Mechanisms Underlying the Renoprotective Effect of GABA against Ischemia/Reperfusion-Induced Renal Injury in Rats

Shuhei Kobuchi, Ryosuke Tanaka, Takuya Shintani, Rie Suzuki, Hideyuki Furusui, Mamoru Ohkita, Kazuhide Ayajiki, and Yasuo Matsumura

Department of Pharmacy, School of Pharmacy, Hyogo University of Health Sciences, Kobe, Hyogo, Japan (S.K., K.A.); Laboratory of Pathological and Molecular Pharmacology, Osaka University Graduate School of Medicine, Takatsuki, Osaka, Japan (S.K., R.T., T.S., R.S., M.O., Y.M.); and Laboratory of Clinical Pharmacology, Faculty of Pharmacy, Osaka Ohtani University, Tondabayashi, Osaka, Japan (H.T.)

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ABSTRACT

The excitation of the renal sympathetic nervous system plays an important role in the development of ischemic acute kidney injury (AKI) in rats. We have reported that intravenous treatment with GABA has preventive effects on ischemia/reperfusion (I/R)-induced renal dysfunction with histological analysis in rats; however, detailed mechanisms of the actions of GABA on the renal injury were still unknown. Therefore, in the present study, we aimed to clarify the detailed mechanisms of GABA in ischemic AKI in rats. Ischemic AKI was induced after clamping the left renal artery and vein for 45 min. Thereafter, the kidney was reperfused to produce I/R-induced injury. Renovascular or intracerebroventricular treatment with 3-[[[(3,4-dichlorophenyl)methyl]amino]propyl]dithiooxymethyl] phosphinic acid (CGP52432), a GABAB receptor antagonist, abolished the suppressive effects of intravenously-applied GABA on enhanced renal sympathetic nerve activity during ischemia, leading to the elimination of the renoprotective effects of GABA. Intracerebroventricular treatment with GABA or intravenous treatment with baclofen, a selective GABAB receptor agonist, prevented I/R-induced renal injury in rat kidney. Intravenous treatment with GABA, however, intravenous treatment with bicuculline, a GABAA receptor antagonist, tended to affect the preventive effects of GABA on ischemic AKI. Therefore, we demonstrated the novel finding that the preventive effect of GABA on ischemic AKI through the suppression of enhanced renal sympathetic nerve activity induced by renal ischemia is presumably mediated via the GABAB receptor stimulation in the central nervous system and peripheral GABAB receptors.

Introduction

Acute kidney injury (AKI) is encountered commonly in the hospital and is associated with a high rate of mortality (Thadhani et al., 1996). Ischemia, followed by reperfusion, is one of the major causes of AKI (Thadhani et al., 1996). Reperfusion of previously ischemic renal tissue induces complex cellular events that result in the injury and eventual death of renal cells due to a combination of apoptosis and necrosis (Lieberthal and Koppel, 1996). It has been reported that several causal factors (ATP depletion, reactive oxygen species, phospholipase activation, neutrophil infiltration, vasoactive peptides, etc.) contribute to the pathogenesis of this renal damage (Edelstein et al., 1997; Lien et al., 2003). However, the mechanisms underlying ischemia/reperfusion (I/R)-induced renal injury are not fully understood. We found that enhancement of renal sympathetic nerve activity (RSNA) and its consequent effect on norepinephrine (NE) overflow from nerve terminals are considered to be involved in the development of I/R-induced AKI and that RSNA is augmented significantly during renal ischemia in the rats (Fujii et al., 2003; Kurata et al., 2006). In addition, we noted that ischemic AKI is ameliorated by renal denervation or ganglionic blockade and that the effect is accompanied by the suppression of elevated NE levels in the renal vein after reperfusion (Fujii et al., 2003).

GABA, an inhibitory neurotransmitter in the central nervous system (CNS), also is found in peripheral tissues (Gladekovich et al., 2006). GABA is known to suppress electrical renal nerve stimulation-induced NE release from isolated rat
kidney without affecting basal release (Fujimura et al., 1999). These findings indicate that GABA can modulate peripheral neurotransmission as well as that in the CNS. It has been reported that GABA has renoprotective effects against glycerol-induced AKI (Kim et al., 2004). We recently reported that preischemic treatment with GABA exerted a suppressive effect on the enhancement of RSNA and caused a consequent elevation of NE levels in the renal vein observed in ischemic AKI rats, suggesting that GABA has renoprotective effects on I/R-induced renal injury (Kobuchi et al., 2009). However, a previous report lacks more precise mechanisms of beneficial action of GABA on AKI and/or I/R-induced renal injury, especially in the subtype of receptors and the site of action of GABA.

Therefore, we investigated the effects of intravenous treatment with baclofen, a GABA receptor agonist, and intravenous treatment with 3-[[3,4-dichlorophenyl)methyl]amino(propyl) diethoxymethyl) phosphinic acid (CGP52432), a GABA receptor antagonist, on the renoprotective effects of GABA to clarify the site receptors responsible for the effects. Furthermore, we examined the effects of intracerebroventricular treatment with CGP52432 on the renoprotective effects of GABA to clarify the site of action. The effects of intracerebroventricular treatment with baclofen, a GABA receptor agonist, and intracerebroventricular treatment with GABA on ischemic AKI were examined.

### Materials and Methods

#### Animals and Experimental Design

Male Sprague-Dawley rats (10 weeks of age; Japan SLC, Shizuoka, Japan) were used. The animals were housed in a fully controlled room, a 12-h light/dark cycle and were allowed free access to food and water. Experimental protocol and animal care methods in the experiments were approved by the Experimental Animal Committee of the Osaka University of Pharmaceutical Sciences (Osaka, Japan). Two weeks before the study (8 weeks of age), the right kidney was removed through a small flank incision under pentobarbital anesthesia (50 mg/kg i.p.) in the left kidney of the rats anesthetized with pentobarbital (50 mg/kg i.p.) for sham-operated control studies. Under pentobarbital anesthesia (50 mg/kg i.p.), surgical preparation of the animals and basic experimental protocols were repeated for all experiment groups. The kidneys then were preserved in phosphate-buffered 10% formalin, separated by centrifugation and used for the measurement of renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a protected least significant difference comparison tests in nerve recording, way repeated measures analysis of variance followed by Fisher’s protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies.

#### Statistical Analysis

All of the values were expressed as means ± S.E.M. Relevant data were processed by Instat (GraphPad Software Inc., San Diego, CA). Nerve recording studies were analyzed by one-way repeated measures analysis of variance followed by Dunnett’s multiple range test for within-group data. For among-group data, we used two-way repeated measures analysis of variance followed by Fisher’s protected least significant difference comparison tests in nerve recording, renal function, or NE concentration studies. Histological data were analyzed using a Kruskal-Wallis nonparametric test combined with a Steel-type multiple comparison test. For all of the comparisons, differences were considered significant at P < 0.05.
Results

Effects of Intravenous Treatment with Bicuculline or CGP52432 on GABA-Induced Improvement against Ischemic AKI. The preischemic treatment with GABA (50 \( \mu \)mol/kg i.v.) markedly suppressed enhanced RSNA during the ischemic period (Fig. 1, A, B, and D). This suppressive effect was dose-dependently inhibited by treatment with CGP52432 (10 and 100 nmol/kg i.v.), a selective GABA\(_A\) receptor antagonist (Fig. 1, C and D). However, treatment with bicuculline (1 and 10 \( \mu \)mol/kg i.v.), a selective GABA\(_B\) receptor antagonist, failed to attenuate the suppressive effect of GABA on RSNA (Fig. 1D).

As shown in Fig. 2, the renal function of rats subjected to 45 min of ischemia showed marked deterioration when measured at 29 h after reperfusion. In comparison with sham-operated rats, vehicle-treated AKI rats showed significant increases in BUN, Pcr, Ccr, and UF and significant decreases in Ccr, indicating renal dysfunction. The intravenous injection of GABA (50 \( \mu \)mol/kg i.v.) to ischemic AKI rats markedly attenuated I/R-induced renal dysfunction. The GABA-induced improvement was reversed by bicuculline or CGP52432 treatment regarding BUN, Pcr, Ccr, and UF.

Fig. 1. Typical responses of RSNA and integrated RSNA to injection of vehicle (0.9% saline intravenous) (A), GABA (50 \( \mu \)mol/kg i.v.) (B), or GABA (50 \( \mu \)mol/kg i.v.) + CGP52432 (100 nmol/kg i.v.) (C) during the ischemic period in anesthetized rats. Each column and bar represents the mean ± S.E.M. (n = 6). ††, \( P < 0.01\), compared with basal point (vehicle-treated AKI rats); ∗∗∗, \( P < 0.001\), compared with vehicle (intravenous)-treated AKI rats.

