Relaxation by urocortin of rat renal arteries: effects of diabetes in males and females

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Abstract

Objective: Urocortin is a peptide structurally related to corticotropin releasing factor (CRF), and the present study was performed to examine the effects of diabetes mellitus on the relaxation by urocortin of renal arteries from males and females. Methods: The response to urocortin was studied in isolated segments, 2 mm long, from renal arteries, from male and female, control (normoglycemic) and streptozotocin-induced diabetic rats. Results: In the renal arterial segments precontracted with endothelin-1, urocortin produced concentration-dependent relaxation, that was not different between males and females. Diabetes reduced the relaxation in renal arteries from females but not in those from males. The potassium channel blocker charybdotoxin (10^-7 M) reduced the relaxation to urocortin of renal arteries from normoglycemic males and females. The cyclooxygenase inhibitor meclofenamate did not modify the relaxation to urocortin in renal arteries from normoglycemic males or females. The inhibitor of nitric oxide synthesis L-nitro-arginine methyl ester (L-NAME, 10^-4 M) reduced the relaxation to urocortin in renal arteries from normoglycemic females, but not in renal arteries from normoglycemic males. Neither charybdotoxin, L-NAME or meclofenamate modified the relaxation to urocortin of renal arteries from diabetic females. Conclusion: These results suggest that urocortin produces a marked vasodilation of renal arteries, which may be mediated by nitric oxide in females and by activation of potassium channels in both genders, and is reduced by diabetes in renal arteries from females.

1. Introduction

Urocortin is a 40 amino acid peptide which has been recently identified in the rat brain [1]. 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 [2] and sauvagine [3], 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 cardiac inotropic action, and produces a potent, long lasting hypotension which may be due to systemic vasodilation [1]. Indeed, it has been shown that urocortin produces relaxation of rat basilar [4], tail [5], and coronary [6,7] arteries. Also, this peptide may have potent vasodilator effects in human saphenous veins [8] and placental circulation [9]. The mechanisms of the relaxation to urocortin are unsettled, and may vary depending on the vascular bed, species and experimental preparation. In isolated rat coronary arteries this relaxation is mediated in part by endothelial nitric oxide release and in part by potassium channel activation [7], whereas in rat perfused heart it is mediated by vasodilator prostanoids but not by nitric oxide [6], in rat basilar artery it is mediated by cyclic AMP and potassium channels but it is endothelium independent [4], and in rat...
Diabetes mellitus is a risk factor for cardiovascular disease, and because of that the study of the vascular function during diabetes has attracted marked attention. This metabolic condition may impair the vascular response to several agents (see Ref. [10]), but it is not known whether it affects the response to urocortin. As urocortin may play a role in cardiovascular function in normal and pathologic conditions, this peptide might be also involved in the vascular complications of diabetes. As the renal circulation is a frequent localization for diabetic complications, the present study was performed to examine the effects of diabetes on the response to urocortin in renal arteries from rats made diabetic by injection of streptozotocin. Also, as the vascular effects of diabetes may differ between males and females [11–14], the present study was performed in arteries from both male and female rats. The role of potassium channels, of nitric oxide and of prostanoids in the vascular response to urocortin during diabetes was analyzed.

2. Methods

Sixteen male and 22 female Sprague–Dawley rats, weighting 200–350 g at the beginning of the study, were used. This investigation conforms 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). In one group of male or female rats, diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg, dissolved in citrate buffer, pH 4.5), and in a second group of age-matched control rats received only the vehicle. 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 2 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) followed by exsanguination, and the renal arteries were carefully dissected. 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; KH₂PO₄, 1.2; MgSO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 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 consists of passing through the lumen of the vascular segment two fine tungsten wires 100 μm in diameter. One wire is fixed to the organ bath wall, while the other is 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 (AIDInstruments). An optimal passive tension of 0.75 g was applied to the vascular segments, and then they were allowed to equilibrate for 60–90 min. This optimal tension was determined in preliminary experiments, by stretching the segments to different passive tensions and recording the contraction to 5-hydroxytryptamine (10⁻⁵ M).

Cumulative concentration–response curves to urocortin (10⁻¹²–10⁻⁸ M) were recorded in renal arteries from male and female, normoglycemic and diabetic rats. This was performed in the arteries precontracted with endothelin-1 (10⁻⁹ M). To analyze the mechanisms involved in this response, the relaxation to urocortin was recorded in renal arteries from normoglycemic male and female rats, in control conditions and after pretreatment with the inhibitor of nitric oxide synthase Nω-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). Also, as diabetes modified the response to urocortin in arteries from female rats, these antagonists were also studied in renal arteries from diabetic female rats.

The relaxation to urocortin is expressed as percentage of the active tone, achieved with endothelin-1, and calculated as means±S.E.M. The pD₂ of each curve was calculated as the negative logarithm of the concentration producing 50% of the maximal response by geometric interpolation. The pD₂ and the maximal response in the arteries from male and female, normoglycemic and diabetic animals, were analyzed by two-way analysis of variance (ANOVA). The pD₂ and the maximal response in the absence and in the presence of the blockers were compared by one-way ANOVA followed by Dunnett’s test to analyze what comparisons were statistically significant. A probability of less than 0.05 was considered as significant.

