Role of Adenosine A1 Receptor in Angiotensin II- and Norepinephrine-Induced Renal Vasoconstriction

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ABSTRACT

We investigated the contributions of adenosine A1 receptors to angiotensin II- and norepinephrine-induced renal vasoconstriction. Intrarenal administrations of angiotensin II (3, 10, and 30 ng) or norepinephrine (100 and 500 ng) produced dose-dependent renal vasoconstriction in anesthetized dogs. Under resting conditions, angiotensin II (30 ng) and norepinephrine (500 ng) significantly decreased renal blood flow by −43 ± 3 and −19 ± 2%, respectively (n = 21). Intra-arterial infusion of adenosine (5 μg/kg/min) significantly augmented renal blood flow responses to both angiotensin II and norepinephrine (−64 ± 4 and −45 ± 14%, n = 7). Renal blood flow responses to angiotensin II and norepinephrine were also augmented by inhibition of cellular uptake of adenosine with dipyridamole (10 μg/kg/min, n = 6). Blockade of adenosine A1 receptors with 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902; 10 μg/kg/min) did not alter basal renal blood flow but significantly attenuated angiotensin II- and norepinephrine-induced renal vasoconstriction (−34 ± 6 and −9 ± 3%, n = 7). Furthermore, KW-3902 completely prevented augmentation of renal blood flow responses to angiotensin II and norepinephrine produced by adenosine or dipyridamole (n = 7 and 6, respectively). Administrations of angiotensin II (30 ng) or norepinephrine (500 ng) into the common carotid artery significantly decreased carotid blood flow by −20 ± 5 and −41 ± 10%, respectively; however, neither adenosine (5 μg/kg/min) nor KW-3902 (10 μg/kg/min) affected the carotid blood flow responses to angiotensin II and norepinephrine (n = 5, respectively). Adenosine concentrations in dialysates were not significantly changed by administrations of angiotensin II (from 19 ± 3 to 24 ± 4 nM, n = 6) or norepinephrine (from 16 ± 3 to 19 ± 3 nM, n = 6). These results suggest that basal interstitial adenosine levels influence both angiotensin II and norepinephrine-induced vasoconstriction via A1 receptors in the kidney but not in the area drained by the common carotid artery. The responses of adenosine to angiotensin II- and norepinephrine-induced renal vasoconstriction may not be mediated through de novo intrarenal adenosine accumulation due to angiotensin II- and norepinephrine-induced renal vasoconstriction.

Adenosine exerts a critical role in the paracrine regulation of renal hemodynamics (Navar et al., 1996; Siragy and Linden, 1996; Miura et al., 1999; Nayeem et al., 1999; Zou et al., 1999; Jackson and Dubey, 2001). Substantial experimental evidence supports the existence of a synergistic interaction between adenosine and the renin-angiotensin system in regulating renal hemodynamics (Hall et al., 1985; Hall and Granger, 1986; Wang et al., 1992; Munger and Jackson, 1994; Navar et al., 1996; Traynor et al., 1998). Early studies showed that suppression of the renin-angiotensin system by feeding a high-sodium diet (Osswald et al., 1975) or administration of an angiotensin-converting enzyme inhibitor (Hall et al., 1985) blunts the renal vasoconstrictor action of adenosine. Micropuncture studies (Munger and Jackson, 1994) have shown that angiotensin II type 1 receptor blockade with losartan markedly attenuates afferent arteriolar vasodilation induced by a selective adenosine A1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine. Similarly, Weihprecht et al. (1994) reported that an adenosine A1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX), significantly attenuated the renal vasoconstrictor action of angiotensin II in isolated afferent arterioles of the rabbit. They also showed that peritubular infusion of an angiotensin II receptor antagonist, saralasin, attenuated the fall in stop-flow pressure caused by selective adenosine A1 receptor agonist, N6-cyclohexyladenosine (CHA). Furthermore, recent studies have demonstrated that angiotensin II type 1A receptor knockout

