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Urocortin does not reduce the renal injury and dysfunction caused by experimental ischaemia/reperfusion

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Abstract

Recent evidence indicates that activators of the serine/threonine kinase pathway protect against ischaemia/reperfusion. Here, we investigate the effects of renal ischaemia/reperfusion on the degree of renal dysfunction and injury with urocortin in rats. Rats treated with urocortin or its vehicle (saline) were subjected to bilateral renal artery occlusion (45 min) and reperfusion (6 h). At the end of experiments, the following indicators and markers of renal injury and dysfunction were measured: plasma urea, creatinine and aspartate aminotransferase, urine flow and creatinine clearance. Urocortin (1 or 15 μg/kg i.v.), administered 5 min prior to reperfusion, was not able to significantly reduce plasma urea, creatinine and aspartate aminotransferase indicating a non-protective effect on the renal dysfunction and reperfusion-injury caused by ischaemia/reperfusion. In addition, 15 μg/kg urocortin significantly depressed urine flow and creatinine clearance, which was associated with a significant depression in mean arterial pressure, indicating reduced renal perfusion. Thus, we propose that the pharmacological application of urocortin does not reduce the renal injury caused by bilateral renal ischaemia/reperfusion.

Keywords: Kidney; Reperfusion-injury; Renal dysfunction; Urocortin

1. Introduction

In hospitalized patients, ischemic acute tubular necrosis remains a significant medical problem and a major cause of acute renal failure, with mortality rates remaining unacceptably high and has not improved in more than 50 years (Kelly and Molitoris, 2000; Sheridan and Bonventre, 2001). Ischaemia of the kidney is one of the most common causes of acute renal failure, initiating a complex and interrelated sequence of events resulting in injury to and the eventual death of renal cells (Lameire and Vanholder, 2001; Lieberthal and Levine, 1996). This prognosis is complicated by the fact that reperfusion, although essential for the survival of ischaemic renal tissue, causes additional damage (reperfusion injury) (Weight et al., 1996). It is important to understand which pathways play critical roles in the determination of cell fate, since it is still relatively unclear which signalling pathways can be modulated due to cellular injury caused by conditions associated with ischaemia/reperfusion (Datta et al., 1999; Su and Karin, 1996).

One such pathway involves a serine/threonine kinase called Akt. Experiments involving multiple cell lines have shown that Akt is a critical mediator of survival signals that is able to protect cells from apoptosis (Brunet et al., 1999), via many different mechanisms. For instance, the pro-apoptotic protease caspase-9 has been shown to be directly inhibited by Akt (Kennedy et al., 1999). Akt is also able to activate nuclear factor-κB to facilitate in cell survival, since nuclear factor-κB regulates the expression of anti-apoptoic genes (Ozes et al., 1999). In addition, the phosphatidylinositol 3-kinase (PI3-kinase)–Akt pathway has been shown to act as a survival signal and plays a key role in the regulation of apoptotic change in mesangial cells principally via nuclear factor-κB (Shimamura et al., 2003). The phosphor-

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ylation of Akt via PI3-kinase also results in the attenuation of ischaemia/reperfusion injury of the liver (Muller et al., 2003) and brain (Noshita et al., 2001).

Urocortin is a 40-amino acid peptide, which is closely related to corticotrophin-releasing factor (Latchman, 2002). Cultured cardiac cells exposed to hypoxia/reoxygenation (Okosi et al., 1998), as well as ischaemia of the intact heart (Brar et al., 2000), have been shown to reduce cell death and infarct size, respectively when treated with urocortin. This protective effect of urocortin has been shown to be dependent on the activation of the p42/p44 MAP kinase pathway (Brar et al., 2000) and PI3-kinase–Akt pathway (Brar et al., 2002). Therefore, this study was designed to investigate the possible beneficial effects of urocortin on the renal dysfunction and injury caused by ischaemia/reperfusion of the rat kidney in vivo.

