Endogenous Adenosine Restrains Renin Release in Conscious Rats

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The purpose of this study was to test the hypothesis that endogenous adenosine functions to restrain the renin release response to pharmacological and pathophysiological stimuli. To achieve this objective, we examined the effects of an adenosine receptor antagonist, 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX), on the renin release response induced by acute administration of hydralazine or by chronic clipping of the left renal artery (renovascular hypertensive rats). In conscious, unrestrained rats, DPSPX significantly increased plasma renin activity (PRA) in control rats, in rats treated with hydralazine, and in renovascular hypertensive rats. The effect of DPSPX on PRA was significantly greater in rats treated with hydralazine or in renovascular hypertensive rats compared with control rats. DPSPX did not influence arterial blood pressure in any group, did not affect the measurement of PRA, and did not alter the elimination of renin activity from the circulation. Additional experiments were performed in the in situ autoperfused kidney so that the effects of DPSPX on renal hemodynamics and renal excretory function could be assessed. In this experimental model, DPSPX also increased PRA in hydralazine-treated rats and in renovascular hypertensive rats without affecting arterial pressure, renal blood flow, or sodium excretion. In a final set of studies in conscious, unrestrained rats, adenosine deaminase increased PRA in a dose-dependent manner in hydralazine-treated rats and significantly increased the slope of the relation between PRA and the depressor response to hydralazine. We conclude: 1) Although the kidney has both $A_1$ and $A_2$ adenosine receptors mediating inhibitory and stimulatory actions, respectively, on renin release, the dominant effect of endogenous adenosine on renin release is inhibitory. 2) Even under basal physiological conditions, endogenous adenosine tonically inhibits renin release. 3) This inhibitory effect is augmented whenever the renin-angiotensin system is stimulated regardless of the approach used to activate renin release. 4) Endogenous adenosine negatively modulates renin release by a direct effect on juxtaglomerular cells. (Circulation Research 1990;66:637–646)

Exogenously administered adenosine can inhibit renin release in vivo$^{1-4}$ and in vitro$^{5-7}$ most likely via a direct action on renin-secreting juxtaglomerular cells.$^{8,9}$ The rank order of potency of adenosine analogues with respect to inhibition of renin release indicates that the inhibition of renin release is mediated by $A_1$ adenosine receptors.$^7$ Although $A_2$ adenosine receptors also exist in the kidney and stimulate renin release,$^7$ the affinity of $A_1$ receptors for adenosine, which is in the nanomolar range, is about 100- to 1,000-fold higher than the affinity of $A_2$ receptors for adenosine. Therefore, if endogenous adenosine participates in the control of renin release, it probably inhibits, rather than stimulates, renin release. Inasmuch as renal production of adenosine increases in certain stress conditions such as during intra-aortic infusions of hypertonic saline$^{10}$ and renal ischemia due to clamping of the renal artery,$^{11,12}$ it is possible that endogenous adenosine is very important in attenuating renin release in various physiological and pathophysiological conditions.

Despite these theoretical arguments supporting an important role for endogenous adenosine as a negative regulator of renin release, few experiments have directly assessed this hypothesis. Recently, we began testing this hypothesis$^{13}$ by examining the effect of an adenosine receptor antagonist, 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX)$^{14}$ on renin release in the in situ autoperfused kidney model.$^{15}$ DPSPX contains a sulfonic acid group, which is negatively

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charged at physiological pH, and a bulky phenyl group. Because of its negative charge, DPSPX does not easily penetrate into the intracellular space, and this characteristic should prevent it from non-specifically perturbing intracellular cyclic nucleotide and calcium levels, two common problems associated with the use of theophylline. Also, this compound is 20- to 60-fold more potent than theophylline and has a high water solubility, two characteristics that make DPSPX particularly suitable for in vivo studies.

interestingly, we discovered that administration of DPSPX increased renin release in response to sodium restriction. Even in animals fed a normal sodium diet, DPSPX tended to increase plasma renin activity (PRA). Assuming that the effect of DPSPX treatment on renin release was due to blockade of adenosine receptors, our results indicate that endogenous adenosine has physiological significance in regulating basal renin release and the renin release response to sodium restriction. In this same study, we found that the increase in renin release induced by treatment with DPSPX was not attenuated by pretreatment with propranolol, which indicated that the sympathetic nervous system did not mediate the effect of DPSPX on renin release. Also, DPSPX-induced changes in renin release could not be explained by changes in renal blood flow, glomerular filtration rate (GFR), filtration fraction, or electrolyte excretion. Therefore, our conclusion was that endogenous adenosine attenuates the renin release response to salt restriction by a direct effect on juxtaglomerular cells.

