Urocortin Protects Coronary Endothelial Function During Ischemia-Reperfusion: A Brief Communication

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Urocortin is a vasodilator peptide related to corticotrophin-releasing factor, which may protect myocardium during coronary ischemia-reperfusion. To study whether urocortin also protects coronary endothelial function during ischemia-reperfusion, hearts from Sprague-Dawley rats were perfused at constant flow and then exposed to 15 mins ischemia followed by 15 mins reperfusion. In one series of experiments, we found that the coronary relaxation to urocortin (10^{-11} to 10^{-8} *M*) was reduced by ischemia-reperfusion (51 ± 4% vs. 79 ± 4% of the active tone, for the 10^{-10} *M* dose). In other series of experiments, we observed that ischemia-reperfusion reduced the coronary relaxation to a test dose of acetylcholine (10^{-6} *M*) (25 ± 2% vs. 54 ± 9% of active tone), without modifying the relaxation to sodium nitroprusside (10^{-6} *M*). Treatment with a low threshold concentration of urocortin (10^{-11} *M*), administered before ischemia and during reperfusion, partly improved the coronary relaxation to acetylcholine (36 ± 3% of active tone). These results suggest that ischemia-reperfusion impairs the coronary vasodilation to urocortin and produces endothelial dysfunction and that this endothelial dysfunction may be improved by urocortin. Exp Biol Med 229:118–120, 2004

Key words: acetylcholine; heart; Langendorff; sodium nitroprusside

Urocortin is a 40–amino acid peptide that has a high degree of structural homology with the peptide corticotrophin-releasing factor and has marked cardiovascular effects (1). In the heart, exogenous urocortin increases heart rate and cardiac output and also produces coronary vasodilation (2). In addition, exogenous urocortin may protect myocardial cells during coronary ischemia, as it increases the survival of cultured cardiac cells exposed to simulated ischemia (3) and also reduces the infarct area in the ischemic and reperfused rat heart (4).

It has been demonstrated that urocortin messenger RNA and protein are produced in the heart, and its production may be increased in cardiac cells exposed to ischemia (3). Therefore, urocortin may act as an endogenous protective substance in the myocardium exposed to ischemia-reperfusion. The *in vitro* protective effect of urocortin on isolated cardiac myocytes may be mediated by activation of mitogen-activated protein kinases (4), but because urocortin has a marked coronary vasodilator effect, there is the possibility that this effect also contributes to its myocardium protective effect during ischemia-reperfusion. Ischemia-reperfusion may also affect endothelium-dependent coronary relaxation because of endothelial dysfunction (5–7), and this dysfunction may contribute to the adverse cardiac effects of ischemia-reperfusion. Therefore, another possible mechanism of cardiac protection by urocortin during ischemia-reperfusion may be maintaining endothelial function (8) because it has been demonstrated for other substances such as adenosine (9), heparin (10), or calcitonin gene-related peptide (11).

Therefore, one aim of the present study was to analyze the effects of ischemia-reperfusion on the coronary vasodilation to urocortin, and the other objective was to study whether treatment with urocortin attenuates the adverse effects of ischemia-reperfusion on the endothelium-dependent coronary vasodilation.

The present work was performed in the heart from rats perfused according to the Langendorff procedure, which is an experimental model frequently used for the study of ischemia-reperfusion (12). Hearts were obtained from 30 male Sprague-Dawley rats (weight 300–350 g) after injection of pentobarbital sodium (40 mg/kg) and heparin (1000 IU). The use of animals in this study has been conducted in compliance with applicable laws and regulations as well as the principles expressed in the National Institutes of Health, USPHS, Guide for the Care and Use of Laboratory Animals. Use of animals has been approved by the institution's Animal Care and Use Committee.

