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Peptidergic modulation of the sympathetic contraction in the rabbit ear artery: effects of temperature

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1 The effects of neuropeptide Y, endothelin-1, arginine-vasopressin and angiotensin II on the vascular contraction to sympathetic nerve stimulation were studied in isolated segments, 2 mm long, from the rabbit central ear artery, a cutaneous vessel, during changes in temperature (24°–41°C).

2 Transmural electrical stimulation (1–8 Hz, at supramaximal voltage) produced frequency-dependent contraction, and this response, partially blocked by tetrodotoxin (1 μM) and phentolamine (1 μM), was reduced by cooling (30°C–24°C) and was not modified by warming (41°C), as compared to that recorded at 37°C.

3 Pretreatment with neuropeptide Y (10, 30 and 100 nM) increased in a concentration-dependent manner the vascular contraction to sympathetic stimulation at every temperature studied, but this potentiation was greater during cooling (34°C–24°C) than at 37°C or warming (41°C).

4 Pretreatment with endothelin-1 (3 and 10 nM) or vasopressin (0.1, 0.3 and 1 nM) increased in a concentration-dependent manner the vascular contraction to sympathetic stimulation during cooling (34°C–24°C), but not at 37°C or warming (41°C).

5 Pretreatment with angiotensin II (0.1, 0.3 and 1 μM) did not modify the contraction to sympathetic stimulation at any temperature studied.

6 These results suggest that neuropeptide Y, endothelin-1 and vasopressin, but not angiotensin II, modulate the cutaneous vasoconstriction to sympathetic nerve stimulation by potentiating this vasoconstriction during cooling.

Keywords: Cutaneous arteries; endothelin-1; neuropeptide Y; arginine-vasopressin; cooling

Introduction

Experimental data suggest that some vasoactive peptides (neuropeptide Y, endothelin-1, angiotensin II, vasopressin) modulate the vascular response to sympathetic stimulation under normal conditions. Neuropeptide Y, a co-neurotransmitter in perivascular sympathetic nerve endings (Lundberg & Tatemoto, 1982), may produce prejunctional inhibition of noradrenaline release (Pernow *et al*., 1986) and postjunctional potentiation of the constriction of some types of vessels to sympathetic stimulation (Ekblad *et al*., 1984). Endothelin-1, a peptide released from the vascular endothelium (Yanagisawa *et al*., 1988), may also produce prejunctional inhibition and/or postjunctional potentiation of the constriction of rat mesenteric arteries (Tabuchi *et al*., 1990) and of canine coronary arteries (Aarnio *et al*., 1993) to sympathetic stimulation. Angiotensin II, a peptide circulating in plasma, has been found to potentiate sympathetic constriction in rat mesenteric artery (Jonsson *et al*., 1993). With regard to vasopressin, this hormone may interact with the sympathetic regulation of the vascular system as it may potentiate the sympathetic-mediated baroreceptor reflex (Cowley *et al*., 1984). However, the possible interaction of this hormone at the peripheral level between perivascular nerve terminals and blood vessels reactivity is little known.

Our interest is focused in studying the possible peptidergic modulatory role in reactivity of cutaneous vasculature during changes in temperature, as it could be of relevance for understanding the physiology and physiopathology of the regulation of the cutaneous circulation, and consequently of body temperature regulation. The cutaneous circulation is adrenergically innervated and is involved in body temperature regulation and exposure of body surface to a cold environment induces cutaneous vasoconstriction, thus reducing cutaneous blood flow and preventing heat loss (Vanhoutte, 1980). It has been shown that cooling may increase the response of cutaneous blood vessels to adrenergic stimulation 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). Recently, we have found that in the rabbit central ear artery cooling may increase the release of nitric oxide, which may potentiate the cholinergic relaxation (Fernández *et al*., 1994) and may inhibit the response of this artery to adrenergic stimulation (García-Villalón *et al*., 1992).

The present study was conducted to investigate the possible peptidergic modulation in the sympathetic nerve response of cutaneous vasculature during changes in temperature. This was performed by recording *in vitro* the effects of neuropeptide Y, endothelin-1, vasopressin and angiotensin II on the response of the central ear artery from rabbits to field electrical stimulation, after exposure of this artery to normal (37°C), increased (41°C) and decreased (34°C, 30°C, 27°C and 24°C) temperatures. The central ear artery was selected because it is a superficial artery that is easily accessible and used as a model of cutaneous blood vessels (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988).

Methods

Thirty two 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

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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 (Micro pH 2001, Crison Instruments). 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 previously determined resting passive tension of 0.5 g was applied to the vascular segments, and then they were 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 at 24°C, 27°C, 30°C, 34°C, 37°C or 41°C, and the arteries remained at the chosen temperature throughout the duration of the experiments.

Transmural electrical stimulation (1, 2, 4 and 8 Hz, 0.2 ms pulse duration, at a supramaximal voltage of 70 V, during 5 s) was applied to the arteries with two platinum electrodes placed at 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 recovery from the response, and the stimulation trains were initially repeated until the responses were reproducible during at least 40 min under control conditions. Then, the effects of neuropeptide Y (10, 30 and 100 nM), endothelin-1 (3 and 10 nM), vasopressin (0.1, 0.3 and 1 nM) and angiotensin II (0.1, 0.3 and 1 μM) on the arterial response to electrical stimulation were studied by adding these peptides in a cumulative manner to the organ bath. These peptide concentrations were selected because they produced small or no alterations in resting arterial tension. Two stimulation series from 1 to 8 Hz were applied to the arteries after each dose of the peptide, and each arterial segment was treated with every dose of the corresponding peptide. It was observed that, from the two stimulation series applied, the maximal effects of neuropeptide Y and vasopressin were observed in the first series, whereas those of endothelin-1 were found in the second series; angiotensin II did not produce an effect in either series. Therefore, the effects of neuropeptide Y, vasopressin and angiotensin II were measured in the first stimulation series (5 min after adding the peptide), and those of endothelin-1 were measured in the second series (20 min after adding endothelin-1). Preliminary studies indicated that in a third stimulation series no further effects of these peptides were observed. The effects of these peptides on the response to electrical stimulation were studied in the arterial segments at 24°C, 27°C, 30°C, 34°C, 37°C or 41°C; each arterial segment was tested at only one of these temperatures.

