Preserved macula densa-dependent renin secretion in A₁ adenosine receptor knockout mice

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Schweda, Frank, Charlotte Wagner, Bernhard K. Krämer, Jürgen Schnermann, and Armin Kurtz. Preserved macula densa-dependent renin secretion in A₁ adenosine receptor knockout mice. Am J Physiol Renal Physiol 284: F770–F777, 2003. First published December 10, 2002; 10.1152/ajprenal.00280.2002.—Recent studies demonstrated that the influence of the macula densa on glomerular filtration is abolished in adenosine A₁ receptor (A₁AR) knockout mice. Inasmuch as the macula densa not only regulates glomerular filtration but also controls the activity of the renin system, the present study aimed to determine the role of the A₁AR in macula densa control of renin synthesis and secretion. Although a high-salt diet over 1 wk suppressed renin mRNA expression and renal renin content to similar degrees in A₁AR+/+, A₁AR−/−, and A₁AR−/− mice, stimulation of Ren-1 mRNA expression and renal renin content by salt restriction was markedly enhanced in A₁AR−/− compared with wild-type mice. Pharmacological blockade of macula densa salt transport with loop diuretics stimulated renin expression in vivo (treatment with furosemide at 1.2 mg/day for 6 days) and renin secretion in isolated perfused mouse kidneys (treatment with 100 μM bumetanide) in all three genotypes to the same extent. Taken together, our data are consistent with the concept of a tonic inhibitory role of the A₁AR in the renin system, whereas they indicate that the A₁AR is not indispensable in macula densa control of the renin system.

The kidneys play a key role in maintenance of fluid and electrolyte balance of the body as well as in blood pressure regulation. Multiple extra- and intrarenal factors cooperate in the adjustments of renal function that underlie body fluid homeostasis. A specific intrarenal control element for NaCl excretion is the juxtaglomerular apparatus, the anatomic substrate of a mechanism in which changes in tubular salt delivery are sensed and translated to changes in afferent arteriolar tone (tubuloglomerular feedback [TGF]) and renin synthesis and secretion (macula densa-mediated renin release). An increase in NaCl concentration at the macula densa results in vasoconstriction of the afferent arteriole, reducing glomerular filtration rate and tubular salt load, and inhibition of the renin-angiotensin system; a decrease in macula densa NaCl concentration has the opposite effect. The nature of the extracellular signaling events between macula densa cells and vascular smooth muscle or renin-producing effector cells is still a matter of debate. Besides cyclooxygenase-2-derived prostanoids (3, 9, 10, 12, 16, 31), the nucleoside adenosine has been proposed to be centrally involved in macula densa control of the renin system and glomerular filtration, inasmuch as adenosine is an inhibitor of the renin system as well as a vasoconstrictor of the afferent arteriole (13, 14), both effects being mediated by the A₁ adenosine receptor (A₁AR). The inhibitory effect of the A₁AR on the renin system has been demonstrated in vitro and in vivo, inasmuch as selective A₁AR agonists suppress renin secretion and pharmacological blockade of the A₁AR results in stimulation of renin secretion (1, 5, 6, 17, 24). The putative role of adenosine in the salt-dependent regulation of the renin system is underlined by several studies suggesting a relationship between tubular salt load and adenosine concentration in the kidney. Infusion of hypertonic saline or a high dietary sodium intake, both of which are associated with an inhibition of the renin system, led to elevated adenosine concentrations in the kidney (23, 27, 37). In contrast, dietary sodium restriction, known to stimulate the renin system, resulted in reduced renal interstitial concentrations of adenosine (27). Therefore, an increase in adenosine concentrations due to a high tubular salt load could mediate vasoconstriction and inhibition of the renin system, whereas a decrease in renal adenosine concentration resulting from a reduced salt load could cause vasodilatation and stimulation of the renin system. The A₁AR agonist cyclohexyladenosine (CHA) suppressed stimulation of renin secretion in response to a perfusion medium containing a low NaCl concentration in the isolated juxtamedullary apparatus, and blockade of the A₁AR diminished the reduction in renin secretion caused by high luminal NaCl concentrations (35), supporting the involvement of adenosine in macula densa control of the renin system. However, in a...
similar experimental setup, application of exogenous adenosine did not fully mimic the inhibitory effects of increasing tubular NaCl concentration on renin secretion (19), which would be expected from a mediator of macula densa control of the renin system.

