

Roles of cyclic AMP and Ca^{2+} -activated K^+ channels in endothelium-independent relaxation by urocortin in the rat coronary artery

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Abstract

Objective: Urocortin possesses cardioprotective properties against the damaging effects of ischemia/reperfusion injury. Our previous study demonstrated that urocortin can induce both endothelium-dependent and -independent coronary relaxation. However, the mechanisms whereby urocortin triggers endothelium-independent relaxation have not been investigated. The present study aimed to examine the role of cyclic AMP and Ca^{2+} -activated K^+ channels in the relaxant response to urocortin in the isolated endothelium-denuded rat left anterior descending coronary arteries. **Methods:** Changes in vessel tension were measured by using a force transducer built in a Multi Myograph System. **Results:** In 9,11-dideoxy-11 α ,9 α -epoxy-methanoprostaglandin $\text{F}_{2\alpha}$ (U46619)-contracted rings, urocortin-induced relaxation (pD_2 : 8.40 ± 0.04) was significantly reduced by cyclic AMP-dependent protein kinase (PKA) inhibitors, Rp-cAMPS triethylamine (Rp-cAMPS) and KT 5720. Treatment with the large-conductance Ca^{2+} -activated K^+ channel blockers, iberiotoxin or tetraethylammonium ions (TEA^+) attenuated urocortin-induced relaxation; this effect was abolished in the presence of 200 nmol/l KT 5720. In contrast, apamin (small-conductance Ca^{2+} -activated K^+ channel blocker), glibenclamide (ATP-sensitive K^+ channel blocker), or BaCl_2 (inwardly rectifier K^+ channel blocker) had no effect. Urocortin-induced relaxation was reduced in rings contracted with increasing concentrations of extracellular K^+ (35 and 50 mmol/l). Treatment with TEA^+ or Rp-cAMPS inhibited the relaxant effect of urocortin in 35 mmol/l K^+ -contracted rings. Combined treatment with TEA^+ and Rp-cAMPS had no additional effect. Similarly, forskolin produced significantly less relaxant response in 50 mmol/l K^+ -contracted than U46619-contracted rings. Forskolin-induced relaxation was attenuated by pretreatment with 3 mmol/l TEA^+ . **Conclusion:** Urocortin relaxed the rat coronary artery in substantial part via activation of the vascular Ca^{2+} -activated K^+ channels and this effect appears to be primarily mediated through PKA-dependent intracellular mechanisms.

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Keywords: Arteries; K-channel; Signal transduction; Vasoconstriction/dilation

1. Introduction

Urocortin is a peptide of 40 amino acids and belongs to the hypothalamic corticotropin-releasing factor (CRF) peptide family, which also include urotensin and sauvagine. CRF polypeptides play biologically diverse

roles in the stress responses by acting on central neurons expressing CRF receptors. CRF agonists acting on the peripheral CRF receptors contribute to the regulation of cardiovascular and inflammatory responses. Abnormal CRF receptor-mediated cellular signaling might be closely associated with the pathophysiology of stress-related centrally controlled disorders such as anxiety, depression and impaired cardiovascular function. Urocortin, first identified

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in rat [1] and later in man [2,3], is the second mammalian member of the CRF family. Urocortin selectively binds to CRF-R2 with over 100-fold greater affinity than CRF and it is therefore thought to be an endogenous agonist for CRF-R2 [4]. CRF-R2 mRNA is widely expressed in peripheral tissues including cardiac myocytes [5,6]. Two subtypes of CRF-R2 are cloned from cardiac tissues, CRF-R2 α in human and CRF-R2 β in the rat [7,8]. Activation of CRF-R2 elevates the cellular contents of cyclic AMP mainly through the G_s protein-adenylate cyclase pathway [9]. Urocortin also stimulates the phosphorylation of cyclic AMP response element-binding protein (CREB) in cells that express CRF-R2, and the PKA inhibitor blocks formation of phosphorylated CREB [10].

Urocortin exerts both positive chronotropic and inotropic actions in the heart and elevates coronary blood flow [11,12]. These effects appear to be associated with elevated formation of cyclic AMP in the cardiac tissue [13]. Urocortin is more potent than CRF in producing the cardiac effect [14]. Furthermore, urocortin fails to enhance cardiac performance and to reduce blood pressure in CRF-R2-knockout mice, thus supporting a central role of CRF-R2 in urocortin-induced peripheral haemodynamic effects [15]. CRF-R2 may also contribute to cardiovascular homeostasis because the CRF-R2-knockout mice have elevated basal blood pressure [15]. Urocortin mRNA is detected in both cultured neonatal cardiac myocytes and the adult heart of rats, and urocortin protects the intact rat heart against the damaging effects of ischemia/reperfusion injury [16,17]. All these indicate a considerable importance of urocortin in the (patho)physiology of the cardiovascular system.

Urocortin produces a potent and long-lasting hypotensive action in conscious rats [1], probably due to reduced peripheral vascular resistance. Its vasodilator effect is also reported in the human perfused placenta [18] and the rat cerebral artery [19]. Urocortin is found to decrease coronary perfusion pressure in the isolated rat heart via coronary vasodilation. We have demonstrated that urocortin induced both endothelium-dependent and -independent relaxation in isolated rat coronary arteries and our results show that endothelial nitric oxide and Ba²⁺-sensitive K⁺ channels may underlie the endothelium-dependent effect [20]. The positive inotropic and coronary dilator effects of urocortin may highlight its potential usefulness in the treatment of congestive heart failure. However, the mechanism responsible for the endothelium-independent coronary effect of urocortin has not been established. Urocortin is a poor dilator in artery rings contracted by elevated extracellular potassium concentration, suggesting that direct inhibition of voltage-gated Ca²⁺ channels is a minor mechanism [20]. The present study was therefore intended to investigate whether K⁺ channels and cyclic AMP-dependent pathway may be involved in the urocortin-induced endothelium-independent relaxation of the rat coronary arteries.