Fig. 2. Effects of intravenous treatment with bicuculline or CGP52432 on renal protection by intravenous treatment with GABA. Parameters of renal function are BUN (A), Pcr (B), Ccr (C), and UF (D) at 29 h after reperfusion. After bicuculline or CGP52432 was given 10 min before ischemia, vehicle or GABA was given 5 min before ischemia. Each column and bar represents the mean ± S.E.M. (n = 6). ††, \( P < 0.01\), compared with vehicle (intravenous)-treated AKI rats; †, \( P < 0.05\); ‡‡‡, \( P < 0.001\), compared with vehicle (intravenous)-treated AKI rats.
intravenous treatment with CGP52432 at the higher dose (100 nmol/kg), whereas the renoprotective effect of GABA was not affected by treatment with bicuculline (1 and 10 µmol/kg) or CGP52432 at the lower dose (10 nmol/kg).

Histological examination revealed severe lesions in the kidneys of vehicle-treated AKI rats at 29 h after reperfusion. These changes were characterized by proteinaceous casts in tubuli in the inner zones of the medulla (Fig. 3B, G, and L), medullary congestion, and hemorrhage in the outer zone inner stripes of the medulla (Fig. 3G), and tubular necrosis in the outer zone outer stripes of the medulla (Fig. 3L) in comparison with kidneys from sham-operated rats (Fig. 3, A, F, and K). Intravenous injection of GABA to ischemic AKI rats significantly attenuated the development of all of these lesions (Fig. 3, C, H, and M; Table 1). Treatment with CGP52432 (Fig. 3, E, J, and O) at the higher dose (100 nmol/kg) negated the above improvement induced by GABA. However, intravenous treatment with bicuculline at the higher dose (10 µmol/kg) failed to affect the GABA-induced action (Fig. 3, D, I, and N).

**Effects of Intracerebroventricular Treatment with CGP52432 on GABA-Induced Improvement against Ischemic AKI.** As shown in Fig. 4, the suppressing effect of GABA (50 µmol/kg) on RSNA was attenuated partially by the lower dose of intracerebroventricularly applied CGP52432 (0.1 nmol/kg), and the higher dose of CGP52432 (1 nmol/kg) almost abolished the effect of GABA.

As shown in Fig. 5, the GABA-induced improvement of renal dysfunction was attenuated partially by intracerebroventricular treatment with CGP52432 at the higher dose (100 nmol/kg). A table showing the effects of bicuculline or CGP52432 on renal tissue protection by intravenous treatment with GABA is also provided. The table indicates that treatment with bicuculline (10 µmol/kg) failed to affect the GABA-induced action. Additionally, the data show that GABA (50 µmol/kg) significantly attenuated the development of all of these lesions in comparison with kidneys from sham-operated rats. A significance level of P < 0.01 is used to indicate statistical significance.
Effects of Intravenous Treatment with Baclofen or Intracerebroventricular Treatment with GABA on Ischemic AKI. The enhanced RSNA during the ischemic period was dose-dependently and markedly suppressed by intravenous treatment with baclofen (0.2 and 1 μmol/kg), a selective GABAB receptor agonist, or intracerebroventricular treatment with GABA (0.1 and 0.5 μmol/kg) (Fig. 6).

As shown in Table 2, intravenous treatment with baclofen (0.2 and 1 μmol/kg) significantly prevented I/R-induced renal dysfunction. The effectiveness of the higher dose of baclofen (1 μmol/kg) was equivalent to that of GABA (50 μmol/kg).

As shown in Table 2, intracerebroventricular treatment with GABA (0.1 and 0.5 μmol/kg) dose-dependently attenuated I/R-induced renal dysfunction. The effectiveness of intracerebroventricular treatment with GABA (0.5 μmol/kg) was similar to that of intravenous treatment with GABA (50 μmol/kg).