ABBREVIATIONS: CPX, 8-cyclopentyl-1,3-dipropylxanthine; CHA, N6-cyclohexyladenosine; KW-3902, 8-(noradamantan-3-yl)-1,3-dipropylxanthine; RBF renal blood flow; HPLC, high-performance liquid chromatography.
mice showed a markedly reduced constrictor response to CHA in the kidney (Traynor et al., 1998). Despite the evidence suggesting synergistic interactions between adenosine and angiotensin II, other studies performed in the hydronephrotic kidney (Dietrich et al., 1991), in situ perfused kidney (Rossi et al., 1987), and juxtamedullary preparation (Carmines and Inscho, 1994) fail to show these interactions.

Several investigators suggested that renal interstitial adenosine levels are an important determinant of vascular responsiveness of angiotensin II (Hall et al., 1985; Carmines and Inscho, 1994; Weihprecht et al., 1994; Navar et al., 1996). Furthermore, recent studies have documented that renal interstitial fluid adenosine concentrations are significantly increased during ischemia (Nishiyama et al., 1999, 2001b). Therefore, the possibility exists that accumulation of renal interstitial adenosine by angiotensin II-induced ischemia amplifies the vasoconstrictor responses to angiotensin II.

The primary objective of this study was to explore further the role of adenosine A1 receptors in angiotensin II-induced renal vasoconstriction. Accordingly, responses of angiotensin II to renal blood flow were examined during treatments with 1) exogenous adenosine; 2) dipyridamole, which blocks the cellular uptake of adenosine resulting in an increase in endogenous adenosine level (Heistad et al., 1981; Ballarin et al., 1991; Wang et al., 1992); and 3) a selective adenosine A1 receptor antagonist, 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902) (Nonaka et al., 1996; Aki et al., 1997; Nishiyama et al., 2001a). Using a renal microdialysis method (Siragy and Linden, 1996; Nishiyama et al., 1999, 2000, 2001b; Zou et al., 1999). The dialysis membrane is made from cuprophan fiber, measuring 15 mm in length, with a 5500-Da transmembrane diffusion cut-off (Toyobo Co. Ltd., Otsu, Japan). Microdialysis probe was implanted into the renal superficial cortex. The probes were connected to a CMA/100 microinfusion pump (Carnegie Medicine, Stockholm, Sweden) and were perfused with saline solution containing iodotubercidin (10 μM), an inhibitor of adenosine kinase, and 2-chloro-4-hydroxy-3-nonyl/adenine (100 μM), an inhibitor of adenosine deaminase, at a perfusion rate of 10 μl/min (Nishiyama et al., 1999, 2001b). Samples were stored at −40°C before analysis. At a perfusion rate of 10 μl/min, the relative equilibrium rate of adenosine was 16 ± 4%, as previously described (Nishiyama et al., 1999, 2001b). At the end of each experiment, the kidney was removed and the location of the microdialysis membrane was confirmed by surgical exposure of the probe. Previous studies showed that dialysate concentrations of adenosine were elevated immediately after the implantation of the microdialysis probe, and that these concentrations were decreased within the first 30 min but remained stable thereafter for 60 min (Nishiyama et al., 1999, 2001b). Therefore, all in vivo collection experiments were started 90 min after the implantation of the microdialysis probe.

Experimental Protocols

Group 1: Effects of KW-3902 on Angiotensin II- or Norepinephrine-Induced Renal Vasoconstriction. After renal blood flow responses to increasing doses of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were determined during vehicle infusion (0.1% DMSO + 0.001 N NaOH), intrarenal infusion of KW-3902 (10 μg/kg/min) (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) was initiated (n = 7). The dose of KW-3902 was chosen on the basis of results from previous studies in dogs (Aki et al., 1997). Furthermore, preliminary experiments also showed that intrarenal infusion of KW-3902 (10 μg/kg/min) prevented renal vasoconstriction induced by exogenous administrations of adenosine (n = 4; data not shown). After a 30-min initiation of KW-3902 infusion, administrations of angiotensin II (3, 10, and 30 ng) or norepinephrine (100 and 500 ng) were repeated. Each dose of injection was separated by at least 10 min. Preliminary experiments showed no differences in RBF responses to repeat administrations of angiotensin II (3, 10, and 30 ng) or norepinephrine (100 and 500 ng) (n = 3; data not shown).

