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Relaxation of rat arteries by urocortin: effects of gender and diabetes

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Abstract

Urocortin is a peptide recently identified, structurally related to corticotropin releasing factor (CRF). We have compared the effects of urocortin in different vascular beds, and have investigated whether there are gender differences in these effects or whether they are altered by diabetes. We have studied the response of isolated segments (2-mm long) from basilar, coronary and tail arteries to urocortin. The segments were obtained from male and female, normoglycaemic and streptozotocin-induced diabetic rats. In the arterial segments precontracted with endothelin-1, urocortin produced concentration-dependent relaxation, and the order of sensitivity was: tail > basilar > coronary. This relaxation was similar in arteries from male and female, diabetic and normoglycaemic rats. In tail arteries from normoglycaemic male rats, the cyclooxygenase inhibitor meclofenamate (10^{-5} M) increased the relaxation to urocortin, and the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) or the potassium-channel-blocker charybdotoxin (10^{-7} M) did not modify it. In tail arteries from normoglycaemic female rats meclofenamate, charybdotoxin or L-NAME did not modify the relaxation to urocortin. These results suggested that urocortin produced vasodilation which showed regional differences between basilar, coronary and tail arteries, but was not affected by diabetes. The mechanisms underlying this relaxation in tail arteries might differ between males and females.

Introduction

Urocortin is a 40 amino acid peptide, which has been recently identified in the rat brain (Vaughan et al 1995). This peptide has a high degree of structural homology with the peptide corticotropin-releasing factor (CRF), and belongs to a group of structurally related peptides which include, in addition to urocortin and CRF, urotensin I (Lederis et al 1982) and sauvagine (Erspamer et al 1980), isolated from fish neurosecretory cells and frog skin, respectively. In addition to their role as neurotransmitters in the central nervous system, CRF and urocortin may have peripheral effects, particularly in the cardiovascular system. In rats, urocortin has a cardiac inotropic action, and produces potent and long lasting hypotension, which may be due to systemic vasodilation (Vaughan et al 1995). Indeed, it has been shown that urocortin produced relaxation of rat basilar (Schilling et al 1998), tail (Lubomirov et al 2001), and coronary (Terui et al 2001; Huang et al 2002) arteries. Also, this peptide may have potent vasodilator effects in human saphenous veins (Sanz et al 2002) and placental circulation (Leitch et al 1998). The mechanisms of the relaxation to urocortin are unsettled, and may vary depending on the vascular bed, species and experimental preparation. In rat basilar artery it was mediated by cyclic AMP and potassium channels but it was endothelium independent (Schilling et al 1998), and in rat tail artery it was mediated by cyclic AMP production (Lubomirov et al 2001). The mechanism of action of urocortin in coronary arteries may differ from those in cerebral and tail arteries, as it has been shown that in isolated rat coronary arteries this relaxation was mediated in part by endothelial nitric oxide release and in part by potassium channel activation (Huang et al 2002; Yao et al 2002), whereas in rat perfused heart it was mediated by vasodilator prostanoids but not

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by nitric oxide (Terui et al 2001). In human saphenous veins the relaxation to urocortin was independent of nitric oxide, dependent on potassium channel activation and modulated by vasoconstrictor prostanoids (Sanz et al 2002).

The study of the differences in vascular function between males and females has been the subject of intensive study. It is known that cardiovascular diseases are less frequent in premenopausal women than in men (Douglas 1997; Hayward et al 2000), and this has been related to the protective effects of ovarian hormones (Mendelsohn & Karas 1999), which may increase the expression and/or activity of endothelial nitric oxide synthase (Kauser & Rubanyi 1997), although gender differences may involve endothelium-independent mechanisms (Nevala et al 1996; Barber & Miller 1997; Kähönen et al 1998; Mayhan et al 2002).

Urocortin is produced in the human (Nishikimi et al 2000) and the rat (Okosi et al 1998) heart, and increases in failing hearts (Nishikimi et al 2000). This peptide may have a protective effect during myocardial ischaemia (Brar et al 2000), and participate in the regulation of arterial pressure in normal conditions (Coste et al 2000). Thus, urocortin may play a role in cardiovascular function in normal and pathologic conditions. However, it is not known whether there are gender differences in the response to urocortin. Therefore, the main objective of this study was to analyse whether the vascular relaxation to urocortin showed gender differences. This study was performed in basilar and coronary arteries, because of the physiopathological importance of these two vascular beds, and in tail arteries, which are cutaneous arteries frequently used for studying vascular reactivity. Previous studies from our laboratory showed that in rats diabetes mellitus may affect the contraction of basilar and coronary arteries to vasopressin (García-Villalón et al 2003), the contraction of coronary and tail arteries to U46619 (Sanz et al 2003a), and the relaxation of tail arteries to sodium nitroprusside and acetylcholine (Sanz et al 2003a). Urocortin may be produced in the brain (Kozicz et al 1998), heart (Okosi et al 1998) and skin (Slominski et al 2000). Diabetes mellitus is a risk factor for cardiovascular disease, but the vascular impairment produced by this metabolic alteration may show gender differences, as it is relatively more marked in premenopausal women than in men (Farmer & Gotto 1997). Therefore, the effects of urocortin were studied in arteries from male and female rats in which diabetes was induced by injection of streptozotocin.