2. Methods

2.1. Vessel preparation

All experiments described below were approved by the Animal Research Ethics Committee, the Chinese University of Hong Kong. A total of 85 male Sprague–Dawley rats (supplied by the Laboratory Animal Service Center, Chinese University of Hong Kong) were used in this study. This investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Male rats weighing between 250 and 300 g were sacrificed by cervical dislocation and bled. The heart was rapidly removed and placed on a dissecting plate containing ice-cold Krebs solution. Krebs solution contained (mmol/l): 119 NaCl, 4.7 KCl, 2.5 CaCl₂, 1 MgCl₂, 25 NaHCO₃, 1.2 KH₂PO₄, and 11 D-glucose. After fatty connective tissues were trimmed off, each left anterior descending coronary artery was cut into two to three ring segments of 2 mm in length. Each arterial ring was mounted in a Multi Myograph System (Danish Myo Technology A/S, Denmark). Briefly, two tungsten wires (each of 40 μ m in diameter) were passed through the ring's lumen and fixed to jaws of myograph. The Krebs solution (5 ml) in the chamber bath was continually oxygenated with a gas mixture of 95% O₂ plus 5% CO₂, and its temperature was maintained at 37 °C using a built-in heat-changer device. The pH of Krebs solution was between 7.3 and 7.5, and monitored by a pH meter (Bechman, USA). The ring was stretched until the optimal tension of 1 mN and then allowed to equilibrate for about 90 min before the start of the experiment. Each experiment was performed on rings prepared from different rats. Only the endothelium-denuded rings were used in this study. The endothelium was mechanically removed by rubbing the luminal surface of the ring several times with a small stainless steel wire (40 μ m in diameter). The functional removal of the endothelium was verified if the ring failed to relax in response to 3 μ mol/l acetylcholine. High K⁺-containing solution was prepared by replacing NaCl with KCl on an equimolar basis in order to retain a constant ionic strength.

2.2. Protocols

Thirty minutes after being set up in the bathing chambers, each ring was initially contracted by 100 nmol/l U46619. After adding acetylcholine, the rings were washed with pre-warmed Krebs solution several times until baseline tone restored. Relaxations induced by urocortin or forskolin were studied in endothelium-denuded rings pre-constricted by U46619, the concentration of which (30–100 nmol/l) was titrated to produce contractions of similar magnitude. U46619-induced tone did not decline within 3

h ($n=3$). There was no difference in the evoked tone among different experimental groups. Once stable vessel tone was obtained, the vasodilator was applied cumulatively (0.1–30 nmol/l for urocortin and 1–1000 nmol/l for forskolin) to the bathing solution to determine concentration–response relationships. In the first set of experiments, the rings were exposed to the following agents: 30 μ mol/l Rp-cAMPS triethylamine, 200 nmol/l KT 5720, and 200 nmol/l KT 5823 for 30 min, contracted again by U46619 and the concentration–response for the vasodilator was obtained.

In the second group of experiments, the rings were incubated for 30 min with various K^+ channel blockers (100–200 nmol/l iberiotoxin, 1–3 mmol/l TEA⁺, 200 nmol/l apamin, or 30 μ mol BaCl₂) before addition of U46619, urocortin-induced relaxations were then determined. The effect of TEA⁺ was also tested in the presence of 200 nmol/l KT 5720. Since glibenclamide antagonized U46619-induced tone, its effect on the urocortin response was examined in rings precontracted by 10 nmol/l endothelin I.

In the third set of experiments, the relaxant response to urocortin was examined on rings precontracted by elevated extracellular K^+ (35–50 mmol/l). The effects of Rp-cAMPS, TEA⁺ and tetrodotoxin (TTX) were tested on urocortin-induced relaxation in 35 mmol/l-containing Krebs solution.

In the final set of experiments, the K^+ blockers were tested on the relaxant responses to forskolin in comparison with their effects on urocortin-induced relaxations. Lastly, the effect of cyclopiazonic acid (CPA, 10 μ mol/l) was examined on urocortin-induced relaxant responses.

2.3. Drugs

The following drugs were used: urocortin (human), 9,11-dideoxy-11 α ,9 α -epoxy-methanoprostaglandin F_{2 α} (U46619), endothelin I, acetylcholine hydrochloride, tetraethylammonium ions, iberiotoxin, apamin, cyclopiazonic acid, forskolin, tetrodotoxin, pinacidil, nifedipine, glibenclamide (Sigma, St. Louis, MO, USA), Rp-cAMPS triethylamine (RBI, Natick, MA, USA), KT 5720, KT 5823 (Tocris Cookson Ltd., UK). U46619, forskolin, pinacidil, nifedipine, glibenclamide were dissolved in dimethyl sulfoxide (DMSO). 0.2% DMSO did not affect U46619-induced tension. Stock solution of urocortin was prepared in 0.1 N HCl and desired dilution was made daily before experiments. Other drugs were prepared in distilled water.

2.4. Data analysis

The results are mean \pm S.E.Ms and n refers to the number of arterial rings examined. Several rings prepared from the same artery were studied in parallel, and a concentration–response curve was established in each ring. The relaxant effect of urocortin was calculated as the

percentage reduction of the initial tone in each ring precontracted by the agonist. Concentration–relaxation curves were constructed based on responses to cumulative concentrations of urocortin and analyzed by non-linear curve fitting using Graphpad software (Version 3.0). The negative logarithm of the relaxant concentration that caused 50% of the maximum relaxation (pD_2) and the maximum relaxation (E_{max}) were estimated. For statistical analysis, Student's t -test or one-way analysis of variance followed by Newman–Keuls test was used when more than two treatments were compared. The results were considered statistically significant when P -value was less than 0.05.

