Adenosine A<sub>1</sub> Receptor Antagonist KW-3902 Prevents Hypoxia-Induced Renal Vasoconstriction<sup>1</sup>

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ABSTRACT

Studies were carried out to determine the intrarenal adenosine production during hypoxia, and the protective effects of a selective adenosine A<sub>1</sub> receptor antagonist 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902) on hypoxia-induced renal hemodynamic changes. We used an in vivo microdialysis method and measured the renal interstitial concentration of adenosine in response to hypoxic exposure in anesthetized mechanically ventilated rabbits. Normocapnic systemic hypoxemia (PaO<sub>2</sub> = 32 ± 6 mm Hg) caused a significant decrease in renal blood flow and increase in renal vascular resistance, indicating a renal vasoconstriction. The basal interstitial concentration of adenosine in the cortex was 293 ± 70 nM, which was significantly higher than that in the medulla (170 ± 23 nM). Five minutes after beginning hypoxia, the renal interstitial concentration of adenosine approximately tripled in the cortex and doubled in the medulla. During treatment with KW-3902, hypoxemia caused a similar increase in the adenosine concentration compared with that in the absence of KW-3902. The administration of KW-3902, however, significantly attenuated hypoxia-induced reduction in renal blood flow. These results suggest that adenosine was involved in hypoxia-induced renal vasoconstriction via its effects on adenosine A<sub>1</sub> receptors, and that KW-3902 had a partial protective effect against renal vasoconstriction during hypoxemia.

Renal insufficiency and failure are associated with hypoxemia (Finn et al., 1975; Myers and Moran, 1986). The mechanisms of hypoxia-induced renal dysfunction have been studied extensively but remain elusive. In animal models as well as in humans, it has been demonstrated that acute normocapnic hypoxemia results in an immediate decrease in renal blood flow (RBF) and glomerular filtration rate (Busija, 1984; Wiesel et al., 1990; Pedrotti et al., 1992; Huet et al., 1997). Various mediators such as the sympathetic nervous system (Malpas et al., 1996), catecholamines (Hirakawa et al., 1997), and the renin-angiotensin system (Robillard et al., 1981; Ritthaler et al., 1997) have been implicated in renal hemodynamic changes during hypoxia; however, the mechanism by which this occurs is poorly understood.

Adenosine is a byproduct of normal ATP hydrolysis or cellular energy-dependent processes (Sparks and Bardenheuer, 1986; Meghji et al., 1988) and the adenosine production is increased by a reduction in O<sub>2</sub> availability for oxidative phosphorylation in a variety of renal cell types (Beach et al., 1991; Reyes et al., 1995). Indeed, adenosine rapidly increases during ischemia (Osswald et al., 1977; Miller et al., 1978; He et al., 1995) or hemorrhage in the kidney (Nagashima and Karasawa, 1996). Churchill and Bidani (1982) have proposed that a possible candidate mediator of hypoxia-induced renal dysfunction is adenosine. In isolated perfused rat kidneys, hypoxia-induced renal vasoconstriction is preventable by the administration of the adenosine A<sub>1</sub> receptor antagonist 1,3-diprophyl-8-(2-amino-4-chlorophenyl)xanthine (Ramos-Salazar and Baines, 1986). Gouyon and Guignard (1988) have shown that treatment with the nonselective adenosine receptor antagonist theophylline can prevent reductions of RBF and glomerular filtration rate induced by acute systemic hypoxia. These studies, which have used adenosine receptor blockade, suggest that adenosine production and/or release in the kidney may be involved in renal vasoconstriction during hypoxia. However, to date, no direct measurements of intrarenal adenosine during hypoxia have been carried out in an in vivo setting, due to the technical difficulties.

Several other researchers and also our department have recently measured the renal interstitial concentration of adenosine with a microdialysis method (Baranowski et al., 1994; Siragy and Linden, 1996; Nishiyama et al., 1998). This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture of Japan. Part of this work was presented at the 31st Annual Meetings of the American Society of Nephrology, Philadelphia, PA, 1998.

