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Sex differences in ischemia/reperfusion-induced acute kidney injury are dependent on the renal sympathetic nervous system

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ABSTRACT

Resistance to ischemic acute kidney injury has been shown to be higher in female rats than in male rats. We found that renal venous norepinephrine overflow after reperfusion played important roles in the development of ischemic acute kidney injury. In the present study, we investigated whether sex differences in the pathogenesis of ischemic acute kidney injury were derived from the renal sympathetic nervous system using male and female Sprague-Dawley rats. Ischemia/reperfusion-induced acute kidney injury was achieved by clamping the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal function was impaired after reperfusion in both male and female rats; however, renal dysfunction and histological damage were more severe in male rats than in female rats. Renal venous plasma norepinephrine levels after reperfusion were markedly elevated in male rats, but were not in female rats. These sex differences were eliminated by ovariectomy or treatment with tamoxifen, an estrogen receptor antagonist, in female rats. Furthermore, an intravenous injection of hexamethonium (25 mg/kg), a ganglionic blocker, 5 min before ischemia suppressed the elevation in renal venous plasma norepinephrine levels after reperfusion, and attenuated renal dysfunction and histological damage in male rats, and ovariectomized and tamoxifen-treated female rats, but not in intact females. Thus, the present findings confirmed sex differences in the pathogenesis of ischemic acute kidney injury, and showed that the attenuation of ischemia/reperfusion-induced acute kidney injury observed in intact female rats may be dependent on depressing the renal sympathetic nervous system with endogenous estrogen.

1. Introduction

Ischemia/reperfusion injury is a leading cause of acute kidney injury, which is a frequent clinical syndrome with high morbidity and mortality (Ympa et al., 2005). Recent epidemiologic findings have suggested that the incidence of acute kidney injury is increasing, affecting as many as 5% of hospitalized patients (Lakhal et al., 2011; Waikar et al., 2006; Xue et al., 2006). A previous study also suggested that clinical acute kidney injury may initiate the onset of progressive renal disease (Venkatachalam et al., 2010). If both of these assumptions are correct, then acute kidney injury could, paradoxically, be a leading cause of chronic kidney failure (Ishani et al., 2009; Lo et al., 2009). Structural changes in post-ischemic kidneys are characterized by vasoconstriction or necrosis with desquamation of the tubular epithelial cells into the tubular lumen. The molecular mechanisms underlying this renal injury are not fully understood, although several causal factors, such as reactive oxygen species, neutrophil infiltration, vasoactive peptides, and ATP depletion, have been shown to contribute to the pathogenesis of ischemia/reperfusion-induced acute kidney injury (Edelstein et al., 1997).

Although ischemic acute kidney injury is a leading killer of both men and women, sex differences in renal ischemia/reperfusion injury have been well established in humans and experimental animals (Hutchens et al., 2008). Males are more susceptible to ischemia/reperfusion-induced kidney injury with worsened renal function than females. A few factors were found to be associated with sex differences in ischemia/reperfusion-induced acute kidney injury in rodent models: mRNA expression of endothelin-1, a causal factor for ischemic acute kidney injury, was shown to be increased in the early phase following ischemia/reperfusion in male rats, but was not in female rats (Takaoka and Matsumura., 2003; Müller et al., 2002); the enzyme activity of Na^+, K^+-ATPase after ischemia/reperfusion treatment was higher in female rats

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than in male rats (Fekete et al., 2004); heat shock protein 72 exhibited higher basal and postischemic levels in the kidneys of female rats (Fekete et al., 2006). Thus, an investigation into the mechanisms underlying sex differences may provide a new approach to therapy for ischemic acute kidney injury.

We found that renal sympathetic nerve activity and norepinephrine concentrations in renal venous plasma were markedly elevated with ischemia/reperfusion-induced acute kidney injury in rats (Fujii et al., 2003; Kurata et al., 2006). These results indicated that an increase in norepinephrine overflow from the nerve endings induced by overactivation of the renal sympathetic nerve has been considered as a possible causal factor of ischemic acute kidney injury. In addition, we noted that renal ischemia/reperfusion injury was ameliorated by renal denervation, ganglionic blockade, or inhibitory neurotransmitters, and that this amelioration was accompanied by the suppression of elevated renal venous norepinephrine levels after reperfusion (Fujii et al., 2003; Kobuchi et al., 2011). In the present study, we investigated whether the renal sympathetic nervous system was involved in sex differences in the pathogenesis of ischemic acute kidney injury.