3. Results

3.1. Effect of cyclic AMP-dependent modulators on urocortin-induced contraction

Urocortin induced concentration-dependent relaxations in endothelium-denuded rings with a pD_2 of 8.40 ± 0.04 ($n=20$). Treatment with the PKA inhibitors, Rp-cAMPS (30 μ mol/l) or KT 5720 (200 nmol/l) attenuated urocortin-induced relaxation without an effect on the maximum response ($n=6$, $P < 0.05$ as compared with control, Fig. 1a and b). Both inhibitors did not change baseline tone ($n=6$). Fig. 1b summarizes the pD_2 values for the urocortin effect under different experimental conditions. In contrast, pretreatment with 200 nmol/l KT 5823 (an inhibitor of cyclic GMP-dependent protein kinase, PKG) did not affect urocortin-induced relaxation. (pD_2 : 8.40 ± 0.04 in control and 8.36 ± 0.08 in KT 5823, $n=4$, data not shown).

3.2. Effect of iberiotoxin, tetraethylammonium and apamin on urocortin-induced relaxation

The representative traces in Fig. 2 show the inhibitory effects of inhibitors of large-conductance Ca^{2+} -activated K^+ channels (BK channels) or elevated extracellular K^+ on urocortin-induced relaxation. Treatment with iberiotoxin (IBX, 100–200 nmol/l) inhibited urocortin-induced relaxation with a reduction in the maximum response (Fig. 3a). Similarly, the relaxant effect of urocortin was attenuated by TEA⁺ (1–3 mmol/l, Fig. 3b). Baseline tone was elevated by TEA⁺ (1.54 ± 0.56 mN and 1.96 ± 0.41 mN for 1 and 3 mmol/l TEA⁺, respectively, $n=6$) and by IBX (3.65 ± 0.63 mN and 4.28 ± 0.35 mN for 100 and 200 nmol/l IBX, respectively, $n=6$), indicating a high level of basal BK channel activity in the rat coronary artery. Nifedipine at 30 nmol/l reduced the contractile response to 3 mmol/l TEA⁺ by $91.2 \pm 3.7\%$ ($n=4$). Apamin, a blocker of small-conductance Ca^{2+} -activated K^+ channels had no effect on baseline tone or on urocortin-induced relaxation (Fig. 3c). The inhibitory effect of 1 mmol/l TEA⁺ was absent in rings pretreated with 200 nmol/l KT 5720 (pD_2 :

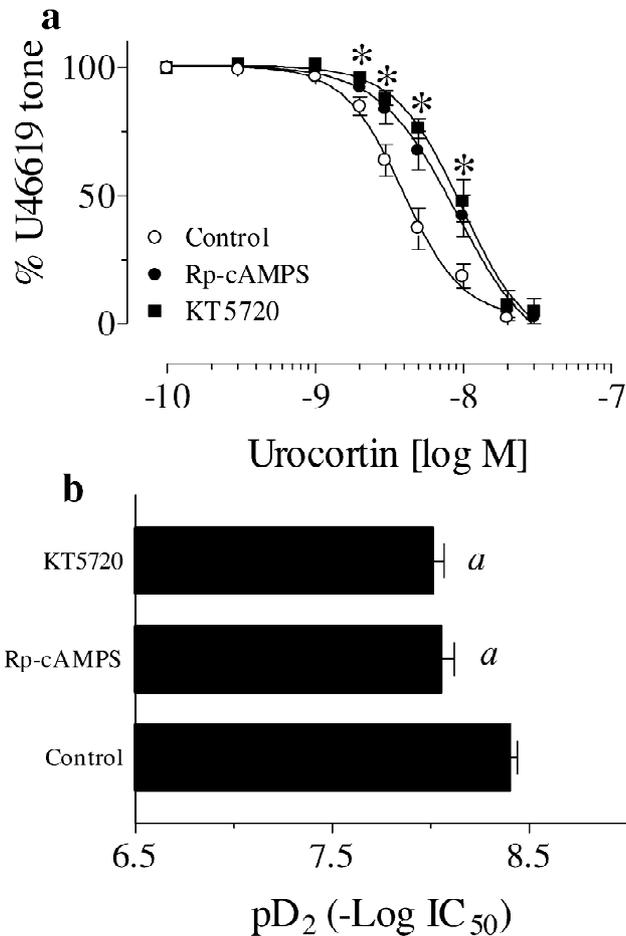


Fig. 1. (a) Log concentration–response curves for urocortin-induced relaxation in control (\circ , $n=20$) and in the presence of 30 $\mu\text{mol/l}$ Rp-cAMPS (\bullet , $n=6$) or 200 nmol/l KT 5720 (\blacksquare , $n=5$) in endothelium-denuded rat coronary artery rings precontracted by U46619. * Significantly different from control ($P<0.05$). (b) pD_2 values were calculated as the negative logarithm of the urocortin concentration that produced 50% the maximal relaxation following various pharmacological interventions. Data are means \pm S.E.M.'s of n experiments. a indicates significant difference from the control group ($P<0.05$).

7.97 ± 0.15 in TEA^+ , 8.01 ± 0.06 in KT 5720, and 7.93 ± 0.16 in KT 5720 plus TEA^+ , $n=6$, $P>0.05$, Fig. 3d). The pD_2 and E_{max} values obtained from various experiments were presented in Table 1.

3.3. Effect of high K^+ on urocortin-induced relaxation

In 35 mmol/l K^+ -contracted rings, urocortin produced significantly less relaxant effect and urocortin-induced relaxation was further inhibited in the presence of 50 mmol/l K^+ (Fig. 4a). Urocortin-induced relaxation was unaffected by tetrodotoxin (TTX, 1 $\mu\text{mol/l}$) in 35 mmol/l K^+ -containing solution (Fig. 4a). The relaxant effect of urocortin was identical in 35 mmol/l K^+ -contracted rings pretreated with 3 mmol/l TEA^+ and in 50 mmol/l K^+ -contracted rings (Fig. 4a), whilst TEA^+ at 3 mmol/l did not modify the urocortin-induced relaxation in 50 mmol/l