The substances used were: charybdotoxin; meclofenamic acid (2-[(2,6-dichloro-3-methyl-phenyl)amino]benzoic acid) sodium salt; L-NAME; and urocortin rat; all from Sigma; and endothelin-1 (human, porcine) from Peninsula Laboratories Europe, Ltd.

3. Results

Six weeks after treatment with streptozotocin, male and female rats showed higher glycemia values (P<0.001) and
lower body weight ($P<0.001$) than age-matched control rats (Table 1). Body weight was higher in male than in female, control and diabetic rats ($P<0.001$), but glycemia values in control rats, or in streptozotocin-treated rats, were similar in the corresponding male and female animals (Table 1).

The level of active tone induced by endothelin-1 in renal arteries was not significantly different between the experimental groups. In arteries from normoglycemic male rats the tone was: in control conditions $1.2\pm0.16$ g, in the presence of L-NAME $0.8\pm0.14$ g, of meclofenamate $1.1\pm0.28$ g and of charybdotoxin $1.2\pm0.28$ g. In arteries from diabetic male rats the tone was $1.3\pm0.24$ g. In the arteries from normoglycemic female rats the tone was: in control conditions $0.9\pm0.11$ g, in the presence of L-NAME $0.8\pm0.16$ g, in the presence of meclofenamate $0.8\pm0.11$ g, and of charybdotoxin $0.9\pm0.19$ g. And in the arteries from diabetic female rats the tone was: in control conditions $1.0\pm0.1$ g, in the presence of L-NAME $0.9\pm0.18$ g, of meclofenamate $1.2\pm0.2$ g and in the presence of charybdotoxin $1.0\pm0.35$ g. In the precontracted arterial segments from normoglycemic animals, urocortin produced concentration-dependent relaxation, and this relaxation was not significantly different between males and females. In precontracted renal arteries from diabetic female rats, but not in those from diabetic male rats the maximal relaxation to urocortin was reduced ($P<0.001$) without altering the $pD_2$ values as compared to those in renal arteries from normoglycemic animals (Fig. 1 and Table 3).

In renal arteries from normoglycemic male rats, charybdotoxin reduced ($P<0.05$) the sensitivity ($pD_2$) to urocortin (Table 2), without modifying the maximal effect,
Table 3
Maximal relaxation (% of active tone) to urocortin in arteries from male and female, normoglycemic and diabetic rats, in the absence (control) and in the presence of L-NAME (10^{-6} M), meclofenamate (10^{-5} M) or charybdotoxin (10^{-7} M)

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<tr>
<th></th>
<th>Normoglycemic</th>
<th>Diabetic</th>
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<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87±6 (11)</td>
<td>89±6 (5)</td>
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<tr>
<td>L-NAME</td>
<td>87±6 (6)</td>
<td></td>
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<tr>
<td>Meclofenamate</td>
<td>85±9 (5)</td>
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<tr>
<td>Charybdotoxin</td>
<td>87±5 (5)</td>
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<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>98±5 (12)</td>
<td>39±8 (10)*</td>
</tr>
<tr>
<td>L-NAME</td>
<td>57±14 (5)††</td>
<td>51±17 (6)</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>89±7 (6)</td>
<td>64±12 (6)</td>
</tr>
<tr>
<td>Charybdotoxin</td>
<td>67±13 (6)†</td>
<td>53±12 (5)</td>
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Values are means±S.E.M. * Significant difference compared with normoglycemic control (P<0.0001). †; †† significant difference compared with control († P<0.05; †† P<0.01). Number of animals and of vascular segments given in parentheses.

and L-NAME or meclofenamate did not modify the response to this peptide. In renal arteries from normoglycemic female rats, L-NAME (P<0.01) and charybdotoxin (P<0.05) reduced the maximal response to urocortin without modifying the sensitivity (Table 3), and meclofenamate did not modify the sensitivity or the maximal effect to this peptide. In renal arteries from diabetic female rats, neither charybdotoxin, L-NAME or meclofenamate modified the relaxation (maximal effect or sensitivity) to urocortin. Figs. 2 and 3 summarize the response to urocortin in renal arteries from normoglycemic and diabetic rats, in the absence and in the presence of charybdotoxin, L-NAME or meclofenamate.

Fig. 2. Summary of the relaxation to urocortin (10^{-12}–10^{-8} M) of renal arteries precontracted with endothelin-1 (10^{-9} M) from normoglycemic male (left) and female (right) rats in the absence (control) and in the presence of L-NAME (10^{-6} M), meclofenamate (10^{-5} M) or charybdotoxin (10^{-7} M).

Fig. 3. Summary of the relaxation to urocortin (10^{-12}–10^{-8} M) of renal arteries precontracted with endothelin-1 (10^{-9} M) from diabetic female rats in the absence (control) and in the presence of L-NAME (10^{-6} M), meclofenamate (10^{-5} M) or charybdotoxin (10^{-7} M).
4. Discussion

The present results suggest that urocortin is a potent vasodilator in renal arteries from rat. Renal circulation receives an important percentage of the cardiac output, and has a central role in the regulation of arterial pressure. Therefore, it may be suggested that the dilatation of the renal vascular bed produced by urocortin may be relevant for the cardiovascular regulation. Plasma levels of urocortin in humans are in the $10^{-12}$ M range [15], but as this peptide may be produced in peripheral tissues, it may reach higher local concentrations.

