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Preventive mechanisms of agmatine against ischemic acute kidney injury in rats

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ABSTRACT

The excitation of renal sympathetic nervous system plays an important role in the development of ischemic acute kidney injury in rats. Recently, we found that agmatine, an adrenaline α_2/imidazoline I_1-receptor agonist, has preventive effects on ischemic acute kidney injury by suppressing the enhanced renal sympathetic nerve activity during renal ischemia and by decreasing the renal venous norepinephrine overflow after reperfusion. In the present study, we investigated preventive mechanisms of agmatine against ischemic acute kidney injury in rats. Ischemic acute kidney injury was induced by clamping the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after the contralateral nephrectomy. Pretreatment with efaroxan (30 μmol/kg, i.v.), an α_2/I_1-receptor antagonist, abolished the suppressive effects of agmatine on the enhanced renal sympathetic nerve activity during renal ischemia and on the elevated norepinephrine overflow after reperfusion, and eliminated the preventing effects of agmatine on the ischemia/reperfusion-induced renal dysfunction and histological damage. On the other hand, pretreatment with yohimbine (6 μmol/kg, i.v.), an α_2-receptor antagonist, eliminated the preventing effects of agmatine on the ischemia/reperfusion-induced renal injury and norepinephrine overflow, without affecting the lowering effect of agmatine on renal sympathetic nerve activity. These results indicate that agmatine prevents the ischemic renal injury by sympathoinhibitory effect probably via I_1 receptors in central nervous system and by suppressing the norepinephrine overflow through α_2 or I_1 receptors on sympathetic nerve endings.

1. Introduction

Agmatine is a polycationic amine synthesized from L-arginine, by the enzyme arginine decarboxylase, and has been identified as an endogenous clonidine-displacing substance in the mammalian brain (Li et al., 1994). Moreover, agmatine may serve as a neurotransmitter in the central nervous system (Reis and Regunathan, 2000). Agmatine has several biologic effects including neuroprotective (Kim et al., 2004, 2006), antidepressant and anxiolytic effects (Zomkowski et al., 2002; Aricioglu and Altunbas, 2003). Most recently, we noted that agmatine overcame ischemia/reperfusion-induced acute kidney injury in rats (Sugiura et al., 2008).

Acute kidney injury is a frequent clinical syndrome with high morbidity and mortality (Ympa et al., 2005). The precise mechanisms underlying the ischemia/reperfusion-induced acute kidney injury are not fully understood, but it has been reported that several causal factors (e.g., ATP depletion, reactive oxygen species, phospholipase activation, neutrophil infiltration, and vasoactive peptides) are contributive to the pathogenesis of this renal damage (Edelstein et al., 1997). In addition, enhancement of renal sympathetic nerve activity and its consequent effect on norepinephrine overflow from nerve endings are considered to be involved in the development of the ischemia/reperfusion-induced acute kidney injury (Ogawa et al., 2002; Kurata et al., 2006). We have found that renal sympathetic nerve activity is significantly augmented during renal ischemia. In addition, we noted that ischemic acute kidney injury is ameliorated by renal denervation or ganglionic blockade and that the effect is accompanied by suppression of elevated renal venous norepinephrine levels after reperfusion (Fujii et al., 2003). In our recent study, the preischemic treatment with agmatine exerted the suppressive effect on the enhancement of renal sympathetic nerve activity and consequent elevation of renal venous norepinephrine levels observed in ischemic acute kidney injury rats (Sugiura et al., 2008).

Agmatine was firstly isolated as an endogenous agonist for imidazoline I receptors (Li et al., 1994). There is accumulating evidence that agmatine affects imidazoline I and adrenaline α_2 receptors (Piletz et al., 1995; Pinthong et al., 1995; González et al., 1996; Li et al., 2001; Li and He, 2001). Imidazoline I receptors have been subclassified into 3 major groups, based largely upon ligand selectivities and subcellular distribution (Eglen et al., 1998), and I_1 subtype is known to exist principally and to play a role in controlling sympathetic nerve activity in the rostral ventrolateral medulla (Ernsberger et al., 1990; Mayorov et al., 2001). Agmatine administered intravenously was reported to