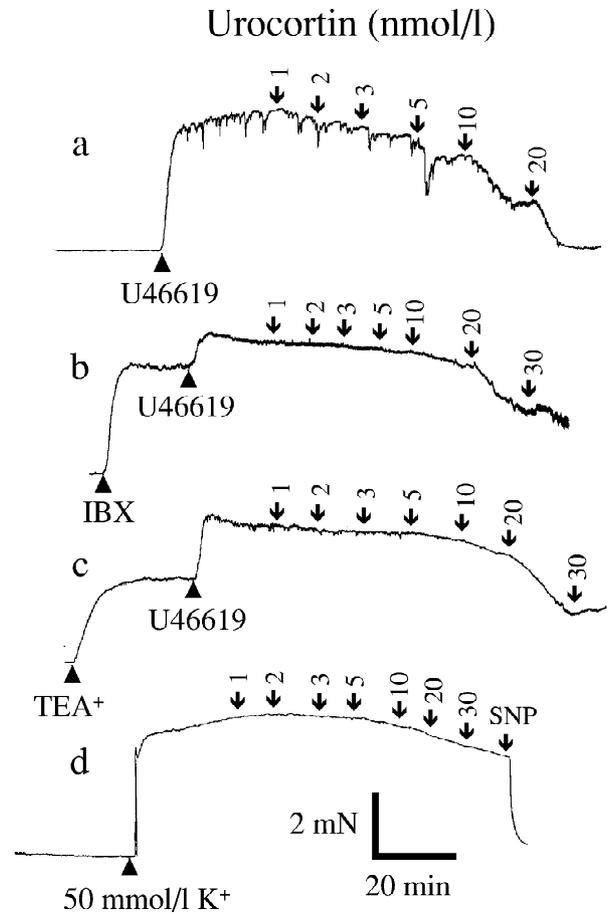


Fig. 2. Traces showing the relaxant effect of urocortin in control (a), in the presence of 100 nmol/l iberiotoxin (IBX, b) or 3 mmol/l TEA^+ (c) in U46619-contracted rings and in 50 mmol/l K^+ -contracted rings (d). Scale bars apply to all records.

K^+ -contracted rings (Fig. 4b). Nifedipine at 3 nmol/l relaxed 50 mmol/l K^+ -contracted rings by 13.8 ± 3.8 ($n=7$). The presence of 3 nmol/l nifedipine did not inhibit urocortin-induced relaxation of high K^+ -contracted rings ($n=4$, Fig. 4b). 50 mmol/l K^+ -induced tone did not decline in control or in the presence of 3 nmol/l nifedipine within a time course required to construct a concentration–response curve for urocortin ($n=3$). Rp-cAMPS (30 $\mu\text{mol/l}$) had a similar effect as to TEA^+ on urocortin-induced relaxation in 35 mmol/l K^+ -contracted rings. Combined treatment with Rp-cAMPS and TEA^+ did not produce a further inhibitory effect (Fig. 4c). Fig. 4d shows 30 nmol/l urocortin-induced relaxation among various treatment groups. Nifedipine (40 nmol/l) abolished 50 mmol/l K^+ -induced contraction but pinacidil (1 $\mu\text{mol/l}$) showed no effect ($n=4$, in each case, data not shown).

