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Role of the purinergic and noradrenergic components in the potentiation by endothelin-1 of the sympathetic contraction of the rabbit central ear artery during cooling

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1 To examine the role of the purinergic and noradrenergic components in the potentiation of endothelin-1 on the vascular response to sympathetic nerve stimulation, we recorded the isometric response of isolated segments, 2 mm long, from the rabbit central ear artery to electrical field stimulation (1–8 Hz) under different conditions, at 37°C and during cooling (30°C).

2 Electrical field stimulation produced frequency-dependent contraction, which was reduced during cooling (about 60% for 8 Hz). Both at 37°C and 30°C, phentolamine (1 μM) or blockade of α_1-adrenoceptors with prazosin (1 μM) reduced, whereas blockade of α_2-adrenoceptors with yohimbine (1 μM) increased, the contraction to electrical field stimulation. This contraction was increased after desensitization of P2-receptors with α,β-methylene adenosine 5′-triphosphate (α,β-meATP, 3 μM) at 37°C but not at 30°C, and was not modified by blockade of P2-receptors with pyridoxalphosphate-6-azophenyl-2,4′-disulphonic acid (PPADS, 30 μM) at either temperature.

3 Endothelin-1 (1, 3 and 10 nM) at 37°C did not affect, but at 30°C it potentiated in a concentration-dependent manner the contraction to electrical field stimulation (from 28±6 to 134±22%, for 8 Hz). At 37°C, endothelin-1 in the presence of phentolamine or prazosin, but not in that of yohimbine, α,β-meATP or PPADS, potentiated the contraction to electrical stimulation. At 30°C, phentolamine or yohimbine reduced, prazosin or PPADS did not modify and α,β-meATP slightly increased the potentiation by endothelin-1 of the response to electrical stimulation.

4 The arterial contraction to ATP (2 mM) and the α_2-adrenoceptor agonist BHT-920 (10 μM), but not that to (−)-noradrenaline (1 μM), was potentiated by endothelin-1 at both 37°C and 30°C.

5 These results in the rabbit central ear artery suggest that the sympathetic response: (a) at 37°C, could be mediated mainly by activation of α_1-adrenoceptors, with low participation of P2-receptors, (b) is diminished during cooling, probably by a reduction in the participation of α_1-adrenoceptors, and in this condition the response could be mediated in part by P2-receptors, and (c) is potentiated by endothelin-1 during cooling, probably by increasing the response of both postjunctional α_2-adrenoceptors and P2-receptors.

Keywords: Cutaneous arteries; temperature; α-adrenoceptor-mediated vasoconstriction; purinergic vasoconstriction

Introduction

Endothelin-1, a 21 amino acid peptide that may be produced by endothelial cells, is a powerful vasoconstrictor (Yanagisawa *et al*., 1988), and may play a neuromodulatory role in the sympathetic vascular response. Endothelin-1 increases the constriction to sympathetic nerve stimulation in rat renal circulation (Reid, 1993), guinea-pig pulmonary artery (Wiklund *et al*., 1989), and rabbit saphenous (Mutafova-Yambolieva & Radomirov, 1994), jejunal (La & Rand, 1993) and central ear (Wong-Dusting *et al*., 1991) arteries. The mechanisms of the potentiation by endothelin-1 of the sympathetic stimulation are not clear. It is known that sympathetic vasoconstriction has two main components, a noradrenergic component, due to the release of noradrenaline, and a purinergic component probably due to the release of adenosine 5′-triphosphate (ATP) from perivascular nerve endings (Burnstock, 1986; Kennedy *et al*., 1986). In addition, these neurotransmitters (noradrenaline and ATP) may act on different subtypes of receptors, as the noradrenergic response is mediated by the α_1-adrenoceptors in deep arteries, and by postjunctional α_2-adrenoceptors in superficial (cutaneous) vessels (Vanhoutte & Flavahan, 1986; Borbujo *et al*., 1989). With regard to the effects of endothelin-1, it has been shown that the potentiation of the sympathetic response in rabbit jejunal (La & Rand, 1993) and saphenous (Mutafova-Yambolieva & Radomirov, 1994) arteries is mainly due to the facilitation of the purinergic response. However, as endothelin-1 can potentiate the contraction to exogenous noradrenaline (Yang *et al*., 1990), the noradrenergic component may also be involved in the interaction between endothelin-1 and the sympathetic vascular response.

In our laboratory we have recently observed that cooling blunts the contraction to sympathetic stimulation and that endothelin-1 potentiates this contraction in the rabbit central ear artery during cooling, but not during normotemperature and warming (Padilla *et al*., 1997). The present study was designed to analyse the possible involvement of the noradrenergic and purinergic components in the potentiation by endothelin-1, of the contraction of the rabbit central ear artery to sympathetic stimulation during cooling. The experiments were carried out in isolated segments from rabbit central ear artery (cutaneous artery) where the effects of endothelin-1, on electrical field stimulation, and of adrenoceptor and P2-receptor agonists were isometrically recorded at 37°C and 30°C (cooling). The temperature 30°C was selected as it was within the range 34–24°C, where the maximal potentiating effects of endothelin-1 on electrical field stimulation in rabbit central ear artery were found (Padilla *et al*., 1997). In this study, we also analysed the role of the noradrenergic and purinergic components in the effects of cooling on the arterial response to sympathetic stimulation. The rabbit central ear artery has been used previously as a model of cutaneous blood vessels (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Van-

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houtte, 1988), and has a large population of α_1-adrenoceptors compared to α_2-adrenoceptors (Harker & Vanhoutte, 1988; García-Villalón *et al*., 1992).

Methods

Thirty one male New Zealand White rabbits, weighing 2–2.5 kg, were killed by intravenous injection of sodium pentobarbitone, 100 mg kg^{-1}. Central ear 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 with the following composition (mM): 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% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4, which was measured with a pH-meter micropH 2001 (Crison Instruments). Briefly, the method consisted of passing two fine, stainless steel pins, 150 μm in diameter, 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 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 previously determined resting passive tension of 0.5 g was applied to the vascular segments, and they were then allowed to equilibrate for 60–90 min before any drug was added. The temperature of the bath was adjusted from the beginning of the experiment to 37°C or 30°C (cooling), and the arteries remained at the chosen temperature throughout the duration of the experiment.

