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Nickel inhibits urocortin-induced relaxation in the rat pulmonary artery

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Abstract

Urocortin relaxes rat pulmonary arteries partly through a cyclic AMP-dependent but Ca^{2+} channel-independent mechanism. However, other participating mechanisms are relatively unknown. The present study was designed to examine whether the forward mode of Na^{+}–Ca^{2+} exchangers play a role in the relaxant responses to urocortin in isolated rat small pulmonary arteries. Endothelium-denuded rings were mounted on small vessel myographs for measurement of changes in isometric tension. Urocortin inhibited 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619)-induced contraction in a concentration-dependent manner and this inhibition was reversed by astressin, a corticotropin-releasing factor receptor antagonist. Micromolar concentrations of nickel (Ni^{2+}) chloride, a putative inhibitor of the Na^{+}–Ca^{2+} exchanger, reduced the relaxant responses to urocortin. Urocortin-induced relaxation was abolished in a Na^{+}-free solution, a condition that eliminates influence of the forward mode of Na^{+}–Ca^{2+} exchanger. In contrast, the relaxant responses to atrial natriuretic peptide or forskolin were unaffected by Ni^{2+} or with removal of extracellular Na^{+}. The present results provide indirect evidence suggesting that stimulation of Na^{+}–Ca^{2+} exchangers may contribute to urocortin-induced endothelium-independent pulmonary artery relaxation.

Keywords: Urocortin; Nickel; Relaxation; Pulmonary artery

1. Introduction

Urocortin, a potent vasodilator identified first in rats (Vaughan et al., 1995) and later in human (Donaldson et al., 1996), is a member of the hypothalamic corticotropin-releasing factor (CRF) family. CRF and peripheral CRF receptors are involved in the regulation of cardiovascular function (Coste et al., 2000). Systemic administration of urocortin or CRF-related peptides lowers blood pressure (Kubler et al., 1994; Vaughan et al., 1995); these peptides also relax many systemic arteries in vitro (Schilling et al., 1998; Sanz et al., 2002; Huang et al., 2002, 2003).

The hypertrophic hearts have higher levels of urocortin mRNA and urocortin-like immunoreactivity than healthy hearts (Nishikimi et al., 2000). It is unknown whether locally produced urocortin in the hypertrophic cardiomyocytes is involved in the regulation of pulmonary blood flow. Urocortin induces pulmonary artery relaxation only in part via a cyclic AMP-dependent mechanism; urocortin-induced relaxation does not involve the endothelium, activation of voltage-gated Ca^{2+} channels, Ca^{2+}-activated or voltage-sensitive K^{+} channels (Chan et al., in press). Other endothelium-independent mechanisms have not been studied. In this study, we demonstrated that urocortin-induced pulmonary artery relaxation is reduced or abolished by pharmacological interventions that are used to inhibit the activity of vascular Na^{+}–Ca^{2+} exchangers.

2. Materials and methods

Male Sprague–Dawley rats (8–10 weeks old, 250–300 g) were sacrificed by cervical dislocation. The lungs were removed and placed in ice-cold Krebs solution. Second order intralobal pulmonary arteries were isolated and cut into two ring segments (~ 1 mm long). As previously described (Chan et al., in press), the artery rings were mounted on wires attached to force transducers in a Multi Myograph System (Danish Myo Technology) for measurement of changes in isometric force. Vessels were incubated

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in 5-ml chambers filled with Krebs solution of the following compositions (in mM): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4 and 11 D-glucose. The bathing solution was gassed with 95% O_2–5% CO_2 at 37 °C (pH 7.2–7.4). Vessels were allowed to stabilize for 60 min at 1 mN optimal resting tension. The endothelial layer of each ring was mechanically denuded; functional removal of the endothelium was confirmed by obtaining no relaxant responses to acetylcholine (3 μM). All experiments were performed on endothelium-denuded vessels.

After 60-min equilibration, two consecutive contractions of similar amplitude to 60 mM K^+ were induced with a 30-min interval. In the first series of experiments, concentration-dependent contractions to 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619, 3–500 nM) were obtained in the absence and presence of urocortin (30 nM). The effect of nickel was examined on urocortin-inhibition of U46619 (3–500 nM)-induced contraction. The effect of Ni^{2+} (30-min incubation time) was also tested on urocortin-induced relaxation in precontracted vessels. In the second set of experiments, the effect of urocortin was tested on U46619-contracted rings in 20 mM Na^+-containing or Na^+-free Krebs solution. The U46619-induced tone remained unchanged for two hours in normal Krebs solution but it was declining in a Na^+-free solution. Therefore, the time-dependent reduction in U46619-induced tone was subtracted when the effect of urocortin was determined. The relaxant responses to atrial natriuretic peptide or forskolin were also examined in the presence of Ni^{2+} or in a Na^+-free solution.