3.4. Effect of glibenclamide and BaCl_2 on urocortin-induced relaxation

Treatment with glibenclamide (10 $\mu\text{mol/l}$) did not alter

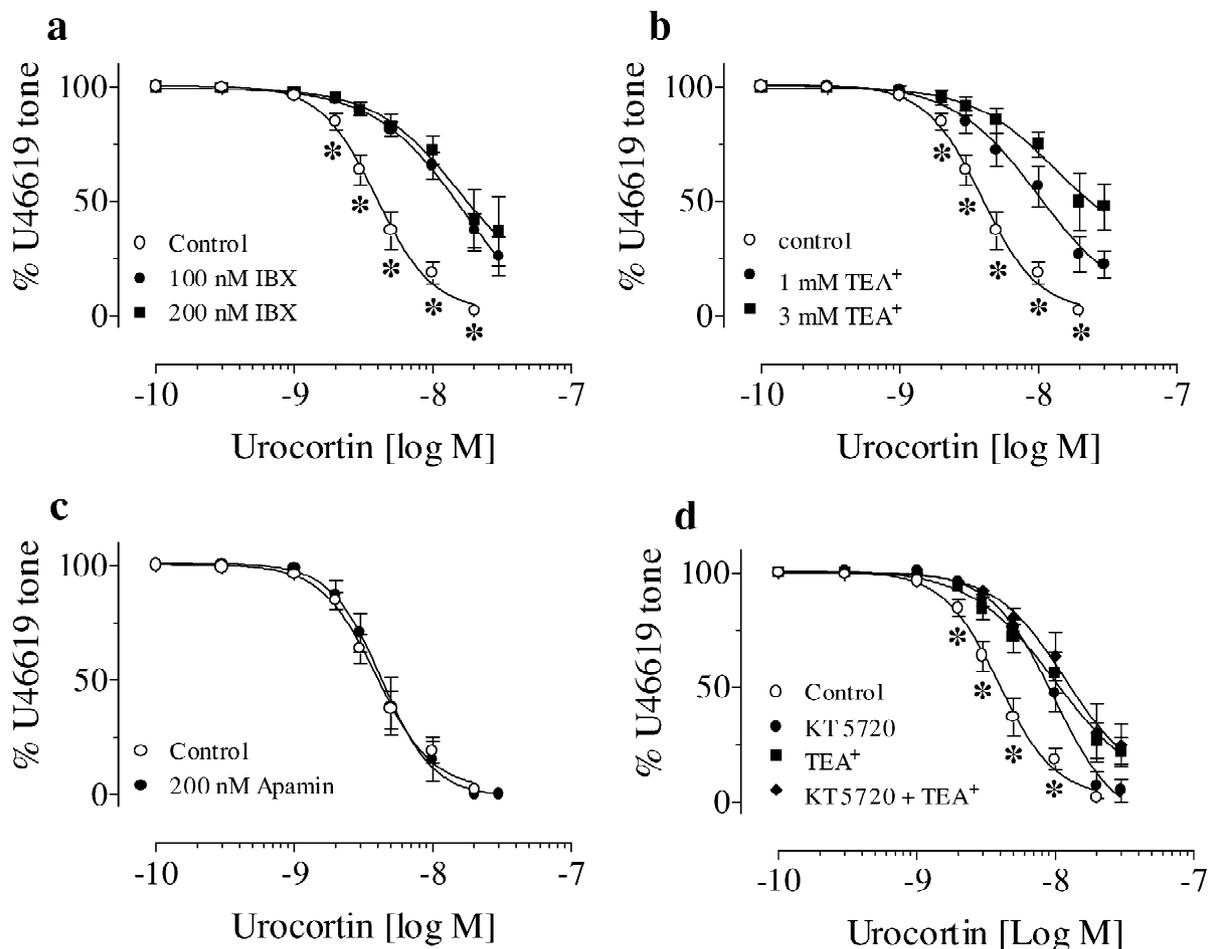


Fig. 3. (a) Log concentration–response curve for urocortin-induced relaxation in rings pretreated by IBX (○, $n=20$ in control; ●, $n=5$ in 100 nmol/l IBX; and ■, $n=6$ in 200 nmol/l IBX). (b) Log concentration–response curve for urocortin-induced relaxation in rings pretreated by TEA⁺ (○, $n=20$ in control; ●, $n=6$ in 1 mmol/l TEA⁺; and ■, $n=6$ in 3 mmol/l TEA⁺). (c) Log concentration–response curve for urocortin-induced relaxation in rings pretreated by apamin (○, $n=20$ in control; ●, $n=6$ in 200 nmol/l apamin). (d) Lack of effect of 1 mmol/l TEA⁺ in the presence of 200 nmol/l KT 5720 ($n=6$). * Significantly different from control ($P<0.05$). Data are means±S.E.M.'s of n experiments.

urocortin-induced relaxation of endothelin I (10 nmol/l)-contracted rings (pD_2 : 8.09 ± 0.05 in control and 8.04 ± 0.05 in glibenclamide, $P>0.05$, $n=5$, Fig. 5a). In U46619-contracted rings, the relaxant response to urocortin were unaffected by 30 μ mol/l BaCl₂ (pD_2 : 8.40 ± 0.04 in control; 8.13 ± 0.22 in BaCl₂, $n=6$, $P>0.05$, Fig. 5b). Both blockers did not affect baseline tone.

Table 1
Effects of K⁺ channel blockers on urocortin-induced relaxation

Treatment	pD_2	E_{max} (%)	n
Control	8.40 ± 0.04	97.8 ± 1.1	20
1 mmol/l TEA ⁺	$7.97\pm0.15^*$	$77.5\pm5.8^*$	6
3 mmol/l TEA ⁺	$7.89\pm0.22^*$	$52.6\pm10.0^*$	6
100 nmol/l IBX	$7.76\pm0.25^*$	$79.0\pm9.9^*$	6
200 nmol/l IBX	$7.82\pm0.12^*$	$61.8\pm15.0^*$	6
200 nmol/l Apamin	8.39 ± 0.04	100	6

Values are means±S.E.M.'s of n experiments. Significant difference (* $P<0.05$) between the control and treatment groups.

3.5. Effect of K⁺ blockers on forskolin-induced relaxation

Forskolin produced significantly less relaxant responses in 50 mmol/l K⁺-contracted rings (Fig. 6a, b and d). Pretreatment with TEA⁺ (3 mmol/l, Fig. 6c) or IBX (100 nmol/l) attenuated forskolin-induced relaxation ($n=6$, Fig. 6e). Fig. 6f summarizes the pD_2 values for forskolin-induced relaxation in the absence and presence of K⁺ channel blockers ($n=6$). TEA⁺ and IBX elevated baseline tone by 3.05 ± 0.32 mN and 3.11 ± 0.64 mN, respectively ($n=6$).

3.6. Effect of cyclopiazonic acid on urocortin-induced relaxation

In order to examine whether urocortin could inhibit intracellular Ca²⁺ release from the endoplasmic reticulum, the effect of cyclopiazonic acid (CPA) was tested. In