The present study had four objectives. First, we wished to determine whether endogenous adenosine attenuates the release of renin regardless of how renin secretion is induced. That is, we wanted to determine whether endogenous adenosine has a role in modulating renin release in general, as opposed to only a role in modulating the renin response to salt depletion. Our second objective was to determine whether endogenous adenosine regulates renin release in a physiological setting where the results are not skewed by anesthesia or surgical manipulations. Inasmuch as our previous study was conducted in anesthetized, surgically manipulated animals, it could very well be that our prior conclusions do not apply to normal physiology. The third goal of this investigation was to test alternative possibilities for DPSPX-induced increases in PRA. Specifically, in our former study it is possible that DPSPX might have increased PRA by affecting the renin assay or by reducing the clearance of renin. The latter possibility is of particular concern since hepatic adenosine receptors are known to affect hepatic blood flow and since plasma renin is metabolized mainly by the liver. Finally, because DPSPX may have effects on renin release that are not mediated by blockade of adenosine receptors, we thought it important to further test the hypothesis that endogenous adenosine regulates renin release by using alternative pharmacological probes.

Materials and Methods

The effects of DPSPX on hydralazine-induced and renal clipping–induced renin release were studied by using two different experimental situations. First, we investigated the effects of DPSPX on renin release in conscious, unrestrained rats. This approach had the advantage that the role of adenosine in controlling renin release could be assessed under physiological conditions. However, the disadvantage of this approach was that inferences as to whether DPSPX directly increased renin release or increased renin release indirectly via changes in renal function were not possible. Therefore, we also examined the effects of DPSPX on renin release in the in situ autoperfused rat kidney. Although this experimental model was not as physiological as the conscious rat model, this approach afforded the opportunity to examine the effects of DPSPX on relevant renal function parameters. By conducting experiments under both experimental situations, we were then able to address both the physiological relevance and mechanism of DPSPX-induced alterations in renin release. Because a diversity of preparations and maneuvers was used in these investigations, for clarity of presentation, all of the protocols are listed in outline form in Table 1.

Study 1: DPSPX on Hydralazine-Induced Renin Release

In the experiment using the conscious animal model, the day before the scheduled experiment, male Sprague-Dawley rats (Sasco, Omaha, Nebraska) weighing 350–400 g were anesthetized with pentobarbital (50 mg/kg i.p.; Sigma Chemical, St. Louis, Missouri). The left jugular vein and left carotid artery were cannulated with a PE-50 (Clay Adams, Parsippany, New Jersey) catheter. The catheters were tunneled subcutaneously to the back of the neck and protected with a jacket-tether–swivel system (Medical Arts, Los Angeles, California). After the rats regained consciousness, they could freely move in the cage and had free access to rat chow (Wayne Lab Blox, Allied Mills, Memphis, Tennessee) and tap water. On the next day, the arterial catheter was connected to a pressure transducer (model 1280, Hewlett Packard, Palo Alto, California) and mean arterial blood pressure (MABP) was recorded continuously with a physiograph (model 7758A, Hewlett-Packard). The rats were randomized to receive a continuous intravenous infusion of either DPSPX (10-mg bolus in 0.5 ml of 0.9% saline +0.15 mg/min in 0.02 ml/min of 0.9% saline) or 0.9% saline (0.5-ml bolus +0.02 ml/min). Thirty minutes after the start of the intravenous infusion, rats received a bolus intravenous injection of either hydralazine (3 mg/kg dissolved in 0.9% saline [1 ml/kg]; Sigma Chemical) or 0.9% saline (1 ml/kg). Therefore, there were four groups of rats: 1) no DPSPX (control group) plus no hydralazine, 2) no DPSPX (control group) plus hydralazine, 3) DPSPX plus no hydralazine, and 4) DPSPX plus hydralazine.
TABLE 1. Outline of Experiments