In some cases, the highest concentrations of endothelin-1 or vasopressin produced contraction of the arterial segments, but usually this contraction diminished after electrical stimulation. If the contractile tone induced by endothelin-1 and vasopressin did not return to less than 25% of the maximal contraction achieved with electrical stimulation, the data of these particular segments were discarded.

In a series of separate experiments the effects of tetrodotoxin (1 μM) on the arterial response to electrical stimulation were studied at 37°C and 30°C to determine whether that response was neurogenic in origin. The effects of phentolamine (1 μM) were also studied at 37°C and 30°C to determine whether the response to electrical stimulation is mediated by adrenergic mechanisms. Tetrodotoxin or phentolamine were added 20 min before applying the electrical stimulation (1–8 Hz).

Statistics

The values of the arterial contraction are expressed as means ± s.e.mean, and were evaluated by analysis of variance applied to each group of data, followed by paired Student's t test to compare each experimental condition with its control. A probability value of less than 0.05 was considered significant.

Drugs used

Neuropeptide Y (porcine), arginine-vasopressin (acetate salt), angiotensin II (human, acetate salt) and tetrodotoxin (Fugu poison) and phentolamine hydrochloride were obtained from Sigma; endothelin-1 (human porcine) was obtained from Peninsula Laboratories Europe, Ltd.

Results

Effects of temperature on the response to electrical stimulation

Electrical stimulation (1–8 Hz) produced frequency-dependent contraction of the vascular segments at every temperature tested. At 41°C and 34°C, the response was not significantly different from that at normotemperature (37°C), but at 30°C the arterial response was significantly ($P<0.01$) reduced in relation to that at 37°C, at every frequency of electrical stimulation applied. With further degrees of cooling (27°C and 24°C), the arterial response was also reduced, this being not significantly ($P<0.05$) different from that obtained at 30°C.

Figure 1 shows representative recordings displaying the effects of electrical stimulation on segments of rabbit central ear artery at 37°C and 30°C, and Figure 2 summarizes these effects at the different temperatures tested.

Effects of tetrodotoxin and of α-adrenoceptor blockade on electrical stimulation

Tetrodotoxin (1 μM) reduced the arterial contraction to electrical stimulation at 37°C (82% at 8 Hz; $P<0.01$) and 30°C (69% at 8 Hz; $P<0.01$) as compared with that produced in the absence of this substance (Table 1).

Phentolamine (1 μM) also reduced the arterial contraction to electrical stimulation at 37°C (66% at 8 Hz; $P<0.01$) and 30°C (40% at 8 Hz; $P<0.01$) in comparison to that recorded in the absence of this antagonist for α-adrenoceptors (Table 1). The blocking effect of phentolamine was significantly lower at 30°C than at 37°C ($P<0.01$).

Effects of neuropeptide Y

Pretreatment with neuropeptide Y (10, 30 and 100 nM) produced a concentration-dependent increase ($P<0.01$) in the arterial contraction to each frequency of electrical stimulation applied, and at every temperature studied. This potentiation was smaller during normotemperature (37°C) ($P>0.05$) and warming (41°C), but it was greater ($P<0.05$) during cooling (34°C, 30°C, 27°C and 24°C) than during normotemperature or warming, it being maximal at 30°C and 27°C (Figure 3).

Effects of endothelin-1

At 37°C and 41°C, addition of endothelin-1 (3 and 10 nM) did not modify significantly ($P>0.05$) the contraction, but at lower temperatures (34°C, 30°C, 27°C and 24°C) endothelin-1 produced a concentration-dependent increase ($P<0.01$) in the response to every frequency of electrical stimulation applied. This increase induced by endothelin-1 was maximal at 30°C, and lower, although still present, at 34°C, 27°C and 24°C (Figure 4).

Effects of vasopressin

The presence of vasopressin (0.1, 0.3 and 1 nM) did not modify ($P>0.05$) significantly the arterial contraction to electrical stimulation at any frequency applied during normo-