Electrical field stimulation (1, 2, 4 and 8 Hz, 0.2 ms pulse duration, at a supramaximal voltage of 70 V, for 5 s) was applied to the arteries via two platinum electrodes placed either side of the artery and connected to a CS-14 stimulator (Cibertec). An interval of at least 5 min was imposed between stimulation periods to allow the response to recover and the trains of stimulation were repeated in each case until the responses were reproducible for at least 40 min under control conditions. The effects of endothelin-1 (1, 3 and 10 nM) on the arterial response to electrical field stimulation were studied by cumulative addition to the organ bath. Electrical field stimulation (1–8 Hz) was applied twice after the addition of each concentration of the peptide. Each arterial segment was treated with three concentrations of endothelin-1. The effect of this peptide on the response to electrical stimulation was studied in the arterial segments at 37°C or 30°C, and each arterial segment was tested at only one of these temperatures. In some cases, the highest concentrations of endothelin-1 produced contraction of the arterial segments, but usually the level of this contraction diminished after electrical stimulation. If the contractile tone induced by endothelin-1 did not return to less than 25% of the maximal contraction achieved with electrical stimulation, the data of these particular segments were discarded.

To analyse the relative contribution of the noradrenergic and purinergic components to the sympathetic contraction of rabbit ear arteries, and the modulation by endothelin-1 of this contraction, the arterial response to electrical stimulation, and the effects of endothelin-1 on this response, were studied in the presence of phentolamine (1 μM), of the α_1-adrenoceptor antagonist prazosin (1 μM) of the α_2-adrenoceptor antagonist yohimbine (1 μM), of α, β, methyleneadenosine 5′-triphosphate (α,β-meATP, 3 μM) which produces desensitization of P2-receptors, of pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS 30 μM), a P2-receptor antagonist, of phentolamine (1 μM) plus α,β-meATP (3 μM) and of phentolamine (1 μM) plus PPADS (30 μM). Cumulative concentration-response curves for exogenous noradrenaline (0.01–100 μM) and adrenosine 5′-triphosphate (ATP, 0.1–10 mM) were also recorded at 37°C and 30°C in the absence and presence of phentolamine, α,β-meATP or PPADS, at the concentrations stated above.

After reproducible responses to electrical stimulation (1–8 Hz) had been obtained for 40 min, one of the antagonists was added to the organ bath and, then, 1 to 8 Hz stimulation applied twice in the presence of the antagonist. After this particular series of stimuli, endothelin-1 (1–10 nM) was also added to the organ bath cumulatively and the responses to electrical stimulation were recorded again in the arteries in the presence of each concentration of endothelin-1 plus the antagonist previously applied. As a control, one vascular segment, which was treated with endothelin-1, but not with any antagonist, was studied at each temperature.

To examine the site of action of endothelin-1 on the arterial response to electrical stimulation, the response of ear arteries to exogenous noradrenaline, ATP or the α_2-adrenoceptor agonist, BHT-920, were studied at 37°C and 30°C, in the absence (control) and in the presence of endothelin-1 (1, 3 and 10 nM). The response to BHT-920 was studied always in the presence of prazosin (1 μM) to block a possible α_1-adrenoceptor-mediated effect of this agonist. A single concentration, close to the EC_{50}, of noradrenaline (1 μM), BHT-920 (10 μM) or ATP (2 mM) was used, and it was first applied to the arteries every 15 min, each time followed by washing, until a repetitive response was obtained for at least 40 min. After this period, endothelin-1, at increasing concentrations (1, 3 and 10 nM), was added to the bath, and 15 min after the addition of each concentration of endothelin-1, the responses to noradrenaline, BHT-920 or ATP were again tested, this being followed by washing and addition of the next endothelin-1 concentration.

Contraction amplitudes are expressed as mean ± s.e.mean and were evaluated by two-way analysis of variance applied to each group of data, followed by paired Student's *t* test adjusted by the Bonferroni method for multiple comparisons with the same control. A probability value of less than 0.05 was considered significant when a single pair of means was compared, and 0.05 divided by the number of comparisons when multiple means were compared with control.

Drugs used were: adenosine 5′-triphosphate, disodium salt (ATP); (−)-arterenol, bitartrate salt ((−)-noradrenaline); α, β-methyleneadenosine 5′-triphosphate lithium salt (α,β-meATP); phentolamine hydrochloride; prazosin hydrochloride and yohimbine hydrochloride, all from Sigma; endothelin-1 (human, porcine) from Peninsula Laboratories Europe, Ltd; pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS tetrasodium salt) from Tocris Cookson Ltd. 5-Allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5]-dazepin hydrochloride (BHT-920) was a gift from Europharma S.A.

Results

Responses to electrical field stimulation, noradrenaline and ATP

Electrical field stimulation (1–8 Hz) produced frequency-dependent contraction of the vascular segments at 37°C and 30°C, but at 30°C the response was significantly lower ($P<0.001$) than at 37°C at each frequency of stimulation (0.52 ± 0.05 vs 1.24 ± 0.1 g for 8 Hz) (Figure 1). Both exogenous noradrenaline (0.001–100 μM) and ATP (0.1–10 nM) produced concentration-dependent contractions, which were similar at 37°C and 30°C (Figure 1).

Effects of adrenoceptor blockade on the response to electrical field stimulation

Phentolamine (1 μM) markedly reduced the response to electrical stimulation at 37°C and 30°C, and this reduction was significantly greater ($P<0.001$) at 37°C than at 30°C (66 ± 3 vs 39 ± 5% for 8 Hz) (Figure 2a). Phentolamine at this concen-

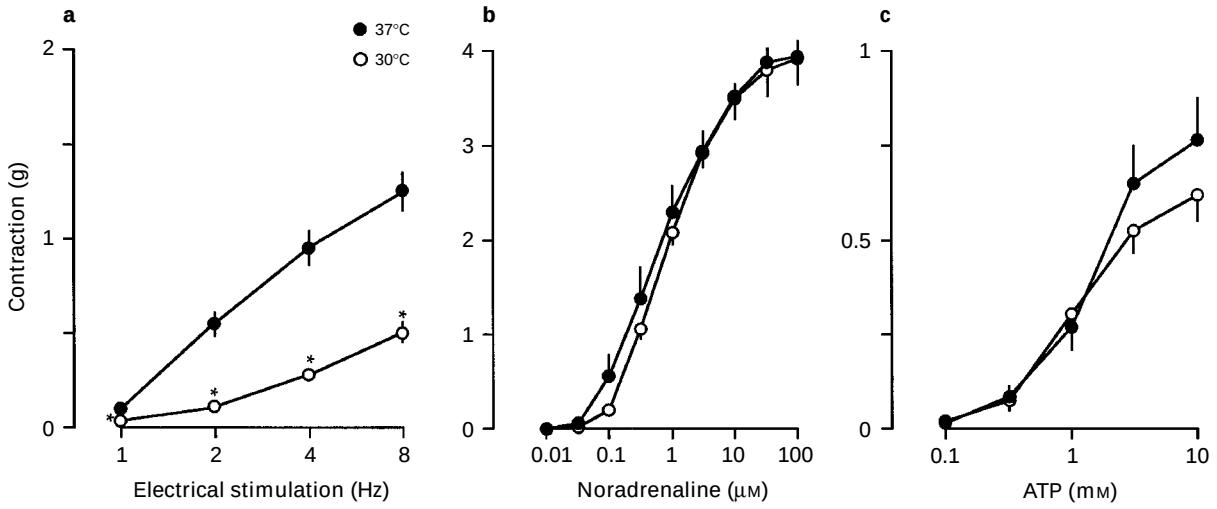

Figure 1 Contraction of rabbit central ear arteries to: (a) electrical field stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s; 19 animals), (b) noradrenaline (0.01–100 μM; 5 animals) and (c) ATP (0.1–10 mM; 7 animals) at 37°C and during cooling (30°C). Points are means and vertical lines show s.e.mean. *Significantly different compared with 37°C ($P<0.01$).

tration (1 μM) shifted to the right in a parallel way the concentration-response curve to exogenous noradrenaline at 37°C (about 10 times) and at 30°C (about 17 times), whereas it did not modify significantly the response to exogenous ATP at 37°C or 30°C (data not shown).

Blockade of α_1-adrenoceptors with prazosin (1 μM) reduced the contraction to electrical stimulation, this reduction being greater ($P<0.001$) at 37°C than at 30°C (62±5 vs 34±6% for 8 Hz) (Figure 2b).

The α_2-adrenoceptor antagonist yohimbine (1 μM) increased the response to electrical stimulation, and this increment, in percentage but not in absolute values, was greater ($P<0.01$) at 30°C than at 37°C (126±22 vs 40±18% for 8 Hz) (Figure 2c).

Effects of P2-receptor blockade on the response to electrical field stimulation

Application of α,β-meATP (3 μM) to desensitize P2-receptors produced a transient contraction at 37°C and at 30°C, which returned to the basal level in 10–15 min even though the agonist was still present in the bath. At 37°C, α,β-meATP increased the contraction (19±3% for 8 Hz; $P<0.001$), whereas at 30°C α,β-meATP did not modify significantly the response to electrical stimulation (Figure 3a). PPADS did not modify the contraction to electrical stimulation at 37°C or 30°C (Figure 3b). At both temperatures, α,β-meATP (3 μM) abolished, and PPADS (30 μM) reduced (maximal effect 0.15±0.08 vs 0.90±0.18 g at 37°C, and 0.20±0.07 vs 0.74±0.09 g at 30°C) the contraction to ATP, both at 37°C and 30°C. However, the contraction to noradrenaline was not modified in the presence of α,β-meATP (maximal effect 3.9±0.17 vs 3.5±0.16 g at 37°C, and 3.9±0.24 vs 3.5±0.18 g at 30°C) or PPADS (maximal effect 4.2±0.45 vs 4.3±0.31 g at 37°C and 3.8±0.45 vs 4.4±0.5 g at 30°C).

Application of both phentolamine (1 μM) and α,β-meATP (3 μM) reduced the response to electrical stimulation at both 37°C (74±3% for 8 Hz) and 30°C (82±2% for 8 Hz) (Figure 3c). The response to electrical stimulation was also reduced by PPADS plus phentolamine at 37°C (82±4% for 8 Hz) and 30°C (71±3% for 8 Hz) (Figure 3d).

Effects of endothelin-1 on the response to electrical field stimulation

Control At 37°C, endothelin-1 (1–10 nM) did not modify significantly the contraction to electrical stimulation, but at 30°C it produced a concentration-dependent increase in this response (Figure 4a).

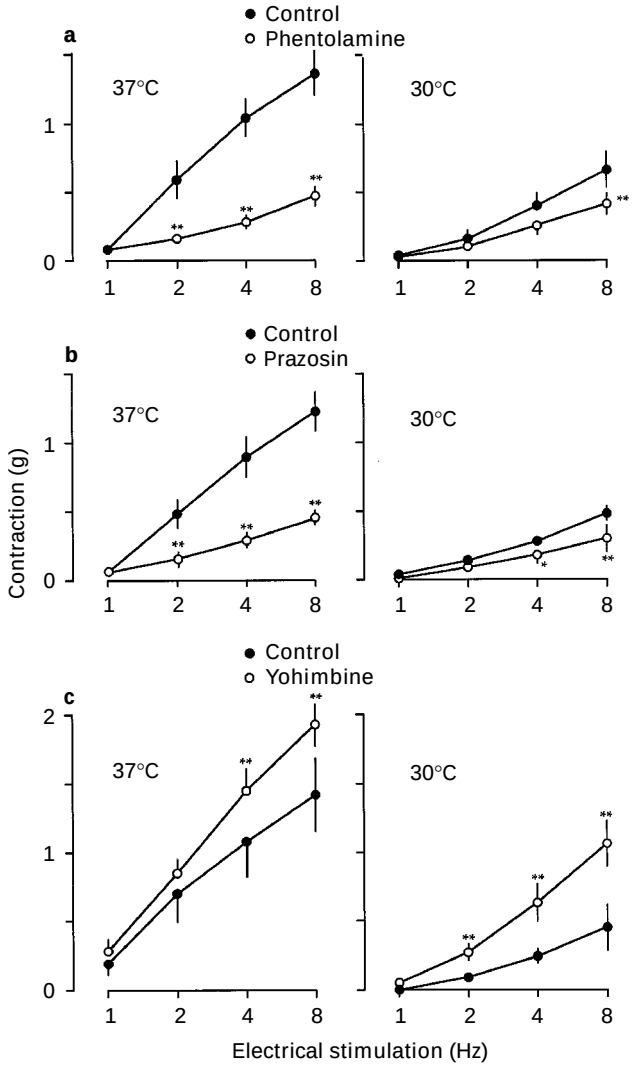

Figure 2 Contraction of rabbit central ear arteries to electrical field stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s): in the absence (control) and presence of: (a) phentolamine (1 μM), (b) prazosin (1 μM) and (c) yohimbine (1 μM), at 37°C and 30°C. Points are means and vertical lines show s.e.mean. *$P<0.05$, **$P<0.01$ significantly different from control. In each case, data were an average from 6–7 animals.

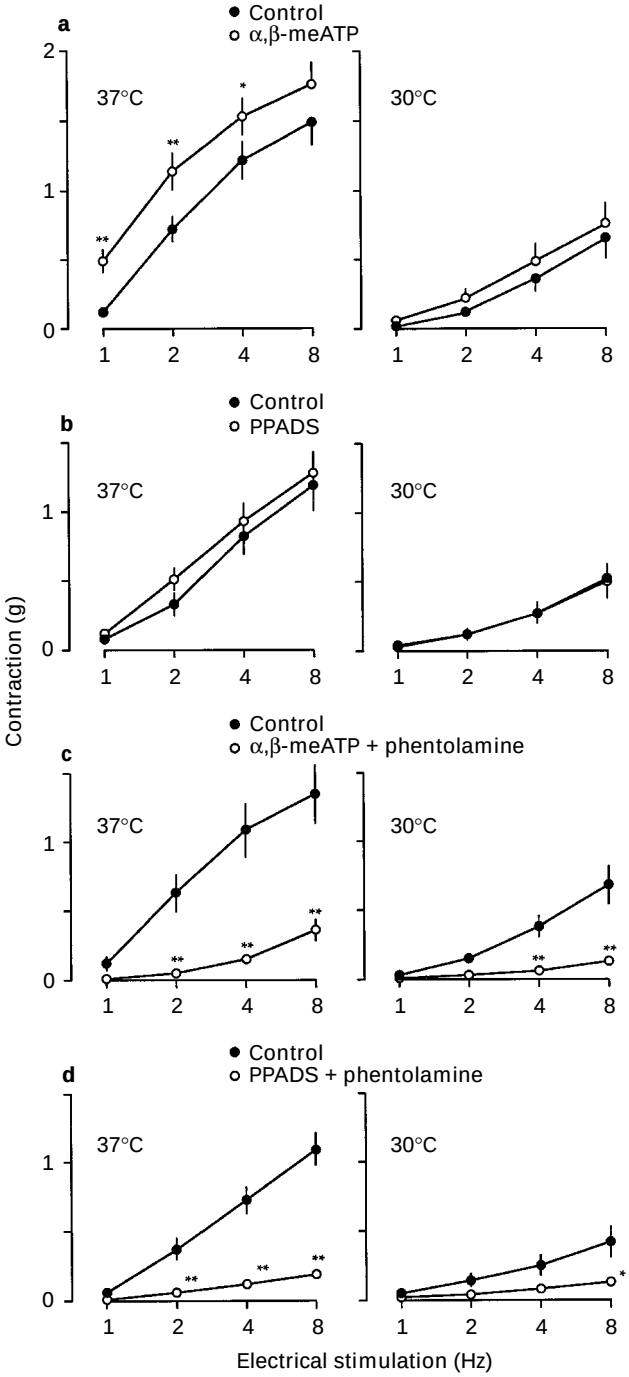

Figure 3 Contraction of rabbit central ear arteries to electrical field stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s) in the absence (control) and presence of: (a) α,β-meATP (3 μM), (b) PPADS (30 μM), (c) α,β-meATP (3 μM) plus phentolamine (1 μM) and (d) PPADS (30 μM) plus phentolamine (1 μM), at 37°C and 30°C. Points are means and vertical lines show s.e.mean. $*P<0.05$, $**P<0.01$, significantly different from control. In each case, data were an average from 6–7 animals.

Adrenoceptor blockade At 37°C, application of endothelin-1 (10 nM) in the presence of phentolamine (1 μM) potentiated the response to electrical stimulation (Figure 4b), in contrast to the lack of potentiation at this temperature in the absence of α-adrenoceptor blockade (Figure 4a). At 30°C, application of endothelin-1 (10 nM) in the presence of phentolamine (1 μM) also potentiated the response to electrical stimulation (Figure 4b), and this potentiation by endothelin-1 was lower ($P<0.001$) than that observed at this temperature in the absence of phentolamine (Figure 4a).

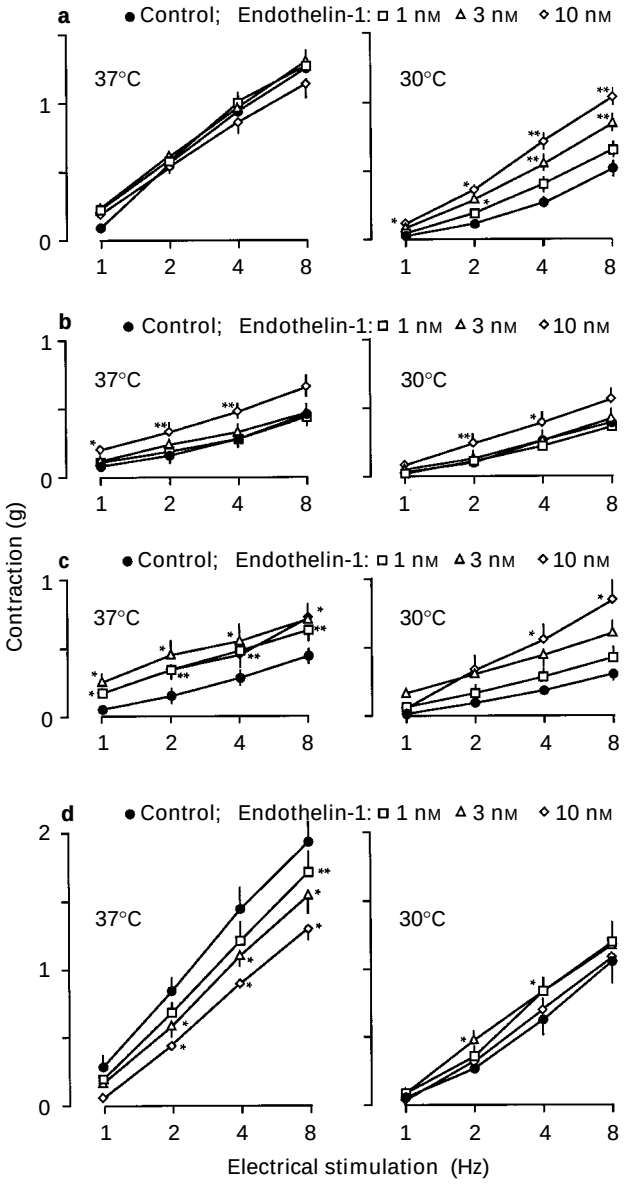

Figure 4 Contraction of rabbit central ear arteries to electrical field stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s) in the absence (control) and in the presence of endothelin-1 (1–10 nM), at 37°C and 30°C: (a) without α-adrenoceptor antagonists, (b) with phentolamine (1 μM), (c) with prazosin (1 μM) and (d) with yohimbine (1 μM). Points are means and vertical lines show s.e.mean. $*P<0.01$, $**P<0.001$, significantly different from that found in the absence of endothelin-1. Data were averaged from 19 animals in (a) and from 6–7 animals in (b), (c) and (d).

In the presence of prazosin (1 μM) endothelin-1 potentiated the contraction to electrical stimulation at 37°C (Figure 4c). At 30°C, the presence of prazosin did not modify the potentiating effects of endothelin-1 to electrical stimulation, as compared to that found in the absence of this α_1-adrenoceptor blocker (Figure 4c).

At 37°C addition of endothelin-1 in the presence of yohimbine reduced the response to electrical stimulation (Figure 4d). At 30°C, addition of endothelin-1 (1 and 3 nM) in the presence of yohimbine (1 μM) increased the response to electrical stimulation (Figure 4d), and this increment was smaller ($P<0.001$) than that observed in the absence of this α_2-adrenoceptor antagonist (Figure 4a).

P2-receptor blockade At 37°C, addition of endothelin-1 in the presence of α,β-meATP reduced ($P<0.001$) the contraction to

electrical stimulation. At 30°C it potentiated the contraction to electrical stimulation, this potentiation being slightly increased ($P<0.01$) in the presence of α,β-meATP (Figure 5a). In the presence of PPADS, endothelin-1 (1–10 nM) at 37°C reduced the contraction to electrical stimulation, and at 30°C it potentiated this contraction as in control conditions (Figure 5b).

Combined adrenoceptor and P2-receptor blockade At 37°C, addition of endothelin-1 in the presence of phentolamine plus α,β-meATP (Figure 5c) or phentolamine plus PPADS (Figure 5d), did not modify the arterial contraction to electrical stimulation. At 30°C, endothelin-1 in the presence of phentolamine plus α,β-meATP (Figure 5c) or phentolamine plus PPADS (Figure 5d) induced an increase in the contraction to 8 Hz stimulation, and this was with only 10 nM endothelin-1.

Effect of endothelin-1 on the contraction to exogenous noradrenaline and ATP

Noradrenaline, at a concentration close to its EC_{50} value (1 μM), contracted the arteries, the magnitude of which was similar at 37°C (2.57 ± 0.13 g) and 30°C (2.27 ± 0.11 g). This response to noradrenaline was similar in the absence (control) and in the presence of endothelin-1 (1–10 nM), both at 37°C and 30°C (Figure 6a).

The α_2-adrenoceptor agonist BHT-920 (10 μM) produced a small contraction at 37°C (0.016 ± 0.002 g) but no apparent response at 30°C. However, in the presence of endothelin-1 (1–10 nM) this α_2-adrenoceptor agonist produced more marked arterial contractions, both at 30°C (0.74 ± 0.06 g) and 37°C (1.0 ± 0.09 g) (Figure 6b).

ATP, at a concentration close to its EC_{50} value (2 mM), contracted the arterial segments similarly at 37°C (0.5 ± 0.02 g) and 30°C (0.51 ± 0.04 g), and these effects were greater in the presence of endothelin-1. The magnitude of the increment evoked by endothelin-1 on the response to ATP was similar at 37°C and 30°C (Figure 6c).