Acetylcholine, U46619, atrial natriuretic peptide, forskolin, astressin and ouabain were from Sigma. The Na^+-free solution contained (in mM): 119 *N*-methyl-D-glucamine chloride, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 choline bicarbonate, 1.2 KH_2PO_4 and 11 D-glucose. The Na^+ (20 mM)-containing solution contained 20 mM NaCl and 99 mM *N*-methyl-D-glucamine chloride.

Results are means ± S.E.M. of *n* artery rings. The contraction was expressed as the percentage of the high K^+-induced tension in each ring (averaged amplitude of two consecutive contractions to 60 mM K^+). Concentration–response relationship was analyzed by non-linear regression curve fitting using GraphPad Prism software (version 3.0). pEC_{50} is the negative logarithm of the U46619 concentration that produced 50% of maximum contraction (E_{max}). Student's *t*-test or analysis of variance followed by Newman–Keuls test was used. $P < 0.05$ was considered significant.

3. Results

U46619 concentration-dependently contracted vessels with a pEC_{50} of 7.45 ± 0.08 and E_{max} of 94.2 ± 3.4% of the contraction induced by 60 mM KCl. Urocortin at 30 nM reduced the U46619 contraction (pEC_{50} of 6.97 ± 0.25 and E_{max} of 40 ± 7.6%, $P < 0.05$). Treatment with Ni^{2+} (300

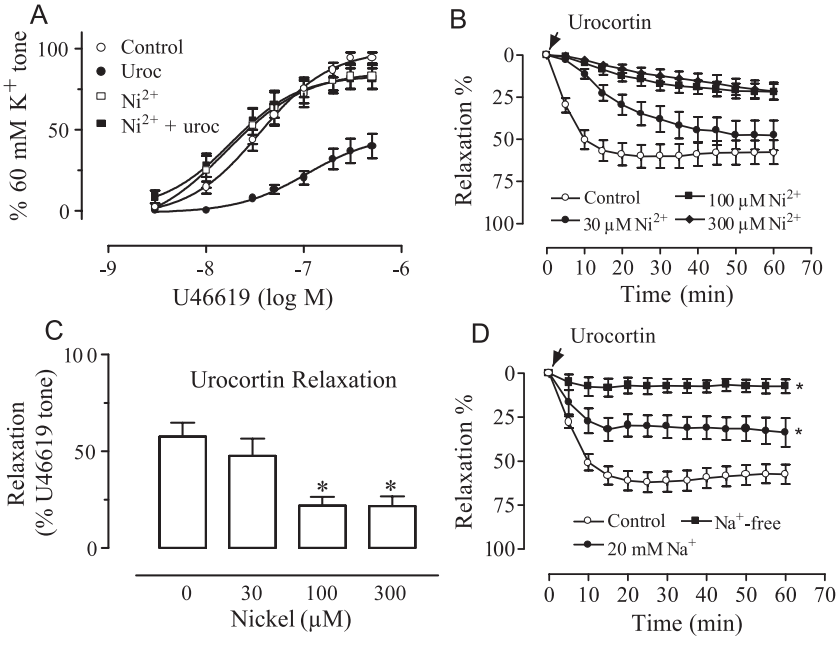

Fig. 1. (A) Effect of 300 μM Ni^{2+} on urocortin-induced reduction of U46619 (3–500 nM)-mediated contraction in isolated endothelium-denuded pulmonary artery rings (○: in control, ●: in urocortin, ■: in Ni^{2+}, □: in Ni^{2+} plus urocortin, $n = 6$). The contractile effect of U46619 was expressed as percentages of averaged amplitude of two 60 mM K^+ contractions. (B) Effect of Ni^{2+} (30–300 μM) on urocortin (30 nM)-induced relaxation in U46619-contracted rings ($n = 6$–12). Relaxation induced by urocortin in the absence and presence of Ni^{2+} (C). (D) Relaxation to 30 nM urocortin in U46619-contracted rings under three ionic conditions (○: in normal Krebs solution, ●: in 20 mM Na^+-containing solution, ■: in Na^+-free solution, $n = 6$–10). *Significant difference ($P < 0.05$) from controls. Values are means ± S.E.M. of *n* experiments.

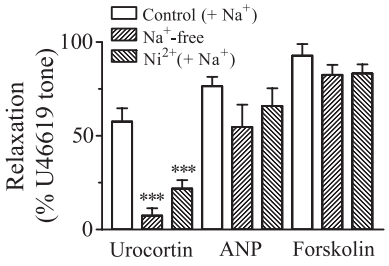

Fig. 2. The relaxation induced by 30 nM urocortin, 100 nM atrial natriuretic peptide or 3 μM forskolin in normal Krebs solution, Na^+-free solution or normal Krebs solution containing 300 μM Ni^{2+} in U46619 (100 nM)-precontracted endothelium-denuded pulmonary artery rings. ***Significant difference ($P<0.001$) from controls. Values are means ± S.E.M. of 6–10 experiments.