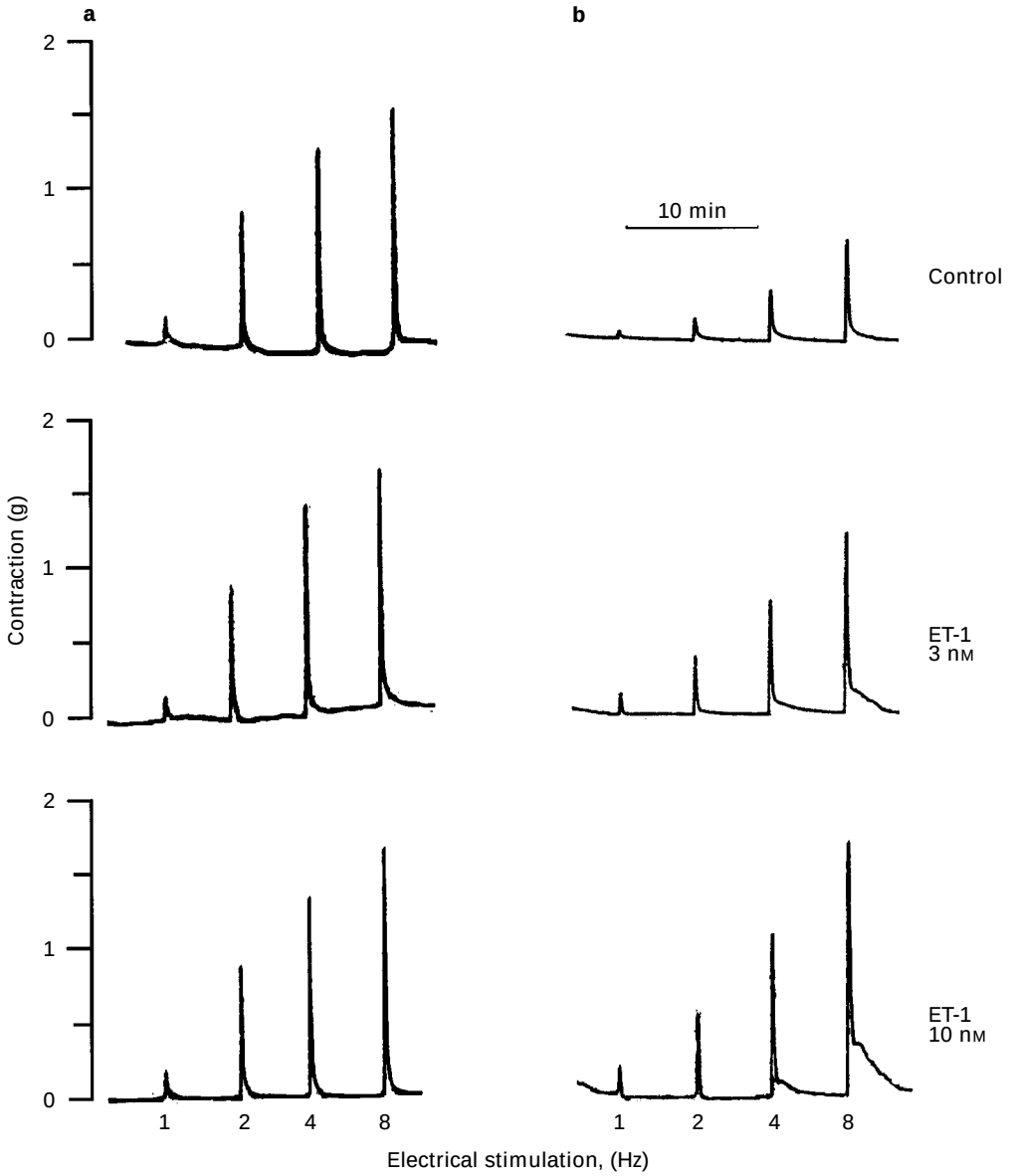

Figure 1 Representative tracings of the effects of transmural electrical stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, during 5 s) on rabbit ear arteries under control conditions and after pretreatment with endothelin-1 (ET-1), at 37°C (a) and 30°C (b).

temperature (37°C), warming (41°C) or slight cooling (34°C). However, it produced a concentration-dependent increase ($P<0.01$) in the arterial contractile response to electrical stimulation during further cooling (30°C, 27°C and 24°C), this increase being maximal at 30°C and 27°C ($P<0.01$) (Figure 5).

Effects of angiotensin II

The presence of angiotensin II (0.1, 0.3 and 1 μM) did not modify ($P>0.05$) significantly the arterial contraction to any frequency of electrical stimulation used, at any temperature studied (not shown).

Discussion

The contraction of rabbit ear arteries in response to transmural electrical stimulation recorded in our experiments at normotemperature (37°C) and cooling (30°C) may be neurogenic in nature as this effect was mostly blocked by tetrodotoxin. The residual contraction that was observed after treatment with tetrodotoxin may be due to direct depolarization of smooth muscle cells during electrical stimulation (Hardebo *et al*., 1986). We found that phentolamine also reduced the arterial contraction to electrical stimulation at the temperatures tested (37°C and 30 °C), suggesting that this response at normotemperature and cooling could be mediated, at least in part, by noradrenaline released from perivascular sympathetic nerve endings which activates postjunctional α-adrenoceptors. This observation is in line with data indicating that the rabbit ear artery is richly innervated by sympathetic nerves (Bevan *et al*., 1972). However, our experiments do not exclude the possibility that other mediators, in addition to noradrenaline, are also involved in the arterial contraction to electrical stimulation; for example ATP (Kennedy *et al*., 1986). There are experiments indicating that the rabbit ear artery is also innervated by sensory nerves, which may inhibit the contractile response to adrenergic and purinergic stimulation (Maynard *et al*., 1990). Therefore, the present data suggest that the contraction of the rabbit ear artery to electrical stimulation is mediated, at least partially, by adrenergic mechanisms. As we have observed that phentolamine produced a smaller inhibition at 30°C than at 37°C in the contraction to electrical stimulation, the possibility exists that adrenergic mechanisms have a lower function during cooling than at normotemperature in mediating the arterial response to sympathetic stimulation.