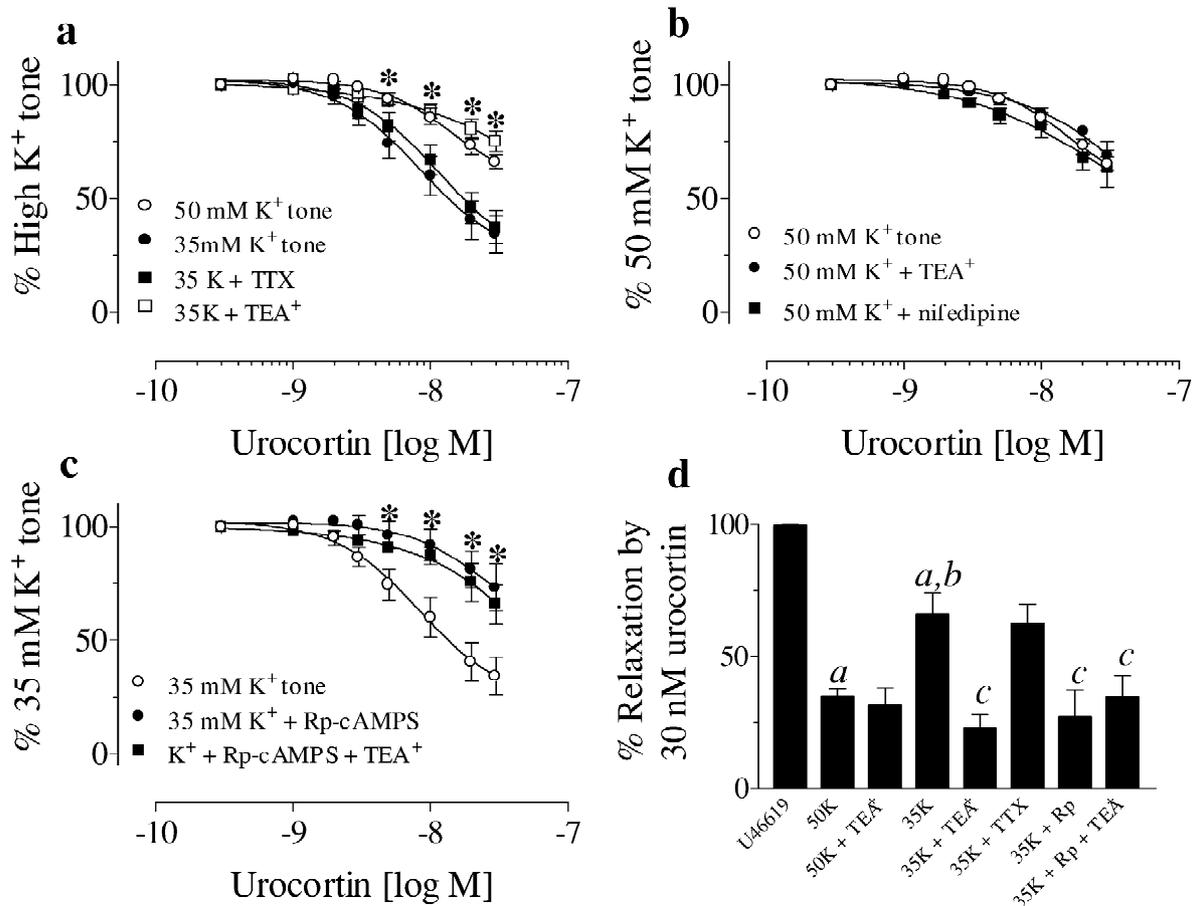


Fig. 4. (a) Log concentration–response curves for urocortin-induced relaxation in rings contracted by 50 mmol/l K⁺ (○, *n*=6) or by 35 mmol/l K⁺ (●, *n*=6) in control, ■, *n*=5 in 100 nmol/l TTX, and □, *n*=6 in 3 mmol/l TEA⁺. (b) The relaxant effect of urocortin on 50 mmol/l K⁺-contracted rings (○, *n*=6 in control and ●, *n*=6 in 3 mmol/l TEA⁺, ■, *n*=4 in TEA⁺ plus 3 nmol/l nifedipine). (c) The relaxant effect of urocortin in 35 mmol/l K⁺-contracted rings (○, *n*=6 in control, ●, *n*=6 in 30 μmol/l Rp-cAMPS, and ■, *n*=5 in Rp-cAMPS plus TEA⁺). * Significantly different from control (*P*<0.05). (d) The percentage relaxation induced by 30 nmol/l urocortin in rings subjected to various treatments. Significant difference (*P*<0.05) between U46619 and high K⁺ groups (a), between 50 and 35 mmol/l K⁺ groups (b), and between 35 mmol/l K⁺ and other treatment groups (c). Data are means±S.E.M.'s of *n* experiments.

U46619-precontracted rings, urocortin induced relaxations in the presence of 10 μmol/l CPA, an inhibitor of the endoplasmic reticulum ATPase with a *pD*₂ value of 8.18±0.26 (*n*=4), which was not different from that in the absence of CPA (*pD*₂: 8.39±0.04, *n*=8, *P*>0.05). The results indicate that stimulation of Ca²⁺ uptake into the endoplasmic reticulum does not contribute to urocortin-induced relaxation.

4. Discussion

The primary objective of this study was to investigate the mechanisms underlying the endothelium-independent relaxant response to urocortin in isolated endothelium-denuded rat coronary arteries. Our results provide several pieces of novel evidence indicating a role of BK channels and PKA-mediated cellular pathway in the urocortin-in-

duced relaxation. These include: (1) urocortin-induced relaxation was inhibited by the PKA inhibitors; (2) urocortin produced significantly less relaxant effect in high K⁺-contracted than U46619-contracted artery rings; (3) urocortin-induced relaxation was attenuated by BK channel blockers and the inhibitory effect of the BK channel blocker was absent in the presence of the PKA inhibitor; and (4) BK channel blockers or elevated extracellular K⁺ inhibited the relaxant response to forskolin, a cyclic AMP-elevating agent.

Upon stimulation of CRF-R2β, cyclic AMP and subsequent PKA-dependent cellular process are proposed to mediate the cardiovascular effects of urocortin and other CRF-related peptides [1,6]. Indeed, the PKA inhibitors, Rp-cAMPS and KT 5720 reduced urocortin-induced relaxation. These results agree with previous reports showing an inhibitory effect on urocortin-induced relaxation by Rp-cAMPS in rat tail artery [21] or by SQ22536 (another PKA

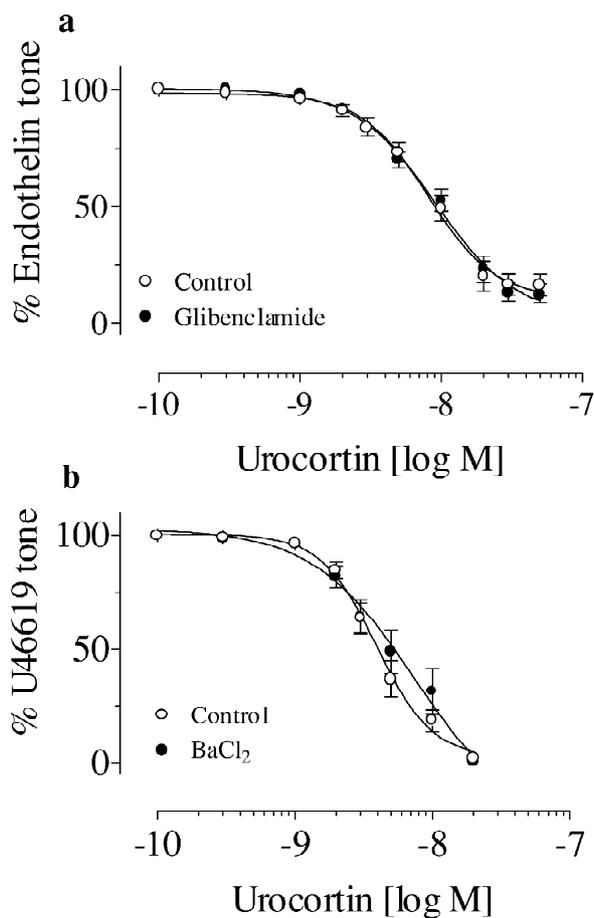


Fig. 5. (a) Lack of effect of glibenclamide (10 $\mu\text{mol/l}$) on urocortin-induced relaxation in endothelin I-contracted rings ($n=5$). Lack of effect of 30 $\mu\text{mol/l}$ BaCl₂ (b, $n=6$) on urocortin-induced relaxation in U46619-contracted rings. Data are means \pm S.E.M.'s of n experiments.

inhibitor) in rat basilar artery [19]. In contrast, the cyclic GMP-dependent mechanism might not be involved since KT 5823 (an inhibitor of PKG) showed no effect.