μM) did not modify U46619-induced contraction, while it abolished the effect of urocortin (Fig. 1A). In U46619-precontracted vessels urocortin induced relaxation by 44.7 ± 8.7%, 57.6 ± 7.1% and 59.0 ± 5.3% in 10, 30 and 50 nM, respectively. The non-selective CRF receptor antagonist, astressin (300 nM) markedly reduced the relaxant effect of 30 nM urocortin (57.6 ± 7.1% relaxation in control and 15.0 ± 2.9% relaxation in astressin, $P<0.05$). Ni^{2+} attenuated urocortin (30 nM)-induced relaxation in a concentration-related manner (Fig. 1B and C). Urocortin (30 nM)-induced relaxation was reduced in 20 mM Na^+-containing solution (57.5 ± 5.4% and 33.7 ± 8.2% relaxation, respectively, in 144 and 20 mM Na^+-containing solution, $P<0.05$, Fig. 1D) and almost eliminated in rings bathed in a Na^+-free solution (7.5 ± 3.9% relaxation, Fig. 1D). The Na^+–K^+-ATPase inhibitor, ouabain at 100 μM did not modify urocortin-induced pulmonary artery relaxation ($n=4$, data not shown). In contrast, the relaxant responses to atrial natriuretic peptide (100 nM) or forskolin (3 μM) were unaffected by Ni^{2+} (300 μM) or in a Na^+-free solution (Fig. 2).

4. Discussion

The main finding of the present study is that urocortin-induced relaxation is partially mediated by activation of a Ni^{2+}-sensitive pathway probably involving the vascular Na^+–Ca^{2+} exchanger in isolated rat small pulmonary arteries. Sarcolemmal Na^+–Ca^{2+} exchange participates in the regulation of Ca^{2+} homeostasis in vascular smooth muscle cells (Motley et al., 1993). A defect in the Na^+–Ca^{2+} exchange translocation pathway may lead to abnormal $[Ca^{2+}]_i$ in the renal arterioles from salt-sensitive hypertensive rats (Bell et al., 2000). Several divalent cations, such as Ni^{2+}, cadmium and manganese were found to be a putative inhibitor of Na^{2+}–Ca^{2+} exchanger in both vascular and non-vascular cells (Frame and Milanick, 1991; Hobai et al., 1997; Tsang et al., 2003).

The activity of the Na^+–Ca^{2+} exchanger is normally coupled to $[Na^+]_i$, which is primarily regulated by the membrane permeability to Na^+ ions and the Na^+–K^+-ATPase activity. Decreased permeability to Na^+ and/or increased activity of Na^+–K^+ pump results in a reduction in $[Na^+]_i$, which then stimulates the forward mode of the Na^+–Ca^{2+} exchanger. The experiments with a Na^+-free ionic condition further support a role of Na^+–Ca^{2+} exchanger in the relaxation. Urocortin-induced effect was attenuated with decreasing extracellular Na^+ concentration and almost abolished in Na^+-free solution, a condition that abolishes the influence of Na^+–Ca^{2+} exchanger (the forward mode) on the regulation of vessel tension. Lack of effect of ouabain, a Na^+–K^+-ATPase inhibitor, suggests that stimulation of this pump is unlikely coupled to urocortin-induced Ni^{2+}-sensitive pulmonary artery relaxation.

The effects of Ni^{2+} or a Na^+-free condition are unlikely non-specific since neither Ni^{2+} nor Na^+-free solution modified the relaxant responses to atrial natriuretic peptide (a peptide dilator) and to forskolin (a non-peptide dilator). We have recently shown that activation of cyclic AMP-dependent protein kinase (PKA) is involved in urocortin-induced vasorelaxation and KT5720 (200 nM, a PKA inhibitor) reduced urocortin-induced steady relaxation by approximately 35% (Chan et al., in press). While, Ni^{2+} induced a maximal 53% inhibition of urocortin-induced relaxation (Fig. 1B). However, nickel did not affect the relaxation induced by the cyclic AMP-elevating agents, atrial natriuretic peptide and forskolin, indicating that PKA-sensitive and Ni^{2+}-sensitive mechanisms may not overlap. Nevertheless, it cannot be ruled out that urocortin binding to vascular CRF receptors may be affected by changes in the extracellular Na^+ concentration.

In summary, this study indicates that stimulation of Na^+–Ca^{2+} exchanger (the forward mode) may be a novel mechanism by which urocortin relaxes isolated rat small pulmonary arteries in vitro. Nevertheless, the physiological importance of urocortin in pulmonary arteries and its potential compensatory role in the regulation of pulmonary circulation in hypertrophic hearts remains to be established.

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