The role of potassium channels, of nitric oxide and prostanoids in the response to urocortin has been studied previously in basilar (Schilling et al 1998) and coronary (Terui et al 2001; Huang et al 2002; Yao et al 2002) arteries, however in cutaneous (tail) arteries these particular mechanisms have not been studied in relation to urocortin response. Therefore, we have studied the effects of urocortin on tail arteries after inhibition of potassium channels and of nitric oxide or prostanoid synthesis. Tail arteries have been used as a model of cutaneous vessels (O'Leary & Wang 1994).

Materials and Methods

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Sixteen male and nineteen female Sprague-Dawley rats (200–350 g) were used. In one group of male or female rats, diabetes was induced by intraperitoneal injection of streptozotocin (60 mg kg^{-1}, dissolved in citrate buffer pH 4.5), and a second group of age-matched control rats received vehicle only. All rats were housed in cages and allowed free access to food and water. The concentration of glucose in plasma was determined from a drop of blood from the tail using Glucostix reactive strips (Bayer Diagnostics). Glucose determination was performed before and two days after streptozotocin injection, and again on the day of the experiment.

Six weeks after streptozotocin or vehicle injection, the rats were killed by pentobarbitone overdose (200 mg kg^{-1}) followed by exsanguination. The basilar (cerebral), anterior interventricular coronary, and ventral caudal (tail) arteries were carefully dissected and removed. Previous studies suggested that six-weeks of the diabetic state might modify the response of these arteries to contracting and relaxing agents (García-Villalón et al 2003; Sanz et al 2003a). As those studies (García-Villalón et al 2003; Sanz et al 2003a) suggested that in our experimental conditions the response of these arteries was not modified by the state of the oestrous cycle, the results from the females in all phases of the cycle were pooled. The arteries were placed in cold isotonic saline solution, cut in 2-mm long segments, and each segment was prepared for isometric tension recording in a 4-mL organ bath at 37 °C, 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; glucose, 11. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Briefly, the method consisted of passing two fine tungsten wires (75-μm diam. for basilar and coronary arteries; 100-μm diam. for tail arteries) through the lumen of the vascular segment. The wires were fixed at both ends to prevent bending during contraction of the vascular segments. One wire was fixed to the organ bath wall, while the other was connected to a strain gauge for isometric tension recording (Universal Transducing Cell UC3 and Statham Microscale Accessory UL5, Statham Instruments, Inc.), thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. Changes in isometric force were recorded on a Macintosh computer by use of Chart v 3.6/s software and a MacLab/8e data acquisition system (ADInstruments). An optimal passive tension (0.25 g for basilar and coronary arteries; 0.75 g for tail arteries) was applied to the vascular segments, and then they were allowed to equilibrate for 60–90 min. These optimal tensions were determined in preliminary experiments, by stretching the segments to different passive tensions and recording the contraction to serotonin (10^{-5} M).

Cumulative concentration–response curves to urocortin (10^{-12}–10^{-8} M) were recorded in basilar, coronary, and tail arteries from male and female, normoglycaemic and diabetic rats. This was performed in the arteries precontracted with endothelin-1 (10^{-9} M). As the response to urocortin was more marked in tail arteries, these arteries were selected to analyse some of the mechanisms of the response. To this, the relaxation to urocortin was recorded in tail arteries from normoglycaemic male and female rats, in control conditions and after pretreatment with the inhibitor of nitric oxide synthase N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), with the cyclooxygenase inhibitor meclofenamate (10^{-5} M) and with the inhibitor of potassium channels charybdotoxin (10^{-7} M). In each experiment, a vascular segment, used as time-control, was precontracted with endothelin-1 but not treated with urocortin, to asses the stability of the contraction. At the end of the experiment, these time-control segments were treated with a concentration of acetylcholine (10^{-6} M) to test the presence of endothelium, and the relaxation was 45% of tone in cerebral, 65% in coronary and 89% in tail arteries, similar to that described by Sanz et al (2003a).

