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Mechanisms of relaxation by urocortin in renal arteries from male and female rats

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1 Urocortin is a peptide recently identified, which is structurally related to the corticotropin-releasing factor (CRF). To analyze the mechanisms that could be involved in its effect on renal arteries from male and female rats, the response to urocortin was studied in isolated segments, 2 mm long, of renal arteries from male and female rats.

2 In renal artery segments precontracted with endothelin-1 (1 nM), urocortin (1 pM–10 nM) produced concentration-dependent relaxation, which was similar in the arteries from male and female rats.

3 This relaxation was reduced by the antagonists of urocortin receptors astressin (1 μM) and α-helical CRF(9–41) (1 μM) in arteries from both male and female rats.

4 In renal arteries from female rats, the relaxation to urocortin was reduced by the inhibitor of adenyl cyclase SQ22536 (300 μM), by 8-bromo-cyclic-ADP-ribose (cADPR; 30 μM), an antagonist of the endogenous activator of sarcoplasmic Ca^{2+} channel cADPR and by ryanodine (1 μM), which produces depletion of sarcoplasmic Ca^{2+}.

5 In renal arteries from male rats, the relaxation to urocortin was increased by ryanodine, and was not modified by SQ22536 or 8-bromo-cADPR.

6 These results suggest that the mechanisms involved in the relaxation to urocortin in renal arteries differ between female and male rats. In female rats, this relaxation may be mediated by the production of cyclic AMP (cAMP), synthesis of cADPR and release of sarcoplasmic Ca^{2+}, whereas in male rats it is not mediated by cAMP.

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Abbreviations: α-helical CRF(9–41), alpha-helical corticotropin-releasing factor fragment (9–41); Br-cADPR, 8-bromo-cyclic adenosine diphosphate ribose; cADPR, cyclic adenosine diphosphate ribose; cAMP, cyclic adenosine monophosphate; CRF, corticotropin-releasing factor; PKA, protein kinase A

Introduction

Urocortin is a recently isolated 40 amino-acid peptide, which has a high degree of structural homology with the peptide corticotropin-releasing factor (CRF) (Vaughan *et al*., 1995), and may produce marked effects on the cardiovascular system (Parkes & May, 2000; Parkes *et al*., 2001). Urocortin induces relaxation of rat basilar (Schilling *et al*., 1998) and tail (Lubomirov *et al*., 2001) arteries and of rat coronary circulation (Terui *et al*., 2001; Huang *et al*., 2002). 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 coronary vascular segments, this relaxation is mediated in part by endothelial nitric oxide release and in part by potassium channel activation (Huang *et al*., 2002), whereas in a rat-perfused heart, it is mediated by the vasodilator prostanoids, but not by nitric oxide (Terui *et al*., 2001); in the rat basilar artery, it is mediated by cyclic AMP and potassium channels, but it is endothelium independent (Schilling *et al*., 1998) and, in the rat tail artery, it is mediated by cyclic AMP production and independent of the endothelium or potassium channels (Lubomirov *et al*., 2001). The vascular effects of urocortin may be mediated by receptors of the CRF-R2 subtype, which predominate in peripheral blood vessels (Parkes *et al*., 2001).

Previous studies from our laboratory suggest that there may exist gender differences in the mechanisms of the relaxation to urocortin (Sanz *et al*., 2003). Although the response to this peptide was similar in the renal arteries from male and female rats, this response was partly dependent on nitric oxide in arteries from females, but not in those from males, and was reduced in renal arteries from diabetic female rats, but not in those from diabetic male rats. Also, the relaxation to urocortin was dependent on the activation of potassium channels of the Ca^{2+}-dependent subtype in renal arteries from both male and female rats, although at high concentrations of urocortin in females and at low concentrations in males. These differences suggest that, although the relaxation to urocortin in control conditions was similar in males and females, there may be differences in the mechanisms of this relaxation between genders, and, therefore, the present study was carried out to

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further analyze the probable gender differences regarding the mechanisms of the relaxation to urocortin in renal arteries.

Several substances relax the vascular and nonvascular smooth muscles by increasing cyclic AMP (cAMP) production, and the relaxing effect of this second messenger has been shown to be mediated, in part, by opening Ca^{2+}-dependent potassium channels and membrane hyperpolarization (Kume *et al*., 1989; Satake *et al*., 1996; Porter *et al*., 1998). cAMP may activate these potassium channels directly (Minami *et al*., 1993), or they may be phosphorylated by cAMP-dependent protein kinase A (PKA) (Sadoshima *et al*., 1988; Kume *et al*., 1989; Wang & Kotlikoff, 1996). A third possible mechanism has been proposed recently: cyclic-ADP ribose (cADPR) is a β-NAD^+ metabolite, which activates the calcium channels in the sarcoplasmic reticulum (Lee *et al*., 1989; Lee, 1997), and may be the endogenous activator of these channels (Galione *et al*., 1991). The plant substance ryanodine also binds to these sarcoplasmic calcium channels, which have been therefore denominated as ryanodine receptors (Sutko *et al*., 1997). It has been shown in the rat pulmonary artery that cAMP may increase cADPR production, which binds to ryanodine receptors and releases Ca^{2+} from the sarcoplasmic reticulum, and this released Ca^{2+} then activates Ca^{2+}-dependent potassium channels (Boittin *et al*., 2003). As this mechanism could also be involved in the relaxation to urocortin, the objective of this study was to analyze whether cADPR and Ca^{2+} release may be involved in the response to urocortin in renal arteries from male and female rats.