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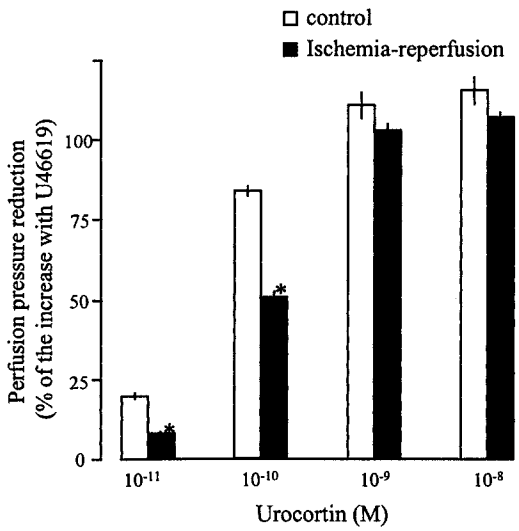

Figure 1. Relaxation to urocortin of the coronary vascular bed of rat-perfused hearts precontracted with U46619 (10^{-8} to 10^{-7} *M*) in control conditions and after ischemia-reperfusion. Data are means ± SEM. *, significant difference with control ($P < 0.01$).

After obtaining the hearts, the ascending aorta was cannulated, and retrograde perfusion of the heart with Krebs-Henseleit buffer (115 m*M* NaCl; 4.6 m*M* KCl; 1.2 m*M* KH_2PO_4; 1.2 m*M* $MgSO_4$; 2.5 m*M* $CaCl_2$; 25 m*M* $NaHCO_3$; 11 m*M* glucose) was initiated in a nonrecirculating Langendorff heart perfusion apparatus (12) at a constant flow of 11–15 ml/min to reach a basal perfusion pressure of 60–70 mm Hg. Perfusion coronary pressure and left intraventricular pressure (recorded with a latex balloon inflated to a diastolic pressure of 5–10 mm Hg) were measured with Statham transducers and recorded in a Grass model 7 polygraph.

After a 15-min equilibration period with perfusion at constant flow, 17 hearts were exposed to 15 mins of global zero-flow ischemia, followed by 15 mins of reperfusion at the same flow rate than before ischemia. Thirteen control hearts were perfused during the same total time (45 mins) at constant flow. In every heart after ischemia-reperfusion or control time-matched perfusion, the coronary vascular bed was precontracted by adding U46619 (10^{-8} to 10^{-7} *M*) to the perfusion buffer, and then the coronary relaxation to urocortin (10^{-11} to10^{-8} *M*) or acetylcholine (10^{-6} *M*) and sodium nitroprusside (10^{-6} *M*) was studied. These concentrations of acetylcholine and sodium nitroprusside were chosen because they produce submaximal relaxation, which is endothelium dependent and independent, respectively. For acetylcholine and sodium nitroprusside, the infusion was maintained 1 min and for urocortin, which produced a slower relaxation, 5 mins. Because the relaxation to urocortin did not recover after each dose, the next dose was injected cumulatively after relaxation reached a steady state.

The concentration-response curves to urocortin were performed in a group of hearts and the response to acetylcholine and sodium nitroprusside in a different group. Sodium nitroprusside was injected after acetylcholine, when the vascular bed had recovered its previous contractile tone. Because the flow was maintained constant, increases in perfusion pressure were considered as contraction and reductions in perfusion pressure as relaxation. The coronary relaxation is expressed as percentage of the contraction (active tone) induced with U46619 and calculated as means ± SEM. The coronary responses in the different experimental conditions were compared by analysis of variance (ANOVA) followed by Bonferroni test to analyze what comparisons were statistically significant.

After equilibration at a basal perfusion pressure of 60–70 mm Hg, addition of U46619 produced a further increase in the perfusion pressure (77 ± 5 mm Hg in control hearts and 70 ± 4 mm Hg in ischemic-reperfused hearts); this did not change in any experimental condition.

In the hearts precontracted with U46619, urocortin produced concentration-dependent coronary relaxation, which was lower ($P < 0.001$ by ANOVA) after ischemia-reperfusion ($n = 5$) than in control conditions ($n = 5$; Fig. 1).

A test dose of acetylcholine (10^{-6} *M*) also produced relaxation of the hearts precontracted with U46619. This relaxation to acetylcholine was lower in untreated ischemia-reperfused hearts ($P > 0.05$; $n = 6$), compared with that in control conditions ($n = 8$), whereas the relaxation to sodium nitroprusside (10^{-6} *M*) was not modified ($P > 0.05$; $n = 5$ in control and $n = 6$ after ischemia-reperfusion).