2. Materials and methods

2.1. Renal ischaemia/reperfusion

Studies were carried out using 35 male Wistar rats (Tuck, Rayleigh, Essex, U.K.) weighing 240–320 g. Rats were allowed access to food and water ad libitum and were cared for in accordance with *Home Office Guidance in the Operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty*'s *Stationery Office,London, U.K.* and the *Guiding Principles in the Care and Use of Animals* published by the American Physiological Society. All rats were anesthetized with sodium thiopentone (Intraval Sodium, 120 mg/kg i.p.; Merial Animal Health, Harlow, Essex, U.K.) and anaesthesia was maintained by supplementary injections (~ 10 mg/kg i.v.) of sodium thiopentone. Anaesthetised rats were placed onto a thermostatically controlled heating mat (Harvard Apparatus, Kent, U.K.) and body temperature was maintained at 38 ± 1 °C by means of a rectal probe attached to a homeothermic blanket. A tracheotomy was performed and a small section of polyethylene tubing was inserted into the airway (internal diameter 1.67 mm, Portex, Kent, U.K.) to maintain airway patency and facilitate spontaneous respiration. The right carotid artery was cannulated (internal diameter 0.58 mm, Portex) and connected to a pressure transducer (Capto SP 844 Physiological Pressure Transducer, AD Instruments, Hastings, U.K.) for the measurement of mean arterial blood pressure and derivation of the heart rate from the pulse waveform, which were displayed on a data acquisition system (PowerLab 8e, Chart v4.04, AD Instruments) installed on a IBM compatible personal computer, throughout the experiment. The jugular vein was cannulated (internal diameter 0.40 mm, Portex) for the administration of saline, vehicle or drug. A midline laparotomy was performed and the bladder was cannulated (internal diameter 0.58 mm, Portex) for the collection of urine. The kidneys were located inside the peritoneum and the renal pedicles, containing the renal artery, vein, and nerve supplying each kidney, were carefully isolated. Upon completion of surgical procedures, rats were divided into the following four groups:

(i) Isch/Rep group: control rats, which underwent renal ischaemia for 45 min followed by reperfusion for 6 h ($n = 10$);
(ii) Isch/Rep Urocortin (1 μg/kg) group: rats that were administered urocortin (1 μg/kg i.v. bolus) 5 min prior to reperfusion ($n = 10$);
(iii) Isch/Rep Urocortin (15 μg/kg) group: rats that were administered urocortin (15 μg/kg i.v. bolus) 5 min prior to reperfusion ($n = 5$);
(iv) Sham group: sham-operated rats, which were subjected to the surgical procedures described above, but were not subjected to renal I/R ($n = 10$).

Rats were allowed to stabilise for 15 min before being subjected to bilateral renal occlusion for 45 min using artery clamps (Dieffenbach Bulldog Clamps, Harvard Apparatus, Kent, U.K.) followed by reperfusion for 6 h. After the renal clamps were removed, the kidneys were observed for a further 5 min to ensure reflow after which 2 ml saline at 37 °C was injected into the abdomen to ensure gut motility. Sham-operated rats underwent identical surgical procedures to rats undergoing ischaemia/reperfusion except that artery clamps were not applied. This model of renal ischaemia/reperfusion has been used for a number of years, is well validated, and we have documented beneficial effects of a number of different interventions in the past 5 years. These interventions include reactive oxygen species—scavengers (Chatterjee et al., 2000a), poly(ADP-ribose) synthetase-inhibitors (Chatterjee et al., 2000b), inducible nitric oxide synthase-inhibitors (Chatterjee et al., 2003) and glitazones (Sivarajah et al., 2003).

In the above experiments, the route of administration and dose of recombinant rat urocortin were based on that of a previously reported experiment in the rat (Schulman et al., 2002). Rats, which did not receive urocortin, were administered 2 ml/kg saline (vehicle for urocortin) at the equivalent time point (5 min prior to reperfusion).

2.2. Measurement of biochemical parameters

At the end of the reperfusion period, 1-ml blood samples were collected via the carotid artery into S/1.3 tubes containing serum gel (Sarstedt, Germany), after which the heart was removed to terminate the experiment. The samples were centrifuged (6000 rpm for 3 min) to separate serum from which biochemical parameters were measured within 24 h (Vetlab Services, Sussex, U.K.). Plasma urea and creatinine concentrations were used as indicators of impaired renal (glomerular) function (Chatterjee et al., 2003). The rise in the plasma levels of

aspartate aminotransferase, an enzyme located in the proximal tubule, was used as an indicator of reperfusion-injury (Thiemermann et al., 2003).

Urine samples were collected during the reperfusion period and the volume of urine produced recorded. Urine concentration of creatinine was measured (Vetlab Services) and was used in conjunction with serum creatinine concentrations and urine flow to calculate creatinine clearance using standard formulae; the latter was used as an indicator of glomerular function (Thiemermann et al., 2003).

2.3. Materials

Unless otherwise stated, all compounds used in this study were purchased from Sigma-Aldrich (Poole, Dorset, U.K.). All solutions used in vivo were prepared using non-pyrogenic saline (0.9% [wt/vol] NaCl; Baxter Healthcare, Thetford, Norfolk, U.K.).