Study 1: DPSPX on hydralazine-induced renin release

| Part A: Conscious rat model | Group 1: Vehicle only (n=6) | Group 2: Hydralazine (n=7) | Group 3: DPSPX (n=7) | Group 4: DPSPX and hydralazine (n=5) |
| Part B: In situ kidney model | Group 1: Hydralazine (n=7) | Group 2: DPSPX and hydralazine (n=7) |

Study 2: DPSPX on renin release in 2K1C renovascular hypertension

| Part A: Conscious rat model | Group 1: Sham operation (n=13) | Group 2: Renal artery clip (n=6) | Group 3: DPSPX and sham operation (n=14) | Group 4: DPSPX and renal artery clip (n=8) |
| Part B: In situ kidney model | Group 1: Renal artery clip (n=15) | Group 2: DPSPX and renal artery clip (n=15) |

Study 3: DPSPX on PRA measurement and PRA elimination

| Part A: Rat plasma model | Group 1: Vehicle only (n=5) | Group 2: DPSPX (n=5) |
| Part B: Anesthetized 2K1C rat model | Group 1: Vehicle only (n=8) | Group 2: DPSPX (n=8) |

Study 4: EHNA and ADA on renin release induced by hydralazine

| Part A: Conscious rat model | Group 1: Hydralazine (n=13) | Group 2: EHNA and hydralazine (n=17) | Group 3: Low-dose ADA and hydralazine (n=13) |
| Part B: Conscious rat model | Group 1: Hydralazine (n=5) | Group 2: High-dose ADA and hydralazine (n=10) |

DPSPX, 1,3-dipropyl-8-(p-sulfophenyl)xanthine; 2K1C, two-kidney, one-clip; PRA, plasma renin activity; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; ADA, adenosine deaminase.

Fourty minutes after the injection of hydralazine, a 1-ml blood sample was taken from the arterial catheter for measurement of PRA.

In the experiments using the in situ autoperfused kidney model, male Sprague-Dawley rats weighing 200–250 g were anesthetized with pentobarbital (50 mg/kg i.p.), and the left kidney was exposed through a midline abdominal incision. The left renal artery was completely dissected from both the surrounding tissue and the left renal vein. A silver clip (0.25-mm gap) was placed around the left renal artery. In rats assigned to sham operation, the silver clip was taken off immediately, whereas in the two-kidney, one-clip (2K1C) group the clip was left on the renal artery. The abdominal cavity was closed with sutures, and rats were allowed to regain consciousness. Rats were fed normal rat chow and given tap water ad libitum for 1 week before they were used in either the conscious animal model or the in situ autoperfused kidney model. The conscious animal model and the in situ autoperfused kidney model were prepared as described for the experiments with hydralazine. Also, the experimental protocols were as described in study 1, except that no hydralazine was administered.

After a 30-minute period of hemostasis, 1,500 units heparin were administered through one of the jugular catheters. The catheter in the carotid artery was connected to the proximal end of an extracorporeal shunt. Along this shunt, there were a cannulating 2-mm flow probe (Transonic Systems, Ithaca, New York) connected to a flow meter (model T201, Transonic Systems), a sampling port, and a port connected to a pressure transducer that was connected to a physiograph. After blood from the carotid artery filled the shunt, the distal end of this extracorporeal shunt was connected to the catheter in the abdominal aorta. Then, the abdominal aorta just above the orifice of the left renal artery was ligated. This procedure created an aortic pouch such that blood flow through the shunt was equivalent to renal blood flow.

After surgery, rats were randomly assigned to receive an intravenous infusion of either DPSPX (10-mg bolus in 0.5 ml of 0.9% saline+0.15 mg/min in 0.05 ml/min of 0.9% saline) or 0.9% saline (0.5-ml bolus+0.05 ml/min). Thirty minutes later, all rats received a bolus intravenous injection of hydralazine (0.15 mg/kg dissolved in 0.9% saline [1 ml/kg]). Starting 10 minutes after the hydralazine injection, urine was collected for 30 minutes. Finally, a 1-ml blood sample for measurement of PRA was taken via the sampling port in the extracorporeal shunt.

