating effect of yohimbine on the response to B-HT 920 found in intact femoral arteries. Yohimbine, by blocking the α_2 -adrenoceptors in the endothelium, could reduce the release of relaxant factors from the endothelium, and thus unmask the direct effects of B-HT 920 on α -adrenoceptors located on smooth musculature. The influence of the endothelium on the adrenergic vasoconstriction has been observed in some

vessels (Cocks & Angus, 1983; Carrier & Shite, 1985; Miller & Vanhoutte, 1985) but not in others (Rimele & Vanhoutte, 1983; Singer & Peach, 1983; Nava-Hernández *et al.*, 1991). α_2 -Adrenoceptor activation seems to be very sensitive to the endothelium modulatory effect, because activation of α_2 -adrenoceptors in the endothelium strongly stimulates nitric oxide release (Vanhoutte & Miller, 1989).

It is accepted that cooling depresses the contractile response of both cutaneous and non cutaneous blood vessels by direct activation of vascular smooth muscle with KCl or BaCl₂, an effect that has been considered to be due mainly to a decrease in permeability of vascular smooth muscle membrane for calcium ions (Vanhoutte, 1980). The inhibitory effect of cooling on the response of ear arteries to KCl has been described previously (Monge *et al.*, 1991) and was also observed in ear and femoral arteries (not shown). The present results also show that cooling inhibits the contraction of the ear and femoral arteries to α -adrenergic activation. Most of the results indicate that cooling potentiates the adrenergic contraction of cutaneous blood vessels and this has been related to the relative predominance of the α_2 -adrenoceptors in these vessels (Vanhoutte & Flavahan, 1986; Borbujo *et al.*, 1989; Harker *et al.*, 1991). The lack of a potentiating effect of cooling on adrenergically induced contraction in the ear artery could be related to the relative paucity of the α_2 -adrenoceptor subtype in this artery, a feature also observed by Harker & Vanhoutte (1988). The reduction of the adrenergic contraction by cooling found in the femoral artery is in accordance with that described in deep, non-cutaneous blood vessels (Vanhoutte & Flavahan, 1986).

Our results suggest that mechanisms underlying the inhibitory effects of cooling on the adrenergic response differ in the ear and femoral arteries. In femoral arteries endothelium removal did not affect the action of cooling, whereas in ear arteries endothelium removal reversed the inhibitory effects of cooling on the α -adrenoceptor response. This suggests that the inhibitory action of cooling on the adrenergic constriction in the femoral artery is the result of the reduced sensitivity of the α -adrenoceptors located in the smooth musculature, whereas in the ear artery it is mediated by an endothelium-dependent mechanism. In the ear artery the endothelium inhibits the contraction to α -adrenoceptor activation during cooling as derived from the results in this artery with and without endothelium. Treatment with L-NAME, an inhibitor of the nitric oxide synthesis (Moore *et al.*, 1990; Rees *et al.*, 1990), also increased the sensitivity of ear arteries to noradrenaline and B-HT 920 at 24°C, but not at 37°C, as occurred in the endothelium-denuded ear arteries. This suggests that the inhibitory effect of cooling on the response of the ear artery to α -adrenergic activation is probably due to the increased effects of endothelium-released nitric oxide at a low temperature. These increased effects of nitric oxide may counteract the response of cutaneous arteries to direct adrenergic activation during cooling. Under resting conditions L-NAME at the concentration used (10^{-5} M) did not modify the basal tension of the arteries. This feature was also observed when higher concentrations of L-NAME (10^{-4} M) were used (Monge *et al.*, 1991), and both of them suggest that the endothelium of these arteries does not release or releases insufficient nitric oxide to produce a vasodilator tone under resting conditions after applying a passive tension of 0.5 g. With regard to the observation that L-NAME reduced the maximal contraction of ear arteries to noradrenaline, but not to phenylephrine or B-HT 920 we have no explanation for this feature. Recently we have found that the contraction of the rabbit ear artery to endothelin-1 is also inhibited, probably by an augmented production in the endothelium or an increased half-life of nitric oxide, during cooling (Monge *et al.*, 1991). In that study (Monge *et al.*, 1991) it was found that the relaxation of the ear artery to nitroprusside, a compound that relaxes vascular smooth muscle in a similar way to nitric oxide (Ignarro & Kadowitz, 1985), was independent of the endothelium and was