2.4. Statistical analysis

All values described in the text and figures are expressed as mean ± standard error of the mean (S.E.M.) for *n* observations. Each data point represents biochemical measurements obtained from five to 10 separate animals. For histological scoring, each data point represents analysis of kidneys taken from 5 to 10 individual animals. One-way analysis of variance with Dunnett's post-test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA www.graphpad.com) and a *P*-value of less than 0.05 was considered to be significant.

3. Results

3.1. Effect of urocortin on renal dysfunction caused by ischaemia/reperfusion

When compared to sham-operated rats, ischaemia/reperfusion caused a significant increase in the plasma levels of urea and creatinine in control rats (Fig. 1A and B), suggesting a significant degree of renal dysfunction. Administration of urocortin (1 or 15 μg/kg 5 min prior to reperfusion, Fig. 1A and B) did not attenuate the renal dysfunction caused by ischaemia/reperfusion in control rats. In another set of experiments, urocortin (3 μg/kg) was administered 15 min prior to ischaemia and did not attenuate the renal dysfunction caused by ischaemia/reperfusion in control rats (data not shown).

When compared to sham-operated rats, renal ischaemia/reperfusion caused a reduction in urine flow (Fig. 1C). The administration of urocortin to rats subjected to renal ischaemia/reperfusion caused a dose-dependent reduction in the production of urine (Fig. 1C), which was significant at the higher dose of urocortin (15 μg/kg).

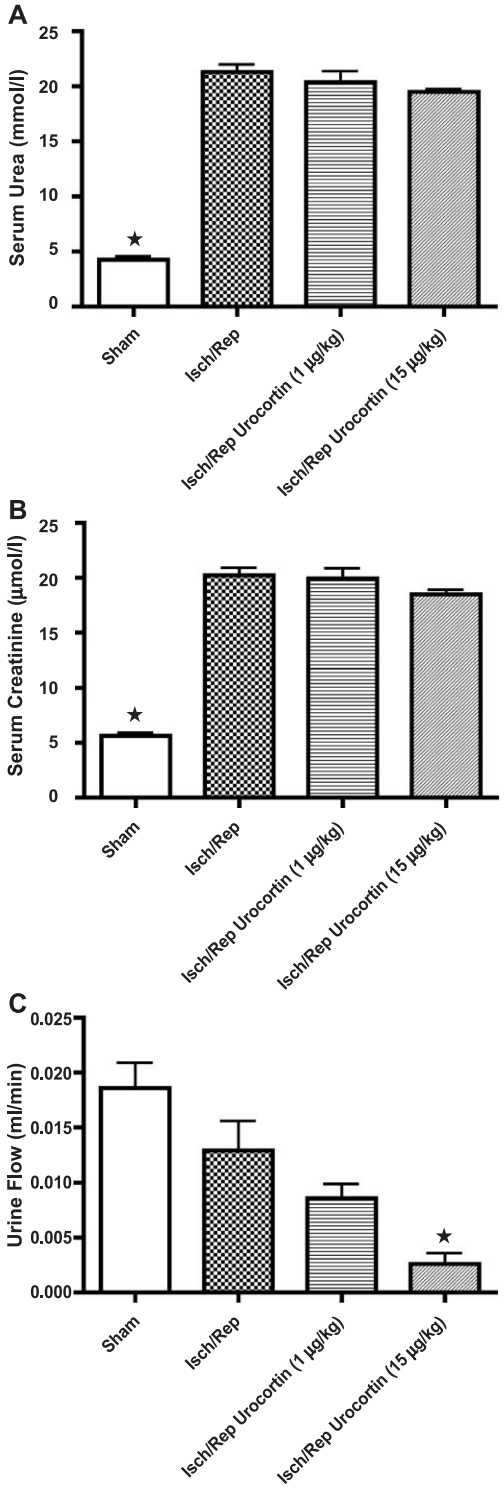

Fig. 1. Effect of urocortin on renal function; serum urea (A) and creatinine (B) levels, and urine flow (C) were subsequent to sham-operation (sham, $n = 10$) or renal ischaemia/reperfusion (Isch/Rep, $n = 10$; Isch/Rep Urocortin [1 μg/kg 5 min prior to reperfusion], $n = 10$; Isch/Rep Urocortin [15 μg/kg 5 min prior to reperfusion], $n = 5$). Data represent mean ± S.E.M. for *n* observations, ★$P < 0.05$ vs. Isch/Rep group.