Study 2: DPSPX on Renin Release in Two-Kidney, One-Clip Renovascular Hypertensive Rats

Male Sprague-Dawley rats weighing 200–250 g were anesthetized with pentobarbital (50 mg/kg i.p.), and the left kidney was exposed through a midline abdominal incision. The left renal artery was completely dissected from both the surrounding tissue and the left renal vein. A silver clip (0.25-mm gap) was placed around the left renal artery. Rats were allowed to regain consciousness. Rats were fed normal rat chow and given tap water ad libitum for 1 week before they were used in either the conscious animal model or the in situ autoperfused kidney model. The conscious animal model and the in situ autoperfused kidney model were prepared as described for the experiments with hydralazine. Also, the experimental protocols were as described in study 1, except that no hydralazine was administered.
Study 3: The Effect of DPSPX on PRA Measurement and PRA Elimination

A male Sprague-Dawley rat was anesthetized with pentobarbital (50 mg/kg i.p.), and a 10-ml blood sample was collected from the abdominal aorta via a PE-90 catheter placed into a test tube containing 150 units heparin. This blood sample was immediately centrifuged in a cold room, and the plasma fraction was then distributed into 10 samples of 0.5 ml plasma each. To half of them, we added 150 μg DPSPX each. To the other half, we added the vehicle, 0.9% saline, only. The amount of DPSPX added provided the highest possible plasma concentration (300 μg/ml) achieved in vivo, if DPSPX is not metabolized and is distributed in the extracellular space only. Most likely, this concentration was not achieved in vivo and, therefore, represents a worst case scenario with respect to interference with the renin assay.

To test the possibility that any increase in PRA induced by DPSPX was due to a decrease in elimination of renin instead of an increase in renin release, we examined the effect of DPSPX treatment on the elimination of renin. Male Sprague Dawley rats that had received a renal clip 1 week before the experiment were anesthetized with pentobarbital (50 mg/kg i.p.). The trachea was cannulated to maintain a patent airway, the left jugular vein was cannulated with two PE-50 catheters, the left carotid artery was cannulated with a PE-90 catheter, and the abdominal cavity was opened through a midline incision. The rats were randomized to receive an intravenous infusion of either DPSPX (10-mg bolus in 0.5 ml of 0.9% saline + 0.15 mg/min in 0.05 ml/min of 0.9% saline) or 0.9% saline (0.5 ml + 0.05 ml/min), and these infusions were sustained till the end of the experiment. Forty minutes after the start of the infusion, a 1-ml blood sample was taken from the arterial catheter to measure PRA, and a bilateral nephrectomy was performed. Thirty minutes and 90 minutes later, a 1-ml blood sample was taken to measure PRA. Volume loss was replaced by infusing 1 ml of 0.9% saline into the animal after each blood sample.

Study 4: EHNA and ADA on Renin Release Induced by Hydralazine

A group of male Sprague-Dawley rats weighing 250–300 g were prepared for the conscious animal model as described above (study 1). On the day of the experiment, the carotid catheter was connected to a pressure transducer and a physiograph to record MABP continuously. After a 15-minute stabilization period, a bolus of hydralazine (3 mg/kg dissolved in 0.9% saline [1 ml/kg] i.v.) was administered. Ten minutes later, the rats were randomly assigned to receive one of the following three treatments: 1) erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), a generous gift from Burroughs Wellcome, Research Triangle Park, North Carolina (intravenous bolus of 2 mg/kg EHNA + 0.05 ml/min i.v. of 0.9% saline for 30 minutes), 2) control infusion (0.05 ml/min i.v. of 0.9% saline for 30 minutes), 3) adenosine deaminase (ADA; Sigma type X, 4 units/min in 0.05 ml/min of 0.9% saline for 30 minutes through a Y-shaped branch of the arterial catheter). A 1-ml blood sample was taken at the end of these infusions.