Materials

The substances used were charybdotoxin, meclofenamic acid (2-[(2,6-dichloro-3-meth yl-phenyl)amino]benzoi c acid) sodium salt, N^{ω}-nitro-L-arginine methyl ester (L-NAME), and rat urocortin, all from Sigma (St Louis, MO). Endothelin-1 (human, porcine) was from Peninsula Laboratories Europe, Ltd (St Helens, UK).

Statistical analysis

The relaxation to urocortin was expressed as percentage of the active tone, achieved with endothelin-1, and calculated as means ± s.e.m. In basilar and tail arteries, the pD_2 of each curve was calculated as the negative logarithm of the concentration producing 50% of the maximal response by geometric interpolation. As in coronary arteries the maximal response was not reached with the concentrations of urocortin used, in these arteries pD_2 values were not calculated. The pD_2 and the maximal response in the arteries from male and female, normoglycaemic and diabetic animals, were analysed by two-way analysis of variance. The pD_2 and the maximal response in the absence and in the presence of the blockers were compared by one-way analysis of variance followed by Dunnett's test. A probability of less than 0.05 was considered as significant.

Results

Six weeks after treatment with streptozotocin, male and female rats showed higher glycaemia values ($P < 0.01$) and lower body weight ($P < 0.01$) than age-matched control rats (Table 1). Body weight was higher in male than in female, control and diabetic rats ($P < 0.01$), but glycaemia values in control rats, or in streptozotocin-treated rats, were similar in the corresponding male and female animals (Table 1).

Table 1 Body weight and values for glycaemia from male and female rats, in control conditions and in diabetic rats (six weeks after diabetes had been induced by streptozotocin injection).

	Glycaemia (mg dL^{-1})	Body weight (g)	n
Male			
Normoglycaemic	83 ± 3	354 ± 11	11
Diabetic	360 ± 22†	238 ± 14†	5
Female			
Normoglycaemic	93 ± 5	250 ± 6*	13
Diabetic	368 ± 13†	186 ± 11†	6

Values are means ± s.e.m. *$P < 0.001$, males compared with females. †$P < 0.001$, diabetic rat compared with normoglycaemic rat.

The level of active tone reached with endothelin-1 was 0.35 ± 0.03 g in basilar, 0.23 ± 0.02 g in coronary and 0.77 ± 0.06 g in tail arteries, and it was not significantly different between the experimental groups. In the arterial segments precontracted with endothelin-1, urocortin produced concentration-dependent relaxation. The order of the sensitivity (pD_2) was: tail > basilar, and the order of the maximal effect was: tail > basilar > coronary. This relaxation was not significantly different between males and females in any type of arteries studied, and it was also similar in basilar, coronary and tail arteries from diabetic rats, compared with their normoglycaemic controls, both in male and female animals. Time-control experiments, which were contracted with endothelin-1 but not treated with urocortin, showed a progressive loss of tone, which was lower than the relaxation produced by urocortin. Figure 1 and Table 2 summarize the relaxation to urocortin in basilar, coronary and tail arteries from male and female, normoglycaemic and diabetic rats, and the tone diminution in the time-control segments.

In tail arteries from normoglycaemic male rats, treatment with meclofenamate (10^{-5} M) increased the relaxation to urocortin, whereas treatment with charybdotoxin (10^{-7} M) or with L-NAME (10^{-4} M) did not modify it. In tail arteries from normoglycaemic female rats, neither meclofenamate, charybdotoxin nor L-NAME modified the relaxation to urocortin. Figure 2 and Table 2 summarize the effects of meclofenamate, charybdotoxin and L-NAME on the relaxation to urocortin of tail arteries from normoglycaemic male and female rats.

Discussion

The main objective of this study was to compare the effect of urocortin in different vascular beds, and the results suggested that urocortin may be a potent vasodilator in cutaneous (tail), coronary and basilar arteries of the rat. Our results in the basilar artery were similar to those found by Schilling et al (1998), and both studies indicated that this artery exhibited a vasodilatation to urocortin in a

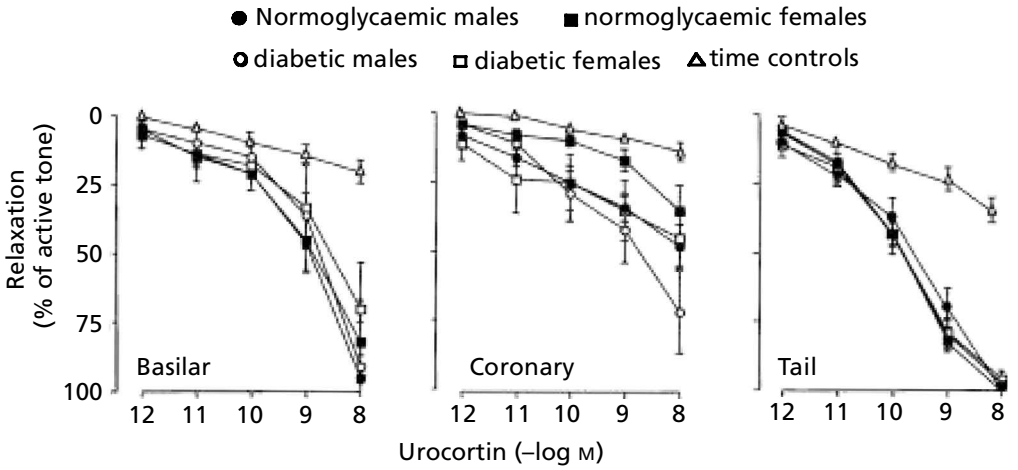

Figure 1 Summary of the relaxation to urocortin (10^{-12}–10^{-8} M) of basilar, coronary and tail arteries precontracted with endothelin-1 (10^{-9} M) from male and female, normoglycaemic and diabetic rats.

concentration range of 10^{-11}–10^{-8} M. Our results in coronary arteries may partly compare with the studies of Huang et al (2002), who observed relaxation to urocortin with a sensitivity similar to that found in this study, although in their study (Huang et al 2002) the maximal relaxation was higher.

Although urocortin produced a marked relaxation in coronary, basilar and tail arteries, there were marked differences between these vascular beds in the relaxation to urocortin, as tail arteries showed a greater response to urocortin compared with the other vascular types studied, and coronary arteries were the least responsive to urocortin. Urocortin has shown protective effects of the myocardium during coronary ischaemia (Brar et al 2000), and it has attracted attention as a potential therapeutic agent during myocardial infarct (Latchman 2001), however its hypotensive effect may limit its possible use in this condition. The results suggested that indeed, systemic injection of urocortin during coronary ischaemia might have adverse effects by producing greater vasodilation in the

Table 2 pD_2 and maximal relaxation (% of active tone) to urocortin in arteries from male and female, normoglycaemic and diabetic rats, in the absence (control) and in the presence of L-NAME (10^{-4} M), meclofenamate (10^{-5} M), or charybdotoxin (10^{-7} M).

	Normoglycaemic		Diabetic	
	pD_2	Maximal relaxation	pD_2	Maximal relaxation
Basilar				
Males	9.04 ± 0.15	95 ± 22 (5)	8.87 ± 0.12	91 ± 16 (5)
Females	9.19 ± 0.19	82 ± 15 (6)	8.96 ± 0.22	70 ± 18 (6)
Coronary				
Males		47 ± 9 (5)		71 ± 17 (5)
Females		34 ± 10 (6)		44 ± 11 (6)
Tail				
Males				
Control	9.7 ± 0.2	98 ± 2 (11)	10.43 ± 0.39	97 ± 2 (5)
L-NAME	9.54 ± 0.19	93 ± 2 (6)		
Meclofenamate	10.44 ± 0.18*	88 ± 9 (6)		
Charybdotoxin	9.1 ± 0.18	91 ± 8 (6)		
Females				
Control	9.85 ± 0.17	100 ± 4 (13)	9.98 ± 0.16	96 ± 4 (6)
L-NAME	10.15 ± 0.3	99 ± 2 (7)		
Meclofenamate	9.49 ± 0.22	98 ± 3 (6)		
Charybdotoxin	9.69 ± 0.15	97 ± 1 (6)		

Values are means ± s.e.m. *$P < 0.05$, compared with control. In parenthesis, number of animals and of vascular segments.