Six hearts subjected to ischemia-reperfusion were treated with a threshold concentration (10^{-11} *M*) of urocortin, which was present in the perfusion buffer 5 mins before ischemia and during the reperfusion period. In these hearts subjected to ischemia-reperfusion and treated with urocortin ($n = 6$), the relaxation to acetylcholine was higher than that in untreated, ischemia-reperfused hearts ($P < 0.01$; $n = 6$), and the relaxation to sodium nitroprusside was not changed ($P > 0.05$; $n = 6$ in each condition; Fig. 2).

These results indicated that ischemia-reperfusion impairs coronary endothelium-dependent relaxation because it reduced the relaxation to acetylcholine without modifying that to sodium nitroprusside. This agreed with results of other studies (see Ref. 13). However, the effects of ischemia-reperfusion in the relaxation to urocortin have not been previously described. Our results suggest that ischemia-reperfusion also impairs the coronary relaxation to urocortin, although the mechanism of this impairment is unclear. It also may be due to endothelial damage because the relaxation to urocortin in coronary arteries may be, in part, endothelium dependent (14). This impairment in the relaxation to urocortin may be due to a reduction in the production of vasodilator prostanoids and not to a reduction of nitric oxide production because the coronary relaxation to urocortin in the perfused rat heart may be mediated by vasodilator prostanoids but not by nitric oxide (15).

Although the ability of urocortin to increase coronary perfusion after ischemia-reperfusion may be impaired, this peptide may have beneficial effects on coronary perfusion by protecting the endothelial function. This suggestion is based

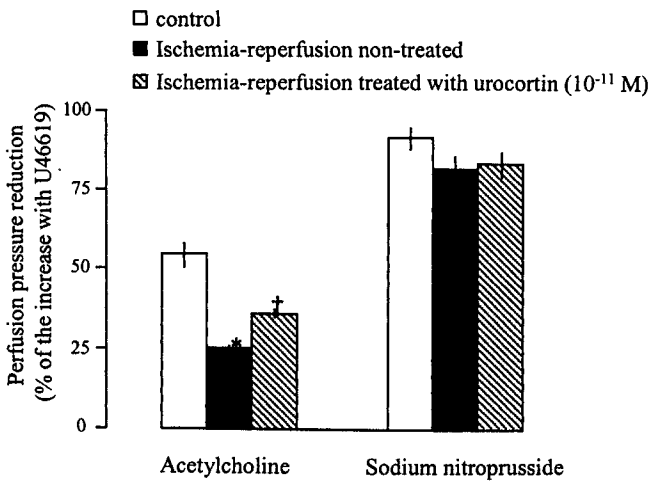

Figure 2. Relaxation to acetylcholine (10^{-6} *M*) and sodium nitroprusside (10^{-6} *M*) of the coronary vascular bed of rat-perfused hearts precontracted with U46619 (10^{-8} to 10^{-7} *M*) in control conditions and after ischemia-reperfusion nontreated or treated with a low concentration of urocortin (10^{-11} *M*). Data are means ± SEM. *, significant difference, compared with control ($P < 0.05$); †, significant difference, compared with ischemia-reperfusion nontreated ($P < 0.01$).

on the observation that urocortin improved the reduced relaxation to acetylcholine after ischemia-reperfusion, therefore partly reverting the impaired coronary vasodilation caused by this condition. It is remarkable that this protective effect of urocortin was observed by using low concentrations of this peptide, which may be close to its concentrations in human plasma (16). Moreover, because urocortin may be produced in cardiac tissue and this production may increase during ischemia-reperfusion (3), this peptide may reach locally higher concentrations than those in plasma and may act as an endogenous cardioprotective substance during coronary ischemic episodes. The present results suggest that urocortin may perform this function in part by protecting the coronary endothelium from ischemic damage, thus improving coronary vascular regulation and perfusion.

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