ABBREVIATIONS: RBF, renal blood flow; KW-3902, 8-(noradamantan-3-yl)-1,3-dipropylxanthine; MAP, mean arterial pressure; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; RVR, renal vascular resistance; ANG II, angiotensin II; NE, norepinephrine.
al., 1997; Zou et al., 1999). This method is well suited for studies of intrarenal adenosine under various conditions because the adenosine receptors are located on the surface of the cell membrane (Spielman and Thompson, 1982). Recently, we established an in vivo renal microdialysis method in anesthetized, mechanically ventilated rabbits (Nishiyama et al., 1999) and, therefore, were able to measure the dynamics of renal interstitial concentration of adenosine during systemic hypoxia.

The aim of the present study is to define the role of intrarenal adenosine and the putative protective effects of a selective adenosine A1 receptor antagonist in hypoxia-induced renal hemodynamic changes. This study was, therefore, undertaken to determine if systemic hypoxia increases the renal interstitial concentration of adenosine, and to determine the ability of the highly selective adenosine A1 receptor antagonist 8-(noradaman-3-yl)-1,3-dipropylxanthine (KW-3902) (Shimada et al., 1992) to prevent renal vasoconstriction in anesthetized, mechanically ventilated rabbits under normocapnic systemic hypoxia.

Materials and Methods

Experimental Protocols

Effects of Hypoxia on Renal Hemodynamics and Renal Interstitial Concentration of Adenosine. Following two 5-min control periods with room air, rabbits were ventilated with a gas mixture containing 8% O2/92% N2 (hypoxic gas) for 15 min (n = 11). MAP, heart rate, and RBF were measured continuously and the dialysates were collected at 5-min intervals. Three additional 5-min collection periods were performed at 15, 30, and 60 min after the cessation of the hypoxic exposure. In five rabbits, we performed a time control of this protocol in which samples were collected for 120 min.

Effects of Hypoxia on Renal Hemodynamics and Renal Interstitial Concentration of Adenosine during Treatment with KW-3902. We used KW-3902 (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan), a highly selective adenosine A1 receptor antagonist (Shimada et al., 1992), to investigate whether selective blocking of the adenosine A1 receptors would modify renal hemodynamics and/or the renal interstitial concentration of adenosine during hypoxia. KW-3902 was dissolved in the lactated Ringer's solution containing 1% dimethylsulfoxide and 0.01 N NaOH. In preliminary experiments (n = 3), we had found that i.v. administration of KW-3902 (priming dose, 0.1 mg/kg; sustaining dose, 0.01 mg/kg/min) inhibited the renal vasoconstrictor response to the intrarenal arterial injection of exogenous adenosine (1 and 10 μg). It also was confirmed that the vehicle (lactated Ringer's solution containing 1% dimethyl sulfoxide and 0.01 N NaOH) did not affect any of the parameters studied (n = 2).

After two 5-min sampling periods, KW-3902 (priming dose, 0.1 mg/kg; sustaining dose, 0.01 mg/kg/min) was administered i.v. in anesthetized rabbits (n = 11) and two additional sampling periods were performed at 10-min intervals. At 20 min following KW-3902 administration, the rabbits were ventilated with a gas mixture containing 8% O2/92% N2 (hypoxic gas) for 15 min and the dialysates were collected in the same manner as described above.

We performed a time control of this protocol with KW-3902 alone in five rabbits in which samples were collected for 100 min following the administration of KW-3902.

Microdialysis Probe

In this study, a newly developed microdialysis probe constructed in our laboratory was used (He et al., 1995). The microdialysis membrane (Toyobo, Otsu, Japan) is made from cuprophan fiber, measuring 15 mm in length with a 0.22-μm o.d. and with a 5000-Da transmembrane diffusion cutoff. Steel needles were inserted into both sides of the cuprophan fiber. The efficiency of the microdialysis probe was determined as follows. The probe was placed in a beaker containing an isotonic saline solution into which different quantities of adenosine were added. We perfused the probes with isotonic saline solution with heparin (30 U/ml) containing NaNH (10 μM) and EHL (100 μM) at a rate of 5 μl/min. The dialysate was collected and the recovery rate of adenosine was calculated by dividing the concentration in the dialysate by the concentration in the medium. At a perfusion rate of 5 μl/min, the recovery rate of adenosine was 30 ± 4%. These recovery rates were higher than those obtained with a commercially available microdialysis probe measuring 2 mm in length and 0.65 mm in diameter, with a 10-kDa transmembrane diffusion cutoff. Based on these results, we considered that a perfusion rate at 5 μl/min was suitable for this experiment.