To check whether the intra-arterial dosage of ADA used above (4 units/min for 30 minutes) was high enough to be fully effective, a higher dosage of ADA (33.3 units/min) was also used. In these experiments, it was necessary to dissolve this high dose of ADA in a solution consisting of 2 parts of 50% glycerol and 50% potassium phosphate (5 mM) and 1 part of 0.9% saline. Since the vehicle could alter renin release, we administered vehicle to the control rats in these experiments. Male Sprague Dawley rats weighing 250–300 g were prepared for the conscious animal model as described above (study 1). Rats were randomly assigned to receive either the high dosage of ADA or its vehicle (0.05 ml/min for 30 minutes) through the Y-shaped branch of the carotid catheter. These infusions started immediately after the intravenous bolus of hydralazine (3 mg/kg dissolved in 0.9% saline). Thirty minutes after the hydralazine, a 1-ml arterial blood sample was taken to measure PRA. Hematocrit was also measured for the vehicle group. One milliliter of 0.9% saline was infused to replace the volume loss. After another 30 minutes, that is, 60 minutes after hydralazine injection, a second 1-ml blood sample was taken for PRA measurement.

DPSPX Synthesis, Assays, and Statistical Analysis

We synthesized DPSPX following a standardized procedure. The chemical identity was verified by comparing our material with commercially available DPSPX (Research Biochemical, Natick, Massachusetts) using microbore high-pressure liquid chromatography, ultraviolet spectroscopy, infrared spectroscopy, and nuclear magnetic resonance. In a previous study, we found that the dosage of DPSPX used in this study 1) abolished the bradycardic response to adenosine, 2) caused a rightward shift of the adenosine dose-response curve with respect to the hypotensive effect of adenosine, and 3) abolished the characteristic adenosine-induced biphasic change in renal blood flow.

Samples for PRA were centrifuged immediately in a cold room. The plasma was stored at −20°C, and PRA was measured within 3 weeks by a previously described method. GFR was calculated from the creatinine clearance. Creatinine levels in urine and plasma were determined using a creatinine analyzer 2 (Beckman Instruments, Fullerton, California). Electrolyte levels in urine were measured by flame photometry (model 343, Instrumentation Laboratory, Lexington, Massachusetts). Statistical analyses were calculated with the Statistical Package for Social Science (SPSSX) installed on a VAX 8800 computer. The statistical significance level was set at p < 0.05. Data were analyzed by unpaired or paired Student’s t test, two-factor analysis of variance (ANOVA), and
linear regression. All data were presented and graphed as mean±SEM.

Results

Study 1: DPSPX on Hydralazine-Induced Renin Release

In the conscious animal model, hydralazine decreased MABP similarly in the control group (from 108±4 to 73±5 mm Hg; p<0.01 by paired t test) compared with the DPSPX-treated group (from 110±4 to 68±2 mm Hg; p<0.01 by paired t test). Although the stimulus for renin release was the same, the group of rats receiving DPSPX and hydralazine had a significantly (p<0.001 by unpaired t test) higher PRA (Figure 1) compared with control rats receiving only hydralazine. In rats that did not receive the hydralazine injection, the treatment with DPSPX had no effect on MABP (112±4 mm Hg vs. 112±5 mm Hg for the DPSPX group and control group, respectively). However, the treatment with DPSPX increased basal PRA (p<0.05 by unpaired t test; Figure 1). Two-factor ANOVA indicated that the effect of hydralazine on PRA was significantly greater in rats treated with DPSPX compared with control rats. This was evidenced by the significant interaction (p<0.01) between the effect of hydralazine injection and the effect of DPSPX treatment.

As in the previous protocol, in the in situ autoperfused kidney model, treatment with DPSPX did not change the decrease in MABP induced by hydralazine (Figure 2), and the MABP was maintained at a stable low level throughout the 30-minute urine collection period. In hydralazine-treated rats, treatment with DPSPX did not affect GFR, filtration fraction, sodium excretion rate, potassium excretion rate, or renal blood flow (Figure 3). However, the group of rats that received DPSPX had a significantly higher PRA (Figure 3) compared with control rats.