Methods

In all, 34 male (body weight = 342 ± 7 g) and 35 age-matched female (body weight = 257 ± 4 g) Sprague–Dawley rats were used in this study. This investigation conforms to the Guide for the Care and Use of Laboratory Animals (NIH publication no 85-23, revised 1996). The rats were killed by pentobarbitone overdose (200 mg kg^{-1}), followed by exsanguination, and the renal arteries were carefully dissected. The arteries were placed in a cold isotonic saline solution, cut into 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 stainless-steel pins, 100 μm in diameter, through the lumen of the vascular segment. 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 using Chart v 3.6/s software and a MacLab/8e data acquisition system (ADInstruments). A previously determined optimal passive tension of 7.5 mN was applied to the vascular segments, and then they were allowed to equilibrate for 60–90 min.

Cumulative concentration–response curves to urocortin (1 pM–10 nM) were recorded in segments from renal arteries after precontracion with endothelin-1 (1 nM). The relaxation to urocortin was recorded in the renal arteries from male and female rats, in the absence (control) and in the presence of antagonists of CRF receptors astressin (1 μM) and α-helical CRF(9–41) (1 μM), the inhibitor of adenyl cyclase SQ22536 (300 μM), the antagonist of cADPR, 8-bromo-cADPR (Br-cADPR, 30 μM), and ryanodine (1 μM). As in the arteries pretreated with SQ22536, the contractile tone obtained with endothelin-1 (1 nM) was lower than in control arteries; in a group of control arteries, a lower concentration of endothelin-1 was used (300 pM) to reach a lesser contraction, which was similar to that obtained in the arteries treated with SQ22536. As ryanodine might have unspecific effects on the vascular relaxation, in precontracted vascular segments from six male and six female rats, the relaxation to sodium nitroprusside was recorded in the absence and in the presence of ryanodine (1 μM). Also, as in the present study, the arteries were precontracted with endothelin-1, and to discard the presence of different endothelin receptor subtypes in males and females, which might influence the relaxation to urocortin, vascular segments from six male and six female rats were challenged with endothelin-1 (1 nM–1 μM) in the absence or presence of the antagonist of endothelin ET_A receptors BQ123 (1 μM).

The relaxation to urocortin is expressed as a percentage of the active tone achieved with endothelin-1, and is calculated as the mean ± s.d. To compare the results between male and female groups, two-way ANOVA followed by unpaired Student's *t*-test was performed, and the results in the absence and presence of the blockers used were compared by two-way ANOVA, followed by one-way ANOVA and Dunnett's test to know which concentrations were significant. To compare the effects of the antagonists in males and females, a two-way ANOVA was performed, in which one factor was the absence or the presence of the antagonist and the other was gender. A probability of less than 0.05 was considered as significant.

The substances used were α-helical CRF(9–41); CRF fragment 12–41 (astressin); 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536); Br-cADPR; ryanodol 3-(1H-pyrrole-2-carboxylate) (Ryanodine) and urocortin rat; all from Sigma (St Louis, MO, U.S.A.).

Results

Endothelin-1 (1 nM) contracted every arterial segment, and the level of this contraction in control conditions was 9.1 ± 4.2 mN for arteries from males (28 animals) and 7.4 ± 3.2 mN for arteries from females (29 animals). This contraction was not significantly different in the arteries pretreated with astressin (8.3 ± 2.4 mN in males, seven animals; and 6.3 ± 2.1 mN in females, seven animals), α-helical CRF(9–41) (7.6 ± 2.2 mN in males, six animals; and 11 ± 3.9 mN in females, six animals), Br-cADPR (7.8 ± 3.7 mN in males, six animals; and 8.2 ± 3.1 mN in females, five animals) or ryanodine (10 ± 3.4 mN in males, eight animals; and 10 ± 2.4 mN in females, seven animals), but it was significantly lower (4.3 ± 1.5 mN in males, six animals; and 5.2 ± 2.7 mN in females, six animals) in the arteries treated with SQ22536. The contractile concentration–response curve to endothelin-1 was similar ($P > 0.05$) in males (maximal effect = 14.6 ± 5.4 mN and EC_{50} = 8.9 nM) and females (maximal effect = 15.5 ± 3.5 mN and EC_{50} = 11 nM), and was shifted to the right