decreased during cooling. Thus, cooling may increase the availability of endothelial nitric oxide not only when the ear artery is activated with endothelin-1, but also when it is adrenergically stimulated. These effects of cooling on the endothelial nitric oxide might be specific for cutaneous blood vessels as a cooling-induced inhibitory effect of the endothelium on the adrenergic contraction was not observed in femoral arteries. It is remarkable that in the study of Monge *et al.* (1991) 10^{-4} M concentrations of L-NAME were needed to increase the reactivity of the rabbit ear artery to endothelin-1 during cooling, whereas in the present study 10^{-5} M concentrations of this inhibitor of nitric oxide production were sufficient to increase the reactivity of this artery to noradrenaline or B-HT 920. This lower concentration of L-NAME was found to be capable of reducing the relaxation of precontracted ear arteries to acetylcholine at 37°C and 24°C (unpublished observations), thus suggesting that 10^{-5} M concentration of L-NAME is sufficient for blocking the production of endothelial nitric oxide. It can be suggested, therefore, that the inhibitory effect of cooling on the response of the ear artery by increasing the effects of endothelial nitric oxide may be more sensitive to adrenergic stimulation than to endothelin-1.

In conclusion, the main observation of the present study

indicates that cooling inhibits the contraction of the ear and femoral arteries of the rabbit to adrenergic activation by different mechanisms. In the ear artery cooling inhibits this contraction, probably by increasing the availability of endothelial nitric oxide, whereas in the femoral artery by decreasing the sensitivity of α -adrenoceptors of smooth musculature. If the results in the ear artery could be extended to other cutaneous vessels, the endothelial function might be of relevance to thermoregulatory function of the cutaneous circulation as well as to the physiopathology of some pathological situations. Patients with Raynaud's phenomenon, which is in part characterized by intense cold-induced cutaneous vasospasm (Harper & LeRoy, 1987), present alterations in the endothelium of finger arteries (Rodnan *et al.*, 1980). Thus, we can speculate that damage of the cutaneous vascular endothelium in these patients may underlie the augmented response of cutaneous vessels by lack of the inhibitory function of the endothelium during cooling.