The present results show that the response of the ear artery to sympathetic stimulation at 37°C persists with similar intensity during warming at 41°C or slight cooling (34°C), but it consistently decreases during cooling to lower temperatures (30°C–24°C). Thus indicates that cooling to 30°C–24°C decreases, whereas warming to 41°C does not affect, the constriction of ear artery to sympathetic activation. It has been previously shown that cooling reduces the vascular contraction to many vasoactive stimuli by unspecifically inhibiting the contractile ability of vascular smooth muscle (Vanhoutte, 1980). Although this unspecific effect may be also present in our experiments, it may be not the sole cause of the reduced response to sympathetic stimulation. The residual contraction found in the presence of tetrodotoxin was similar at 37°C and 30°C, suggesting that at least at this cooling, there is little inhibition of smooth muscle contractility to non-neurogenic stimulation. Therefore, it is suggested that during cooling, the vascular contraction to sympathetic stimulation is inhibited, and that the myogenic component may be more important than the adrenergic component of the contraction in response to electrical stimulation. The mechanism of these cooling effects is unclear. As cooling could slow down enzymatic processes, we can hypothesize that a reduced enzymatic activity at low temperature might result in a lower synthesis and release of neurotransmitter and/or a less efficient postreceptorial mechanism, thus explaining our results during cooling. However, this hypothesis may not be right, as the decrease of enzymatic activity would be expected to be progressive during temperature reduction, instead we observed a rather sharp threshold effect when the temperature was lowered from 34°C to 30°C. Also, a reduction in the enzymatic breakdown of the neurotransmitter would be expected during cooling, thus balancing the hypothetical reduction in synthesis or release of neurotransmitter. Another possibility is that at low temperatures the protein conformation and/or membrane properties might be modified, thus changing the receptor binding characteristics during cooling. The explanation for the sharp threshold effect in the response when temperature changes from 34°C to 30°C is at present unknown, and we suggest that some mechanism, sensitive to small changes in this range of temperatures, might be inhibited by moderate cooling.

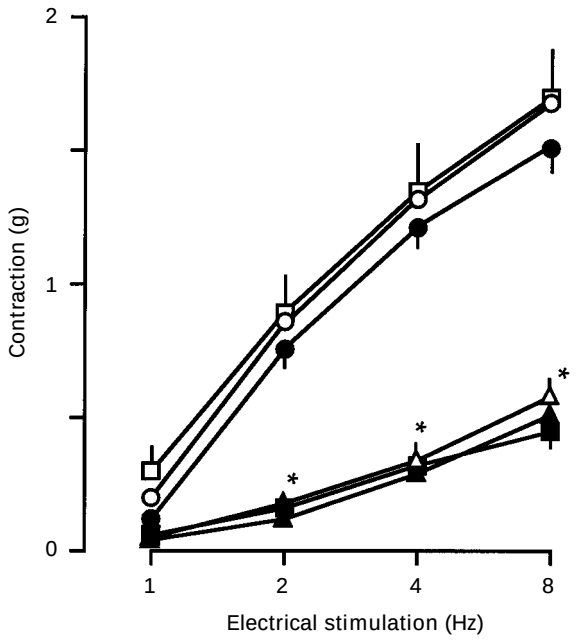

Figure 2 Effects of temperature (41°C (○), 37°C (●), 34°C (□), 30°C (■), 27°C (△) and 24°C (▲)) on the contractile responses of rabbit central ear arteries to transmural electrical stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, for 5 s). Points are means and vertical lines show s.e.mean. *Statistically significant difference from data at 37°C ($P<0.01$). Data are average from at least 9 animals.

Our results in the rabbit central ear artery during cooling contrast with those obtained in other blood vessels considered as cutaneous such as human (Harker *et al*., 1994) and canine (Flavahan & Vanhoutte, 1986) saphenous veins where cooling increased the vasoconstriction to sympathetic nerve stimulation. The reason for this discrepancy may be related to differences between arteries and veins, skin vascular regions and perhaps species. One of these differences may reside in the characteristics of the adrenergic neurotransmission in blood vessels. It has been shown that in cutaneous blood vessels equipped with a predominance of the α_2-adrenoceptor over the α_1-adrenoceptor subtype (Flavahan & Vanhoutte, 1986; Borbujo *et al*., 1989) that cooling may increase the response to adrenergic stimulation by augmenting the sensitivity of postjunctional adrenoceptors of the α_2 subtype (Flavahan *et al*., 1985; Harker *et al*., 1994) or both α_1- and α_2-adrenoceptors (Gómez *et al*., 1991). As the rabbit ear artery has a relatively small population of the α_2-adrenoceptor subtype (Harker & Vanhoutte, 1988; García-Villalón *et al*., 1992), this feature may explain the lack of potentiating effects of cooling on the adrenergic response of this artery (García-Villalón *et al*., 1992; present results). In this respect, it is possible that the rabbit ear artery, which has been used as a model of cutaneous blood vessels (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988), differs from other cutaneous vessels, and that body thermoregulation might be accomplished by mechanisms that differ subtly depending on the skin vascular territory.

