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Moxonidine prevents ischemia/reperfusion-induced renal injury in rats

Hidenobu Tsutsui [a,b], Takahiro Sugiura [a], Kentaro Hayashi [a], Mamoru Ohkita [a], Masanori Takaoka [a], Tokihito Yukimura [b], Yasuo Matsumura [a,*]

[a] Laboratory of Pathological and Molecular Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[b] Laboratory of Clinical Pharmacology, Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-kita, Tondabayashi, Osaka, 584-8540, Japan

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ABSTRACT

Enhancement of renal sympathetic nerve activity during renal ischemia and its consequent effect on norepinephrine overflow from nerve endings after reperfusion play important roles in the development of ischemic acute kidney injury. In the present study, we evaluated whether moxonidine, an α_2-adrenaline/I_1-imidazoline receptor agonist which is known to elicit sympathoinhibitory action, would prevent the post-ischemic renal injury. Ischemic acute kidney injury was induced by clamping the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Intravenous (i.v.) injection of moxonidine at a dose of 360 nmol/kg to ischemic acute kidney injury rats suppressed the enhanced renal sympathetic nerve activity during the ischemic period, to a degree similar to findings with intracerebroventricular (i.c.v.) injection of moxonidine at a dose of 36 nmol/kg. On the other hand, suppressive effects of the i.v. treatment on renal venous norepinephrine overflow, renal dysfunction and tissue injury in the post-ischemic kidney were significantly greater than those elicited by the i.c.v. treatment. These results suggest that renoprotective effects of moxonidine on ischemic acute kidney injury probably result from its suppressive action on the ischemia-enhanced renal sympathetic nerve activity followed by norepinephrine spillover from the nerve endings of the post-ischemic kidney.

1. Introduction

Ischemic acute kidney injury is a frequent clinical syndrome with high morbidity and mortality (Thadani et al., 1996). Reperfusion of previously ischemic renal tissue initiates complex cellular events that result in injury and the eventual death of renal cells due to a combination of apoptosis and necrosis (Lieberthal and Levine, 1996). The molecular mechanisms underlying the ischemia/reperfusion-induced renal injury are not fully understood, although it has been reported that several causal factors, such as ATP depletion, reactive oxygen species, phospholipase activation, neutrophil infiltration, vasoactive peptides, are contributive to the pathogenesis of this renal damage (Edelstein et al., 1997). In addition to these causal factors, the renal sympathetic nervous system and circulating catecholamines are considered to be involved in the development of ischemic acute kidney injury (Baines, 1983; Iaina and Eliahou, 1983).

β-Adrenoceptor antagonist propranolol (Solez et al., 1977a,b; Chevalier and Finn, 1980) and α2-adrenoceptor agonist clonidine (Solez et al., 1980) are known to lessen post-ischemic acute kidney injury. Renal denervation before the ischemia is also reported to attenuate the decreased responses of glomerular filtration rate after the ischemia/reperfusion (Ogawa et al., 2002). Moreover, we have revealed that renal sympathetic nerve activity is significantly augmented during renal ischemia, and renal venous norepinephrine levels are markedly increased immediately after reperfusion following 45-min ischemia (Fujii et al., 2003). Most recently, we found that enhanced renal sympathetic nerve activity during ischemia, norepinephrine overflow into the renal vein and renal injury after reperfusion were suppressed by central and peripheral administration of agmatine (Sugiura et al., 2008), which is reported to suppress sympathetic nervous system (Sun et al., 1995; Raasch et al., 2003). It is therefore reasonable to consider that ischemia-induced enhancement of renal sympathetic nerve activity and its consequent effect on norepinephrine overflow from nerve endings have crucial roles in the development of ischemic acute kidney injury.

The purpose of the present study is to further evaluate whether an agent having sympathoinhibitory actions would prevent the post-ischemic acute renal injury. To attain this, we utilized a clonidine-like α2-adrenoceptor agonist moxonidine having an imidazoline structure. This compound is known to bind to I1-imidazoline receptors with a higher affinity than α2-adrenoceptors in the ventrolateral medulla (Ernsberger et al., 1993), in which activation of I1-imidazoline receptors with moxonidine reduces arterial pressure by decreasing sympathetic nerve activity (Morris and Reid, 1997; Van Zwieten, 1997). Thus, we compared the effects of intravenous (i.v.) injection of

* Corresponding author. Tel./fax: +81 72 690 1051.
E-mail address: matumrh@gly.oups.ac.jp (Y. Matsumura).