Group 2: Effects of Exogenous Adenosine on Angiotensin II- or Norepinephrine-Induced Renal Vasoconstriction. After renal blood flow responses to increasing doses of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were determined during vehicle infusion, intrarenal infusion of adenosine (5 μg/kg/min) was initiated (n = 7). Thirty minutes after starting the adenosine infusion, administrations of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were repeated. Then, KW-3902 (10 μg/kg/min) was added to adenosine infusion. Thirty minutes after starting the adenosine plus KW-3902 infusion, administrations of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were repeated. Each dose of injection was separated by at least 10 min.

Group 3: Effects of Dipyridamole on Angiotensin II or Norepinephrine-Induced Renal Vasoconstriction. After renal blood flow responses to increasing doses of angiotensin II (3, 10, and 30 ng)
and norepinephrine (100 and 500 ng) were determined during vehicle (0.005 N HCl in 5% glucose solution) infusion, intrarenal infusion of dipyridamole (10 μg/kg/min) was initiated (n = 6). Thirty minutes after starting the dipyridamole infusion, administrations of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were repeated. In a separate group of animals (n = 7), we examined the effects of dipyridamole on renal interstitial fluid concentrations of adenosine. The experimental protocol was started with dialysate fluid collections for two consecutive 5-min periods. At the end of the second control collection, dipyridamole was infused intra-arterially at a rate of 10 μg/kg/min. After 30 and 60 min of dipyridamole infusion, 5-min dialysate samples were collected, respectively.

**Group 4: Effects of Exogenous Adenosine or KW-3902 on Renal Interstitial Concentrations of Adenosine in Responses to Angiotensin II or Norepinephrine-Induced Cerebral Vasconstriction.** In these groups of animals, an electromagnetic flow probe was placed around the common carotid artery for measurement of carotid blood flow. Furthermore, angiotensin II, norepinephrine, adenosine, and KW-3902 were administered directly into the common carotid artery. After carotid blood flow responses to increasing doses of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were determined during vehicle infusion, intra-arterial infusions of adenosine (5 μg/kg/min, n = 5) or KW-3902 (10 μg/kg/min, n = 5) were initiated. Thirty minutes after starting the administrations of adenosine or KW-3902, administrations of angiotensin II (3, 10, and 30 ng) and norepinephrine (100 and 500 ng) were repeated. Each dose of injection was separated by at least 10 min.

**Group 5: Changes in Renal Interstitial Concentrations of Adenosine in Responses to Angiotensin II and Norepinephrine Administrations.** The dialysate was collected during two 5-min control periods. Thereafter, angiotensin II (3, 10, and 30 ng) was administered consecutively at 5-min intervals and the dialysate was collected (n = 6). In a separate group of animals, effects of norepinephrine (100 and 500 ng) on renal interstitial concentrations of adenosine were examined in a manner similar to that of angiotensin II (n = 6).

**Analytical Procedures**

Adenosine in the dialysate was measured as previously described (Nishiyama et al., 1999, 2000, 2001b). Briefly, 45 μl of dialysate were transferred into a microcentrifuge tube, and 130 μl of 1 mM acetate buffer (pH 4.0) and 4.5 μl of 40% chloroacetaldehyde were added. The preparation was incubated at 80°C for 1 h to allow for the conversion of adenosine to ethenoadenosine. For HPLC, a reverse-phase HPLC column (Develosil ODS HG-5, 150 × 4.6 mm i.d.) was maintained at 40°C with a column oven (655A-52; Hitachi, Tokyo, Japan). An isocratic elution with 7.5% acetonitrile in 50 mM potassium phosphate buffer (pH = 3.0) at a flow rate of 1.0 ml/min was performed with a HPLC pump (L-600; Hitachi). Then, 50 μl of sample were injected, and the elution was monitored using a fluorescence spectrometer (F-1000; Hitachi) at an excitation wavelength of 310 nm and an emission wavelength of 400 nm.