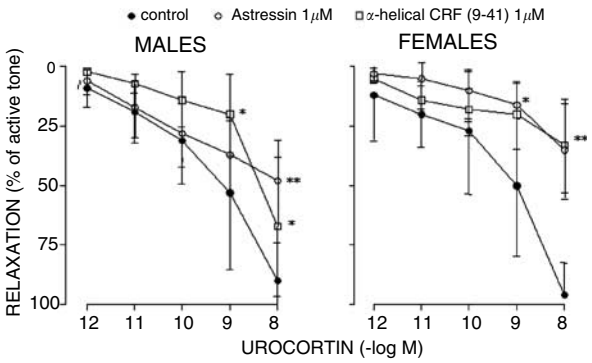

Figure 1 Summary of the relaxation to urocortin (1 pM–10 nM) of renal arteries precontracted with endothelin-1 (1 nM) from male and female rats in the absence (control) and in the presence of astressin (1 μM) or α-helical CRF(9–41) (1 μM). *,**Points are means $\pm$ s.d. of the mean. Significantly different compared to control (*$P<0.05$; **$P<0.01$).

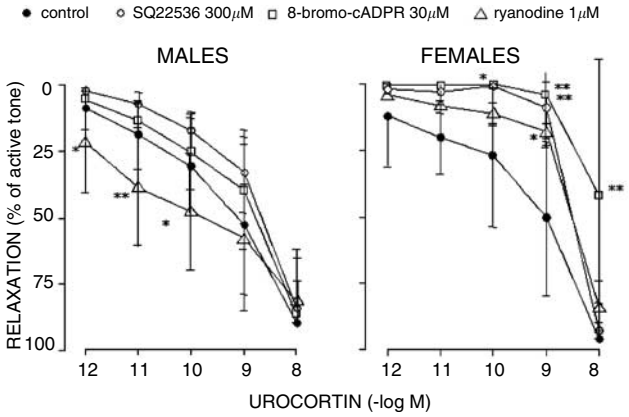

Figure 2 Summary of the relaxation to urocortin (1 pM–10 nM) of renal arteries precontracted with endothelin-1 (1 nM) from male and female rats in the absence (control) and in the presence of SQ22536 (300 μM), Br-cADPR (30 μM) or ryanodine (1 μM). Points are means $\pm$ s.d. of the mean. *,** Significantly different compared to control (*$P<0.05$; **$P<0.01$).

in a parallel way similarly ($P>0.05$) in males (7.4 $\pm$ 2.8 times) and in females (5.7 $\pm$ 3.1 times) (not shown).

In these precontracted vascular segments, urocortin produced concentration-dependent relaxation. In control conditions, this relaxation was similar in arteries from male and female rats.

Both the antagonists of CRF receptors astresssin and α-helical CRF(9–41) reduced the relaxation to urocortin, and this reduction was higher for both antagonists in females than in males (Figure 1).

The inhibitor of adenyl cyclase SQ22536 reduced the relaxation to urocortin in renal arteries from females, but it did not modify this relaxation in renal arteries from males significantly (Figure 2). As the contractile tone induced by endothelin was lower in the arteries treated with SQ22536 than in control arteries, a group of seven control vascular segments was precontracted with a lower concentration of endothelin-1 (300 pM) to achieve a tone (5.0 $\pm$ 0.8 mN in males and 6.1 $\pm$ 1.8 mN in females) similar to that obtained in the segments treated with SQ22536 (4.3 $\pm$ 1.5 mN in males and 5.2 $\pm$ 2.7 mN in females). When the arterial relaxation to urocortin in the presence of SQ22536 was compared to that found in control segments with a tone similar to the treated segments, SQ22536 also reduced the relaxation to urocortin in arteries from females, and it did not modify this relaxation in the arteries from males (not shown).

Ryanodine reduced the relaxation in the arteries from females and increased the response in those from males (Figure 2), and the relaxation of precontracted arteries to sodium nitroprusside was not modified by ryanodine in males or females (not shown). Br-cADPR also reduced this relaxation in arteries from females, abolishing the relaxation in some segments completely (two from five), without modifying it in males (Figure 2).

Discussion

Our study indicates that urocortin produces marked dilatation of rat renal arteries, and that the magnitude of this relaxation was not different in arteries from males and females, thus confirming previous studies from our laboratory (Sanz *et al*., 2003). Our results suggest, however, that the mechanisms underlying this relaxation may vary between genders.