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moxonidine with those of its intracerebroventricular (i.c.v.) injection on the post-ischemic acute renal injury. Furthermore, we evaluated effects of the above treatment with moxonidine on the ischemia-enhanced renal sympathetic nerve activity and on norepinephrine overflow into the renal vein after reperfusion.

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 (50 mg/kg, i.p.). After a 2-week recovery period, uninephrectomized rats were divided into four groups: (1) sham-operated control, (2) vehicle-treated acute kidney injury, (3) moxonidine-treated (i.v.) acute kidney injury, (4) moxonidine-treated (i.c.v.) acute kidney injury. Five animals were provided for each group (total animals: $n=20$). 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. Moxonidine or vehicle (0.9% saline) was injected 5 min before ischemia into the left external jugular vein in a volume of 1 ml/kg or into the right lateral cerebral ventricle in a volume of 10 µl/kg. The i.c.v. injection was performed by a 30-gauge stainless steel cannula implanted into the right lateral cerebral ventricle (stereotaxic coordinates: 0.9–1.0 mm posterior to bregma; 1.4–1.6 mm lateral to midline; 3.2–3.3 mm ventral to dura), as described by Paxions and Watson (1998). The position of the cannula was confirmed by the staining of all four ventricles after injection of 2% pontamine sky blue (5 µl) at the end of each experiment. 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. Blood samples were drawn from the thoracic aorta at the end of the urine collection period under pentobarbital anesthesia (50 mg/kg, i.p.). The plasma was separated by centrifugation at 3000 rpm for 15 min at 4 °C. These samples were used for measurement of renal functional parameters. The left kidneys were excised and used for light microscopic observation.

In separate experiments, we examined the effect of moxonidine on changes of norepinephrine level in renal venous plasma after reperfusion. Under pentobarbital (50 mg/kg, i.p.) anesthesia, an abdominal midline incision of uninephrectomized rats was made, and the left kidney was exposed. A curved 26-gauge needle was inserted into the left renal vein for venous blood sampling. Each blood sample was taken at baseline (sham), immediately after the reperfusion 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 at 3000 rpm for 15 min at 4 °C. These samples were stored at −80 °C until the assay for norepinephrine concentration. Five animals were provided for each group (total animals: $n=40$).

2.2. Renal nerve recording

In another set of experiments, electrical signals of renal neural activity were directly recorded for evaluation of changes in renal sympathetic nerve activity during the 45-min ischemic period. In this experiment, uninephrectomized rats were divided into five groups: (1) vehicle-treated acute kidney injury, (2) moxonidine-treated (36 nmol/kg, i.v.) acute kidney injury, (3) moxonidine-treated (360 nmol/kg, i.v.) acute kidney injury, (4) moxonidine-treated (3.6 nmol/kg, i.c.v.) acute kidney injury, (5) moxonidine-treated (36 nmol/kg, i.c.v.) acute kidney injury. Five animals were provided for each group (total animals: $n=25$).

For the measurement of renal sympathetic nerve activity, uninephrectomized rats were anesthetized with pentobarbital (50 mg/kg, i.p.). Renal sympathetic nerve activity was recorded from the left renal nerve branch before and during the 45-min ischemic period, as previously described by Shokoji et al. (2003). 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, Osaka, Japan) 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, Castle Hill, Australia). For the quantification of renal sympathetic nerve activity, the height of integrated nerve discharge was measured for 30 s in each experiment. The changes in nerve activity were expressed as percentages of control resting spontaneous nerve activity.

2.3. Analytical procedures

Blood urea nitrogen and plasma creatinine levels were determined using a commercial kit, the BUN-test-Wako and Creatinine-test-Wako (Wako, Osaka, Japan), respectively. Urine and plasma sodium concentrations were determined using a flame photometer (205D, Hitachi, Ibaraki, Japan). Fractional excretion of sodium (FE_{Na}, %) was calculated from the following formula: $FE_{Na}=U_{Na}V/(P_{Na}\times Ccr)\times 100$, where $U_{Na}V$ is urinary excretion of sodium and P_{Na} is the plasma sodium concentration.

Norepinephrine concentration in renal venous plasma was measured by high-performance liquid chromatography with an amperometric detector (EC-100; 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 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 change (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). Evaluations were made by an observer who was blind to the treatment origin of the tissue.

2.5. Drugs

Moxonidine was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). It was dissolved in saline (0.9%). Other chemicals were obtained