The main objective of this study was to analyse the possible peptidergic modulation of the sympathetic effects on cutaneous vasculature during changes in temperature. At normotemperature (37°C), neuropeptide Y potentiated the contraction to electrical stimulation, which agrees with the results obtained by others in rat femoral (Lundberg *et al*., 1985) or rabbit ear (Budai *et al*., 1989) arteries, and suggests that neuropeptide Y may facilitate the vasoconstriction to sympathetic activation during normotemperature. This potentiating effect of neuropeptide Y on the vascular response has also been found by others with electrical stimulation and several vasoconstrictors (Edvinsson *et al*., 1984; Ekblad *et al*., 1984). Ekblad *et al*. (1984) found that neuropeptide Y does not produce a potent contractile effect in isolated vessels, but that

Table 1 Summary of the contractions (g) of rabbit isolated ear arteries to electrical field stimulation obtained in the absence (control) and in the presence of tetrodotoxin (TTX, 1 μM) and phentolamine (Phen, 1 μM), at 37°C and 30°C

	Electical field stimulation (Hz)							
	1		*2*		*4*		*8*	
	37°C	*30°C*	*37°C*	*30°C*	*37°C*	*30°C*	*37°C*	*30°C*
Control (12)	0.06±0.017	0.04±0.009	0.60±0.04	0.15±0.02	0.90±0.09	0.34±0.03	1.14±0.06	0.62±0.04
TTX (12)	0.007±0.005*	0.005±0.003*	0.05±0.017*	0.06±0.013*	0.12±0.02*	0.12±0.02*	0.20±0.017*	0.19±0.03*
Control (7)	0.08±0.04	0.03±0.008	0.58±0.13	0.19±0.06	1.04±0.15	0.41±0.11	1.36±0.17	0.67±0.015
Phen (7)	0.08±0.04	0.03±0.014	0.16±0.05*	0.10±0.03	0.28±0.05*	0.26±0.06	0.46±0.07*	0.40±0.08*

Values are means±s.e.mean. Number of arterial segments used at each temperature is shown in parentheses. *$P<0.01$ compared with its control.

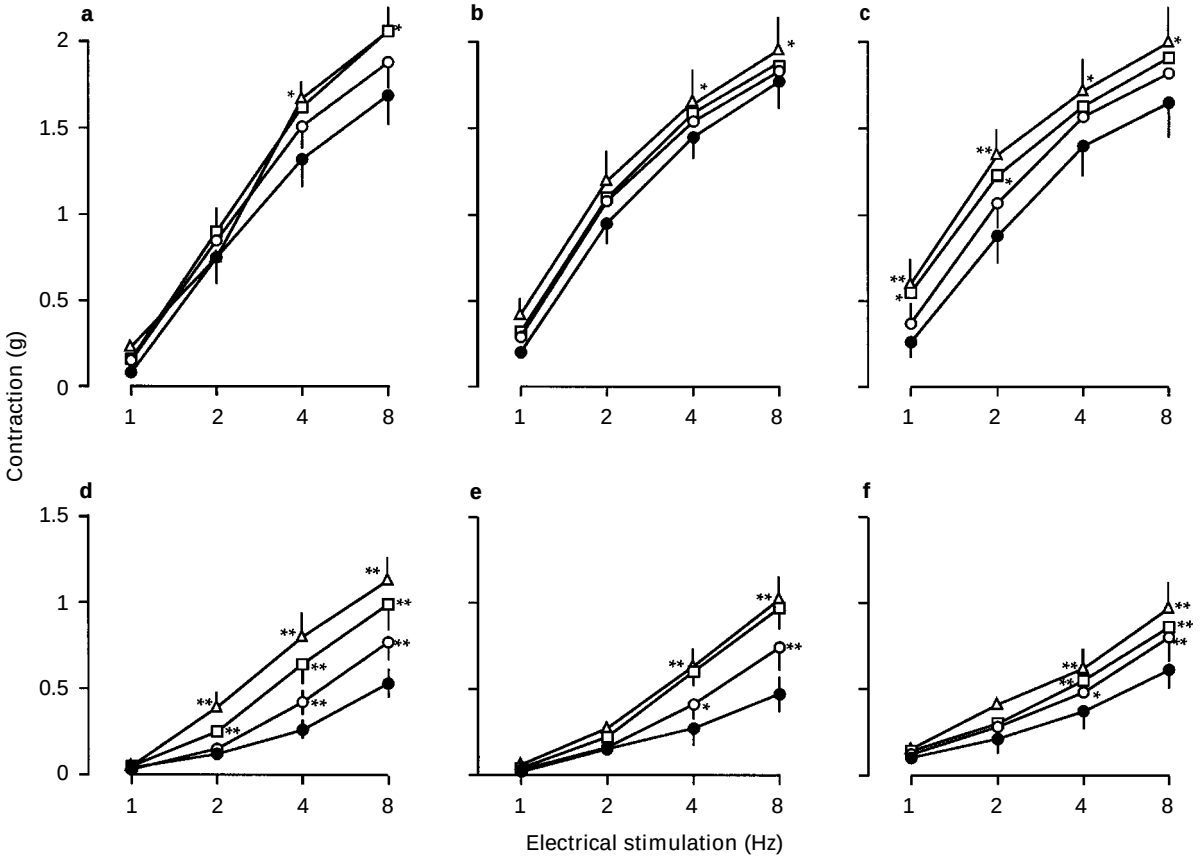

Figure 3 Contractions of rabbit central ear arteries to transmural electrical stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, during 5 s) in the absence (control, ●) and in the presence of neuropeptide Y (10 nM (○), 30 nM (□), 100 nM (△)) at the different temperatures tested: (a) 41°C, $n=5$; (b) 37°C, $n=7$; (c) 34°C, $n=5$; (d) 30°C, $n=6$; (e) 27°C, $n=5$; (f) 24°C, $n=7$). Points are means and vertical lines show s.e.mean. Statistically significant difference from control, $^{*}P<0.05$, $^{**}P<0.01$. n=number of animals.