**Statistical Analysis**

Renal vasoconstriction induced by each stimulus was expressed as the percentage change calculated by the following formula: (\[(\text{minimum RBF achieved by the vasoconstrictors} - \text{preinjection RBF})/\text{preinjection RBF}\]). The values are presented as means ± S.E. Statistical comparisons of the differences were performed using the one-way or the two-way analysis of variance for repeated measures combined with Fisher's protected least significant difference. P < 0.05 was considered statistically significant.

**Results**

In all protocols, intra-arterial administrations of angiotensin II, norepinephrine, KW-3902, adenosine, and dipyridamole did not alter mean arterial pressure (data not shown).

**Effects of KW-3902 on Renal Vasoconstriction Induced by Angiotensin II or Norepinephrine.** Intrarenal administrations of angiotensin II or norepinephrine produced dose-dependent decreases in renal blood flow (Figs. 2 and 3). Consistent with previous studies (Aki et al., 1997), intrarenal injection of KW-3902 (10 μg/kg/min) increased renal blood flow transiently from 3.70 ± 0.23 to 4.07 ± 0.43 ml/min/g (at 3 min) but soon returned to the preinfusion level (3.82 ± 0.26 ml/min/g at 10 min) (Fig. 1A). As shown in Fig. 2 renal blood flow responses to both angiotensin II and norepinephrine were significantly attenuated by treatment with KW-3902.

**Results of Adenosine and KW-3902 on Renal Vasoconstriction Induced by Angiotensin II or Norepinephrine.** Intrarenal administrations of angiotensin II or norepinephrine produced dose-dependent decreases in renal blood flow (Fig. 3). Intrarenal infusion of adenosine (5 μg/kg/min) evoked a transient reduction in renal blood flow, which rapidly waned with renal blood flow returning to a value above the control level (Fig. 1B). Thereafter, renal blood flow became stable at a level that was 34% higher than control renal blood flow (from 3.47 ± 0.24 to 4.65 ± 0.31 ml/min/g at 15 min). During adenosine infusion, RBF responses to angiotensin II or epinephrine were significantly augmented (Fig. 3). After addition of KW-3902 to adenosine infusion, renal blood flow was further increased to 5.92 ± 0.39 ml/min/g. As shown in Fig. 3, KW-3902 completely prevented augmentation of

Fig. 1. Effects of intrarenal infusion of KW-3902 (10 μg/kg/min; A) and adenosine (5 μg/kg/min; B) on RBF. * P < 0.05 versus 0 min. n = 7, respectively.
renal blood flow responses to angiotensin II and norepinephrine produced by administration of adenosine.

**Effects of Dipyridamole and KW-3902 on Renal Vasocostriction Induced by Angiotensin II or Norepinephrine.** As shown in Fig. 4A, intrarenal infusion of dipyridamole for 30 min significantly increased adenosine concentrations in dialysates from 15 ± 2 to 24 ± 2 nM (n = 7). Dipyridamole did not alter renal blood flow significantly (from 3.28 ± 0.13 to 3.20 ± 0.16 ml/min/g). However, renal blood flow responses to angiotensin II or norepinephrine were significantly augmented by dipyridamole (Fig. 4, B and C). Addition of KW-3902 did not alter basal renal blood flow (from 3.25 ± 0.31 to 3.20 ± 0.16 ml/min/g); however, dipyridamole-induced augmentation of renal blood flow responses to angiotensin II and norepinephrine were reversed by KW-3902 (Fig. 4, B and C).

**Effects of Adenosine or KW-3902 on the Constriction of the Vascular Beds Drained by Common Carotid Artery Induced by Angiotensin II or Norepinephrine.** Intrarenal administrations of angiotensin II or norepinephrine into the common carotid artery produced dose-dependent decreases in carotid blood flow (Fig. 5). Intra-arterial infusion of adenosine (5 µg/kg/min) significantly increased carotid blood flow from 93 ± 12 to 222 ± 47 ml/min, but did not modify the vasoconstrictor actions of angiotensin II or norepinephrine (Fig. 5).

