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Urotensin II and urotensin II-related peptide (URP) in cardiac ischemia-reperfusion injury

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ABSTRACT

Circulating urotensin II (UII) concentrations and the tissue expression of its cognate receptor (UT) are elevated in patients with cardiovascular disease (CVD). The functional significance of elevated plasma UII levels in CVD is unclear. Urotensin-related peptide (URP) is a paralog of UII in that it contains the six amino acid ring structures found in UII. Although both peptides are implicated as bioactive factors capable of modulating cardiovascular status, the role of both UII and URP in ischemic injury is unknown. Accordingly, we provide here the first report describing the direct cardiac effects of UII and URP in ischemia-reperfusion injury. Isolated perfused rat hearts were subjected to no-flow global ischemia for 45 min after 30 min preconditioning with either 1 nM rUII or 10 nM URP. Both rUII- and URP-induced significant vasodilation of coronary arteries before (both $P < 0.05$) and after ischemia (both $P < 0.05$). Rat UII alone lowered contractility prior to ischemia ($P = 0.053$). Specific assay of perfusate revealed rUII and URP both significantly inhibited reperfusion myocardial creatine kinase (CK) release ($P = 0.012$ and 0.036, respectively) and atrial natriuretic peptide (ANP) secretion ($P = 0.025$). Antagonism of the UT receptor with 1 μM palosuran caused a significant increase in perfusion pressure (PP) prior to and post-ischemia. Furthermore, palosuran significantly inhibited reductions in both PP and myocardial damage marker release induced by both rUII and URP. In conclusion, our data suggests rUII and URP reduce cardiac ischemia-reperfusion injury by increasing flow through the coronary circulation, reducing contractility and therefore myocardial energy demand, and inhibiting reperfusion myocardial damage. Thus, UII and URP present as novel peptides with potential cardioprotective actions.

1. Introduction

Urotensin II (UII) is a cardiovascular peptide with an array of biological effects that are species and tissue specific [12,30]. Thus, UII has been shown to constrict and dilate vascular tissue [3,23,27], increase and decrease blood pressure and contractility *in vivo* [1,18,40,42], stimulate vascular smooth muscle cell proliferation [39], and promote cardiomyocyte hypertrophy [38,44]. Blood levels of UII and the tissue expression of its cognate G protein-coupled receptor (UT) are both elevated in patients with congestive heart failure (CHF) and other cardiovascular diseases [13,21,25,32,33].

Recently a precursor cDNA peptide encoding the same conserved six amino acid rings as UII was isolated from rat, mouse and human tissues. This UII paralog has been named urotensin-related peptide (URP) and is a second endogenous

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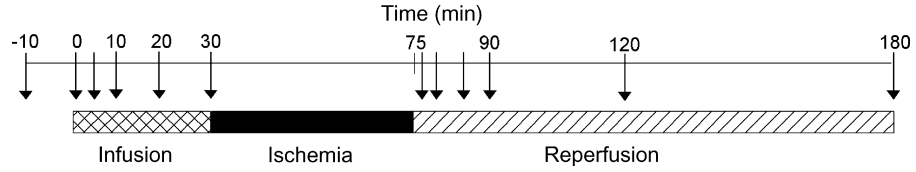

Fig. 1 – Experimental protocol of ischemia-reperfusion. Rat hearts underwent preconditioning infusion (30 min), followed by no-flow global ischemia (45 min), and a reperfusion period (105 min). Arrows indicate hemodynamic and hormonal sampling time points.

ligand for the UT receptor [8,35], with studies administering URP both *in vitro* and *in vivo* reporting biological actions similar to UII [8,35]. The distribution of prepro-URP in human and rat tissue is widespread, including brain, and the cardiovascular system [35]. However, its biological effects in these systems are unclear.