A markedly reduced vasorelaxant effect of urocortin in elevated extracellular K⁺ solution raises a possibility that urocortin may hyperpolarize coronary smooth muscle cells via increasing K⁺ conductance. One of the principal effects of increasing extracellular K⁺ is to reduce the electrochemical driving force for K⁺ efflux, so that K⁺ channel activation would have minimal effect on the cell membrane potential. Urocortin-induced relaxation was attenuated to a similar extent by IBX, a potent blocker of BK channels and by TEA⁺ at concentrations that inhibited single arterial BK channels [22]. The concentration–relaxation curves for urocortin in 35 mmol/l K⁺-contracted rings appear identical in the presence of 3 mmol/l TEA⁺ and in rings precontracted by 50 mmol/l K⁺ (see Fig. 4a). In the human saphenous veins it has also been observed that the relaxation to urocortin was markedly reduced by TEA⁺ [23]. In contrast, apamin, a blocker of small-conductance Ca²⁺-activated K⁺ channels showed no effect.

Urocortin produced significantly less relaxant effect in rings contracted by 50 mmol/l than 35 mmol/l K⁺. TEA⁺ at 3 mmol/l further inhibited urocortin-induced relaxation in rings contracted by 35 mmol/l K⁺ but not in rings contracted by 50 mmol/l K⁺ (see Fig. 4a and b). The urocortin-induced relaxation was unaffected by TTX, suggesting that Na⁺ channel-dependent and action potential-mediated release of peripheral neuronal factors are unlikely involved. These new results support the notion that urocortin may activate arterial BK channels and subsequent membrane hyperpolarization would inhibit Ca²⁺ influx via voltage-gated Ca²⁺ channels. This effect is apparently different from that of nifedipine (a blocker of L-type voltage-gated Ca²⁺ channels) as nifedipine at 40 nmol/l entirely relaxed the high K⁺-contracted rings as shown in the present study.

Both ATP-sensitive (K_{ATP}) and BK channels are activated by PKA stimulation in porcine or rabbit coronary artery smooth muscle cells [24,25]. Urocortin-induced relaxation is unaffected by glibenclamide at a concentration that blocks vascular K_{ATP} channels [26], indicating that K_{ATP} channels play no role. This is in agreement with previously reported results in the rat cerebral artery [19]. The present study clearly demonstrated a considerable involvement of PKA in BK channel-dependent urocortin-induced relaxation. There is no additive effect in the inhibitory action of KT 5720 and 1 mmol/l TEA⁺. Similarly, Rp-cAMPS (another inhibitor of PKA) and TEA⁺ equieffectively inhibited urocortin-induced relaxation in 35 mmol/l K⁺-contracted rings. It appears that a larger portion of urocortin-induced relaxation is mediated by the receptor-coupled adenylate cyclase stimulation, which results in activation of BK channels. A similar observation is reported for urocortin in the rat cerebral artery [19]. Similarly, both TEA⁺ and IBX significantly reduced the relaxant response to forskolin, which relaxes blood vessel primarily through stimulation of cyclic AMP-dependent mechanisms. In consistence with this effect, the relaxant effect of forskolin was markedly attenuated in depolarized smooth muscle cells bathed in 50 mmol/l K⁺-containing solution. In single non-vascular smooth muscle cells, urocortin was found to cause concentration-dependent increase in Ca²⁺-activated K⁺ currents. This effect was reduced by inhibitors of adenylate cyclase and PKA, but not by inhibitors of guanylate cyclase and PKG [27]. Activation of inward rectifier K⁺ (K_{IR}) channels may contribute to urocortin-induced endothelium-dependent coronary relaxation [20]. However, BaCl₂ does not modulate urocortin-induced endothelium-independent relaxation at a concentration that selectively inhibits the activity of K_{IR} channels in coronary vascular smooth muscle cells [28].

Urocortin at 30 nmol/l was still able to induce partial relaxation (34%) in 50 mmol/l K⁺-contracted rings which was insensitive to TEA⁺ and higher concentration of IBX