this peptide greatly enhances the *in vitro* vascular response to adrenergic nerve stimulation probably by a postsynaptic mechanism. These authors (Ekblad *et al*., 1984) also found that neuropeptide Y greatly potentiates the *in vitro* vascular response to exogenous adrenaline, noradrenaline and histamine. Also, Edvinsson *et al*. (1984) found that neuropeptide Y potentiated the contraction to noradrenaline and to histamine in rabbit femoral, gastro-epiploic and basilar arteries, but not in ear arteries, whereas it did not modify the response to potassium in any of these blood vessels. In contrast to that observed by us with neuropeptide Y at normotemperature, we found that endothelin 1, vasopressin or angiotensin II at the concentrations used did not modify the arterial contraction to electrical stimulation at 37°C, suggesting that these peptides may be not involved in modulation of sympathetic vasoconstriction during normotemperature. It has been shown that at 37°C 0.01–0.3 nM endothelin-1 potentiates the contraction to nerve stimulation in rat renal circulation (Reid, 1993) and rabbit ear (Wong-Dusting *et al*., 1989) arteries. In saphenous arteries, concentrations of endothelin-1 higher than 0.3 nM were required to potentiate the response to neural stimulation (Mutafova-Yambolieva & Radomirov, 1994). On the other hand, there are data indicating that 0.01 mM angiotensin II increased the neurogenic response in the perfused mesenteric bed from hypertensive, but not from normotensive rats (Jonsson *et al*., 1993). The concentrations of endothelin-1 and angiotensin II used in these studies are lower (Wong-Dusting 1989; Reid, 1993; Jonsson *et al*., 1993), or similar (Mutafova-Yambolieva & Radomirov, 1994) to those used in our experiments. Therefore, the discrepancy between our results at 37°C and these studies may be due to differences in vascular beds (Jonsson *et al*., 1993; Reid, 1993; Mutafova-Yambolieva & Radomirov, 1994), species used (Jonsson *et al*., 1993; Reid, 1993) or experimental procedure (Wong-Dusting *et al*., 1989; Jonsson *et al*., 1993; Reid, 1993). In some of these studies (Wong-Dusting *et al*., 1989; Jonsson *et al*., 1993; Reid, 1993), the duration of stimulation pulses (1 or 2 ms) was longer than that used in our experiments (0.2 ms), which may modify the characteristics of the neurogenic response. The study of Mutafova-Yambolieva and Radomirov (1994), which used a shorter pulse duration (0.3 ms) only showed potentiation at a rather high endothelin-1 concentration (10 nM). Moreover, in the studies of Wong-Dusting *et al*. (1989), performed in the same type of arteries as the present study, the potentiation was observed after an incubation time (60–90 min) longer than that used in our study. We did not use higher concentrations of endothelin-1 or vasopressin because they caused a marked contraction, a condition that reduced the magnitude of the arterial contraction to electrical stimulation and masked the potentiating effects of these peptides during cooling. Nevertheless, although endothelin-1 and vasopressin at the concentrations used did not affect the response to sympathetic stimulation at 37°C, they did potentiate this response during cooling, thus suggesting that the modulatory role by these two peptides in the arterial response to sympathetic stimulation may differ with temperature. However, angiotensin II failed to modify the arterial response to sympathetic stimulation at all temperatures tested in spite of it being used at relatively high concentrations.

The role of neuropeptide Y, endothelin-1, vasopressin and angiotensin II in the sympathetic response of ear arteries during warming at 41°C may be similar to that at 37°C as we found that this warming did not modify the potentiating effects of neuropeptide Y, or the negligible effects of endothelin-1, vasopressin or angiotensin II on the arterial response to electrical stimulation. This suggests that the presence of these