Discussion

In a recent study from our laboratory, we found that in the rabbit central ear artery, cooling reduces the contraction to sympathetic stimulation, and that endothelin-1 potentiates this contraction during cooling but not at normotemperature and warming (Padilla *et al*., 1997). The present study confirms

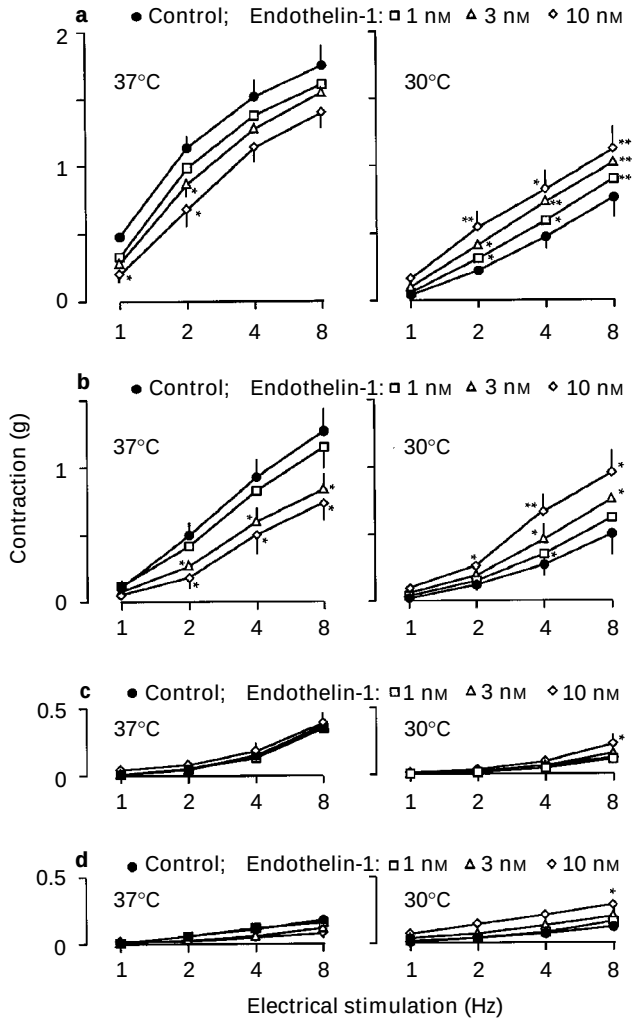

Figure 5 Contraction of rabbit central ear arteries to electrical field stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s) in the absence (control) and in the presence of endothelin-1 (1–10 nM), at 37°C and 30°C: (a) with α,β-meATP (3 μM), (b) with PPADS (30 μM), (c) with α,β-meATP (3 μM) plus phentolamine (1 μM) and (d) with PPADS (30 μM) plus phentolamine (1 μM). Points are means and vertical lines show s.e.mean. $*P<0.01$, $**P<0.001$, significantly different from that found in the absence of endothelin-1. In each case, data were averaged from 6–7 animals.

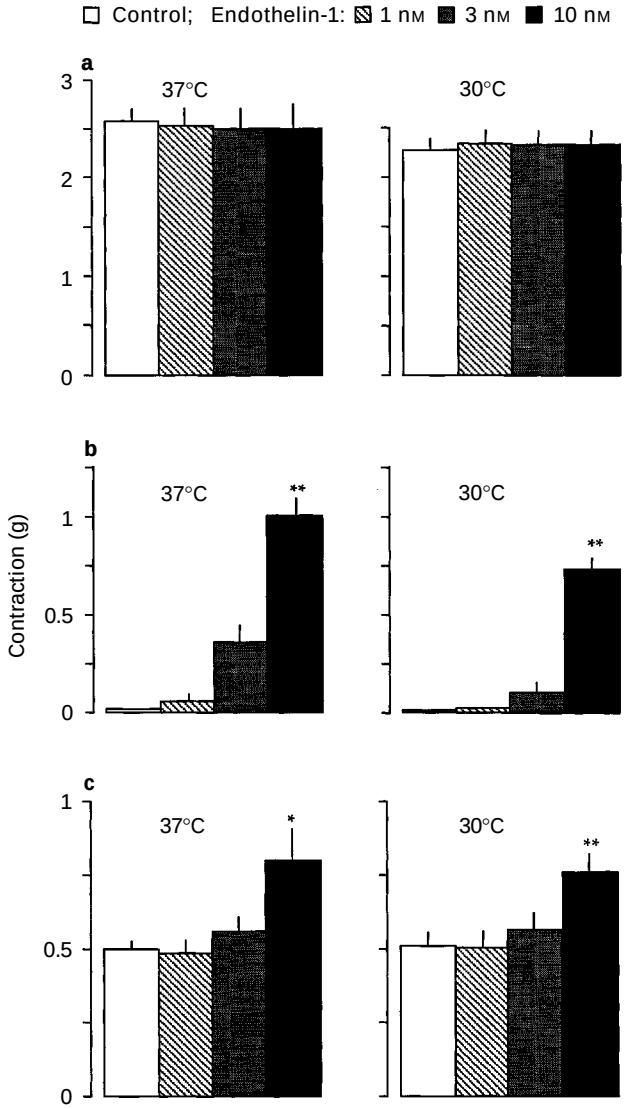

Figure 6 Contraction of rabbit central ear arteries to: (a) noradrenaline (1 μM), (b) BHT-920 (10 μM) in the presence of prazosin (1 μM) and (c) ATP (2 mM). These responses were obtained in the absence (control) and presence of endothelin-1 (1–10 nM), at 37°C and 30°C. Values are means±s.e.mean. $*P<0.01$, $**P<0.001$, significantly different from control. In each case, data were averaged from 6 animals.

these observations and provides data suggesting that endothelin-1 potentiates the arterial contraction to sympathetic stimulation during cooling by, at least in part, increasing the responsiveness of postjunctional α_2-adrenoceptors and P2-receptors. Before discussing the results with electrical field stimulation in the presence of endothelin-1, we will discuss firstly the mechanisms by which cooling blunts the arterial response to this stimulation.