Study 2: DPSPX on Renin Release in 2K1C Renovascular Hypertensive Rats

In conscious, renovascular hypertensive rats, DPSPX treatment significantly increased PRA (p<0.001 by unpaired t test; Figure 4) but did not alter MABP (121±7 mm Hg, control group; 114±1 mm Hg, DPSPX group; p<0.38 by unpaired t test). In rats that received a sham operation, treatment with DPSPX also increased PRA (p<0.05 by unpaired t test). Two-factor ANOVA indicated that the effect of clipping on PRA was greater in rats treated with DPSPX compared with control rats. This conclusion was supported by the significant (p<0.017) interaction between effect of clipping the renal artery and DPSPX treatment.

In the in situ autoperfused kidney model, treatment with DPSPX also increased PRA significantly in 2K1C rats (p<0.01 by unpaired t test; Figure 5). DPSPX treatment did not affect sodium excretion or renal blood flow. However, GFR, filtration fraction, and potassium excretion were significantly decreased by the treatment with DPSPX (Figure 5) in these 2K1C rats.

Study 3: The Effect of DPSPX on PRA Measurement and PRA Elimination

The two groups of PRA samples from the same pool of plasma had almost the same PRA values (26.8±2.9 ng angiotensin I/ml/hr, saline group; 28.6±2.3 ng angiotensin I/ml/hr, DPSPX group; p<0.64 by unpaired t test; n=5 for each group). Therefore, DPSPX did not interfere with the measurement of PRA.

Just before bilateral nephrectomy, another group of renovascular hypertensive rats receiving DPSPX also had PRA levels that were higher compared with
control renovascular hypertensive rats (Figure 6). Thirty minutes after bilateral nephrectomy, PRA levels in both the control and DPSPX-treated groups dropped to the same extent in terms of the percentage of their initial levels (52±5% control group vs. 54±8% DPSPX group). At 90 minutes after nephrectomy, there was still no evidence that DPSPX treatment decreased the elimination of PRA (38±6% control group; 24±2% DPSPX group).

Study 4: EHNA and ADA on Renin Release Induced by Hydralazine

EHNA and low-dose ADA did not significantly affect MABP either during the baseline period (118±4 mm Hg, EHNA; 122±3 mm Hg, control; 118±3 mm Hg, ADA) or at 40 minutes after hydralazine administration (77±1 mm Hg, EHNA; 76±3 mm Hg, control; 82±3 mm Hg, ADA). The PRA levels in the EHNA group tended to be lower than the control group, and the PRA levels of the ADA group were significantly (p<0.01) higher than the control group (Figure 7). Trend analysis by ANOVA indicated that PRA increased stepwise from the EHNA group to control group to ADA group (p<0.07). Linear regression analysis of the relation between PRA levels and the decrease of MABP induced by hydralazine indicated a significant linear relation in the control group and the ADA group (Table 2). No significant linear relation was found between these variables in the EHNA group. The slope of the regression equation for the ADA group was significantly (p<0.05 by t test) steeper than that for the control group (Table 2, Figure 8). When a higher dosage of ADA was tested, the high-dose ADA group had a significantly greater PRA compared with the vehicle group at 30 minutes and at 60 minutes after the hydralazine injection (Figure 9). In this experiment there were no significant differences in MABP between the two groups during either the control period (124±2 mm Hg, vehicle; 124±4 mm Hg, ADA), the period 30 minutes after hydralazine (86±4 mm Hg, vehicle; 82±3 mm Hg, ADA), or the period 60 minutes after hydralazine (79±5 mm Hg, vehicle; 74±2 mm Hg, ADA). Hematocrit measured for the vehicle group at 30 minutes after hydralazine was 44±1% (n=4), indicating that the vehicle did not cause significant hemolysis.

Discussion

Although exogenously administered adenosine can inhibit renin release, excluding our studies there is little experimental support for the hypothesis that endogenous adenosine can accumulate to an effective concentration and diffuse to target cells to inhibit renin release. The exact role of endogenous adenosine as a modulator of renin release is made even more uncertain by the fact that A2 receptors mediate...
stimulatory effects on renin release, and many adenosine analogues have a biphasic action on renin release, that is, inhibitory at low concentrations and stimulatory at high concentrations.5,7 The main objective of our experimental program is to determine whether endogenous adenosine modulates renin release and, if so, whether the regulation of renin secretion by endogenous adenosine is negative or positive. Inasmuch as endogenous adenosine levels fluctuate with physiological conditions,10-12 we thought it important to address the hypothesis that endogenous adenosine serves a physiologically significant role in regulating renin release under a variety of clinically significant situations including sodium restriction, administration of a vasodilator, and renovascular hypertension.