Analytical Procedures

Adenosine in the dialysate was measured according to the method developed by Zhang et al. (1991). The procedure is briefly described as follows. Twenty-five microliters of dialysate is transferred into a microcentrifuge tube and 72.5 μl of 1 mM acetate buffer (pH 4.0) and 2.5 μl of 40% chloroacetaldehyde are added. The preparation is incubated at 80°C for 1 h to allow for the conversion of adenosine to ethanoladenosine. For HPLC, a reversed-phase HPLC column (Develosil ODS HG-5, 150 × 4.6 mm i.d.) is maintained at 40°C with a
after the cessation of hypoxic exposure (Fig. 1). The concentrations of adenosine returned to the respective basal level soon after the cessation of hypoxic exposure. These parameters remained at the same level during hypoxic exposure. The concentration of adenosine increased to 940 nM in the cortex and 356 nM in the medulla (Fig. 2). At 5 min after beginning hypoxia, RBF had returned to the control level and this recovery time also was shortened. The KW-3902 treatment did not affect the renal interstitial concentration of adenosine, but hypoxia caused a similar increase in adenosine levels compared with that in the absence of KW-3902 (Figs. 3 and 4).

In five rabbits, we performed a time control experiment of this protocol. Normoxic control rabbits showed no changes in arterial pH, PaCO₂, MAP, heart rate, or RBF during 120 min. At 120 min after starting sampling, the adenosine concentration was 314 ± 62 nM in the cortex and 172 ± 34 nM in the medulla, and were not significantly different from basal adenosine concentrations (346 ± 68 nM in the cortex and 191 ± 39 nM in the medulla).

**Results**

**Effects of Hypoxia on Renal Hemodynamics and Renal Interstitial Concentration of Adenosine.** Three minutes after changing to the gas mixture containing 8% O₂/92% N₂ (hypoxic gas), control arterial PaO₂ (98 ± 11 mm Hg) decreased significantly to 32 ± 6 mm Hg, but the control arterial pH (7.41 ± 0.05) and PaCO₂ (39.9 ± 3.4 mm Hg) was unchanged (7.43 ± 0.09 and 28.1 ± 3.3 mm Hg, respectively). These parameters remained at the same level during hypoxic exposure.

Within only 3 min after initiating hypoxia, RBF had significantly decreased from 2.84 ± 0.37 to 1.27 ± 0.29 ml/min/g. MAP slightly increased from 88 ± 4 to 92 ± 4 mm Hg, but this change was not significance (Table 1). The calculated renal vascular resistance (RVR), which had increased significantly from 33.0 ± 6.8 to 78.9 ± 9.8 mm Hg/ml/min/g, indicated a renal vasoconstriction. At 15 min after beginning hypoxia, MAP and RBF had returned to the respective control levels. The basal adenosine concentration in the renal interstitial space, which was measured at 90 min after the implantation of the microdialysis probe, was 293 ± 70 nM in the cortex and 170 ± 23 nM in the medulla. The adenosine concentration of the medulla was significantly higher than that of the cortex (p < .05). Five minutes after beginning hypoxia, the adenosine concentration increased to 940 ± 222 nM in the cortex and 356 ± 103 nM in the medulla, and remained at the same level during hypoxic exposure. The concentrations of adenosine returned to the respective basal level soon after the cessation of hypoxic exposure (Fig. 1).

In five rabbits, we performed a time control experiment of this protocol. Normoxic control rabbits showed no changes in arterial pH, PaCO₂, MAP, heart rate, or RBF during 120 min. At 120 min after starting sampling, the adenosine concentration was 314 ± 62 nM in the cortex and 172 ± 34 nM in the medulla, and were not significantly different from basal adenosine concentrations (346 ± 68 nM in the cortex and 191 ± 39 nM in the medulla).