Despite a number of reports documenting cardiovascular actions of UII [1,11,31,42] its role in cardiac ischemia-reperfusion injury is unclear. Experimental animal models of heart failure suggest UII may have a pathological role, as antagonism of UT results in reduced mortality and improved recovery of heart function in rats [4,38]. In contrast, patients with significantly elevated plasma UII levels have improved outcomes post-myocardial infarction, suggesting UII may play a cardioprotective role [24,43]. Furthermore, any role for URP in cardiac ischemia-reperfusion injury is unreported. Accordingly, we provide here the first documentation of the species-specific actions of rUII and URP in cardiac ischemia-reperfusion injury and their comparative effects upon contractile and cardioendocrine function.

2. Materials and methods

2.1. Materials

All animals used in this study were male Sprague–Dawley rats (250–400 g) obtained from the Christchurch School of Medicine, Christchurch, New Zealand. Rats had free access to standard rat chow and water and were housed under controlled temperature (21 °C), humidity (~40%) and natural day length.

Rat urotensin II (rUII) and URP were obtained from Phoenix Pharmaceuticals (Belmont, USA). UII receptor antagonist palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt) was a generous gift from Dr. M. Iglarz, Actelion Pharmaceuticals Ltd. (Switzerland) [9]. All peptides and palosuran were diluted in distilled water, aliquoted and stored at −20 °C prior to use.

2.2. Langendorff isolated heart preparation

Isolated heart perfusion was carried out as previously described [29,30]. Briefly, rats were anesthetized with sodium-pentobarbitol (50 mg/kg, i.p.). The heart was exposed and cannulated above the aortic valve with oxygenated (95% O_2/5% CO_2) buffer comprising (mM): 123 NaCl, 22.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.1 $MgSO_4{\cdot}7H_2O$, 1.5 $CaCl_2{\cdot}H_2O$ and 11.0 glucose (final pH 7.40). Buffer was maintained at 37 °C. The heart was rapidly excised and attached to the Langendorff apparatus perfused retrograde at 12 ml/min. The left atrium was removed allowing an ethanol-filled balloon (attached to a pressure transducer) to be inserted into the left ventricle, enabling the measurement of left ventricular developed pressure (DP) and rate of change in pressure (±dp/dt). A side-arm cannula attached to a pressure transducer was inserted into the rubber aortic cannula measuring perfusion pressure (PP), a direct measure of coronary artery resistance. All data were recorded using a Powerlab Chart 5 System (ADInstruments). All hearts were paced at 330 bpm using a Digitimer DS2A-Mk. II stimulator placed on the right atrium. In all studies hearts were allowed to equilibrate for 30 min before any experimental procedure was started. All experiments in the current study were approved by the University of Otago Animal Ethics Committee.

2.3. Ischemia-reperfusion protocols

Hearts were allowed to stabilize before being infused with either 1 nM rUII, 10 nM URP, 1 μM palosuran or vehicle continuously for 30 min (0.5 ml/min). These doses were employed based on our previous work [30]. Infusion of the drug/vehicle was then stopped and hearts underwent no-flow global ischemia for 45 min with pacing halted. Hearts were then reperfused with pacing for 105 min following ischemia (Fig. 1).

2.4. Analysis of atrial natriuretic peptide (ANP) secretion and myocardial creatine kinase (CK) release

Perfusate samples were collected at specific time points after passing through the heart (Fig. 1). ANP release was measured by extracting perfusate samples using SepPak columns and measured by radioimmunoassay (RIA) as previously described [28]. Perfusate creatine kinase (CK) concentrations were determined on an Abbot Aeroset (Canterbury Health Labs, Christchurch, New Zealand).

2.5. Statistical analysis

All data are presented as mean + S.E.M. Analysis of changes in cardiac hormones and hemodynamics measured were performed on SPSS using a two-way ANOVA with repeated measures, with Bonferroni's multiple comparison test, post hoc. Cumulative data comparisons were made using a paired Student's *t*-test. In all statistical tests a value of $P < 0.05$ was considered significant.