The relaxation to urocortin may be mediated by activation of CRF receptors in renal arteries from both males and females, as this relaxation was blocked in both sexes by astressin and also by α-helical CRF(9–41). These two substances are antagonists of CRF receptors (Kishimoto *et al*., 1985; Gulyas *et al*., 1995), and we observed that they inhibited the response to urocortin at concentrations similar to those used for inhibiting responses mediated by these types of receptors (Ardati *et al*., 1999; Smart *et al*., 1999; Brauns *et al*., 2001). The blocking effect of astressin and α-helical CRF(9–41) was higher in females than in males, which may mean that these receptors have different affinities in both genders. Also, we have observed previously (Sanz *et al*., 2003) that the relaxation to urocortin in renal arteries from males and females is mediated, at least in part, by the activation of Ca^{2+}-dependent potassium channels, as it was reduced by charybdotoxin in both genders. These potassium channels may also be involved in the relaxation to urocortin of the coronary (Huang *et al*., 2002) and basilar (Schilling *et al*., 1998) arteries from rats. However, the mechanisms by which the activation of CRF receptors may open Ca^{2+}-dependent potassium channels in renal arteries is not known.

The present results suggest that stimulation of urocortin receptors might activate Ca^{2+}-dependent potassium channels in renal arteries from female rats by a mechanism different from that in renal arteries from males. In the arteries from females, the relaxation may be dependent on the synthesis of cAMP, as it was reduced by SQ25536, which is an inhibitor of adenyl cyclase. The relaxation in females was also inhibited by Br-cADRP, which is an antagonist of cADPR. This mediator activates the ryanodine receptor and induces release from the sarcoplasmic reticulum of Ca^{2+}, which may activate Ca^{2+}-dependent potassium channels in the plasmatic membrane. The relaxation to urocortin in renal arteries from female rats may at least be partly dependent on the release of Ca^{2+} from the sarcoplasmic reticulum, as this relaxation was reduced by ryanodine, which depletes sarcoplasmic Ca^{2+} deposits, and this effect may be specific for urocortin, as ryanodine did not modify the relaxation to sodium nitroprusside. Therefore,

based on previous (Sanz *et al*., 2003) and the present results, the following mechanism may be hypothesized for the relaxation to urocortin in renal arteries from females: urocortin binding to the CRF receptor activates adenyl cyclase, which increases cAMP. This activates the synthesis of cADPR; then this mediator opens ryanodine receptors in the sarcoplasmic reticulum, and the released Ca^{2+} activates Ca^{2+}-dependent potassium channels. A similar mechanism has been proposed for the β-adrenergic relaxation of rat pulmonary arteries (Boittin *et al*., 2003).

In renal arteries from males, the mechanisms of the relaxation to urocortin may differ from that in arteries from females. In males, the response to urocortin may not be mediated by cAMP or sarcoplasmic Ca^{2+} release, as this response was not inhibited by SQ25536, Br-cADPR or ryanodine. Our results do not allow one to clarify the mechanism operating in arteries from males. However, as previous observations from our laboratory (Sanz *et al*., 2003) suggest that Ca^{2+}-dependent potassium channels may be involved in this relaxation in renal arteries from male and female rats, we may hypothesize that, in males, activation of CRF receptors by urocortin may stimulate these channels through a mechanism independent of cAMP. Beta-adrenoceptors may activate potassium channels independently of cAMP, due to the direct action of a protein G on the channel (Scornik *et al*., 1993; Ahn *et al*., 1995). Also, the present study shows that in renal arteries from males ryanodine increased the relaxation to urocortin, whereas in females this blocker reduced this relaxation. These results differ from those of Huang *et al*. (2003), who did not find an effect on urocortin relaxation of interfering with the sarcoplasmic reticulum function, and this discrepancy may be due to differences between the coronary (Huang *et al*., 2003) and renal arteries (present results). Ryanodine may have opposite effects of vascular reactivity as the sarcoplasmic reticulum may either release or uptake cytoplasmic Ca^{2+}, and this blocker may increase or reduce the contraction of the vascular smooth muscle (Asano & Nomura, 2000). These differences between males and females in the mechanism of potassium channel activation by urocortin may also be related to the differences found in a previous study from our laboratory (Sanz *et al*., 2003), where it has been shown that inhibition of these channels reduced the relaxation to urocortin at high concentrations in females and at low concentrations in males.

Other mechanisms, in addition to those previously described, may be involved in the relaxing effect of urocortin, as the blockers used in the present study reduced, but did not abolish this relaxation completely. Indeed, it has been described that this peptide may produce the relaxation of rat tail arteries by mechanisms not related to membrane potential changes, reducing the sensitivity to the contractile apparatus for calcium (Lubomirov *et al*., 2001).

In summary, our results suggest that the mechanism of urocortin relaxation in rat renal arteries may differ in males and females. In females, this relaxation may be mediated by the release of sarcoplasmic Ca^{2+} due to cAMP-dependent production of cADPR, whereas in males this relaxation may be mediated by a mechanism independent of cAMP or sarcoplasmic Ca^{2+} release.

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