The main objective of the present study was to analyze whether the vascular effects of urocortin are affected by diabetes, and whether there are gender differences in these effects of diabetes. There is wide interest in the differences between genders in the vascular function, and several studies have found different responsiveness of arteries from females compared to those from males. In the present study, however, we have not found differences in the reactivity of renal arteries to urocortin between normoglycemic male and female rats. Some studies have shown that the vasodilation to acetylcholine is higher in arteries from females compared to those from males (see Refs. [16,17]). However, for other vasodilating agents the effects of gender may vary, as the relaxation of rat mesenteric arteries to isoproterenol [18], of rat coronary arteries to calcitonin gene-related peptide [19], of pig coronary arteries to prostacyclin [20], and of human internal mammary arteries to sodium nitroprusside [21] were similar in males and females.

Regarding the mechanisms of the relaxation to urocortin, we have found that in renal arteries from both normoglycemic males and females the relaxation to urocortin may be mediated by activation of potassium channels of the Ca$^{2+}$-dependent subtype, as this relaxation was modified by charybdotoxin. Also, our results suggest that the manner of the antagonism produced by charybdotoxin on the relaxation to urocortin may be different in arteries from males and from females, as in males charybdotoxin reduced the sensitivity (pD$_2$) and in females the maximal effect. Therefore there may be gender differences in the participation of potassium channels in the relaxation of renal arteries to low and high concentrations of urocortin. Our results in renal arteries agree with those found in rat basilar arteries [4] and in human saphenous veins [8], in which potassium channels of the Ca$^{2+}$-dependent subtype may also mediate the relaxation to urocortin. However, in rat coronary arteries potassium channels of a different subtype may be involved [7], thus suggesting that regional differences may exist in this aspect of urocortin effects.

Nitric oxide may also participate in the relaxation to urocortin of renal arteries from normoglycemic female rats, as in this case L-NAME reduced this relaxation. In renal arteries from normoglycemic male rats L-NAME did not modify the relaxation to urocortin, suggesting that in this case nitric oxide may not be involved in this relaxation. Therefore, the role of nitric oxide in the relaxant effect of urocortin in renal arteries may differ between genders. Prostanoids may not participate in the relaxation of renal arteries to urocortin, as this relaxation was not modified by meclofenamate in renal arteries of males or females.

Regarding the effects of diabetes on the vascular response to urocortin, this condition reduced the relaxation in renal arteries from females, but not in renal arteries from males. The impairment of the relaxation to urocortin in renal arteries from diabetic females may be related to a reduction of nitric oxide release, as in this case L-NAME did not modify this relaxation whereas this inhibitor of nitric oxide synthesis did inhibit the relaxation of renal arteries from normoglycemic females. This agrees with evidence indicating that diabetes may reduce the release of nitric oxide in several vascular beds (see Ref. [10]). In the present study, an impairment of potassium channel activation may be also involved in the reduction of the relaxation to urocortin in renal arteries from diabetic females, as in this case charybdotoxin did not modify this relaxation, contrarily to that found in renal arteries from normoglycemic females. Diabetes may impair the relaxation mediated by activation of potassium channels in rat aorta [22,23] and in tail [24], coronary [25], and carotid [26] arteries from rats. Prostanoids may not be involved in the reduced relaxation to urocortin in renal arteries from diabetic female rats, as meclofenamate did not modify this relaxation in arteries from normoglycemic or diabetic female animals.

This observed gender difference in the effects of diabetes on vascular response to urocortin may be related at least in part to the different role played by nitric oxide in this response. In renal arteries from normoglycemic females the participation of nitric oxide in the relaxation to urocortin may be greater than in those from males, as only in renal arteries from normoglycemic females the relaxation was significantly reduced by L-NAME, and also only in these arteries the relaxation was reduced by diabetes. As diabetes may impair the role of nitric oxide in the effects of urocortin, and the relaxation to urocortin may be independent of nitric oxide in the renal arteries from normoglycemic males, that might contribute to explain why diabetes does not affect the response of these latter arteries. The observed impairing effect of diabetes on urocortin action in renal arteries from females agrees with other studies showing that diabetes produces a relatively greater impairment in females than in males in the relaxation to acetylcholine of rat basilar artery [11] and aorta [12] and in the relaxation to methacholine of human leg circulation [13].

In summary, our results suggest: (1) that urocortin has a vasodilatory effect in renal arteries from both genders, (2) that this vasodilatation may be mediated by nitric oxide in
females, and by activation of potassium channels in both genders, and (3) that this vasodilation may be impaired by diabetes in renal vessels from females. Therefore, although urocortin may produce vasodilation in both genders, the mechanism involved in this vasodilation and also the effects of diabetes on this vasodilation may differ between vascular beds and genders.

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