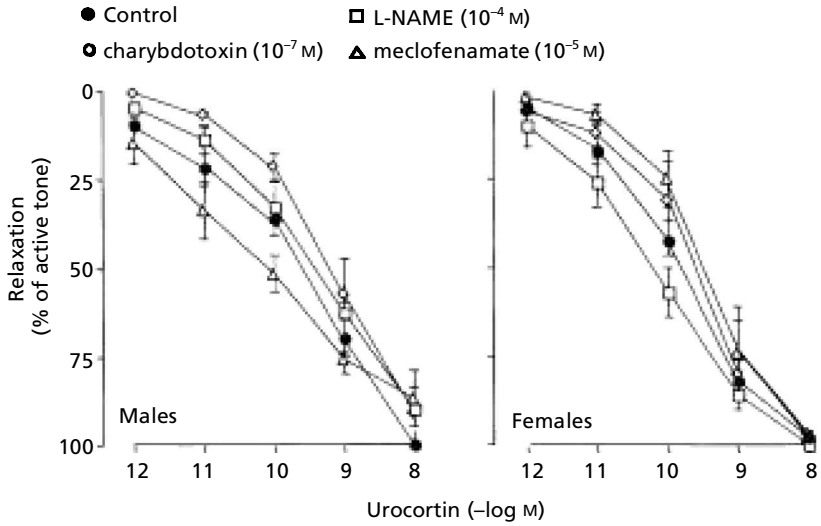

Figure 2 Summary of the relaxation to urocortin (10^{-12}–10^{-8} M) of tail arteries precontracted with endothelin-1 (10^{-9} M) from normoglycaemic male and female rats in the absence (control) and in the presence of L-NAME (10^{-4} M), meclofenamate (10^{-5} M) or charybdotoxin (10^{-7} M).

systemic (e.g. cutaneous) than in the coronary circulation and therefore may divert blood flow from the ischaemic myocardium. However, in basilar arteries the relaxing effect of urocortin was more marked than in coronary arteries, and although the sensitivity to this peptide at low concentrations was lower in basilar than in tail arteries, the maximal relaxation at high concentrations was nearly as high in basilar as in tail arteries. This suggested that urocortin might be useful as a cerebral vasodilator.

As the effects of urocortin were more pronounced in tail arteries than in coronary and basilar arteries, we studied some possible mechanisms of the relaxation to urocortin in tail arteries. We found that this relaxation was not dependent on nitric oxide, vasodilating prostanoids or calcium-dependent potassium channels, as it was not reduced by L-NAME, meclofenamate or charybdotoxin. Our results may agree with those of Lubomirov et al (2001), who found that urocortin produced relaxation of rat tail artery in the presence of a high concentration of extracellular potassium, which would inhibit the effects of potassium channels. Also, this relaxation was not modified by endothelium removal, therefore it was probably not dependent on endothelial nitric oxide. Lubomirov et al (2001) proposed that urocortin may produce relaxation by reducing the sensitivity to calcium of the contractile apparatus of the smooth muscle.

We did not find any differences in the reactivity of basilar, coronary and tail arteries to urocortin between male and female rats, nor between diabetic and normoglycaemic rats. This is in contrast to a previous study from our laboratory (Sanz et al 2003b), which found that in rat renal arteries, the response to urocortin was reduced by diabetes in females but not in males. This difference could be related to the fact that in renal arteries from females the response to urocortin was partly dependent on nitric oxide, whereas in tail arteries this response may be independent of nitric oxide as it was not modified by inhibition of nitric oxide synthesis (present results). Diabetes may affect vascular reactivity mainly by reducing endothelial nitric oxide release, whereas endothelium-independent relaxation is often unaffected in this condition (Pieper 1998). Thus diabetes may affect preferentially the reactivity in vessels where nitric oxide plays a main role in modulating vascular reactivity.

Although we did not find gender differences in the relaxation to urocortin in any vascular bed studied, there may exist some differences between males and females regarding the involvement of vasoconstrictor prostanoids in the relaxation to urocortin. In tail arteries from males, but not in those from females, cyclooxygenase inhibition with meclofenamate increased the relaxation to urocortin, suggesting that in these particular arteries from males there may be release of vasoconstricting prostanoids, which may counteract the relaxation to urocortin. A similar phenomenon has been observed for the response to urocortin in human saphenous veins (Sanz et al 2002). Therefore, the role played by prostanoids in the vascular effects of urocortin may differ between males and females, as vasoconstrictor prostanoids may participate in vessels from males but not in those from females. It has been shown that the release of vasoconstrictor prostanoids may be increased in mesenteric arteries from rats after ovariectomy (Dantas et al 1999). Also, vasoconstrictor cyclooxygenase products may reduce the relaxation to acetylcholine in mesenteric arteries from male rats (Kähönen et al 1998), or the relaxation to bradykinin in coronary arteries from male pigs (Barber & Miller 1997).

In summary, our results suggested that urocortin was a potent vasodilator in basilar, coronary and cutaneous (tail) arteries. The mechanisms underlying this vasodilation in cutaneous arteries may show gender differences, as there may be a greater release of vasoconstrictor prostanoids, which modulate the relaxation to urocortin, in tail arteries from males but not in those from females.

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