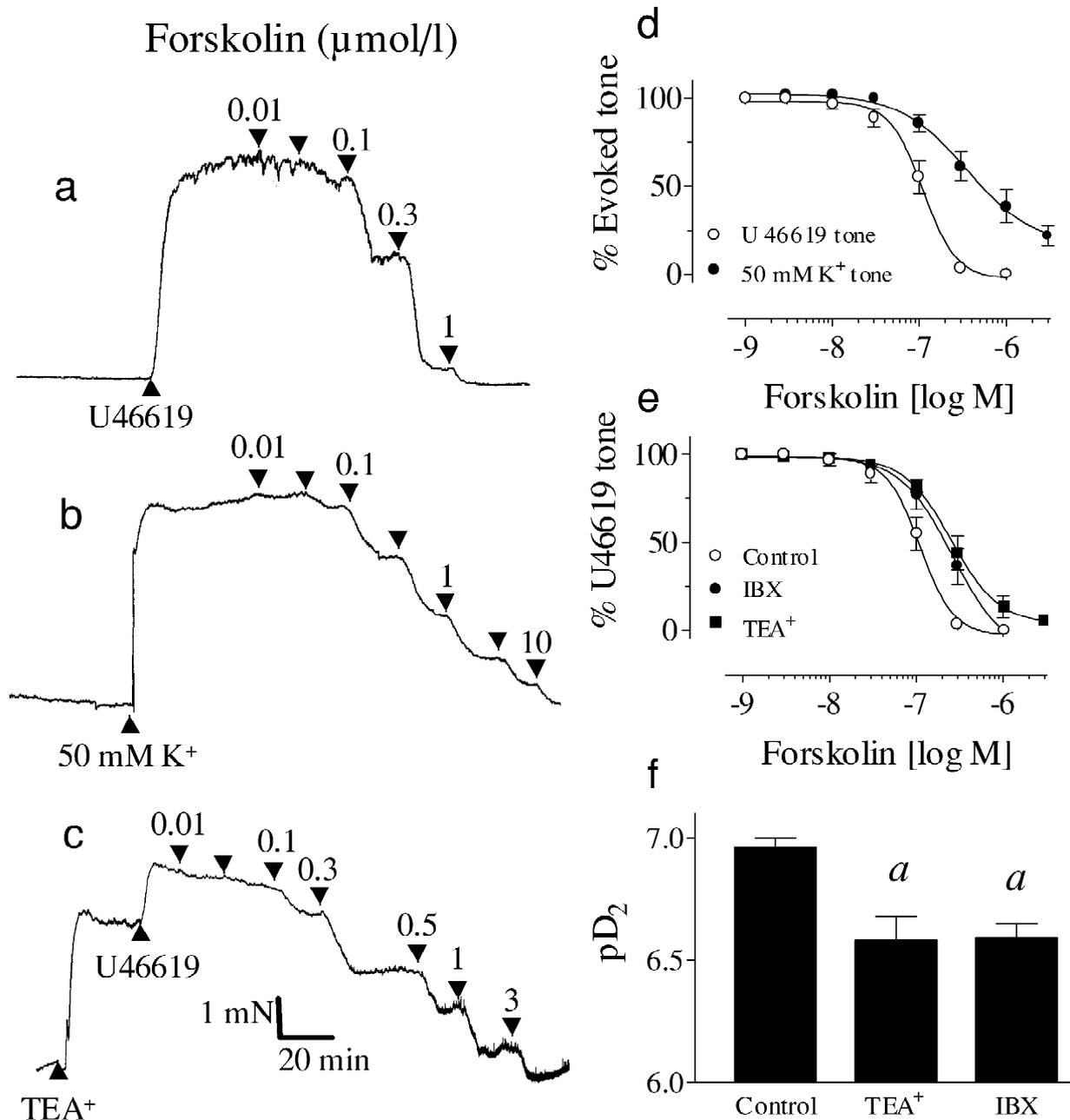


Fig. 6. Traces showing the relaxant effect of forskolin in rings contracted by U46619 (a), U46619 in the presence of 3 mmol/l TEA⁺ (b), or by 50 mmol/l K⁺ (c). (d) Concentration–response curve for forskolin in rings contracted by U46619 and high K⁺ ($n=6$). (e) Concentration–response curve for forskolin in the presence of 3 mmol/l TEA⁺ or 100 nmol/l IBX ($n=6$). (f) The pD_2 values for forskolin-induced relaxation calculated under three conditions. *a* indicates significant difference from the control ($P<0.05$). Data are means \pm S.E.M.'s of n experiments.

did not produce greater inhibitory effect in U46619-contracted rings, suggesting that other mechanisms may also be involved. Stimulation of thromboxane A₂ receptor by U46619 results in activation of G protein-coupled phospholipase C, which leads to generation of two intermediate second messenger molecules, inositol triphosphate (IP₃) and diacylglycerol [29]. IP₃ mobilizes intracellular Ca²⁺ stores and diacylglycerol activates endogenous protein kinase C that jointly cause contraction of vascular smooth muscle cells. However, neither U46619 nor caffeine

produced significant contraction in a Ca²⁺-free Krebs solution (personal communication), indicating that Ca²⁺ from intracellular stores or protein kinase C should play little role in the U46619-induced steady tone in the rat coronary artery. These results together with lack of effect of CPA suggest that a possible inhibition of internal Ca²⁺ mobilization may make small contribution to urocortin-induced relaxation. It appears that direct inhibition of Ca²⁺ channels may account for urocortin-induced partial relaxation in high K⁺-contracted rings. It would be expected that

the effect of urocortin should be reduced in the presence of Ca^{2+} channel blockers if urocortin relaxes blood vessels partly through direct inhibition of Ca^{2+} influx. However, our recent data are not in favor of this possibility since a partial blockade of the voltage-gated Ca^{2+} channel by nifedipine (3 nmol/l) had no effect on urocortin-induced relaxation in high K^+ -containing solution (see Fig. 4b). Nevertheless, the possible effect on arterial Ca^{2+} channels needs to be verified by the electrophysiological study. However, urocortin did not inhibit inward Ca^{2+} currents in non-vascular smooth muscle [27].

Urocortin plasma concentration is around 1 pmol/l in humans [30]. The present results show that urocortin relaxed rat coronary artery with an IC_{50} of ~ 4 nmol/l, a value comparable with that (~ 2.6 nmol/l) obtained in rat tail artery [21]. However, more potent relaxing effects of urocortin were observed in rat basilar artery [19], human perfused placenta [18], and human saphenous vein [23]. In these vessels, urocortin exerts the relaxing effect at very low concentrations (1–10 pmol/l). It appears that vascular sensitivity for urocortin may be different among various vessels or from different species. Nevertheless, the exact physiological role of urocortin in the cardiovascular system is yet to be established.

In conclusion, The present study demonstrates a significant role of BK channels in urocortin-induced endothelium-independent relaxation in the isolated rat coronary artery. Urocortin may activate BK channels but not other K^+ channels, largely via a PKA-dependent cellular mechanism. The coronary vasodilator effect together with other potential benefits in the cardiovascular system may make urocortin and new CRF-related peptides useful therapeutic agents against the damaging effect of ischemia/reperfusion injury to the heart.

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