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References

- BODELSSON, M., ARNEKLO-NOBIN, B. & TORNEBRANDT, K. (1989). Cooling augments contractile response to 5-hydroxytryptamine via an endothelium-dependent mechanism. *Blood Vessels*, **26**, 347–359.
- BORBUJO, J., GARCIA-VILLALON, A.L., VALLE, J., GOMEZ, B. & DIEGUEZ, G. (1989). Postjunctional α_1 and α_2 adrenoceptors in human skin arteries. An in vitro study. *J. Pharmacol. Exp. Ther.*, **249**, 284–287.
- CARRIER, G.O. & WHITE, R.E. (1985). Enhancement of α_1 - and α_2 adrenergic agonists by removal of endothelium in rat aorta. *J. Pharmacol. Exp. Ther.*, **232**, 682–687.
- COCKS, T.M. & ANGUS, J.A. (1983). Endothelium dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, **305**, 627–630.
- FLAVAHAN, N.A., LINDBLAD, L.E., VERBEUREN, T.J., SHEPHERD, J.T. & VANHOUTTE, P.M. (1985). Cooling and α_1 - and α_2 -adrenergic responses in cutaneous veins: role of receptor reserve. *Am. J. Physiol.*, **249**, H950–H955.
- FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.*, **3**, 2007–2018.
- GOMEZ, B., BORBUJO, J., GARCIA-VILLALON, A.L., NAVA-HERNANDEZ, E., VALLE, J., GARCIA, J.L. & DIEGUEZ, G. (1991). α_1 - and α_2 -adrenergic response in human isolated skin arteries during cooling. *Gen. Pharmacol.*, **221**, 341–346.
- HARKER, C.T., OUSLEY, P.J., BOWMAN, C.J. & PORTER, J.M. (1991). Cooling augments α_2 -adrenoceptor-mediated contractions in rat tail artery. *Am. J. Physiol.*, **260**, H1166–H1171.
- HARKER, C.T. & VANHOUTTE, P.M. (1988). Cooling the central ear artery of the rabbit: Myogenic and adrenergic responses. *J. Pharmacol. Exp. Ther.*, **245**, 89–93.
- HARPER, F.E. & LEROY, E.C. (1987). Raynaud's Phenomenon. In *Clinical Dermatology*, Vol. II. ed. Demis, D.J., Unit 7-36, pp. 1–9. Philadelphia: J.B. Lippincott.
- IGNARRO, L.J. & KADOWITZ, P.J. (1985). The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu. Rev. Pharmacol. Toxicol.*, **25**, 171–191.
- KARAKI, H. & NAGASE, H. (1987). Low temperature augments the endothelium-dependent relaxation in isolated rat aorta. *Eur. J. Pharmacol.*, **142**, 129–132.
- MILLER, V.M. & VANHOUTTE, P.M. (1985). Endothelial α_2 -adrenoceptors in canine pulmonary and systemic blood vessels. *Eur. J. Pharmacol.*, **118**, 123–129.
- MONGE, L., GARCIA-VILLALON, A.L., MONTROYA, J.J., GARCIA, J.L., GOMEZ, B. & DIEGUEZ, G. (1991). Response of rabbit ear artery to endothelin-1 during cooling. *Br. J. Pharmacol.*, **104**, 609–612.
- MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br. J. Pharmacol.*, **99**, 408–412.
- NAVA-HERNANDEZ, E., VALLE, J., GARCIA-VILLALON, A.L., GARCIA, J.L., MONGE, L., GOMEZ, B. & DIEGUEZ, G. (1992). The canine internal carotid system: Regional differences in reactivity to nerve stimulation, noradrenaline and 5-hydroxytryptamine. *Acta Physiol. Scand.*, **144**, 000–000.
- PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.
- PALMER, R.M.J., ASHON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.
- PATTON, J.N. & WALLACE, W.F.M. (1978). Responses of isolated rabbit ear arteries to intra- and extraluminal L- and D-noradrenaline and histamine in the temperature range 37–3°C. *Ir. J. Med. Sci.*, **147**, 313–317.
- RAPOPORT, R.M. & MURAD, F. (1983). Agonist-induced endothelium dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.*, **52**, 352–357.
- REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.*, **101**, 146–152.
- RIMELE, T.J. & VANHOUTTE, P.M. (1983). Effects of inhibitors of arachidonic acid metabolism and calcium entry on responses to acetylcholine, potassium and norepinephrine in the isolated canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **225**, 720–726.
- ROBERTS, M.F. & ZYGMUNT, A.C. (1984). Reflex and local thermal control of rabbit ear blood flow. *Am. J. Physiol.*, **246**, R979–R984.
- RODNAN, G.P., MYEROWITZ, R.L. & JUSTH, G.O. (1980). Morphologic changes in the digital arteries of patients with progressive systemic sclerosis (scleroderma) and Raynaud phenomenon. *Medicine*, **59**, 393–408.
- SINGER, H.A. & PEACH, M.J. (1983). Endothelium dependent relaxations of rabbit aorta I: relaxation stimulated by arachidonic acid. *J. Pharmacol. Exp. Ther.*, **226**, 790–795.
- VANHOUTTE, P.M. (1980). Physical factors of regulation. In *Handbook of Physiology*, Section 2, Circulation, Vol. II, Vascular Smooth Muscle, ed. Bohr, D.F., Somlyo, A.P. & Sparks, H.V. pp. 443–474. Bethesda, Maryland: American Physiological Society.
- VANHOUTTE, P.M. & FLAVAHAN, N.A. (1986). Effects of temperature on α -adrenoceptors in limb veins: role of receptor reserve. *Fed. Proc.*, **45**, 2347–2354.
- VANHOUTTE, P.M. & MILLER, V.M. (1989). α_2 -adrenoceptors and endothelium-derived relaxing factor. *Am. J. Med.*, **87**, (Suppl. 3C), 1S–5S.

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