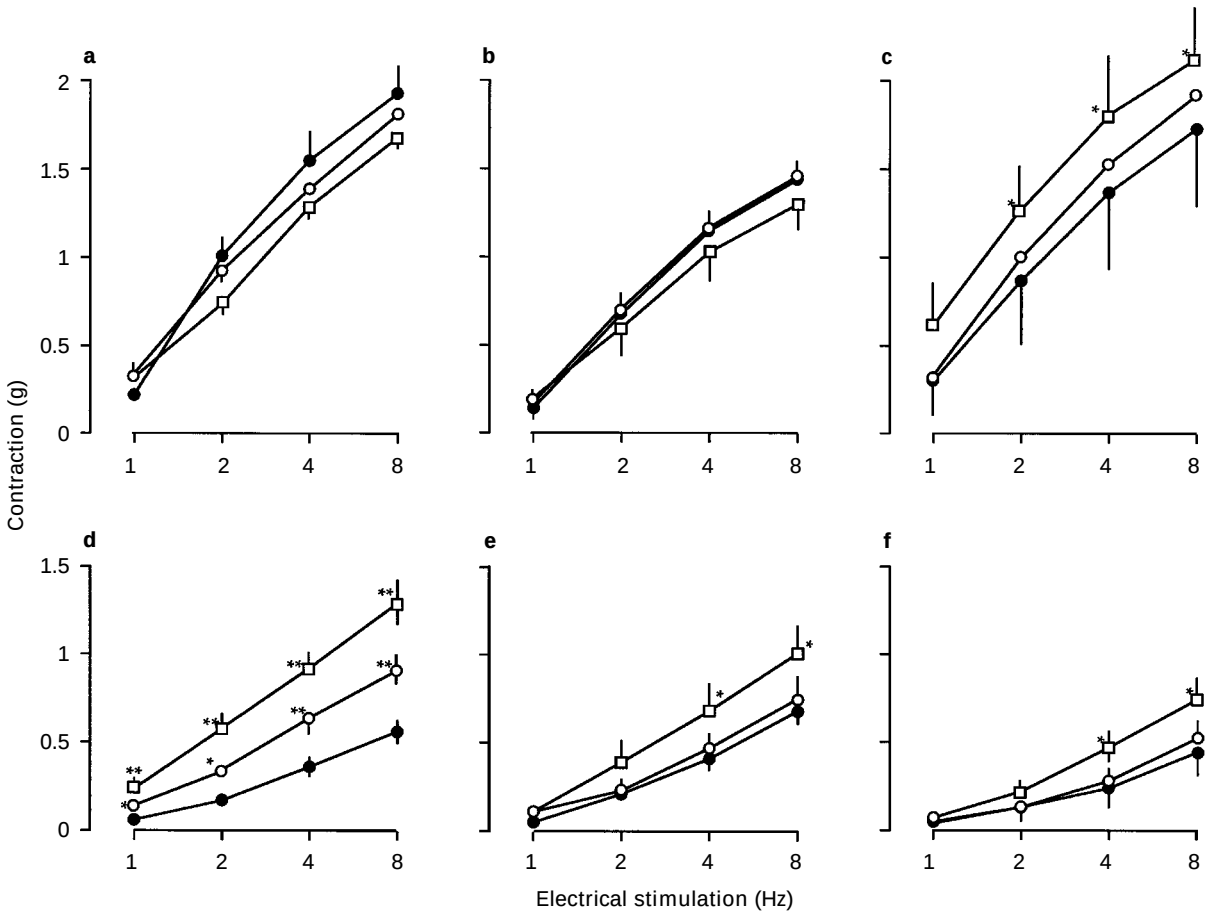

Figure 4 Contractions of rabbit central ear arteries to transmural electrical stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, during 5 s) in the absence (control, ●) and in the presence of endothelin-1 (3 nM (○), 10 nM (□)) at the different temperatures tested: (a) 41°C, $n=5$; (b) 37°C, $n=8$; (c) 34°C, $n=4$; (d) 30°C, $n=6$; (e) 27°C, $n=5$; (f) 24°C, $n=8$. Points are means and vertical lines show s.e.mean. Statistically significant difference from control, $^*P<0.05$, $^{**}P<0.01$. n = number of animals.

peptides will not modify the sympathetic response of cutaneous vasculature during elevation of body or environment temperature. On the contrary, cooling may produce marked changes in the modulatory role of some of these peptides on the sympathetic-induced response of cutaneous vessels. The potentiation induced by neuropeptide Y on the response of the ear artery to electrical stimulation at 37°C was increased during cooling. Endothelin-1 and vasopressin, which did not affect the arterial response to electrical stimulation at 37°C and 41°C, did potentiate this response during cooling, for endothelin-1 it was evident at 34°C, and for vasopressin it required cooling to 30°C. These potentiating effects of neuropeptide Y, endothelin-1 and vasopressin were maximal at 30°C, in comparison to those found at 34°C, 27°C and 24°C. The significance of these differences in the potentiating effects by these peptides at different degrees of cooling are not clear, but as these temperatures are probably reached frequently *in vivo* in superficial blood vessels during exposure of body surface to cold environments, our results may be of functional importance. The rabbit ear artery could be involved in regulation of body temperature (Patton & Wallace, 1978; Roberts & Zygmunt, 1984; Harker & Vanhoutte, 1988), and, although it may differ in some aspects from other cutaneous vessels, its functional response to temperature changes may give insight in the mechanisms by which the cutaneous circulation participates in body thermoregulation. If our results in the rabbit ear artery can be extrapolated to overall cutaneous vasculature, they suggest that neuropeptide Y, endothelin-1 and vasopressin may participate in the regulation of cutaneous circulation during cooling by increasing the cutaneous vasoconstriction to sympathetic response, as a homeostatic mechanism for preventing heat loss. Angiotensin II, as suggested by the present results, is probably not involved in the cutaneous vasoconstriction to sympathetic activation under normotemperature and during cooling. The absence of effects of angiotensin II on the response to sympathetic stimulation suggests that the modulation by endothelin-1, neuropeptide Y and vasopressin is specific for these peptides.

Interestingly, the inhibitory effects of cooling on the sympathetic response of ear artery found in the present study are blunted or reversed by peptidergic modulatory factors. We observed that the reduced arterial contraction to electrical stimulation recorded at 30°C, compared to 37°C, was reversed by endothelin-1 10 nM, so that the response of the ear artery to electrical stimulation was similar at 30°C and 37°C in the presence of endothelin-1. This observation and the fact that endothelin-1 concentrations in plasma are elevated during cooling (Fyhrquist *et al*., 1990) suggest that during cooling this peptide may potentiate the vasoconstrictor action of sympathetic activation. The concentrations of endothelin-1 used in this study are higher than that present in plasma (Zamora *et al*., 1990), but as endothelin-1 is mainly released by the endothelium it is possible that the concentrations of this peptide reached locally on the side of vascular smooth musculature may be higher than that determined in plasma.