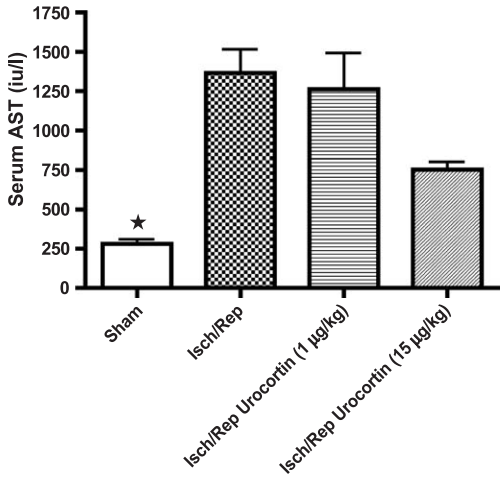

Fig. 2. Effect of urocortin on renal reperfusion-injury; serum aspartate aminotransferase levels were measured as a biochemical marker of reperfusion-injury subsequent to sham-operation (sham, $n=10$) or renal ischaemia/reperfusion (Isch/Rep, $n=10$; Isch/Rep Urocortin [1 μg/kg 5 min prior to reperfusion], $n=10$; Isch/Rep Urocortin [15 μg/kg 5 min prior to reperfusion], $n=5$). Data represent mean ± S.E.M. for n observations, ★$P<0.05$ vs. Isch/Rep group.

3.2. Effect of urocortin on reperfusion-injury caused by ischaemia/reperfusion

When compared to sham-operated rats, ischaemia/reperfusion caused a significant increase in the plasma levels of aspartate aminotransferase (from 280 ± 29.6 to 1365 ± 151.0) in control rats, suggesting significant reperfusion-injury (Fig. 2). Administration of urocortin (1 or 15 μg/kg 5 min prior to reperfusion, Fig. 2) did not attenuate the renal injury (1262 ± 230.1 and 752.8 ± 50.2, respectively) caused by ischaemia/reperfusion in control rats. It should be noted that the administration of urocortin at 15 μg/kg was able to cause a non-significant attenuation in the level of serum aspartate aminotransferase.

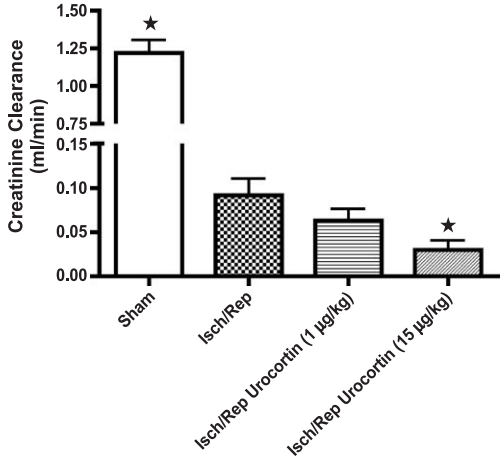

Fig. 3. Effect of urocortin on glomerular function; creatinine clearance was calculated as a measure of glomerular function subsequent to sham-operation (sham, $n=10$) or renal ischaemia/reperfusion (Isch/Rep, $n=10$; Isch/Rep Urocortin [1 μg/kg 5 min prior to reperfusion], $n=10$; Isch/Rep Urocortin [15 μg/kg 5 min prior to reperfusion], $n=5$). Data represent mean ± S.E.M. for n observations, ★$P<0.05$ vs. Isch/Rep group.

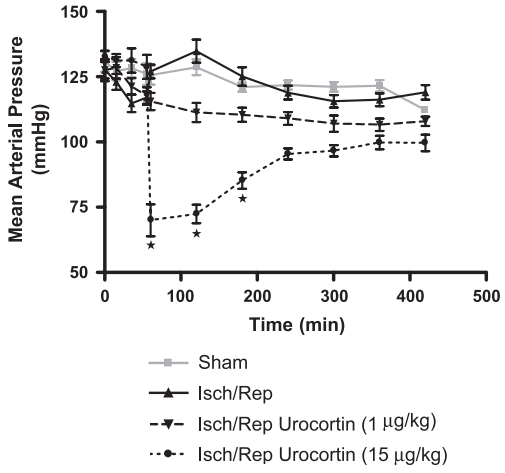

Fig. 4. Effect of urocortin on mean arterial pressure; mean arterial pressure was monitored throughout renal ischemia/reperfusion in rats subjected to sham-operation (sham, $n=10$) or renal ischaemia/reperfusion (Isch/Rep, $n=10$; Isch/Rep Urocortin [1 μg/kg 5 min prior to reperfusion], $n=10$; Isch/Rep Urocortin [15 μg/kg 5 min prior to reperfusion], $n=5$). Data represent mean ± S.E.M. for n observations, ★$P<0.05$ vs. Isch/Rep group.