2. Materials and methods

2.1. Animals and experimental design

Male and female Sprague-Dawley rats (10 weeks of age; Japan SLC, Shizuoka, Japan) weighing 280–320 g were used. Animals were housed in a light-controlled room with a 12-hr light/dark cycle and were allowed ad libitum access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences (Osaka, Japan). Two weeks before the study (at 8 weeks of age), the right kidney was removed through a small flank incision under pentobarbital anesthesia (50 mg/kg, i.p.). Some female rats were ovariectomized one week before the study, others were treated with tamoxifen (10 mg/kg/day, i.p.), an estrogen receptor antagonist, for 3 days before ischemia. To perform gonadectomy, female rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and abdomen incisions were made in both sides of the skin and underlying muscles. The ovaries were isolated, tied off with sterile suture, and removed, and the incisions were then closed. Tamoxifen or vehicle (a mixture of 30% ethanol and 70% polyethylene glycol 400) was administered intraperitoneally (2 ml/kg) with a 26-gauge needle. Male, female, ovariectomized, and tamoxifen-treated female rats were divided into three groups: (1) sham-operated control, (2) vehicle-treated ischemic acute kidney injury, (3) ischemic acute kidney injury treated with hexamethonium bromide (25 mg/kg, i.v.), a ganglionic blocker. To induce ischemic acute kidney injury, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a nontraumatic clamp for 45 min. At the end of the ischemic period, the clamp was released to allow reperfusion. Hexamethonium bromide or vehicle (0.9% saline) was injected into the left external jugular vein (1 ml/kg) with a 26-gauge needle 5 min before ischemia. In sham-operated control rats, the left kidney was treated identically, with the exception of clamping. Animals exposed to 45-min ischemia were housed in metabolic cages 24 h after reperfusion and 5-hr urine samples were collected. At the end of urine collection, blood samples were drawn from the thoracic aorta, and the left kidneys were then excised under pentobarbital anesthesia (50 mg/kg, i.p.). Plasma was separated by centrifugation and was used to measure renal function parameters. The kidneys were used for light microscopic observations.

In separate experiments, we examined changes in norepinephrine levels in renal venous plasma after reperfusion. Under pentobarbital (50 mg/kg, i.p.) anesthesia, an abdominal midline incision was made in uninephrectomized rats and the left kidney was exposed. A 26-gauge needle was inserted into the left renal vein for venous blood sampling. Each blood sample was taken at baseline and immediately after reperfusion following 45-min ischemia. The sampling period (only one sample from each animal) was 2 min in duration. Plasma was immediately separated by centrifugation. These samples were stored at −80 °C until the assay for norepinephrine concentrations.

2.2. Analytical procedures

Blood urea nitrogen and creatinine levels in plasma were determined using the commercial assay kits, the BUN-test-Wako

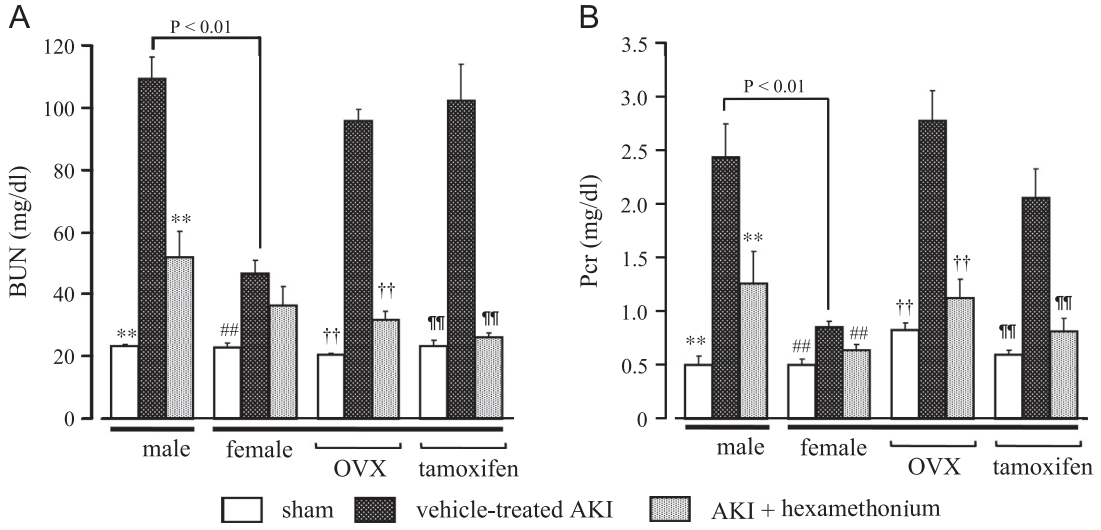

Fig. 1. Blood urea nitrogen (BUN, A) and plasma creatinine (Pcr, B) concentrations 1 day after reperfusion in male, female, ovariectomized or tamoxifen-treated female rats. Hexamethonium bromide (25 mg/kg, i.v.) was given 5 min before ischemia. Each column and bar represent the mean ± S.E.M. ($n=6$). $^{*}P<0.05$, $^{**}P<0.01$, significantly different from AKI male rats. $^{\#\#}P<0.01$, significantly different from AKI female rats. $^{\dagger\dagger}P<0.01$, significantly different from OVX+AKI female rats. $^{\P\P}P<0.01$, significantly different from tamoxifen-treated AKI female rats. OVX, ovariectomy. AKI, acute kidney injury.