Intra-arterial infusion of KW-3902 (10 µg/kg/min) did not alter basal carotid blood flow (from 111 ± 17 to 110 ± 17 ml/min). Furthermore, KW-3902 did not affect the vasoconstrictor actions of angiotensin II or norepinephrine (data not shown).

**Effects of Angiotensin II or Norepinephrine on Renal Interstitial Concentrations of Adenosine.** Intrarenal administrations of angiotensin II or norepinephrine produced dose-dependent decreases in RBF (data not shown). As shown in Fig. 6, basal concentrations of adenosine in dialysates tended to be increased in response to administrations of angiotensin II, but these changes were not statistically significant (by 17 ± 11% from 19 ± 3 to 24 ± 4 nM). Similarly, norepinephrine administrations did not alter adenosine concentrations significantly (by 9 ± 4% from 16 ± 3 to 19 ± 3 nM; Fig. 6B).
The results from the present study confirm previous observations that renal vasoconstrictor actions of angiotensin II were augmented by intra-arterial infusion of exogenous adenosine in anesthetized dogs (Hall and Granger, 1986). Furthermore, the present data extend these observations to indicate that adenosine also augments norepinephrine-induced renal vasoconstriction. These effects of adenosine were completely prevented by a selective adenosine A1 receptor antagonist, KW-3902. These results suggest that adenosine amplifies renal vasoconstrictor effects of both angiotensin II and norepinephrine via adenosine A1 receptors. We also investigated renal vascular responsiveness of angiotensin II and norepinephrine in kidneys treated with dipyridamole that blocks the cellular uptake and deamination of adenosine (Heistad et al., 1981; Ballarin et al., 1991; Wang et al., 1992). Consistent with the results from the studies using a microdialysis method in the heart (Wang et al., 1992), dipyridamole resulted in significant increases in renal interstitial fluid adenosine levels. We also observed that renal vasoconstrictor actions of angiotensin II and norepinephrine were significantly augmented by dipyridamole, and that KW-3902 prevented these effects of dipyridamole. These data support the hypothesis that endogenous adenosine levels influence both angiotensin II and norepinephrine-mediated renal vasoconstriction via adenosine A1 receptors. To support this hypothesis further, it will be necessary to determine the effects of reductions in basal renal interstitial adenosine levels on renal vascular responsiveness of angiotensin II and norepinephrine.

Several investigators suggest that renal interstitial adenosine level reflects vascular responsiveness of angiotensin II (Hall et al., 1985; Carmines and Inscho, 1994; Weihprecht et al., 1994; Navar et al., 1996). Therefore, we hypothesized that accumulation of renal interstitial adenosine due to angiotensin II- and norepinephrine-induced vasoconstriction further augments the vasoconstrictor responses to angiotensin II. In the present study, however, we observed that neither angiotensin II nor norepinephrine administrations altered renal interstitial fluid concentrations of adenosine.
Thus, these data provide no support for the hypothesis that de novo formation of adenosine due to angiotensin II- and norepinephrine-induced ischemia causes additive vasoconstriction or modulates angiotensin II- and norepinephrine-induced vasoconstriction. In the present study, we collected dialysate samples for 5 min. Therefore, it is possible that short dialysate collection fails to detect the actual angiotensin II- and norepinephrine-induced changes in renal interstitial adenosine. It is also possible that the implantation of a microdialysis probe may influence renal interstitial adenosine levels. We recently demonstrated that renal interstitial concentrations of adenosine increased 30-fold after 5 to 10 min of renal ischemia (Nishiyama et al., 2001b). These data suggest that rapid changes in renal interstitial adenosine levels can be detectable using this technique. Furthermore, our recent data showed that dialysate contamination by tubular fluid and plasma is minimal (Nishiyama et al., 2002). However, future experiments are needed to determine the alterations of renal interstitial adenosine levels in response to intra-arterial infusion, instead of injection, of angiotensin II and norepinephrine to increase the collection time. Furthermore, we cannot exclude the possibility that renal interstitial adenosine levels are influenced by micro-injury due to the implantation of microdialysis probes in the present experimental settings.