Neuropeptide Y caused potentiation of the arterial contraction to sympathetic stimulation, which was greater during cooling than at 37°C. This peptide is stored in perivascular sympathetic nerves and may act as a co-neurotransmitter in the sympathetic nervous system (Lundberg & Tatemoto, 1982), and consequently it may be released together with noradrenaline when sympathetic innervation of cutaneous blood

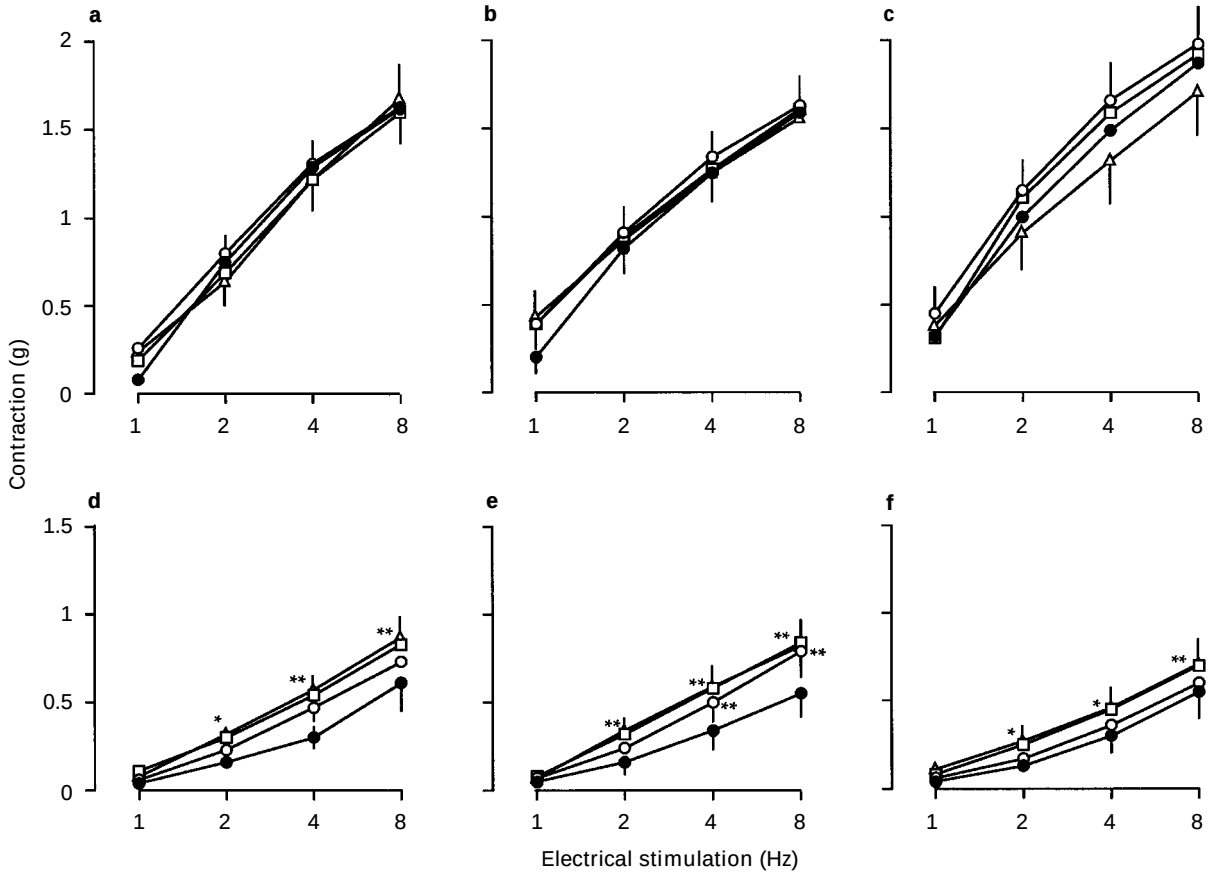

Figure 5 Contractions of rabbit central ear arteries to transmural electrical stimulation (1–8 Hz, 0.2 ms pulse duration, 70 V, during 5 s) in the absence (control, ●) and in the presence of vasopressin (0.1 nM (○), 0.3 nM (□), 1 nM (△)) at the different temperatures tested: (a) 41°C, $n=5$; (b) 37°C, $n=8$; (c) 34°C, $n=5$; (d) 30°C, $n=5$; (e) 27°C, $n=5$; (f) 24°C, $n=8$. Points are means and vertical lines show s.e.mean. Statistically significant difference from control, $*P<0.05$, $**P<0.01$. n = number of animals.

vessels is stimulated during cooling. Therefore, neuropeptide Y may also contribute to facilitate the cutaneous vasoconstriction during cooling.

Our data with vasopressin also suggest that the presence of this peptide would increase the sympathetic vasoconstriction during cooling. However, the interpretation of these data is difficult because plasma levels of vasopressin may be reduced, rather than increased, during cooling (Wittert *et al*., 1992). Thus, the functional significance of the potentiating action of vasopressin on the sympathetic cutaneous vasoconstriction, as a homeostatic mechanism for preventing heat loss during decreases of body or environment temperature, is uncertain. Vasopressin, instead, is released during hypotensive states and surgical procedures (Cowley & Liard, 1987) and in these situations there may be increased sympathetic activity and cutaneous vasoconstriction. With these circumstances in mind, our results with vasopressin allow us to hypothesize that this peptide might interact with sympathetic innervation of cutaneous blood vessels to produce a pronounced cutaneous vasoconstriction, thus contributing to the regulation of the distribution of cardiac output for preserving perfusion of vital organs, in detriment of other tissues such as skin, during stress.

In summary, the results from the present study suggest that neuropeptide Y, endothelin-1 and vasopressin, but not angiotensin II, may modulate the cutaneous vasoconstriction to sympathetic nerve stimulation by potentiating this vasoconstriction during cooling. This may be of relevance for the homeostatic mechanisms in the cutaneous circulation for body temperature regulation. Our findings also indicate that the mechanisms underlying the effects of each of these peptides in the sympathetic cutaneous vasoconstriction during cooling should be investigated.

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