3.3. Effect of urocortin on glomerular dysfunction caused by ischaemia/reperfusion

When compared to sham-operated rats, renal ischaemia/reperfusion caused a significant decrease in creatinine clearance in control rats, suggesting reduced glomerular function (Fig. 3). Administration of urocortin caused a further decrease in creatinine clearance, which was dose-dependent (Fig. 3); this decrease was not significant (Fig. 3).

3.4. Effect of urocortin on mean arterial pressure caused by ischaemia/reperfusion

When compared to sham-operated rats, renal ischaemia/reperfusion caused no significant alterations in mean arterial pressure (Fig. 4). However, rats subjected to renal ischaemia/reperfusion that were administered the higher dose of urocortin (15 μg/kg) produced a significant fall in mean arterial pressure compared to rats subjected to renal ischaemia/reperfusion alone, which lasted for 3 h into the reperfusion period (Fig. 4). Mean arterial pressure did recover in the final hours of reperfusion; however, they did not rise above 100 mm Hg (Fig. 4). A small decrease in mean arterial pressure was observed with the lower dose of urocortin (1 μg/kg) upon administration (Fig. 4), which was not significant.

4. Discussion

Urocortin is a central mediator of the hypothalamic–pituitary–adrenal axis and stress response in animals, af-

fecting appetite, anxiety and activity (Latchman, 2002). Here, we demonstrate that urocortin is unable to reduce the renal dysfunction and injury caused by bilateral occlusion (45 min) and reperfusion (6 h) in anesthetized rats. Specifically, in rats subjected to renal ischaemia/reperfusion, urocortin (1 or 15 μg/kg given 5 min prior to reperfusion) did not attenuate the (i) renal dysfunction (increases in plasma creatinine and urea) and (ii) reperfusion-injury (increase in plasma aspartate aminotransferase). Additionally, urocortin was unable to restore urine flow and creatinine clearance, which were made significantly worse following the administration of high dose urocortin to rats subjected to renal ischaemia/reperfusion. Moreover, our findings are in agreement with the notion that renal ischaemia/reperfusion causes both renal and glomerular dysfunction (Paller, 1994).

There is good evidence that urocortin reduces the tissue injury (infarct size) caused by ischaemia/reperfusion of the rat heart ex vivo (Brar et al., 2000). In this study, urocortin (10^{-8} M) was added to the perfusate of the isolated perfused rat heart during reperfusion for 30 min after a 35-min ischaemic period to attenuate infarct size. Interestingly, Schulman and colleagues report that only higher doses of urocortin (i.e. 15 μg/kg) reduce myocardial infarct size in vivo. We demonstrate here that this relatively high dose of urocortin causes a significant and substantial fall in mean arterial pressure, and hence renal perfusion pressure. Urocortin has well-documented haemodynamic effects, secondary to vasodilatation, which results in an increase in regional blood flow in the heart, liver, stomach, colon and skin, as well as an overall fall in mean arterial pressure and in total peripheral resistance (Abdelrahman and Pang, 2003). In the kidney, however, this fall in perfusion pressure results in a significant fall in renal blood flow (Abdelrahman and Pang, 2003), which may well result in a fall in urine flow. Indeed, in our study, we document that urocortin causes a significant fall in urine flow and a decline in creatinine clearance. Thus, we propose that any beneficial effect of urocortin in the kidney (i.e. secondary to the activation of certain salvage pathways; Akt pathway) will be abolished by a significant fall in renal perfusion, which would exacerbate renal ischaemia and reduce recovery of renal function after an ischaemic insult. If this conclusion is correct, other corticotrophin-releasing factor receptor agonists (receptor 2 type rather than receptor 1 type), which do not cause a significant fall in blood pressure, may well protect the kidney against ischaemia/reperfusion injury. Two such peptides, closely related to urocortin, have recently been isolated from rodents and humans and named stresscopin and stresscopin-related peptide (Dautzenberg and Hauger, 2002). Both stresscopin and stresscopin-related peptide have been shown to bind exclusively to corticotrophin-releasing factor receptor 2 and not to corticotrophin-releasing factor receptor 1. Thus, these related compounds may therefore have beneficial effects in conditions associated with ischaemia/reperfusion of the kidney.

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