It has been suggested that synergistic interactions between adenosine and angiotensin II are mediated through the cross talk between adenosine A1 receptors and receptors coupled to phospholipase C (Ardaillou et al., 1992; Dickenson and Hill, 1994). Furthermore, recent studies have demonstrated that adenosine increases cytosolic free calcium concentration along the entire length of the afferent arteriole from the rabbit kidney (Gutierrez et al., 1999). Clearly, further studies are needed to determine the intracellular interactions between adenosine and angiotensin II or norepinephrine.

Consistent with previous reports (Heistad et al., 1981; Wang et al., 1992), adenosine administration into the common carotid artery did not show any vasoconstriction but significantly increased carotid blood flow. We also observed that vasoconstrictor actions of angiotensin II or norepinephrine in carotid blood flow were not affected by treatment with adenosine or KW-3902. Thus, it seems likely that angiotensin II- and norepinephrine-induced vasoconstriction of the area drained by the common carotid artery are not modulated by adenosine A1 receptor. Previous studies also showed that exogenously administered adenosine attenuated rather than augmented angiotensin II-induced mesenteric vasoconstriction (Holycross and Jackson, 1989). Collectively, these data suggest the possibility that adenosine A1 receptor-mediated augmentation of angiotensin II-induced vasoconstriction is somehow specific for the renal vascular beds.

In the present study, we observed that both adenosine and dipyridamole augmented norepinephrine-induced reductions in renal blood flow, and these effects of adenosine and dipyridamole were prevented by KW-3902. However, Weihprecht et al. (1994) performed micropuncture studies and showed that peritubular infusion of a selective adenosine A1 receptor antagonist, CPX, did not block the fall in stop-flow pressure induced by norepinephrine, indicating that A1 receptor is not involved in norepinephrine-induced afferent arteriolar constriction. The reason for the discrepancy between these results is not clear; however, it is important to note that adenosine has been shown to inhibit the norepinephrine release evoked by sympathetic nerve stimulation but has a stimulatory effect on the postsynaptic response in the kidney (Hedqvist and Fredholm, 1976). In the present study, we examined experiments in denervated dog kidneys, whereas Weihprecht et al. (1994) performed micropuncture studies in innervated rat kidneys. Therefore, it is possible that adenosine-induced inhibition of norepinephrine release would mask part of the adenosine-induced potentiation of vascular responses to norepinephrine in innervated kidneys.

Consistent with previous reports (Hall and Granger, 1986; Aki et al., 1990), the present data showed that intrarenal infusion of adenosine evoked a transient reduction in renal blood flow that rapidly waned, with renal blood flow returning to a value above the control level. We also observed that renal blood flow was further increased after addition of KW-3902 to adenosine infusion. These results are consistent with in vitro observations that adenosine dilates renal microvasculature in the presence of the adenosine A1 receptor antagonists (Nishiyama et al., 2001a). Recent studies also have shown that afferent (Tang et al., 1999; Nishiyama et al., 2001a) and efferent (Nishiyama et al., 2001a) arteriolar vasodilatory responses to adenosine during adenosine A1 receptor blockade were significantly attenuated by adenosine A2a receptor inhibition. Thus, these data as well as the results
from the present study support the concept that adenosine A3 receptor-mediated vasodilation may buffer adenosine A1 receptor-mediated vasoconstriction in the kidney.

In conclusion, the results from the present study suggest that basal renal interstitial adenosine levels influence vascular responses to angiotensin II and norepinephrine via adenosine A1 receptor in the kidney, but not in the area drained by the common carotid artery. However, adenosine-induced augmentation against angiotensin II- and norepinephrine-induced renal vasoconstriction may not be mediated through de novo adenosine accumulation in the kidney due to angiotensin II- and norepinephrine-induced renal vasoconstriction.

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