Our present results suggest that the mechanisms involved in the constriction of the rabbit central ear artery to sympathetic stimulation are modified during moderate cooling (30°C). At 37°C, the contraction of rabbit central ear arteries to electrical field stimulation may be mainly due to activation of postjunctional α_1-adrenoceptors by noradrenaline released from perivascular sympathetic terminals, with little participation of P2-receptors, as this response was markedly blocked by phentolamine or prazosin, whereas the P2-receptor inhibitors α,β-meATP and PPADS increased it and had no effect, respectively. A purinergic component of the sympathetic response has been found in rat mesenteric arteries (Sjöblom-Widfeldt *et al*., 1990) and rabbit mesenteric (von Kügelgen & Starke, 1985) and saphenous (Burnstock & Warland, 1987) arteries, but not in rabbit central ear arteries (Saville *et al*., 1990). These discrepancies may be related to the experimental protocol used, as the relative participation of the noradrenergic and purinergic components in the vasoconstriction to electrical stimulation may be strongly dependent on the stimulation parameters used (Kennedy *et al*., 1986) and perhaps the vascular bed studied.

During cooling, there may be an impairment of the α_1-adrenoceptor-mediated response, because the contraction to electrical field stimulation was reduced at 30°C, and phentolamine or prazosin produced a smaller reduction of the contraction at 30°C than at 37°C. This impairment may be due to a reduction in the release of noradrenaline from perivascular sympathetic terminals, as cooling did not affect the response to exogenous noradrenaline. This reduced release of noradrenaline might be related to activation by cooling of prejunctional α_2-adrenoceptors, although this hypothesis is not clearly supported by our data, since the increment caused by blockade of α_2-adrenoceptors with yohimbine on the response to electrical stimulation was proportionally greater at 30°C than at 37°C only in percentage but not in absolute values. Moreover a purinergic component in the contraction of ear arteries to electrical stimulation may be present during cooling, because the contraction which remained after blockade of α-adrenoceptors with phentolamine was reduced further by treatment with α,β-meATP or PPADS together with phentolamine, and this reduction was greater at 30°C than at 37°C. This result agrees with the observation that in rat mesenteric arteries cooling potentiates the purinergic component of the sympathetic response (Yamamoto *et al*., 1992).

With regard to the effects of endothelin-1 on the sympathetic vascular contraction, the present results show that this peptide potentiates the contraction of the rabbit central ear artery to electrical field stimulation at 30°C but not at 37°C. This potentiating effect of endothelin-1 at 30°C may be due, in part, to endothelin-1 increasing the response of postjunctional α_2-adrenoceptors, as the observed potentiating effect of endothelin-1 was reduced by phentolamine or yohimbine but was not modified by prazosin. This potentiation probably occurs at the postjunctional level, because endothelin-1 also potentiated the contraction to the α_2-adrenoceptor agonist BHT-920. Previous studies (García-Villalón *et al*., 1992) have suggested that, in rabbit central ear arteries, postjunctional α_2-adrenoceptors have relatively little functional importance and the present results confirm this, as in control conditions (in the absence of endothelin-1) the arterial response to BHT-920 was very small both at 37°C and 30°C. However, following pretreatment with endothelin-1 the response to BHT-920 was higher than in control conditions both at 37°C and 30°C, thus suggesting that during cooling endothelin-1 may increase the functional importance of α_2-adrenoceptors in ear central arteries. A similar phenomenon has been described in canine mesenteric artery and vein (Shimamoto *et al*., 1992) and in rat tail artery (MacLean & McGrath, 1990); these authors suggested that this effect of endothelin-1 is due to an increase in the levels of cytoplasmatic Ca^{2+} (MacLean & McGrath, 1990; Shimamoto *et al*., 1992).

Moreover, not only postjunctional α_2-adrenoceptors, but P2-receptors may also be involved in the potentiating effects of endothelin-1 on the sympathetic response of the rabbit central ear artery during cooling. We observed that the potentiation of the response to electrical field stimulation by endothelin-1, that persisted in the presence of phentolamine, was reduced further by treatment with α,β-meATP and tended to be reduced, although not significantly, by PPADS. Endothelin-1 also potentiated the contraction to exogenous ATP. These observations suggest that endothelin-1 may increase the contraction to electrical stimulation during cooling because at low temperature this peptide may increase the sensitivity of P2-receptors. On the basis that in the rabbit central ear artery noradrenaline acts mainly through α_1-adrenoceptors (García-Villalón *et al*., 1992), the present results suggest that the responsiveness of α_1-adrenoceptors is not affected by endothelin-1 as the contraction to exogenous noradrenaline was not modified by this peptide.

On the other hand, we found that endothelin-1 failed to potentiate the arterial contraction to electrical field stimulation at 37°C in control conditions, in contrast with other studies where it was shown to potentiate this response in rabbit ear (Wong-Dusting *et al*., 1991), saphenous (Mutafova-Yambolieva & Radomirov, 1994) or jejunal (La & Rand, 1993) arteries. The reason for this discrepancy is unclear, but it may be related, at least in part, to the neurotransmission characteristics of the response, which may differ under the different experimental conditions. It has been suggested that endothelin-1 potentiates preferentially the purinergic component of the contractile response to electrical stimulation in the rabbit saphenous (Mutafova-Yambolieva & Radomirov, 1994) and jejunal (La & Rand, 1993) arteries, and in accord with this is our observation that endothelin-1 potentiated the response to exogenous ATP at 37°C. However, as discussed above, in our experimental conditions the contraction to electrical stimulation at 37°C may be mainly mediated by α_1-adrenoceptors with little participation of P2-receptors or α_2-adrenoceptors, a feature that might explain the lack of potentiating effect of endothelin-1 at 37°C in our experiments. We did observe a potentiating effect of endothelin-1 at 37°C after treatment with phentolamine or prazosin, and this effect of endothelin-1 may be due to potentiation of P2-receptors as it was abolished by pretreatment with α,β-meATP or PPADS together with phentolamine. Therefore, at 37°C endothelin-1 may potentiate the purinergic component of the arterial response to electrical stimulation when this component is of functional importance, as occurs in rabbit saphenous (Mutafova-Yambolieva & Radomirov, 1994) or jejunal (La & Rand, 1993) arteries or when the α_1-adrenoceptor-mediated component is reduced by cooling or pharmacologically blocked (present results). A potentiation of α_2-adrenoceptors by endothelin-1 during the arterial response to electrical stimulation may also be present at 37°C, because in our experiments endothelin-1 also increased the contraction to the α_2-adrenoceptor agonist BHT-920.