**Effects of Hypoxia on Renal Hemodynamics and Renal Interstitial Concentration of Adenosine during Treatment with KW-3902.** KW-3902 did not affect MAP or the concentration of adenosine, but increased RBF transiently (Table 2). At 25 min following KW-3902 administration, hypoxic exposure was initiated. The arterial PaO₂, pH, and PaCO₂ achieved in hypoxia after KW-3902 were not significantly different from those attained before KW-3902. During treatment with KW-3902, hypoxia caused a significant decrease in RBF, but this reduction was significantly attenuated compared with that in the absence of KW-3902 (Fig. 2). At 5 min after beginning hypoxia, RBF returned to the control level and this recovery time also was shortened. The KW-3902 treatment did not affect the renal interstitial concentration of adenosine, but hypoxia caused a similar increase in adenosine levels compared with that in the absence of KW-3902 (Figs. 3 and 4).

We performed a time control of this protocol with KW-3902 alone (n = 5). Intravenous administration of KW-3902 (priming dose, 0.1 mg/kg; sustaining dose, 0.01 mg/kg/min) did not alter the renal hemodynamics during the time period of this study.

**Table 2**

<table>
<thead>
<tr>
<th>Hypoxia (min)</th>
<th>MAP (mm Hg)</th>
<th>RBF (ml/min/g)</th>
<th>RVR (mm Hg/ml/min/g)</th>
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<tbody>
<tr>
<td>Control (min)</td>
<td>87 ± 4</td>
<td>2.41 ± 0.22</td>
<td>38.1 ± 5.9</td>
</tr>
<tr>
<td>10</td>
<td>87 ± 4</td>
<td>2.35 ± 0.22</td>
<td>38.9 ± 5.8</td>
</tr>
<tr>
<td>20</td>
<td>89 ± 4</td>
<td>3.24 ± 0.35*</td>
<td>29.8 ± 6.8*</td>
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<tr>
<td>3</td>
<td>89 ± 4</td>
<td>3.01 ± 0.35*</td>
<td>31.2 ± 7.9*</td>
</tr>
<tr>
<td>5</td>
<td>89 ± 4</td>
<td>2.81 ± 0.32*</td>
<td>34.9 ± 8.9</td>
</tr>
<tr>
<td>10</td>
<td>90 ± 4</td>
<td>2.49 ± 0.24</td>
<td>39.1 ± 9.7</td>
</tr>
<tr>
<td>20</td>
<td>90 ± 4</td>
<td>2.44 ± 0.22</td>
<td>38.5 ± 9.4</td>
</tr>
</tbody>
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Values are means ± S.E. (n = 11). *p < .05.

**Table 1**

<table>
<thead>
<tr>
<th>Effects of hypoxia on renal hemodynamics in rabbits</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
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<td>Control (min)</td>
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<tr>
<td>10</td>
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Values are means ± S.E. (n = 11). *p < .05.
modify arterial PaO$_2$, pH, or PaCO$_2$. KW-3902 also did not affect MAP or the concentration of adenosine. RBF increased transiently, but returned to the control level at 10 min after the administration of KW-3902 and remained at the same levels for 90 min (data not shown).

**Discussion**

The present study demonstrates that normocapnic systemic hypoxia increased the renal interstitial concentration of adenosine and caused a renal vasoconstriction in mechanically ventilated anesthetized rabbits. KW-3902, a highly selective A$_1$ adenosine receptor antagonist (Shimada et al., 1992), did not affect the elevation of the adenosine level; however, it significantly attenuated the reduction in RBF during hypoxia. These results suggest that the intrarenal production and/or release of adenosine are involved in hypoxia-induced renal vasoconstriction.

Because endogenously produced adenosine is formed primarily by renal tubular epithelial cells and reaches the renal microvasculature via the interstitial space (Navar et al., 1996), measurements of interstitial adenosine levels could be critical. A microdialysis method has been suggested to be well suited to measure the dynamics of renal interstitial adenosine in the kidney (Baranowski and Westenfelder, 1994; Siragy and Linden, 1996; Nishiyama et al., 1997; Zou et al., 1999). Recently, we were able to minimize tissue injury by making a fiber type probe with a thinner diameter (0.22 mm). In addition, the length of the dialysis membrane is 1.5 cm, which is three to four times longer than that of a regular probe. As a result, the dialysis efficiency of the new probe was better than that of a regular probe (He et al., 1995; Nishiyama et al., 1999). We can perfuse our probe at a high perfusion rate (5 \mu l/min) and shorten the sampling time (5 min). Thus, this newly developed microdialysis probe appears to be a useful tool for monitoring the dynamics of adenosine in the renal interstitial space during hypoxia.