To probe the role of endogenous adenosine as a modulator of renin release, our strategy was to examine the effect of an adenosine receptor antagonist, DPSPX, on renin release. DPSPX is 20- to 60-fold more potent than theophylline and is highly water soluble. More importantly, almost all molecules of DPSPX are negatively charged at physiological pH because of a sulfonic acid group. This characteristic of DPSPX tends to exclude it from the intracellular space,16,17 while allowing full access of DPSPX to adenosine receptors on the cell surface. Therefore, interpretation of data obtained with DPSPX is more straightforward since it is unlikely that DPSPX nonspecifically interferes with intracellular cyclic nucleotide and calcium concentrations.

In a previous study,13 we discovered that treatment with DPSPX enhances renin release in response to sodium restriction. This effect of DPSPX is not dependent on alterations in sympathetic tone or neurotransmission, nor is it due to changes in GFR,
renal blood flow, or electrolyte excretion. However, our previous study left unanswered several important questions: 1) Is the apparent inhibitory effect of endogenous adenosine on renin release a general regulatory mechanism that is operative regardless of how the renin-angiotensin system is activated? 2) Our previous study was done in the in situ autoperfused kidney model in which anesthesia and extensive surgical manipulation might have stimulated the formation of endogenous adenosine. Does endogenous adenosine regulate renin release under more physiological conditions such as in the conscious, unrestrained animal? 3) A higher PRA may be due to either an increased release or a decreased elimination of renin activity. Hepatic adenosine receptors mediate an increase in hepatic blood flow, and renin is cleared primarily by the liver. Is it possible that DPSPX increases PRA by decreasing the elimination of renin activity from the plasma? 4) Is the observed effect of DPSPX on renin release due to the blockade of adenosine receptors; that is, will other maneuvers that affect the activity of endogenous adenosine also change the renin release response to physiological stimuli?

In the conscious, unrestrained rat, treatment with DPSPX markedly augmented the increase in PRA induced by either hydralazine or renovascular hypertension. Inasmuch as DPSPX did not alter the elimination of renin from the circulation, the increase in PRA induced by DPSPX must have been due to an increase in renin secretion. Importantly, even in control rats, DPSPX increased PRA; however, the effect of DPSPX on PRA was less in control animals compared with animals that received a stimulus to renin release. Inasmuch as these results are the same as in sodium restricted rats, we conclude that the inhibitory effect of endogenous adenosine is a general phenomenon that is not unique to a particular stimulus of the renin-angiotensin system. The fact that our results were similar in conscious and anesthetized rats indicates that in our previous study the role of endogenous adenosine was not exaggerated by anesthesia and surgical manipulation.

Our results indicate that although both stimulatory and inhibitory adenosine receptors exist in the kidney, the physiologically significant action of endogenous adenosine on renin release is inhibition. Further, our results demonstrate that although endogenous adenosine inhibits renin release even under normal physiological conditions, this inhibitory input is augmented as stimulatory signals to renin release increase. Presently, it is unclear why the inhibitory effect of endogenous adenosine is augmented when the renin-angiotensin system is activated. It could be due to higher levels of endogenous adenosine in such conditions; alternatively, the import of any given level of endogenous adenosine on renin release may depend on the degree to which renin release is stimulated.