Recent investigations have provided direct evidence that adenosine is required for the vasconstrictive action caused by TGF. Consistent with earlier studies showing that inhibition of adenosine production (29) or selective blockade of the A1AR (26, 36) blunts TGF, mice with a genetic deletion of the A1AR lack the TGF response to an increase in tubular NaCl concentration (2, 28). Inasmuch as the effector cells of the TGF, namely, the vascular smooth muscle cells of the afferent arteriole, are located in the immediate vicinity of the renin-producing juxtaglomerular cells, it is reasonable to assume that adenosine mediating the TGF also influences the renin system. Therefore, the present experiments were performed to determine whether adenosine and A1AR may be centrally involved in macula densa control of the renin system. Utilizing A1AR knockout mice, we determined whether the absence of the A1AR is associated with altered expression or secretion of renin consistent with tonic inhibition of the renin-angiotensin system by adenosine. Furthermore, the macula densa mechanism is believed to be critically involved in adjustment of the renin system to different salt loads of the body, with a high sodium intake inhibiting and a low sodium intake stimulating the renin system (8, 18, 25, 33). Pharmacological blockade of macula densa NaCl transport with loop diuretics is an intervention that, similar to a low-salt diet, stimulates the renin-angiotensin system by diminishing the NaCl transport-dependent, renin-inhibitory signal to the granular cells (11). Therefore, we investigated the influence of a high- and a low-salt diet and furosemide on the renin system in mice with a genetic deletion of the A1AR. Finally, to assess the more acute modulation of renin secretion by the macula densa, we investigated the effects of the loop diuretic bumetanide on the rate of renin secretion in isolated perfused kidneys of A1AR knockout mice and their wild-type controls. The isolated perfused kidney model is ideally suited to investigate renin secretion in the absence of interindividual differences in systemic factors that may influence the renin system, such as variations in blood pressure or renal sympathetic nerve activity, for example.

MATERIALS AND METHODS

A1AR knockout mice. A1AR knockout mice were generated as described by Sun et al. (28). The mice were derived from two heterozygous breeder pairs. For genotyping, tail biopsies were performed, and DNA was extracted and tested for the presence of wild-type and mutant genes using A1AR- and Neo-R-specific PCR primers (28).

Experimental procedures in vivo. In the first set of experiments, 10 mice of each genotype (A1AR+/-, A1AR-/-, and A1AR-/-, 20–24 g body wt) were fed a low-salt (0.02% NaCl) or a high-salt (8% NaCl) diet for 7 days. As controls, 10 mice of each genotype were fed normal mouse chow (0.6% NaCl).

In the second set of experiments, five mice of each genotype were treated with furosemide (1.2 mg/day; Dimazan, Intervert) administered via osmotic minipumps (Alzet, Durect) for 6 days. As controls, five mice of each genotype were infused with physiological saline. Surgical insertion of the pumps was performed under inhalation anesthesia (Sevoflurane, Abbot). The mice had free access to standard mouse chow (0.6% NaCl), tap water, and an electrolyte solution containing 0.9% NaCl and 0.1% KCl.

After the experimental periods, the animals were killed by decapitation, and blood was collected for determination of serum electrolyte concentration by flame photometry (model PFP7, Jenway, Dunmow). Kidneys were removed rapidly, frozen in liquid nitrogen, and stored at –80°C until further processing.

Determination of preprorenin mRNA and cytosolic β-actin by RNase protection assay. After isolation of total RNA from the frozen kidney using the method of Chomczynski and Sacchi (4), renin was measured by an RNase protection assay using an antisense RNA probe suitable for detecting mRNA levels from the Ren-1 and Ren-2 genes as described previously (32). Cytosolic β-actin was measured by an RNase protection assay as described elsewhere (32). For semiquantitative RNase protection assay, samples derived from kidneys to generate angiotensin I according to a modification of the method described by Norling et al. (22). Frozen kidneys were halved, homogenized in 1 ml of homogenization buffer [5% (vol/vol) glycerol, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, and 0.1 mM 4-[2-aminoethyl]benzenesulfonyl fluoride] for 30 s (Ultra-Turrax, IKA Labortechnik), and centrifuged at 4°C at 14,000 g for 5 min. The supernatants were frozen at –20°C and then thawed three times by alternating the temperature between –20°C and 4°C. Supernatants were incubated with saturating concentrations of rat renin substrate, and the generated angiotensin II immunoreactivity signals were related to those obtained for β-actin mRNA. β-Actin mRNA levels were not different between the different genotypes and the different experimental maneuvers (not shown).

Determination of renal renin content. The renal renin content was determined by measuring the capacity of homogenized kidneys to generate angiotensin I according to a modification of the method described by Norling et al. (22). Frozen kidneys were halved, homogenized in 1 ml of homogenization buffer [5% (vol/vol) glycerol, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, and 0.1 mM 4-[2-aminoethyl]benzenesulfonyl fluoride] for 30 s (Ultra-Turrax, IKA Labortechnik), and centrifuged at 4°C at 14,000 g for 5 min. The supernatants were frozen at –20°C and then thawed three times by alternating the temperature between –20°C and 4°C. Supernatants were incubated with saturating concentrations of rat renin substrate, and the generated angiotensin I was assayed with a commercial radioimmunoassay kit (Byk and DiaSorin).

Isolated perfused mouse kidney. Male A1AR+/-, A1AR+/-, and A1AR-/- mice (20–23 g body wt) with free access to commercial pellet chow and tap water were used as kidney donors. The animals were anesthetized with an intraperitoneal injection of 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid (100 mg/kg; Trapanal, Byk Gulden) and ketamine HCl (80 mg/kg; Curamed, Germany) and placed on a heating table. The abdominal cavity was opened by a midline incision, and the aorta was clamped distal to the right renal artery so that the perfusion of the right kidney was disturbed during the subsequent insertion of the perfusion cannula into the abdominal aorta distal to the clamp. The mesenteric artery was ligated, and a metal perfusion cannula (0.8 mm OD) was inserted into the abdominal aorta. After removal of the aortic clamp, the cannula was advanced to the origin of the right renal artery and fixed in this position. The aorta was ligated proximal to the right renal artery, and perfusion was started in situ with an initial flow rate of 1 ml/min. With the use of this technique, a significant ischemic period of the right kidney was avoided. Finally, the right kidney was placed in a thermostated moistening chamber, and perfused at constant pressure (100 mmHg). Perfusion pressure was monitored within the perfusion cannula (Isotec pressure transducer, Hugo Sachs Elektronik), and the pressure signal was used for feedback control (model SCP 704, Hugo Sachs Elektronik) of a peristaltic pump.
Finally, the renal vein was cannulated (1.5-mm-OD polypropylene catheter). The venous effluent was drained outside the moistening chamber and collected for determination of renin activity and venous blood flow.

The basic perfusion medium, supplied from a thermostated (37°C) 200-ml reservoir, consisted of a modified Krebs-Henseleit solution containing all physiological amino acids at 0.2–2.0 mM, 8.7 mM glucose, 0.3 mM pyruvate, 2.0 mM l-lactate, 1.0 mM L-ketoglutarate, 1.0 mM 1-malate, and 6.0 mM urea. The perfusate was supplemented with 6 g/100 ml bovine serum albumin, 1 mL/100 ml vasopressin 8-lysine, and freshly washed human red blood cells (10% hematocrit). Ampicillin (3 mg/100 ml) and flucloxacillin (3 mg/100 ml) were added to inhibit possible bacterial growth in the medium. To improve the functional preservation of the preparation, the perfusate was continuously dialyzed against a solution of the drugs to be tested were added to the dialysate. After constant perfusion pressure was established, perfusate flow rates usually stabilized within 15 min. Stock solutions of the drugs to be tested were added to the dialysate.

For determination of perfusate renin activity, venous effluent was collected over a period of 1 min at intervals of 3 min. The samples were centrifuged at 1,500 g for 10 min, and the supernatants were stored at −20°C until assayed for renin activity. For determination of renin activity, the perfusate samples were incubated for 1.5 h at 37°C with plasma from bilaterally nephrectomized male rats as renin substrate. The generated angiotensin I (ng·mL⁻¹·h⁻¹) was determined by radioimmunoassay (Byk and DiaSorin). Renin secretion rates were calculated as the product of the renin activity and the venous flow rate (ml·min⁻¹·g kidney wt⁻¹).

Statistical analysis. Values are means ± SE. Differences between groups were analyzed by ANOVA and Bonferroni’s adjustment for multiple comparisons. In the isolated perfused kidney experiments, all values obtained within an experimental period (n = 4) were averaged and compared with the average values of an adjoining experimental period. Student’s paired t-test was used to calculate levels of significance within individual kidneys. P < 0.05 was considered statistically significant.

RESULTS

Serum concentrations of sodium, chloride, or potassium were not different between any of the genotypes or the treatment groups (not shown).

Basal renin expression. Basal renal renin content was 1.5-fold higher in A1AR⁻/⁻ mice than in wild-type mice (Fig. 1A).

For determination of the renal renin mRNA abundance, we used an RNase protection assay with an antisense probe that was able to discriminate between Ren-1 and Ren-2 mRNA. Autoradiographic band intensity of the RNase protection assays (Fig. 1B) as well as semiquantification of renin mRNA expression by β-actin correction (Fig. 2C) revealed a similar abundance of Ren-1 mRNA in each of the genotypes. In contrast, Ren-2 mRNA expression showed distinct differences between the genotypes: whereas the expression levels of Ren-1 and Ren-2 were similar in A1AR⁻/⁻ mice, Ren-2 mRNA was not found in A1AR⁻/+ mice. A1AR⁺/⁻ mice showed an intermediate abundance of Ren-2 gene expression: Ren-2 mRNA levels were ~50% of Ren-1 mRNA levels.

Effect of a high-salt diet on renin expression. A high-salt diet for 1 wk resulted in inhibition of Ren-1 mRNA expression irrespective of the genotype (0.6-, 0.54-, and 0.66-fold of control for A1AR⁻/⁻, A1AR⁺/⁻, and A1AR⁺/+ mice, respectively, all P < 0.05; Fig. 2A). Moreover, Ren-2 mRNA levels were suppressed by the high-salt diet in the kidneys of A1AR⁻/⁻ and A1AR⁺/⁻ mice, whereas no Ren-2 mRNA signal was detectable in the
kidneys of A\textsubscript{1}AR\textsuperscript{+/+} mice (Fig. 2B). According to the changes in Ren-1 and Ren-2 gene expression, total renin mRNA expression was significantly suppressed by a high-salt diet in all three genotypes (Fig. 2C). In parallel with the changes in renin gene expression, renal renin content was lowered to 0.6-fold of control by a low-salt diet in A\textsubscript{1}AR\textsuperscript{+/+} mice (Fig. 3A).

**Effect of a low-salt diet on renin expression.** Dietary salt restriction stimulated the renin system in all three groups of animals. However, in contrast to the changes due to a high-salt diet, there were marked differences between the genotypes: stimulation was most pronounced in A\textsubscript{1}AR\textsuperscript{−/−} mice, in which Ren-1 mRNA levels increased 2.5-fold compared with control animals fed a normal-salt diet, whereas in A\textsubscript{1}AR\textsuperscript{+/+} mice only a 1.2-fold increase was detected. A\textsubscript{1}AR\textsuperscript{+/−} mice showed an intermediate stimulation of renin mRNA, with Ren-1 mRNA levels showing a twofold upregulation (Fig. 2A). Similar to the expression of Ren-1, Ren-2 mRNA expression levels were augmented by a low-sodium intake in A\textsubscript{1}AR\textsuperscript{−/−} and A\textsubscript{1}AR\textsuperscript{+/−} mice. In the kidneys of wild-type mice, no Ren-2 signal was detectable, even in animals fed the low-salt diet (Fig. 2B). As a result of the differences in Ren-2 expression, total renin mRNA was significantly higher in A\textsubscript{1}AR\textsuperscript{+/+} than in A\textsubscript{1}AR\textsuperscript{−/−} mice and wild-type controls (Fig. 2B).

In parallel with renal renin gene expression, salt restriction caused a twofold increase in renal renin content in A\textsubscript{1}AR\textsuperscript{−/−} and A\textsubscript{1}AR\textsuperscript{+/−} mice compared with control values. In contrast, in A\textsubscript{1}AR\textsuperscript{+/+} mice, in which total renin gene expression was only slightly stimulated by the low-salt diet, no significant stimulation of renal renin content was detected (Fig. 3A).

**Effect of furosemide on renin expression.** Blockade of thick ascending limb and macula densa salt transport with furosemide administration for 6 days stimulated Ren-1 expression in A\textsubscript{1}AR\textsuperscript{−/−}, A\textsubscript{1}AR\textsuperscript{+/−}, and A\textsubscript{1}AR\textsuperscript{+/+} mice (Fig. 2B). As a result of the differences in Ren-2 expression, total renin mRNA was significantly higher in A\textsubscript{1}AR\textsuperscript{+/+} than in A\textsubscript{1}AR\textsuperscript{−/−} mice and wild-type controls (Fig. 2B).

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mice to similar degrees, so that no differences in *Ren-1* expression exist between genotypes (Fig. 4). Again, *Ren-2* mRNA was not detectable in A1AR<sup>/−/−</sup> mice, whereas it was significantly stimulated in A1AR<sup>/±/±</sup> and A1AR<sup>/+/+</sup> mice (Fig. 4). Total renin mRNA expression was stimulated by furosemide in all groups, with the highest abundance detectable in the A1AR<sup>/+/+</sup> mice. Furosemide also augmented renal renin content in all three groups of mice to a similar extent, so that no significant differences were detected between the different genotypes (Fig. 3C).

**Effect of bumetanide administration on renin secretion in isolated perfused mouse kidneys.** To investigate the acute effects of loop diuretics on renin secretion, we adapted the model of the isolated perfused rat kidney to the anatomic conditions of mice. This model allows us to study the acute regulation of renin secretion without interference by confounding systemic side effects of the experimental drug or systemic counterregulations. Basal renin secretion rates of isolated perfused kidneys were similar in A1AR<sup>/−/−</sup>, A1AR<sup>/+/−</sup>, and A1AR<sup>/+/+</sup> mice (Fig. 5). Blockade of thick ascending limb and macula densa salt transport by bumetanide resulted in significant and comparable increases in renin secretion in A1AR<sup>/−/−</sup>, A1AR<sup>/+/−</sup>, and A1AR<sup>/+/+</sup> mice: 3.1-, 3.0-, and 2.8-fold of control, respectively. During subsequent administration of the A1AR agonist CHA, renin secretion rates returned to basal levels in

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**Fig. 4.** Renin mRNA expression in A1AR<sup>/−/−</sup>, A1AR<sup>/+/−</sup>, and A1AR<sup>/+/+</sup> mice treated with vehicle or furosemide. Values are means ± SE (n = 5). *P < 0.05; **P < 0.001 vs. vehicle.

**Fig. 5.** Effects of blockade of macula densa salt transport and subsequent administration of cyclohexyladenosine (CHA) on renin secretion rates of isolated perfused kidneys in A1AR<sup>/−/−</sup>, A1AR<sup>/+/−</sup>, and A1AR<sup>/+/+</sup> mice. Values are means ± SE (n = 5).
kidneys of A₁AR<sup>+/−</sup> and A₁AR<sup>−/−</sup> mice, whereas CHA was without effect in A₁AR<sup>−/−</sup> mice (Fig. 5).

**DISCUSSION**

The present experiments in A₁AR knockout mice aimed to assess the chronic role of A₁AR in the renal expression of renin under basal conditions as well as in the macula densa control of the renin system. Previous pharmacological studies provided evidence for a direct inhibitory role of adenosine on renin expression and renin secretion, an effect that appeared to be mediated by A₁AR (1, 5, 15, 17, 24, 35). The present observation that renal renin content under basal conditions is elevated in A₁AR knockout mice supports the concept of a tonic inhibition of the renin-angiotensin system through A₁AR mediation. However, besides the direct disinhibition of the renin system by A₁AR deletion, an enhanced sodium excretion reported previously in A₁AR<sup>−/−</sup> mice (2) as well as after acute pharmacological blockade of A₁AR (36) might also account, in part, for the higher renin content in A₁AR<sup>−/−</sup> mice. The finding that A₁AR<sup>−/−</sup> mice possess two renin genes (Ren-1<sup>d</sup> and Ren-2), whereas wild-type mice harbor only one renin gene (Ren-1<sup>+</sup>), and that this discrepancy is related, as discussed in detail below, to the different mouse strains used in the generation of the knockout mice somewhat complicates the straightforward interpretation of our data. Inasmuch as, in general, plasma renin activities and concentrations appear to be markedly higher in two-renin than in one-renin gene strains (20, 34), it is conceivable that the higher renin content in the A₁AR<sup>−/−</sup> animals is the consequence of their expression of Ren-1<sup>d</sup> and Ren-2. However, the differences in Ren-2 expression do not explain the marked enhancement of Ren-1 mRNA stimulation in A₁AR<sup>−/−</sup> animals by a low-salt diet, so this result further supports the concept of a tonic inhibitory role of A₁AR in the renin system.

The main intention of our study was to clarify the specific role of A₁AR in the macula densa control of the renin system. The rationale for the assumption of a central role of the A₁AR in this process was as follows: 1) adenosine inhibits the renin system via the A₁AR, 2) adenosine concentration in the kidney changes in parallel with the sodium load of the kidney, and 3) A₁AR is essentially required for control of glomerular filtration by the macula densa. According to the hypothesis that the macula densa-controlled changes in renin expression and secretion are related to salt-dependent changes in the intrarenal adenosine concentration, the amplitude of the inhibition of the renin system due to a high-salt diet or the stimulation due to a low-salt diet should be attenuated or even blunted in mice lacking the A₁AR. However, our results demonstrate that a high-salt diet suppressed renal renin mRNA expression and renal renin content to the same extent in A₁AR<sup>−/−</sup> and A₁AR<sup>+/−</sup> mice, clearly arguing against a role of the A₁AR in mediation of this process. Moreover, stimulation of the renin system by a low-salt diet was not diminished, but was even enhanced, by the genetic deletion of the A₁AR, a further result that is not compatible with a role of A₁AR in mediation of this stimulation. If stimulation of renal renin content and mRNA expression by the low-salt diet were related to the known decrease in renal adenosine concentration and the subsequent disinhibition of the A₁AR, this should not be possible in mice lacking this receptor. However, as stated above, the pronounced stimulation of Ren-1 mRNA expression due to salt restriction is highly consistent with a tonically inhibitory role of the A₁AR on the renin system, which is absent in A₁AR<sup>−/−</sup> mice. The conclusion that the A₁AR is not causally involved in regulation of the renin system by the macula densa is further supported by the intact stimulation of the renin gene expression and renin content by blockade of the macula densa salt transport with furosemide in A₁AR<sup>−/−</sup> animals. Interpretation of the in vivo data is limited by the fact that salt restriction or furosemide treatment might affect renin expression through pathways independent from or in addition to the macula densa mechanism, for example, by alterations in blood pressure or in sympathetic nervous system activity. We therefore investigated the effects of loop diuretics on renin secretion in the isolated perfused kidney model. In this preparation, administration of loop diuretics would appear to act solely through the macula densa, because perfusion pressure is experimentally controlled and changes in sympathetic nerve activity are unlikely. Even under these experimental conditions, bumetanide stimulated renin secretion to the same extent in kidneys of A₁AR<sup>−/−</sup> mice and their wild-type controls, arguing against a role of this receptor in mediation of this process. However, the complete reversal of the stimulated renin secretion by the selective A₁AR agonist CHA in A₁AR<sup>−/−</sup> and A₁AR<sup>+/−</sup> mice underlines the direct suppressive effects of the A₁AR on renin secretion, as has been demonstrated in previous studies (1, 5, 6, 15, 19). Besides the advantages of constant experimental conditions, the isolated perfused mouse kidney model is suitable for use in investigating the acute effects of an inhibition of macula densa salt transport on renin secretion and, therefore, in examining the renin system in a time frame similar to that used in the studies demonstrating the absence of a TGF response in A₁AR<sup>−/−</sup> mice (2, 28). Because the TGF response has been found to be abolished by pharmacological blockade or genetic deletion of the A₁AR (2, 26, 28, 29, 36), regulation of glomerular filtration rate and control of the renin system by the macula densa appear to follow different pathways.

A further interesting result of our study is the discovery of a linkage between the A₁AR mutation and the renin gene locus that causes homozygosity in the A₁AR knockout genotype to be invariably associated with the two-renin gene constellation. In contrast, the wild-type phenotype, homozygous for the absence of the A₁AR mutation, always contains a single renin gene. The foundation for this linkage is the fact that the genes encoding the A₁AR and renin are localized on chromosome 1 in close vicinity, as first shown in humans (7, 30).
Analysis of available mouse genomic sequences has confirmed that the renin and A1AR genes in the mouse are also located on chromosome 1 in relative close juxtaposition, separated by ~850 kb of DNA containing several putative gene loci. As is commonly done, the embryonic stem cells used for targeted disruption of the A1AR gene were derived from the 129J mouse strain, one of several mouse strains with two renin genes, designated Ren-1° and Ren-2 (21). By propagating the A1AR mutation in the one-renin gene C57BL/6 background, A1AR+/− mice will carry the 129J background in the area of the mutated A1AR gene and will therefore possess two renin genes. On the other hand, A1AR+/+ mice will have to carry the C57BL/6 background in the area of the native A1AR gene and will therefore have only one renin gene, designated Ren-1°. Breeding strategies will be used to segregate the A1AR knockout mutation from the two-renin gene constellation by backcrossing into the C57BL/6 background or knockout mutation from the two-renin gene constellation of data comparing knockout with wild-type mice. The knockout procedure potentially occurs in every knockout mouse, and neighboring gene loci supposedly unaffected by the knockout procedure potentially occurs in every knock- out model derived from different mouse strains, our results emphasize the necessity of careful interpretation of data comparing knockout with wild-type mice.

Taken together, our results support the concept of a tonic inhibitory role of the A1AR on the renin system, but they argue against a role of the A1AR in mediation of the macula densa control of the renin system, as has been demonstrated previously for macula densa control of glomerular filtration.

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