The basal interstitial concentration of adenosine in the cortex was 293 \pm 70 nM, which was significantly higher than that in the medulla (170 \pm 23 nM) in anesthetized rabbits. These findings are inconsistent with those reported by Siragy and Linden (1996) and Zou et al. (1999). They have reported that the renal interstitial concentration of adenosine is higher in the renal medulla than in the cortex in anesthetized rats. The reason for this discrepancy is not clear, however, it might be due to differences in species and experimental conditions. During ischemia, it has been reported that the AMP concentration in the cortex is the highest in the rabbit kidney, whereas the AMP concentration in the outer medulla is the highest in the rat kidney (Zager et al., 1990). Interspecies variation may provide a likely explanation for this discrepancy in adenosine levels. Moreover, we used perfusate containing iodotubercidin (10 \mu M) and EHNA (100 \mu M). Because adenosine metabolism is so fast, it is difficult to detect the exact adenosine level in the renal interstitial space without the use of inhibitors of adenosine deaminase and adenosine kinase (He et al., 1995). Therefore, the composition of the perfusate also might have contributed to this discrepancy.

Normocapnic systemic hypoxia resulted in an immediate decrease in RBF whereby levels initially fell to a minimum at
3 to 5 min after beginning hypoxic exposure (Table 1). These results are consistent with those of previous studies (Busija, 1984; Wiesel et al., 1990; Pedrotti et al., 1992; Huet et al., 1997). RBF tended to return to the control value during hypoxic exposure, whereas renal interstitial adenosine concentrations immediately increased and remained at the same level (Fig. 1). It is possible that the responsiveness of the renal vasculature to adenosine may have contributed to such differential changes because adenosine can act as either a vasoconstrictor or a vasodilator of the renal vasculature (Murray and Churchill, 1984; Navar et al., 1996). Furthermore, the fact that adenosine redistributes RBF with predominant actions in the inner cortex and medulla (Dinour and Brezis, 1991; Agmon et al., 1993; Navar et al., 1996) has led to confusion. Infusion of adenosine directly into the renal interstitium increased medullary PO2 and decreased cortical PO22, suggesting that elevating interstitial adenosine concentration redistributes cortical blood flow to the renal medulla and/or that adenosine decreased medullary oxygen consumption (Dinour and Brezis, 1991). Agmon et al. (1993) reported that the A1 agonist N6-cyclopentyladenosine decreases both cortical and medullary blood flow, whereas the A2 agonist CGS-21680C increases medullary blood flow without changing cortical blood flow significantly. Because four subtypes of adenosine receptors (A1, A2a, A2b, A3) are present in both the renal cortex and the medulla (Zou et al., 1999), changes in renal interstitial adenosine concentrations in the cortex as well as in the medulla may cause hypoxia-induced regional changes in intrarenal blood flow. Further studies are needed to determine the role of adenosine in regulating cortical and medullary blood flow.

It is known that various vasoactive factors such as the sympathetic nervous system (Malpas et al., 1996), catecholamines (Claustre et al., 1985; Schuijers et al., 1986; Hirakawa et al., 1997), and the renin-angiotensin system (Robillard et al., 1981; Hirakawa et al., 1997; Rittenthal et al., 1997) are activated during hypoxia. Recently, we investigated the role of adenosine in modulating the renal vasoconstric- tor action of angiotensin II (ANG II) and norepinephrine (NE) (Aki et al., 1997). The ANG II or NE-induced reduction in RBF was attenuated by the administration of KW-3902. Furthermore, this renal vasoconstriction was enhanced by an intrarenal administration of adenosine, which, in turn, could be diminished by the administration of KW-3902 (Aki et al., 1997). These findings indicate that there was a relationship between adenosine A1 receptor-mediated renal vasoconstric- tion and ANG II or NE. The evidence supporting a synergistic dependence on ANG II for adenosine to exert its renal hemodynamic changes in rabbits is consistent with those of previous studies (Busija, 1984; Rittenthal et al., 1997). These results suggest that intrarenal adenosine was involved in hypoxia-induced renal vasoconstriction and that the adenosine A1 receptor antagonist KW-3902 had a partial protective effect against renal vasoconstriction during hypoxemia.

Acknowledgments

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References


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