The effect of DPSPX treatment on renin release was not secondary to changes in arterial blood pressure, GFR, filtration fraction, renal blood flow, or electrolyte excretion. In hydralazine-treated rats,
none of the monitored parameters of renal function were altered by treatment with DPSPX. The PRA level, however, was significantly increased by DPSPX treatment. In 2K1C renovascular hypertensive rats, neither arterial blood pressure, renal blood flow, nor sodium excretion was altered by treatment with DPSPX. Interestingly, DPSPX decreased GFR, filtration fraction, and potassium excretion in renovascular hypertensive rats. However, these changes were unlikely to be the cause of the increase in PRA, since in studies with hydralazine and sodium restriction13 renal function parameters did not change, yet DPSPX still significantly increased PRA. Although the changes in renal function were not related to the changes in renin levels, these data suggested that certain actions of endogenous adenosine might be critical for maintaining GFR and filtration fraction in 2K1C renovascular hypertensive rats. Presently, how DPSPX decreases GFR in renovascular hypertensive rats is obscure. The reason for the decrease in potassium excretion induced by DPSPX in renovascular hypertensive rats is also unclear. However, changes in potassium excretion were not related to changes in PRA; in the hydralazine study, DPSPX did not alter potassium excretion, and in the sodium restriction study, DPSPX actually increased potassium excretion. Yet in all these studies, DPSPX increased PRA.

In our studies using DPSPX to probe the role of endogenous adenosine, we made the critical assumption that all changes induced by DPSPX were due to blockade of adenosine receptors. Even though it is likely that DPSPX does not significantly enter the intracellular space and does not have effects on renin release other than via blockade of adenosine receptors, it is possible that DPSPX nonspecifically elevates renin release. Therefore, we tested our hypothesis further by employing other pharmacological maneuvers, that is, administration of adenosine deaminase and blockade of endogenous ADA with EHNA. These treatments should decrease or increase the endogenous level of adenosine, respectively.

In hydralazine-treated conscious, unrestrained rats, the PRA values were lower in the EHNA group than in the control group and lower in the control group than in the ADA (low-dose) group (p<0.07; trend analysis by ANOVA) (Figure 7). The order of the three groups was as predicted by our hypothesis. That is, EHNA should have potentiated the amount of endogenous adenosine and reduced renin release, whereas ADA should have attenuated the amount of endogenous adenosine and increased renin release. Additional support for our hypothesis came from the regression analysis (Table 2, Figure 8). A significant linear relation between PRA and the hydralazine-induced depressor response was observed for both the control and ADA groups. However, the slope of the regression equation in the ADA (low-dose) group was significantly steeper compared with the control group. EHNA treatment abolished the relation between PRA levels and the decrease in MABP induced by hydralazine. Finally, the mean PRA level in the ADA (low-dose) group was significantly higher compared with the control group (p<0.01).

The low dose of ADA did not increase PRA in hydralazine-treated rats as much as did DPSPX. This implied that either DPSPX increased renin release by other mechanisms or that the dose of ADA was too low. Therefore, we conducted additional experiments using a ninefold higher dosage of ADA in hydralazine-treated, conscious rats. PRA levels measured at 30 minutes and at 60 minutes after hydralazine were significantly augmented by ADA treatment compared with the vehicle group (Figure 9). Importantly, the magnitude of the ADA-induced increase in PRA was similar to that observed with DPSPX. One caveat regarding the experiment with high-dose ADA is that it was necessary to solubilize the ADA in a vehicle containing a high glyceral content. In hydralazine-treated animals receiving this vehicle, PRA was lower compared with animals that were not treated with a high glyceral content vehicle. However, inasmuch as all rats in this study received the same vehicle, the changes in PRA (compared with the appropriate vehicle-control group) induced by high-dose ADA should be a valid indicator of the importance, with respect to renin release, of endogenous adenosine in this experimental setting.

In summary, we conclude: 1) The primary action of endogenous adenosine on renin release is inhibition. 2) Under basal physiological conditions, endogenous adenosine tonically inhibits renin release. 3) When the renin-angiotensin system is activated, the inhibitory action of endogenous adenosine on renin release is augmented. 4) This inhibitory effect of endogenous adenosine on renin release is not secondary to changes in renal blood flow, GFR, filtration fraction, or electrolyte excretion. These data support our hypothesis that endogenous adenosine is an important physiological mechanism restraining renin release in both basal and stimulated states. One important clinical implication from our studies is the potential augmentation, by caffeine-containing beverages, of the renin release response to physiological and pharmacological stimuli. Inasmuch as caffeine blocks adenosine receptors, it is possible that caffeine could interfere with the therapeutic benefit of antihypertensive measures, such as a low sodium diet or vasodilator therapy, by increasing PRA in response to these antihypertensive interventions.

References


**KEY WORDS** • adenosine • renin
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