In summary, our results in the rabbit central ear artery (cutaneous artery) suggest that the sympathetic response: (1) at 37°C is mainly mediated by activation of α_1-adrenoceptors; (2) is diminished during cooling (30°C), probably due to reduction of the α_1-adrenoceptor-mediated response, and (3) is potentiated during cooling by endothelin-1, probably because in this condition this peptide facilitates the responsiveness of postjunctional α_2-adrenoceptors and P2-receptors.

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References

BORBUJO, J., GARCÍA-VILLALÓN, A.L., VALLE, J., GÓMEZ, B. & DIÉGUEZ, G. (1989). Posjunctional alpha$_1$ and alpha$_2$-adrenoceptors in human skin arteries. An in vitro study. *J. Pharmacol. Exp. Ther.*, **249,** 284–287.

BURNSTOCK, G. (1986). The changing face of autonomic neurotransmission. *Acta Physiol. Scand.*, **126,** 67–91.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). A pharmacological study of the rabbit saphenous artery *in vitro*: a vessel with a large purinergic contractile response to sympathetic nerve stimulation. *Br. J. Pharmacol.*, **90,** 111–120.

GARCÍA-VILLALÓN, A.L., MONGE, L., MONTOYA, J.J., GARCÍA, J.L., FENÁNDEZ, N., GÓMEZ, B. & DIÉGUEZ, G. (1992). Cooling and response to adrenoceptor agonists of rabbit ear and femoral artery: role of the endothelium. *Br. J. Pharmacol.*, **106,** 727–737.

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.

KENNEDY, C., SAVILLE, V.L. & BURNSTOCK, G. (1986). The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. *Eur. J. Pharmacol.*, **122,** 291–300.

LA, M. & RAND, M.J. (1993). Endothelin-1 enhances vasoconstrictor responses to exogenously administered and neurogenically released ATP in rabbit isolated perfused arteries. *Eur. J. Pharmacol.*, **249,** 133–139.

MACLEAN, M.R. & MCGRATH, J.C. (1990). Effects of pre-contraction with endothelin-1 on α_2-adrenoceptor and (endothelium-dependent) neuropeptide Y-mediated contractions in the isolated vascular bed of the rat tail. *Br. J. Pharmacol.*, **101,** 205–211.

MUTAFOVA-YAMBOLIEVA, V.N. & RADOMIROV, R.G. (1994). Modulatory effects of endothelin-1 on purinergic and adrenergic components of sympathetically-mediated contractile activity of rabbit saphenous artery. *Br. J. Pharmacol.*, **112,** 1109–1117.

PADILLA, J., GARCÍA-VILLALÓN, A.L., MONGE, L., GARCÍA, J.L., FERNÁNDEZ, N., GÓMEZ, B. & DIÉGUEZ, G. (1997). Peptidergic modulation of the sympathetic contraction in the rabbit ear artery: effects of temperature. *Br. J. Pharmacol.*, (in press).

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 37–30°C. *Ir. J. Med. Sci.*, **147,** 313–317.

REID, J.J. (1993). Endothelin-1 may be a physiologic modulator of vasoconstriction in rat kidney. *J. Cardiovasc. Res.*, **22,** (Suppl. 8), S267–S270.

ROBERTS, M.F. & ZYGMUNT, A.C. (1984). Reflex and local thermal control of rabbit ear blood flow. *Am. J. Physiol.*, **246,** R979–R984.

SAVILLE, V.L., MAYNARD, K.I. & BURNSTOCK, G. (1990). Neuropeptide Y potentiates purinergic as well as adrenergic responses of the rabbit ear artery. *Eur. J. Pharmacol.*, **176,** 117–125.

SHIMAMOTO, H., KWAN, C.-Y. & DANIEL, E.E. (1992). Pharmacological assessment of Ca^{2+} dependence of endothelin-1 induced response in rat aorta. *Eur. J. Pharmacol.*, **216,** 225–233.

SJÖBLOM-WIDFELDT, N., GUSTAFSSON, H. & NILSSON, H. (1990). Transmitter characteristics of small mesenteric arteries from the rat. *Acta Physiol. Scand.*, **138,** 203–212.

VANHOUTTE, P.M. & FLAVAHAN, N.A. (1986). Effects of temperature on alpha-adrenoceptors in limb veins: role of receptor reserve. *Fed. Proc.*, **45,** 2347–2354.

VON KÜGELGEN, I.V. & STARKE, K. (1985). Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasoconstriction in rabbit mesenteric artery. *J. Physiol.*, **367,** 435–455.

WIKLUND, N.P., OHLEN, A., WIKLUND, C.U. & CEDERQVIST, B. (1989). Adrenergic neuromodulation by endothelin in guinea-pig pulmonary artery. *Neurosci. Lett.*, **101,** 269–274.

WONG-DUSTING, H.K., LA, M. & RAND, M.J. (1991). Endothelin-1 enhances vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline in rabbit ear artery. *Clin. Exp. Phamacol. Physiol.*, **18,** 131–136.

YAMAMOTO, R., TAKASAKI, K. & NICKOLS, G.A. (1992). Purinergic vasoconstrictor component revealed by moderate cooling in the isolated mesenteric vasculature of Sprague-Dawley rats. *J. Pharmacol. Exp. Ther.*, **262,** 1133–1139.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMBOE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332,** 411–415.

YANG, Z., RICHARD, V., VON SEGESSER, L., BAUER, E., STULZ, P., TURINA, M. & LÜSCHER, T.F. (1990). Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries. *Circulation*, **82,** 188–195.

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