Blood Pressure–Dependent Inhibition of Renin Secretion Requires A1 Adenosine Receptors

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Abstract—Renal perfusion pressure (RPP) regulates renin release with a reduction of RPP stimulating and an elevation inhibiting renin secretion. The precise sensing and effector mechanisms by which changes in arterial pressure are linked to the exocytosis of renin are not well-defined. The present experiments were designed to study the potential role of adenosine as a mediator of this renal baroreceptor mechanism. In isolated perfused mouse kidneys a stepwise reduction of RPP from 90 mm Hg to 65 and 40 mm Hg stimulated renin secretion rates (RSR) 1.4-fold and 3.6-fold, whereas stepwise elevations of RPP from 90 mm Hg to 115 and 140 mm Hg suppressed RSR to 64% or 40% of baseline. Inactivation of A1 adenosine receptors by either pharmacological blockade (DPCPX 1 μmol/L) or genetic deletion (A1AR−/− mice) did not modify the stimulation of renin release by a low RPP, but completely prevented the suppression of renin secretion by higher perfusion pressures. In vivo, the induction of arterial hypertension by either acute (single subcutaneous injection) or chronic (osmotic minipump for 72 hours) application of phenylephrine significantly reduced plasma renin concentration (PRC) in wild-type mice to ~40% of control, whereas it did not significantly affect PRC in A1AR−/− mice. Together these data demonstrate that A1 adenosine receptors are indispensable for the inhibition of renin secretion by an increase in blood pressure, suggesting that formation and action of adenosine is responsible for baroreceptor-mediated inhibition of renin release. In contrast, the stimulation of the renin system by a low blood pressure appears to follow different pathways. (Hypertension. 2005;46:780-786.)

Key Words: kidney ◊ phenylephrine ◊ prostaglandins

The renin-angiotensin-aldosterone-system is a central regulator of arterial blood pressure with an activation of the renin-angiotensin-aldosterone system cascade elevating and a suppression lowering it. As part of a homeostatic feedback circuit, blood pressure in turn affects the synthesis and release of renin from the juxtaglomerular cells of the kidney, with an increase of blood pressure inhibiting and a decline of blood pressure stimulating the renin system. So far, the functional mechanisms underlying this so-called renal baroreceptor mechanism are not completely identified.

Previous studies have suggested that either the renin producing juxtaglomerular cells themselves or the macula densa may be the site where perfusion pressure is translated into a signal controlling renin synthesis and secretion. Moreover, several studies suggested that the cyclooxygenase products prostaglandin E2 and prostacyclin are involved in the renal baroreceptor mechanism. However, because blockade of prostaglandin formation did not attenuate the pressure dependent regulation of the renin system in other investigations, the precise role of prostanoids in the renal baroreceptor mechanism is not entirely clear.

The nucleoside adenosine has been shown to mediate macula densa control of glomerular filtration (tubuloglomerular feedback [TGF] mechanism), a mechanism in which high luminal NaCl concentrations cause an increment in glomerular resistance by activation of A1 adenosine receptors (A1AR). Aside from controlling glomerular filtration, the TGF mechanism has also been implicated in autoregulation, the adjustments of renal vascular resistance by changes in perfusion pressure. Because the macula densa controls not only glomerular filtration but also renin secretion, it seems reasonable to speculate that activation of A1AR may be involved in pressure-dependent inhibition of renin secretion. In fact, adenosine is a potent inhibitor of the renin system, an effect directly mediated at the level of the renin producing juxtaglomerular cells by activation A1AR. Moreover, previous reports have demonstrated an inhibitory role of adenosine on plasma renin activity in rats subjected to renovascular hypertension, suggesting an involvement of adenosine in the regulation of the renin system by arterial blood pressure. Therefore, the present study was performed to investigate the role of A1AR in the regulation of the renin secretion by renal perfusion pressure. By using the isolated perfused mouse kidney model, the relation between perfusion pressure and renin secretion could be examined without the application of any vasoactive substance that potentially could...
directly modulate renin release. Moreover, the effect of systemic counter-regulatory responses, for instance, of a change in sympathetic nervous tone resulting from the variations of blood pressure, does not have to be considered in the isolated perfused kidney model.

**Methods**

**A1AR Knockout Mice**

The A1AR knockout mice were generated as described by Sun et al. The homozygous and wild-type mice used in this study were derived from heterozygous breeder pairs. All animal experiments were performed according to the guidelines for the care and use of laboratory animals published by the US National Institutes of Health and were approved by the local ethics committee.

**Isolated Perfused Mouse Kidney**

Homozygous A1AR knockout mice (A1AR<sup>−/−</sup>) and their wild type littermates (A1AR<sup>+/−</sup>) (23 to 30 g body weight) of either sex, or male C57BL/6 mice (Charles River, Germany) were used as kidney donors. The isolated perfused mouse kidney model has been described in detail previously. Briefly, the animals were anesthetized with an intraperitoneal injection of 12 mg/kg xylazine (Rompun, Bayer, Germany) and 80 mg/kg ketamine-HCl (Curamed, Germany), the abdominal aorta was cannulated, the right kidney was excised, placed in a thermostated moistening chamber and perfused at constant pressure (90 mm Hg). Using an electronic feedback control, perfusion pressure could by changed and held constant in a pressure range between 40 and 140 mm Hg. Finally, the renal vein was cannulated and the venous effluent was collected for determination of renin activity and venous blood flow.

The basic perfusion medium consisted of a modified Krebs-Henseleit solution supplemented with 6 g/100 mL bovine serum albumin and with freshly washed human red blood cells (10% hematocrit). Renin activity in the venous effluent was determined by radioimmunoassay (Byk & DiaSorin Diagnostics, Germany) as described previously. Renin secretion rates were calculated as the product of the renin activity and the venous flow rate [mL/min×g kidney weight].

**In Vivo Experiments**

A1AR<sup>−/−</sup> and A1AR<sup>+/+</sup> mice of either sex (25 to 30 g body weight) were used for the in vivo experiments.

In the acute experiments, blood pressure was increased by a single subcutaneous injection of phenylephrine hydrochloride (Sigma) 1.6 mg/kg. Vehicle injected mice (0.9% NaCl) served as controls; 30 minutes after injection the mice were placed into a holding device and venous blood samples (75 μL) were taken from the tail vein into hematocrit tubes containing 1 μL of 125 mmol/L EDTA to prevent clotting. The blood pressure response to subcutaneous phenylephrine injection was measured invasively in anesthetized mice in an additional set of A1AR<sup>−/−</sup> and A1AR<sup>+/+</sup> mice (12 mg/kg xylazine and 80 mg/kg ketamine-HCl, cannulation of the Arteria carotis) because a noninvasive determination by tail-cuff manometry was not applicable because of marked vasoconstriction induced by phenylephrine and the resulting loss of a pulse signal.

To determine chronic effects of an elevation of arterial blood pressure on the plasma renin activity, phenylephrine 1.6 mg/kg per hour was infused via osmotic minipumps (Alzet Corp, purchased via Charles River) inserted subcutaneously into A1AR<sup>−/−</sup> and A1AR<sup>+/+</sup> mice under sevoflurane anesthesia (Sevorane; Abbott) using a standard surgical approach. Blood pressure response to phenylephrine infusion was determined by tail-cuff manometry (TSE, Germany). Mice were conditioned by placing them into a holding device on 5 consecutive days before the first blood pressure determination.

After 72 hours of infusion (40 μg/kg body weight per hour), blood samples were taken from the tail vein for determination of plasma renin concentration. Thereafter, the animals were deeply anesthetized with sevoflurane and killed by surgical dislocation.

For determination of plasma renin concentration, plasma samples were incubated for 1.5 hours at 37°C with plasma from bilaterally nephrectomized male rats as renin substrate. The generated angiotensin I [ng/mL×h<sup>−1</sup>] was determined by radioimmunoassay (Byk & DiaSorin Diagnostics, Germany).

**Statistical Analysis**

Values are given as mean±SEM. Differences between groups were analyzed by ANOVA and Bonferroni adjustment for multiple comparisons. In the isolated perfused kidney experiments, the last 2 values obtained within an experimental period were averaged and used for statistical analysis. Student paired t test was used to calculate levels of significance within individual kidneys. P<0.05 was considered statistically significant.

**Results**

**Role of A1AR in Pressure-Dependent Renin Secretion of Isolated Perfused Mouse Kidneys**

In kidneys from C57BL/6 mice, lowering of the renal perfusion pressure (RPP) from 90 mm Hg (control pressure) to 40 mm Hg caused a prompt stimulation of renin secretion rate by a factor of 3.6 (Figure 1; P<0.001). Subsequent elevation of the perfusion pressure to 65 mm Hg partially reversed this stimulation, but renin secretion rate remained elevated 1.4-fold compared with control pressure (P<0.01). The adjustment of RPP to 90 mm Hg completely reversed renin secretion rates back to control values. A further increase of perfusion pressure to 115 mm Hg and finally to 140 mm Hg suppressed renin secretion rates to 64% (P<0.01) and 40% (P<0.01) of control values, respectively (Figure 1).
To test for the role of A1ARs in the pressure-dependent control of renin secretion, A1ARs were inactivated by either pharmacological blockade (DPCPX 1 μM/L) or genetic deletion (A1AR knockout mice), and renal perfusion pressure was changed in a stepwise fashion from 40 mm Hg to 140 mm Hg as depicted in Figure 1. As shown in Figure 2, the inactivation of A1ARs did not alter the stimulation of renin secretion rates by low perfusion pressures. Thus, lowering the perfusion pressure to 40 or 65 mm Hg stimulated renin secretion rates to the same extent in kidneys treated with 1 μM/L DPCPX (Figure 2, upper panel, black circles) and vehicle-treated controls (white circles). Moreover, at low perfusion pressures, no differences in renin release were detectable between isolated perfused kidneys of A1AR knockout mice and their wild-type littermates (Figure 2, lower panel). In contrast the inhibition of renin secretion rates by a high perfusion pressure was completely abrogated by pharmacological blockade or genetic deletion of A1AR (Figure 2). While the elevation of renal perfusion pressure from 90 to 115 or 140 mm Hg reduced renin secretion rates to 65% or 40% in control kidneys and to 45% or 30% in kidneys of A1AR+/− mice, this marked inhibition of renin release was completely prevented by either pharmacological blockade (Figure 2, upper panel) or genetic deletion (Figure 2, lower panel) of A1ARs.

To confirm the requirement of A1AR for the pressure-dependent inhibition of renin release, additional experiments were performed in which RPP was elevated from the control pressure of 90 mm Hg to either 115 mm Hg (Figure 3 upper) or 140 mm Hg (Figure 3 lower) without previous lowering of the perfusion pressure. Whereas the increase of RPP to 115 mm Hg or 140 mm Hg suppressed renin secretion rates to 60% or 50% of values at control pressure in kidneys of wild-type mice (Figure 3, white circles), this inhibition was blunted in kidneys of A1AR knockout mice (Figure 3, black circles) or by the application of DPCPX (Figure 3, black squares).

These results demonstrate an indispensable requirement of A1AR for pressure-dependent inhibition of the renin secretion, but they also suggest that the stimulation of renin release by a reduction of perfusion pressure appears to be regulated through an independent pathway. Because prostaglandins...
have been suggested to be involved in the stimulatory effects of a low renal perfusion pressure on the renin system, we next examined the effects of a blockade of prostanoid formation using indomethacin (10 μmol/L) in isolated perfused kidneys of wild-type mice (Figure 4, black circles). Indomethacin had no significant effect on renin secretion at the control pressure of 90 mm Hg. However, whereas a lowering of perfusion pressure stimulated renin secretion of untreated kidneys by a factor of 3.6 (P<0.05 compared with control pressure; white circles), this effect was reduced to a factor of 1.9 in indomethacin-treated kidneys of both A1AR+/+/+ and A1AR−/− mice (P<0.05 compared with control pressure; black symbols). In contrast, the blockade of prostanoid formation did not attenuate the inhibition of renin secretion by high renal perfusion pressure in mice with intact A1AR (Figure 4). The combination of a blockade of prostaglandin formation with genetic inactivation of A1AR markedly buffered pressure-dependent renin secretion over the entire pressure range tested (Figure 4, black squares) because the stimulation of renin secretion rate by a reduction of RPP to 40 or 65 mm Hg was significantly attenuated and the inhibition of renin release by a high perfusion pressure was again completely abolished.

Role of A1ARs in the Pressure-Dependent Inhibition of Renin Release In Vivo
There are several well-established models to induce arterial hypertension in experimental animals such as the angiotensin II-dependent 2-kidney 1-clip model, the infusion of angiotensin II, or the blockade of NO synthesis. However, these approaches are not applicable to investigate the specific effects of an increase of blood pressure on renin secretion, because these experimental maneuvers not only inhibit the renin system by increasing blood pressure but also by directly affecting the renin-producing cells. Therefore, an experimental approach that would allow elevating arterial blood pressure without directly inhibiting the renin system was established.

As shown in Figure 5, both angiotensin II (1 nmol/L) as well as the NO synthase inhibitor L-NAME (1 mmol/L) reduced the perfusate flow of isolated kidneys perfused at constant pressure demonstrating their powerful vasoconstrictor effects. However, both agents significantly suppressed renin secretion rates even at constant perfusion pressure, suggesting that both agents exert direct inhibitory effects on the renin-producing cells. In contrast, the α-adrenoceptor

![Graph showing renin secretion rate vs perfusion pressure](http://hyper.ahajournals.org/)

**Figure 4.** Role of prostaglandins in the renal baroreceptor mechanism in isolated perfused kidneys from A1 adenosine receptor knockout mice. Prostaglandin synthesis was inhibited by adding indomethacin 10 μmol/L to the perfusate. Subsequently, renal perfusion pressure was changed in a stepwise manner in isolated perfused kidneys from A1 adenosine receptor wild-type mice (A1AR+/+ Indo, black circles, n=5) and knockout mice (A1AR−/− Indo, black squares, n=5). Kidneys from A1AR wild-type mice without treatment (A1AR+/+ , white circles, also shown in Figure 2) are shown for comparison.

*P<0.05 vs 90 mm Hg. #P<0.05 vs A1AR+/+ without indomethacin.

![Graph showing effects of angiotensin II, L-NAME, or phenylephrine on perfusate flow and renin secretion rates](http://hyper.ahajournals.org/)

**Figure 5.** Effects of angiotensin II, L-NAME, or phenylephrine on perfusate flow and renin secretion rates of isolated perfused mouse kidneys. Either angiotensin II 1 nmol/L (*, n=5), phenylephrine 100 nmol/L (black circles, n=4), or L-NAME 1 mmol/L (white circles, n=4) were infused in isolated kidneys of C57BL/6 mice perfused at a constant pressure of 100 mm Hg. Upper panel shows the perfusate flow rate, lower panel the renin secretion rate. *P<0.05 vs control period.
agonist phenylephrine (100 nmol/L) did not inhibit renin release at constant perfusion pressure (Figure 5), although it induced significant vasoconstriction. Therefore, phenylephrine was used for elevating blood pressure in our in vivo studies.

The plasma renin concentration (PRC) after subcutaneous injection of 40 μL 0.9% NaCl (vehicle) was significantly higher in female and male A1AR−/− mice (females 464±57 ng angI/h×mL, n=12; males 950±60 ng angI/h×mL, n=5) compared with their wild-type littermates (females 297±30 ng angI/h×mL, n=14, P<0.05 versus A1AR−/−; males 590±100 ng angI/h×mL, n=5, P<0.05 versus A1AR−/−) (Figure 6). A single subcutaneous injection of phenylephrine (1.6 mg/kg body weight) significantly elevated the blood pressure to the same extent in A1AR−/− (from 106.9±4.6 to 138.4±5.6 mm Hg, P<0.01) and A1AR−/− (from 101.0±2.6 to 135.6±5.8 mm Hg, P<0.01). This marked increase of blood pressure was paralleled by a significant suppression of plasma renin concentration (PRC) in conscious female A1AR−/− mice (121±22, n=11 versus 297±30 ng angI/h×mL in vehicle injected females, P<0.05) as well as male A1AR+/+ mice (119±41, n=5 versus 590±100 ng angI/h×mL, P<0.01). In contrast in A1AR−/− mice the increase of blood pressure did not significantly suppress PRC (females 359±48, n=12 versus 464±57 ng angI/h×mL; not significant; males 878±96, n=5 versus 950±60 ng angI/h×mL; not significant) (Figure 6).

A more prolonged phenylephrine infusion over 72 hours (1.6 mg/kg per hour) in female mice significantly elevated the blood pressure to the same extent in conscious A1AR−/− (from 127.7±1.7 mm Hg to 135.9±1.8 mm Hg, P<0.05) and A1AR−/− (from 130.5±3.2 mm Hg to 139.5±4.3 mm Hg, P<0.05). However, the arterial hypertension did not suppress plasma renin concentration (PRC) in A1AR knockout mice, whereas it markedly inhibited PRC to 40% of vehicle infused controls in A1AR−/− mice (Figure 7), further underlining the critical requirement of A1AR for the inhibition of renin secretion by high renal perfusion pressure.

**Discussion**

Although pressure-dependent regulation of renin secretion is a well-established phenomenon, the intrarenal mechanisms underlying so-called baroreceptor control of renin release are not well understood. The present study for the first time demonstrates that adenosine, signaling via A1AR, is critical for the inhibition of renin secretion after elevation of renal perfusion pressure both in vitro and in vivo. In isolated perfused mouse kidneys, the suppression of renin secretion by directly increasing the perfusion pressure was completely abrogated by pharmacological blockade or by genetic deletion of A1AR. Moreover, in vivo both acute and prolonged hypertension after application of phenylephrine markedly reduced PRC in wild-type mice, whereas it did not alter PRC in A1AR knockout mice.

The dominant effect of adenosine is an inhibition of renin release that reflects the direct effect of A1AR activation. For example, application of selective A1AR agonists suppress renin secretion in vivo and in vitro,16,21,22 whereas blockade of these receptors stimulate renin release.17,23 Our finding of a higher plasma renin concentration in A1AR receptor knockout mice (A1AR−−) compared with their wild-type littermates is compatible with such an inhibitory effect of adenosine on the renin system and confirms the results of a previous study using an independently generated strain of A1AR knockout mice.24 It is of note, however, that renin secretion rates at a perfusion pressure of 90 mm Hg were not significantly different between isolated perfused kidneys from...
Figure 7. Effects of prolonged phenylephrine infusion on blood pressure and plasma renin concentration of A1 adenosine receptor knockout mice. Phenylephrine (1.6 mg/kg per hour) or 0.9% NaCl as vehicle were infused by osmotic minipumps for 72 hours in female A1AR+/+ (n=5 each treatment) and A1AR−/− (n=6 each treatment). Systolic blood pressure (upper panel) and plasma renin concentration (lower panel) were determined at the last day of phenylephrine infusion.

A1AR+/+ and A1AR−/− mice. Absence of a systematic difference in PRC between A1AR−/− and A1AR+/+ has also been reported in another study.24 Thus, the impact of A1AR deficiency on renin secretion under resting conditions appears to be modulated by uncontrolled factors. Importantly, it has to be noted that A1AR−/− mice carry 2 renin genes, whereas A1AR+/+ only possess 1 renin gene,21,25 a genetic consequence of using embryonic stem cells of a mouse strain harboring 2 renin gene strain (C57BL/6).21 Although the number of renin genes does not systematically correlate with PRC,25 the different number of renin genes between A1AR−/− and A1AR+/+ mice is a striking example for a difference in genetic background that may complicate comparisons of the absolute values of PRC between knockout and wild-type mice. However, because in the present study relative changes were compared within the genotypes, and because the pharmacological blockade of A1AR confirmed the results obtained from A1AR knockout mice, the different number of renin genes does not limit the main conclusion of our study that A1AR are critical for pressure-dependent inhibition of renin release.

Interestingly, the role of adenosine in the renal baroreceptor mechanism seems to be restricted to the inhibitory response, whereas the stimulation of renin release by a low renal perfusion pressure was not attenuated by either pharmacological blockade or genetic deletion of A1AR. The implication of this finding is that A1AR-independent factors must be responsible for the stimulation of renin secretion by a reduction of perfusion pressure. Results of previous investigations indicate that prostaglandins are one of these renin-stimulatory factors activated by a low perfusion pressure.6–10 Similar to these studies, the blockade of prostaglandin formation by indomethacin attenuated the stimulation of renin release by a low perfusion pressure without completely abrogating it. This either might be related to an incomplete pharmacological blockade of cyclooxygenase activity or it may indicate that other renal autacoids, such as nitric oxide,6,26,27 are also involved in this process.

Perspectives

A1ARs are critical for the inhibition of renin release by a high renal perfusion pressure. Because the renin angiotensin aldosterone cascade itself centrally regulates blood pressure, the pathophysiological role of A1AR in the development of the renin dependent forms of arterial hypertension remains to be clarified in future studies. Moreover, our data raise the question for the source of adenosine acting on A1AR and suppressing renin release. Both macula densa cells28 and the renin-producing juxtaglomerular cells themselves29 have been demonstrated to release ATP and a high correlation between renal artery pressure and the interstitial ATP concentration has been found in the renal cortex of dogs.30 Because ATP can be degraded extracellularly to 5′-AMP and finally to adenosine by ecto-5′-nucleotidase,31,32 a signaling pathway involving the release of ATP from either macula densa cells or the juxtaglomerular cells finally resulting in an increase of the adenosine concentration might mediate the inhibition of renin release by an increase of perfusion pressure. Therefore, the identification of the source of adenosine or ATP will be matter of future research.

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References


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