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suppress the sympathetic nerve activity of anesthetized rats (Sun et al., 1995) and to reduce norepinephrine release provoked by preganglionic electrical stimulation in pithed spontaneously hypertensive rats (Raasch et al., 2003). In the current study, in order to investigate the receptor types and related mechanisms involved in the protective effect of agmatine against ischemic acute kidney injury, we evaluated effects of efaroxan, an α_2/I_1-receptor antagonist, and yohimbine, an α_2-receptor antagonist, on the renoprotective effect of agmatine.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague-Dawley rats (10 weeks of age; Japan SLC, Shizuoka, Japan) were used. The animals were housed in a light-controlled room with a 12-h 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 (40 mg/kg, i.p.). After a 2-week recovery period, uninephrectomized rats were divided into sham-operated control, vehicle-treated ischemic acute kidney injury, and drug-treated ischemic acute kidney injury groups. To induce ischemic acute kidney injury, the rats were anesthetized with 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. Each drug used in this study or vehicle (0.9% saline) was administered into the left external jugular vein (1 ml/kg). Agmatine or vehicle was injected 5 min before the start of ischemia, and efaroxan or yohimbine was given 10 min before the ischemia. In sham-operated control rats, the left kidney was treated identically, with the exception of the clamping. The animals exposed to 45-min ischemia were housed in metabolic cages at 24 h after reperfusion and 5-h urine samples were collected. At the end of urine collection, blood samples were drawn from the thoracic aorta, and then the left kidneys were excised under pentobarbital anesthesia (50 mg/kg, i.p.). The plasma was separated by centrifugation and used for measurement of renal function parameters. The kidneys were used for light microscopic observation.

In separate experiments, we examined the effect of efaroxan or yohimbine on the suppression of norepinephrine overflow by agmatine. Under pentobarbital (50 mg/kg, i.p.) anesthesia, an abdominal midline incision of uninephrectomized rats was made 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 baseline and 24 h 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 concentration.

As described below, in another set of experiments, electrical signals of renal neural activity were directly recorded for evaluation of changes in renal sympathetic nerve activity during 45-min ischemic period.

2.2. Renal nerve recording

For the measurement of renal sympathetic nerve activity, uninephrectomized rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and given additional doses as required. Depth of anesthesia was assessed as stability of heart rate and blood pressure, which were continuously monitored with a pressure transducer connected to a polygraph system (RM6000G, Nihon Kohden, Osaka, Japan). Data collection was done when the hemodynamic parameters maintained stable conditions. Surgical preparation of the animals and basic experimental techniques were identical to those described previously (Shokoji et al., 2003). Renal sympathetic nerve activity was recorded from the left renal nerve branch before and during ischemia. The nerve was isolated near the aortic-renal arterial junction through a left flank incision and placed on a Teflon-coated stainless steel bipolar electrode. The renal nerve and electrode were covered with silicone rubber. The renal nerve discharge was amplified using a differential amplifier (AVB-11A; Nihon Kohden) with a band-pass filter (low frequency, 50 Hz; high frequency, 1 kHz). The amplified and filtered signal was visualized on a dual-beam oscilloscope (VC-11; Nihon Kohden) and monitored by an audio speaker. The output from the amplifier was integrated by an integrator (EI601G; Nihon Kohden) with 1-s resetting. The output from the integrator was recorded and analyzed with PowerLab (ML750; ADInstruments Pty Ltd., Castle Hill, Australia). For the quantification of renal sympathetic nerve activity, the height of integrated nerve discharge was measured for 20 s in each experiment. Changes in nerve activity were expressed as percentages of control resting spontaneous nerve activity.

2.3. Analytical procedures

Blood urea nitrogen and creatinine levels in plasma or urine were determined using a commercial assay kit, the BUN-test-Wako, and Creatinine-test-Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively. Creatinine clearance (Ccr, ml/min/kg) was calculated from the formula: Ccr = Ucr × UF/Pcr, where Ucr and Pcr are creatinine concentration in urine and plasma, respectively, and UF is urine flow.

Norepinephrine concentration in renal venous plasma was measured by high-performance liquid chromatography with an amperometric detector (HTEC-500; Eicom, Kyoto, Japan), as previously reported (Hayashi et al., 1991).

2.4. Histological studies

Excised left kidneys were processed for light microscopic observation, according to standard procedures. The kidneys were then preserved in phosphate-buffer 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, cut at 4 μm, and stained with hematoxylin and eosin. Histopathological changes were analyzed for tubular necrosis, proteinaceous casts, and medullary congestion, as described by Caramelo et al. (1996). Tubular necrosis and proteinaceous casts were graded as follows: no damage (0), mild (1; unicellular, patchy isolated damage), moderate (2; damage less than 25%), severe (3; damage between 25 and 50%), and very severe (4; more than 50% damage). The degree of medullary congestion was defined as no congestion (0), mild (1; vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2; vascular congestion with identification of erythrocytes by ×200 magnification), severe (3; vascular congestion with identification of erythrocytes by ×100 magnification), and very severe (4; vascular congestion with identification of erythrocytes by ×40 magnification). The scoring of the histological data was performed by independent observers in a double blind manner.

2.5. Drugs

Agmatine, efaroxan and yohimbine were purchased from Sigma Chemical (St. Louis, MO, USA). These drugs were dissolved in saline (0.9%). Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan).