Adenosine as a mediator of macula densa-dependent inhibition of renin secretion

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Kim, Soo Mi, Diane Mizel, Yuning G. Huang, Josie P. Briggs, and Jurgen Schnermann. Adenosine as a mediator of macula densa-dependent inhibition of renin secretion. Am J Physiol Renal Physiol 290: F1016–F1023, 2006. First published November 22, 2005; doi:10.1152/ajprenal.00367.2005.—Adenosine acting through A₁ adenosine receptors (A1AR) has been shown previously to be required for the vasoconstriction elicited by high luminal NaCl concentrations at the macula densa (MD). The present experiments were performed to investigate a possible role of A1AR in MD control of renin secretion in conscious wild-type (WT) and A1AR-deficient mice. The intravenous injection of NaCl (5% body wt) reduced plasma renin concentration (PRC; ng ANG I ml⁻¹ h⁻¹) from 1,479 ± 129 to 711 ± 77 (P < 0.0001; n = 18) in WT mice but did not significantly change PRC in A1AR⁻/⁻ mice (1,352 ± 168 during control vs. 1,744 ± 294 following NaCl; P = 0.19; n = 17). NaCl injections also caused a significant reduction in PRC in β₁/β₂-adrenergic receptor⁻/⁻ mice (298 ± 47 vs. 183 ± 42; P = 0.03; n = 6). Injections of isotonic NaHCO₃ (5% body wt) elicited significant increases in PRC in both WT and A1AR⁻/⁻ mice. NaCl as well as NaHCO₃ injections were accompanied by transient increases in blood pressure, heart rate, and activity that were similar in WT and A1AR⁻/⁻ mice. The increase in PRC caused by an intraperitoneal injection of furosemide (40 mg/kg) was comparable in WT and A1AR⁻/⁻ mice, and it was accompanied by similar transient increases in blood pressure, heart rate, and activity. Similarly, the stimulation of PRC caused by hydralazine was the same in WT and A1AR⁻/⁻ mice. We conclude that the inhibition of renin secretion in response to an increase in NaCl at the MD requires A1AR and therefore appears to be adenosine dependent, whereas the stimulation of renin secretion during reductions in MD NaCl transport or arterial pressure does not require functional A1AR.

A₁, adenosine receptor; β₁/β₂-adrenergic receptor knockout; telemetry; furosemide; hydralazine; propranolol

THE JUXTAGLOMERULAR APPARATUS (JGA) is an intrarenal regulator of the balance between glomerular filtration rate and tubular reabsorption. The JGA consists of the macula densa (MD) cells in the distal tubule, the glomerular arterioles, and the interposed extraglomerular mesangial cells. The regulatory role of the JGA is twofold, to control the tone of the afferent arteriolar smooth muscle cells through the tubuloglomerular feedback (TGF) mechanism and to regulate renin secretion (27). Recent studies have shown that TGF responses are absent in mice with genetic deletion of A₁ adenosine receptors (A1AR), suggesting that adenosine is a critical component of the extracellular signaling pathway in the JGA, resulting in changes in vasomotor tone (2, 31). The extracellular mediators of MD-mediated renin release are less well defined. Because adenosine can both stimulate renin secretion through A₂ adenosine receptors and inhibit it through A1AR, it is conceivable that, in addition to regulating TGF, adenosine may also participate in affecting MD-dependent renin secretion (6). In fact, studies in an isolated, perfused rabbit JGA preparation have shown that the inhibition of renin secretion produced by an elevation in luminal NaCl was significantly attenuated by the selective A1AR blocker 8-cyclopentyl-1,3-dipropylxanthine (36).

Nevertheless, overall progress in identifying the extracellular mediator of MD-regulated renin release has been slow because renin secretion in the intact organism is affected by several parallel mechanisms, notably the baroreceptor and the sympathetic nervous, which are difficult to separate from the MD effects in vivo. The purpose of the present studies was to assess the possible role of A1AR in MD-mediated stimulation and inhibition of renin secretion in conscious mice. Using wild-type (WT) and A1AR⁻/⁻ mice, we determined the effect of A1AR deficiency on the ability of saline injections to decrease, and of furosemide to increase, renin secretion. The use of these approaches is based on the earlier observations that saline injections increase MD NaCl concentration and thus assess the inhibitory part of the renin regulatory pathway (14). Furosemide, on the other hand, blocks NaCl transport, thereby mimicking a reduction in MD NaCl concentration. We therefore used furosemide as a test of a NaCl concentration reduction from normal to zero (35). Possible changes in plasma renin concentration (PRC) elicited through the baroreceptor were assessed by determining the effect of all interventions on blood pressure in conscious mice, and by evaluating the effect of the blood pressure-lowering drug hydralazine in the presence and absence of A1AR. The role of the sympathetic input was determined by studying the effect of furosemide and saline in β₁/β₂-adrenergic receptor knockout mice.

Our data show that the renin-inhibitory actions of saline administration are fully blocked in A1AR⁻/⁻ mice while the stimulation of renin release by furosemide is maintained. The effects of saline and furosemide were maintained in β₁/β₂-adrenergic receptor-deficient mice. Furthermore, the stimulatory effect of hydralazine was unaltered in A1AR⁻/⁻ mice. Thus our observations are consistent with the notion of an asymmetric action of adenosine in which the nucleoside is restricted to mediating the arm of the MD mechanism that links increments in MD NaCl concentration with inhibition of renin release.

METHODS

Animals

We used male and female mice of the A1AR strain generated in this laboratory (31). Brother–sister mating of heterozygous mice generated WT and ⁻/⁻ genotypes in a mixed 129J/C57BL6 genetic
blood samples were collected 60 min later. All plasma samples were stored at 60°C until assay.

**Study Protocols**

**Saline administration.** NaCl (150 mM), corresponding to 5% of body weight, was slowly injected intravenously as a bolus. Then, tail blood samples were collected 60 min later.

**HCO₃⁻ administration.** HCO₃⁻ (150 mM) was given intravenously in a volume corresponding to 5% of body weight. Blood was collected 60 min later.

**Furosemide.** Mice received a single intraperitoneal injection of 40 mg/kg furosemide (Lasix, Hoechst). Blood was collected 60 min later.

**Propranolol and hydralazine.** Propranolol (10 mg/kg) was given by intraperitoneal injection with blood collection 30 min later, followed by injection of hydralazine (1 mg/kg) with another blood collection 30 min later. All plasma samples were stored at -20°C until assay.

**Blood Pressure Telemetry**

Telemetric transmitters were magnetically activated >24 h before implantation. Mice were anesthetized with ketamine and xylazine (90 and 10 mg/kg, respectively), and the left carotid artery was isolated. The telemetric catheter was inserted into the left carotid artery and advanced to reach the aortic arch, and the body of the telemeter (model TA11PA-C20, Data Sciences International, St. Paul, MN) was placed in a subcutaneous pocket on the right flank (3, 4). One day after surgery, each animal was returned to its home cage with ad libitum food and water for the duration of the study. The telemeter signal was recorded while mice were treated with furosemide, saline, or hydralazine.

**RESULTS**

**NaCl and NaHCO₃ Administration**

An intravenous bolus injection of isotonic saline corresponding to 5% of body weight was used to suppress renin release through the MD mechanism (11, 14). There was a consistent and highly significant reduction in PRC 60 min after the NaCl injection in both male (P < 0.01; n = 12) and female WT mice (P < 0.01; n = 6). In contrast, an identical saline injection had no significant effect on PRC in male (n = 11) or female A1AR−/− animals (n = 6). Because there were no gender differences with regard to changes in PRC during NaCl injections, individual data from all mice are shown in Fig. 1. Overall, PRC (ng ANG I/ml•h⁻¹) fell from 1,479 ± 129 to 711 ± 77 in WT mice (P < 0.01; n = 18), whereas it did not significantly change in A1AR−/− mice (1,352 ± 168 during control vs. 1,744 ± 294 following NaCl; P > 0.05; n = 17).

To assess the impact of a nonspecific effect of volume expansion, we compared the effect of NaCl with that of NaHCO₃ given in an equivalent amount (5% of body wt). In contrast to the PRC-reducing effect of NaCl, administration of NaHCO₃ caused a significant increase in PRC (ng ANG I/ml•h⁻¹) in both WT (from 1,415 ± 149 to 2,250 ± 252; P < 0.01; n = 19) and A1AR−/− mice (1,159 ± 227 to 2,375 ± 402; P < 0.01; n = 18) (Fig. 2).

Changes in MAP, heart rate, and activity in response to the administration of NaCl and NaHCO₃ in WT and A1AR−/− mice are shown in Fig. 3. It can be seen that both NaCl and NaHCO₃ injections induced transient increases in MAP, heart rate, and activity that were comparable in WT and A1AR−/− mice and are probably for the most part the result of a disturbance reaction previously noted in mice (34). All values had returned to baseline 20–30 min after the injection or 30–40 min before blood collection. In control experiments, we found that two successive blood collections taken 60 min apart

![Fig. 1. Plasma renin concentration (PRC) in conscious A1 adenosine receptor (A1AR)−/+ (left; n = 18; 12 male and 6 female) and A1AR−/− mice (right; n = 17; 11 male and 6 female) before and after an intravenous injection of NaCl (5% body wt). Lines connect measurements from individual animals. Dotted lines connect mean values, and vertical lines indicate SE.](http://ajprenal.physiology.org/ by 10.22033.1 on June 23, 2017)
showed similar PRC values (1,172 ± 218 and 1,266 ± 285 ng ANG I·ml⁻¹·h⁻¹; *P* > 0.05; *n* = 5), indicating that exposure to stress at time 0 did not significantly affect PRC 60 min later.

NaCl injections also caused a significant reduction in PRC in β₁/β₂-ADR⁻⁻ mice (298 ± 47 vs. 183 ± 42; *P* < 0.05; *n* = 6), indicating that NaCl injections can affect renin release in the absence of a change in adrenergic β-receptor activation (Fig. 4). Baseline levels of PRC were markedly reduced in β₁/β₂-ADR⁻⁻ mice, showing that basal renin release depends to a major extent on intact β-adrenergic innervation. The effects of NaCl and NaHCO₃ injections on blood pressure and heart rate were greatly blunted in β₁/β₂-ADR⁻⁻ mice (data not shown).

**Furosemide**

As noted previously (5), an intraperitoneal injection of furosemide (40 mg/kg) caused PRC (ng ANG I·ml⁻¹·h⁻¹) to increase from 1,722.5 ± 252 to 8,921 ± 795 in WT (*n* = 16; *P* < 0.01; Fig. 5) and from 2,137 ± 284 to 9,706 ± 947 in A1AR⁻⁻ mice (*n* = 16; *P* < 0.01). Basal and furosemide-stimulated levels of PRC were not significantly different between A1AR⁺⁺ and A1AR⁻⁻ mice. As a consequence, the relative and absolute increments of PRC were comparable between genotypes (ΔPRC = +7,199 ± 730 vs. +7,571 ± 822; *P* > 0.05).

The blood pressure response to furosemide was determined by telemetry to assess whether a blood pressure reduction may contribute to the increase in PRC (Fig. 6A). MAP was not significantly different between WT (*n* = 4) and A1AR⁻⁻ mice (*n* = 5) in the control period (106 ± 3.5 vs. 102 ± 1.8 mmHg). The intraperitoneal furosemide injection caused a transient increase in MAP by ~25 mmHg, heart rate, and activity in both WT and A1AR⁻⁻ mice. Although MAP returned to control within about 45 min, heart rate remained elevated for the observation period of 2 h. The increase in MAP was associated with an increase in motor activity of the mice. As can be seen in Fig. 6B, the effect of a sham saline injection on MAP and activity was similar to that of furosemide both with respect to peak changes and time course of change, whereas the response of heart rate to the sham injection was transient.

To determine whether the effect of furosemide was mediated to a measurable extent by an increase in sympathetic activity, we assessed the effect of furosemide on PRC in...
ute to the stimulation of renin release by hydralazine, we determine to what extent sympathetic activation may contribute to the stimulation of renin release by low blood pressure is not or absent in following the injection in WT mice were markedly dampened after the intravenous injection of NaCl (5% body wt; gray bars). Vertical lines indicate SE.

β1/β2AR−/− mice (Fig. 5). In this series, administration of furosemide (40 mg/kg) increased PRC (ng ANG I·ml⁻¹·h⁻¹) from 1,612 ± 308 to 9,417 ± 1,821 in WT (n = 5) and from 370 ± 64 to 6,108 ± 496 in β1/β2AR−/− mice (n = 7). Again, basal levels of PRC were significantly reduced in β1/β2AR−/− compared with WT mice (P < 0.01), but the response to furosemide was maintained. The response of blood pressure to furosemide in β1/β2AR−/− mice is included in Fig. 6A. The increases in MAP, heart rate, and activity seen following the injection in WT mice were markedly dampened or absent in β1/β2-ADR−/− animals.

Hydralazine

To assess whether the stimulatory effect of an acute blood pressure reduction may be affected by adenosine, we determined the response of PRC to the vasodilator agent hydralazine (1 mg/kg sc). Hydralazine reduced MAP from 103 ± 1.5 mmHg to a nadir of 81 ± 2.8 mmHg at 30 min after the injection (by 21.7 ± 2 mmHg) in WT and from 108 ± 5.8 mmHg to a nadir of 82 ± 4.2 mmHg (by 26.5 ± 4 mmHg) in A1AR−/− mice (Fig. 7). Hydralazine increased PRC (ng ANG I·ml⁻¹·h⁻¹) from 1,557 ± 198 to 4,355 ± 661 in WT (n = 18; P < 0.01) and from 1,497 ± 203 to 5,103 ± 815 in A1AR−/− mice (n = 18; P < 0.01). The hydralazine-induced increase in PRC was not significantly different between WT and A1AR−/− mice (ΔPRC 2,798 ± 635 in WT, and 3,606 ± 764 in A1AR−/− mice; P = 0.42), suggesting that the stimulation of renin release by low blood pressure is not significantly altered by chronic A1AR deficiency (Fig. 8). To determine to what extent sympathetic activation may contribute to the stimulation of renin release by hydralazine, we assessed the effect of the vasodilator in β1/β2AR−/− mice (Fig. 8). Following hydralazine, PRC increased from 181 ± 43 to 889 ± 144 ng ANG I·ml⁻¹·h⁻¹ (n = 12; P < 0.01) in this strain of mice, suggesting again that basal, but not low blood pressure-stimulated renin release is dependent on tonic sympathetic input. As can be seen in Fig. 7, MAP was lower in β1/β2AR−/− than WT mice (92 ± 5 vs. 108 ± 3 mmHg; P < 0.05), and the blood pressure nadir in response to hydralazine was also reduced (−26.5 ± 2.1 vs. −10 ± 4.7 mmHg).

In view of the earlier observation that the effect of hydralazine on PRC was markedly reduced when given together with propranolol, we examined the effect of hydralazine in mice pretreated with 10 mg/kg propranolol (24, 25). As shown in Fig. 9, the β-adrenergic antagonist reduced PRC (ng ANG I·ml⁻¹·h⁻¹) from 1,472 ± 95 to 156 ± 39 (n = 5; P < 0.01). Administration of hydralazine 30 min after propranolol increased PRC to 2,401 ± 329 (P < 0.01 compared with propranolol; ANOVA). Time control experiments without administration of hydralazine showed that the effect of propranolol on PRC was maintained at the 60-min time point (1,147 ± 191 vs. 159 ± 69 ng ANG I·ml⁻¹·h⁻¹; n = 6). Thus genetic deletion as well as acute pharmacological inhibition of β-adrenergic receptors reduced basal renin release without preventing the stimulatory effect of the vasodilator-induced blood pressure reduction.

DISCUSSION

The aim of the present study was to investigate the possible role of adenosine as an extracellular paracrine factor in MD-mediated renin secretion. Under most experimental conditions, adenosine inhibits renin release through activation of A1AR (1, 10, 21, 32). Thus the specific focus of our studies was to examine a possible inhibitory role of adenosine mediated by A1AR in JG control of renin release. The impact of such inhibition on MD-dependent renin secretion would depend on the relationship between MD NaCl concentration and JG interstitial adenosine concentrations. If MD NaCl correlates directly with local adenosine levels, the nucleoside could be directly responsible for the inhibition of renin release caused by an increasing MD NaCl signal. On the other hand, if the relationship between MD NaCl and local adenosine is inverse, adenosine could diminish the stimulatory effect of a decrease in the NaCl signal. Thus the basic strategy of our studies was to compare the effects of increases or decreases in MD signaling on plasma renin in WT mice with those in genetically A1AR-deficient animals. An increase in the MD NaCl signal was produced in conscious mice by an acute intravenous injection of isotonic saline. Extensive studies in the rat have shown that this intervention causes a reduction in PRC, an effect that is specifically dependent on the presence of Cl in the injectate (11, 14) and is therefore reminiscent of the Cl depen-

Fig. 4. Mean PRC in conscious WT (left) and β1/β2-adrenergic receptor-deficient mice (β1/β2AR−/−; right) during control (open bars) and 60 min after the intravenous injection of NaCl (5% body wt; gray bars). Vertical lines indicate SE.

Fig. 5. A: mean PRC in conscious A1AR+/+ (left) and A1AR−/− mice (right) before and after 60 min after the intraperitoneal injection of furosemide (40 mg/kg). B: mean PRC in conscious WT (left) and β1/β2-ADR−/− (right) before and after 60 min after the intraperitoneal injection of furosemide (40 mg/kg). Vertical lines indicate SE.
dency of MD-dependent renin secretion demonstrated in an isolated, perfused JGA preparation (18). Micropuncture studies have shown that there is a concomitant increase in NaCl concentration in the distal tubule that is thought to serve as the renin-inhibitory luminal signal (17). Intrarenal infusion of NaCl, but not of Cl-free solutions, also caused a reduction in renin release and of intrarenal ANG II formation in the dog (8, 37).

Our studies show that saline administration caused the expected reduction in PRC in WT mice but that this effect was completely absent in A1AR-deficient animals. This finding is compatible with the notion that an increase in luminal NaCl concentration is associated with the generation of adenosine in the JGA interstitium, leading to activation of A1AR and inhibition of renin release. Previous results from our laboratory in an isolated, perfused rabbit JGA preparation have shown that the A1AR antagonist 8-cyclopentyl-1,3-dipropylxanthine caused an ~40% reduction in the response of renin secretion to an increase in perfusate Cl concentration from 7 to 125 mM (36). This partial inhibition is compatible with the notion that the action of endogenous adenosine may be restricted to some portion of the effective Cl concentration range. Adenosine probably exerts its effects at the level of the JG cells because the nucleoside has been shown to inhibit renin secretion in primary cultures of JG cells, indicating that A1AR are expressed in JG cells (16). A direct relationship between MD NaCl and JG interstitial adenosine levels has been proposed earlier to be responsible for TGF based on the observation that TGF responses to increased NaCl were absent in A1AR−/− mice (2, 31). Interestingly, recent studies in isolated, perfused

![Fig. 6. Telemetric recordings of the average responses of MAP (top), HR (middle), and activity (bottom) to an intraperitoneal injection of furosemide at time 0 (A) or vehicle (B) in 4 WT (●), 5 A1AR−/− (○), and 5 β1/β2ADR−/− (▲) mice.](image)

![Fig. 7. Telemetric recordings of the average responses of MAP to an intraperitoneal injection of hydralazine (1 mg/kg) in 4 WT (●), 5 A1AR−/− (○), and 4 β1/β2ADR−/− mice (▲).](image)

![Fig. 8. Mean PRC in conscious A1AR+/- (left), A1AR−/− (middle), and β1/β2ADR−/− mice (right) before (open bars) and 60 min after the intraperitoneal injection of hydralazine (1 mg/kg; gray bars). Vertical lines indicate SE.](image)
mouse kidneys have shown that the inhibitory effect of an increase in perfusion pressure on renin secretion was entirely absent in kidneys from A1AR−/− mice (28). Thus the mechanisms responsible for the decrements of renin secretion caused by an increase in NaCl through the MD and by an increase in perfusion pressure through the baroreceptor appear to include an inhibitory role of adenosine mediated by A1AR. Previous data have shown that the regulation of renin expression by high- or low-NaCl diets is maintained in A1AR−/− mice (29). This observation does not appear to be in major conflict with the present data because a predominant regulation of the renin system through the MD mechanism is unlikely during changes in chronic dietary NaCl intake.

As the telemetric blood pressure monitoring shows, handling of the animals during drug injections was accompanied by cardiovascular responses that are most likely related to stress. Thus one may assume that the blood collection procedure elicits comparable blood pressure and heart rate alterations and could thus be the cause for elevations of PRC levels above resting values. We cannot exclude this possibility, although it is unknown how quickly newly released renin gains access to the general circulation to cause measurable changes in plasma renin. Nevertheless, the interpretation of our data would not be affected qualitatively as long as any procedure-induced alteration of PRC would be comparable between the different strains. That this is likely to be the case is supported by the observation that the disturbance reaction was rather uniform in magnitude and duration between WT and A1AR−/− strains. In addition, possible collection artifacts did not prevent us from detecting a reduction in PRC following NaCl injection, although it is conceivable that the magnitude of the change was diminished by the superimposed stress effect.

Inhibition of renin secretion following the injection of saline could be the nongenomic consequence of extracellular volume expansion. However, our observation that an equivalent injection of a NaHCO3 solution did not reduce PRC argues against this possibility. A similar conclusion had been reached earlier in rat studies in which a renin-suppressing effect was only seen with NaCl and NaBr, but not with Cl-free solutions despite the fact that the diuretic and saluretic effects were comparable (11). In contrast to the effect of NaCl, injections of NaHCO3 caused significant increases in PRC in both WT and A1AR−/− mice. A similar increase in plasma renin has previously been reported in rats during the infusion of NaHCO3 (11). The mechanism for this increase is not entirely clear, but it is likely that the injection of NaHCO3 may lead to a reduction in MD Cl concentration similar to that observed following the infusion of a solution in which Cl was replaced with an assortment of anions including HCO3 (17). Furthermore, distal Cl concentration fell when the delivery of NaHCO3 to the loop of Henle was increased by inhibition of proximal HCO3 reabsorption (23). Because MD-regulated renin release appears to be Cl dependent, this reduction in Cl concentration could stimulate renin release (18). An alteration in acid-base status may contribute to the increase in renin secretion, although similar studies in the rat have failed to find a significant change in plasma pH and acid-base status (15). The mild hypokalemia caused by NaHCO3 administration would be expected to decrease rather than increase renin secretion (30).

The injection of saline caused a transient increase in arterial blood pressure (Fig. 3), but we consider it unlikely that the reduction in PRC is a result of this change. A return of blood pressure to baseline was seen within 30 min following the injection, so that any directly pressure-related change in PRC is likely to have dissipated at the time of plasma collection. Furthermore, a similar increase in blood pressure was observed following the injection of NaHCO3, where PRC actually increased. Finally, because a major component in the hemodynamic response to the disturbance appears to depend on sympathetic activation (Fig. 6), the potentially inhibitory effect of the blood pressure increase on PRC will be counteracted by the stimulatory effect of enhanced β-adrenergic input.

Another consequence of an extracellular volume expansion caused by the saline administration may be inhibition of renal sympathetic nerve activity (RSNA) (7, 19). This decrease in RSNA may contribute to the inhibition of renin release caused by saline administration. The effect of a reduction in RSNA on renin release is mediated by β1-adrenoceptors on JG cells, and possibly by presynaptic β2-receptors (13, 20). However, examination of the effect of saline on PRC in genetically β1/β2-deficient mice showed that the relative reduction in PRC was maintained. This observation is consistent with previous reports that the MD-dependent pathway of renin secretion operates independently of renal nervous input, although the absolute magnitude of the renin response is directly related to RSNA (33). Because prostaglandins appear to be important mediators of MD-mediated renin secretion, it is pertinent to point out that the inhibitory effects of cyclooxygenase inhibitors and β-adrenergic blockers on renin secretion appear to be simply additive, further suggesting that the MD pathway does not require adrenergic input (12). Basal values of PRC were greatly reduced in β1/β2-deficient mice, and it is likely that this reflects reduced rates of renin synthesis and renin release under baseline conditions. Renin mRNA levels are markedly reduced in chronically denervated kidneys, suggesting tonic control of renin gene expression by sympathetic input (9).

The effect of furosemide on PRC was found to be comparable between WT and A1AR−/− mice. This observation is in accord with the earlier finding in an isolated, perfused kidney preparation that the stimulatory effect of furosemide on renin secretion was preserved in kidneys harvested from A1AR−/− mice (29). Previous observations have indicated that adenosine
serves as a physiological brake mechanism in several renin-stimulatory pathways, including that activated by furosemide (10, 22). The present data show that this tonic restraint of renin release by A1AR activation cannot be demonstrated when A1AR are chronically absent. There is no evidence that the acute response of PRC to furosemide is significantly modified by changes in arterial pressure because the only pressure change observed was a transient increase in both WT and A1AR−/− mice that had abated well before the blood sample collection. It is interesting, however, that heart rate remained elevated for >1 h following furosemide. This effect is presumably a reflection of β-adrenergic stimulation because it was not seen in β1/β2-deficient mice (Fig. 6), but the exact pathway causing sympathetic activation is unclear.

Hydralazine was used to examine whether the stimulation of renin secretion caused by a decrease in blood pressure is modified in A1AR-deficient mice. Our data show that hydralazine-induced decrements in blood pressure that were the same between genotypes caused similar increases in PRC in WT and A1AR−/− mice. Thus the stimulation in renin secretion following renal baroreceptor unloading does not appear to be dependent on adenosine, a conclusion similar to that derived in studies using isolated, perfused kidneys from WT and A1AR−/− mice (28). Maintenance of the renin-stimulatory effect of hydralazine in β1/β2-adrenergic receptor-deficient mice indicates that a catecholamine-mediated effect is not primarily responsible for the renin secretion caused by the blood pressure reduction. This conclusion is seemingly at variance with the earlier finding that the renin secretory response to hydralazine is markedly reduced in the presence of the β-adrenergic antagonist propranolol (24, 25). However, the design of these earlier studies in which propranolol was given together with hydralazine is compatible with the possibility that the effect of hydralazine was obscured by a reduction in basal PRC caused by the β-blocker. In fact, we show in the present experiments that propranolol markedly reduces basal levels of PRC and that the subsequent administration of hydralazine leads to a virtually normal increase in renin release, thereby more than restoring basal PRC levels (Fig. 9). Thus it would appear that most of the stimulatory effect of hydralazine may be due to a direct action of the blood pressure reduction rather than to reflex activation of the sympathetic nervous system.

In summary, the inhibition of renin secretion caused by an increase in MD NaCl concentration requires A1AR and is therefore adenosine dependent. In contrast, the stimulation of renin secretion caused by a reduction in the MD NaCl signal or by a decrease in blood pressure is not modified in A1AR-deficient mice, suggesting a role for mediators other than adenosine. The present observations together with our earlier studies are consistent with the overall conclusion that levels of adenosine high enough to activate A1AR are attained in the JG interstitium when luminal NaCl concentrations are supranormal and that this activation causes both vasoconstriction and inhibition of renin release.

GRANTS

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