Discussion

The renal sympathetic nerve system and circulating catecholamines are considered to be involved in the pathogenesis of AKI because pharmacological blockade of the sympathetic nervous system exerts an efficient protective effect on AKI (Kurata et al., 2006; Sugiura et al., 2008). We recently found that intravenous treatment with GABA (10 and 50 μmol/kg) in the ischemic AKI rats efficiently suppressed the enhancement of RSNA during the ischemic period and increased NE overflows after reperfusion (Kobuchi et al., 2009). In the present study, we investigated the more precise mechanisms of the renoprotective effects of GABA on AKI, especially on the subtype of receptors and the site of action of GABA.
The receptor subtypes of GABA receptors are well known as GABA<sub>A</sub> and GABA<sub>B</sub> receptors. The GABA<sub>A</sub> receptor is coupled to the ligand-gated chloride channel, whereas bicuculline, a GABA<sub>A</sub> receptor antagonist, failed to affect the suppressive effects of GABA. In addition, intravenous treatment with baclofen, a GABA<sub>B</sub> receptor agonist, prevented renal ischemia-induced renal injury by suppressing enhanced RSNA. These findings suggest that activation of GABA<sub>B</sub> receptors, but not GABA<sub>A</sub> receptor, is responsible for protective effects against the development of I/R-induced renal dysfunction.

It has been reported recently that the concentration of GABA also activates metabotropic GABAB receptors, which are distributed widely within the mammalian CNS and exhibit a differential topographical distribution (Palacios et al., 1981). In the present study, we investigated the receptor subtype involved in the suppressive effects of GABA on I/R-induced enhancement of RSNA and functional and histological renal injury in rats.
GABA in the cerebrospinal fluid is increased by intravenous infusion with GABA itself (Al-Awadi et al., 2006). This finding indicates that intravenous injection of GABA may suppress enhanced RSNA during the ischemic period by acting on the CNS. In the present study, we found that intracerebroventricular treatment with GABA in the cerebrospinal fluid is increased by intravenous infusion with GABA itself (Al-Awadi et al., 2006). This finding suggests that the renoprotective effect of GABA on I/R-induced renal injury.

### Effects of intravenous treatment with baclofen or intracerebroventricular treatment with GABA on BUN, Pcr, Ccr, and UF at 29 h after reperfusion

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>BUN</th>
<th>Per</th>
<th>Ccr</th>
<th>UF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKI + vehicle intravenous</td>
<td>104.1±5.02</td>
<td>2.70±0.28</td>
<td>1.40±0.14</td>
<td>8±8.10</td>
</tr>
<tr>
<td>AKI + baclofen 0.2 μmol/kg i.v.</td>
<td>76.3±5.36**</td>
<td>1.86±0.24*</td>
<td>2.00±0.48</td>
<td>3.8±13.78*</td>
</tr>
<tr>
<td>AKI + baclofen 1 μmol/kg i.v.</td>
<td>53.0±5.62**</td>
<td>1.24±0.08**</td>
<td>2.5±0.39</td>
<td>3.8±7.62**</td>
</tr>
<tr>
<td>AKI + vehicle intracerebroventricular</td>
<td>103.2±6.04</td>
<td>2.57±0.14</td>
<td>1.8±0.18</td>
<td>6±9.12</td>
</tr>
<tr>
<td>AKI + GABA 0.1 μmol/kg i.c.v.</td>
<td>67.6±12.63*</td>
<td>1.84±0.29*</td>
<td>2.03±0.69</td>
<td>7.3±12.93</td>
</tr>
<tr>
<td>AKI + GABA 0.5 μmol/kg i.c.v.</td>
<td>51.3±5.66**</td>
<td>1.18±0.12**</td>
<td>1.18±0.12**</td>
<td>7.6±7.96**</td>
</tr>
</tbody>
</table>

# P < 0.05; ## P < 0.01, compared with vehicle (i.c.v.)-treated AKI rats. * P < 0.05; ** P < 0.01, compared with vehicle (i.v.)-treated AKI rats.

In conclusion, GABA suppressed the enhanced RSNA during ischemia and increased NE overflow after I/R by the activation of the GABAB receptor, but not that of GABAergic input, especially in the CNS rather than the peripheral sympathetic nerves in the sympathetic nervous system. Inhibitory effects are presumably responsible for renoprotection against I/R-induced renal injury.

### Authorship Contributions

- **Participated in research design:** Kobuchi, Tanaka, and Matsumura.
- **Conducted experiments:** Kobuchi, Shindo, Suzuki, Tsutsui, and Okhita.
- **Performed data and text:** Kobuchi.
- **Wrote or contributed to the writing of the manuscript:** Kobuchi, Ayajiki, and Matsumura.

### Reference


Experiment 109–114.

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Address correspondence to: Shuhei Kobuchi, Department of Pharmacy, School of Pharmacy, Hyogo University of Health Sciences, 1-3-6 Minatojima, Chuo-ku, Kobe, Hyogo 650-8530, Japan. E-mail: kobuchi-s@huhs.ac.jp