Modulatory Effects of Endogenous Adenosine on Epinephrine Secretion From the Adrenal Medulla of the Rat

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Abstract The purpose of this study was to examine (1) whether endogenous adenosine receptors inhibit the release of epinephrine and norepinephrine from adrenal medulla in response to physiological and pharmacological stimuli and (2) whether the renin-angiotensin system modulates this effect of endogenous adenosine. We used a conscious animal model to approximate normal physiological conditions. Male Sprague-Dawley rats were treated with a surface adenosine receptor antagonist, 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX) to explore the effect of endogenous adenosine. Plasma epinephrine and norepinephrine levels in response to hydralazine-induced hypotension were measured in these animals. The same protocol was repeated in rats pretreated with either adrenalectomy or captopril. The results showed that DPSPX treatment significantly increased plasma epinephrine and norepinephrine levels at both baseline conditions and after hydralazine-induced hypotension. The results from the adrenalectomized rats showed that the difference in plasma epinephrine level between the control and DPSPX groups originated from the adrenal medulla. Pretreatment with captopril attenuated the rise of plasma epinephrine and norepinephrine levels in DPSPX-treated animals. This result suggests that endogenous adenosine receptors inhibit epinephrine release from the adrenal medulla and suppress plasma norepinephrine levels. When catecholamine release was stimulated by physiological and pharmacological stimuli, this inhibitory function of adenosine receptors was augmented. The renin-angiotensin system is at least partially responsible for the modulatory function of endogenous adenosine on the catecholamine response as demonstrated in this study. (Hypertension. 1994;24:714-718.)

Key Words • catecholamines • adrenal medulla • adenosine • hydralazine • renin-angiotensin system

The endogenous nucleoside adenosine is a neuro-modulator in many autonomic functions. Adenosine is known to be released by nerve terminals after depolarization. After release, adenosine can stimulate specific binding sites known to be coupled to adenylate cyclase. Adenosine is also a potent vasoactive substance known to be involved in the regulation of cerebral, muscle, and cardiac blood flow. Our previous studies have demonstrated that endogenous adenosine plays a role in the central regulation of autonomic outflow to the cardiovascular system, and antagonists such as caffeine can inhibit baroreflex activation.

The adrenal medulla is recognized as the counterpart of postganglionic sympathetic neurons embryologically and physiologically. Both secretory cells of the adrenal medulla and postganglionic neurons are derived from the neural crest of the embryo; both are innervated by preganglionic sympathetic fibers; and both have catecholamine metabolism enzymes and release catecholamines. Stimulation of the sympathetic nerves to the adrenal medulla causes large quantities of epinephrine and norepinephrine to be released into the circulating blood.

Evidence shows that adenosine inhibits sympathetic neurotransmission and norepinephrine release, and adenosine release is also increased in response to sympathetic nerve stimulation. In superfused chromaffin granules from bovine adrenal medulla, catecholamines and the precursor of adenosine, ATP, are released in parallel. An important component of adenosine metabolism, adenosine kinase, has been purified from bovine adrenal medulla. Recently, with the use of a [3H]dipyridamole ligand binding technique, adenosine transporter, one of the key proteins in regulating intracellular and extracellular adenosine concentration, has been found in cultured chromaffin cells. Also, in cultured bovine chromaffin cells, ATP, ADP, and adenosine inhibit the acetylcholine-stimulated catecholamine release. These data strongly suggest that adenosine is present in chromaffin cells of adrenal medulla and that adenosine modulates catecholamine release.

In the present study, we tested the hypothesis that endogenous adenosine restrains catecholamine release from the adrenal medulla. Hypotension caused by intravenous administration of hydralazine was used as a pharmacological stimulus to the adrenal medulla. A conscious rat in vivo model was used to avoid the influence of anesthesia and surgical intervention on the plasma levels of norepinephrine and epinephrine. An adenosine receptor antagonist, 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX), was used to explore the effect of endogenous adenosine. Plasma levels of epi-
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Methods

Effect of DPSPX on Epinephrine and Norepinephrine Release in Response to Hydralazine-Induced Hypotension

A conscious rat model was prepared as described previously. Briefly, the day before the scheduled experiment, male Sprague-Dawley rats (National Defense Medical Center, Taipei, Taiwan) were anesthetized with pentobarbital sodium (Sigma Chemical Co, 30 mg/kg IP). Both the left carotid artery and left jugular vein were cannulated with a PE-50 catheter (Clay Adams). These two catheters were tunneled subcutaneously to the back of the rat’s neck and extended out of the cage and while protected by a jacket-tether-swivel system (Medical Arts). After these rats regained consciousness, they could freely move in the cage and had unlimited access to normal rat chow and tap water. On the next day before the experiment was started, the arterial catheter was connected to a pressure transducer (P231D, Gould Instruments) and a physiological recorder (RS3800, Gould). Mean arterial blood pressure and heart rate were recorded continuously. The venous catheter was used for intravenous infusion of DPSPX or vehicle (normal saline).

Fourteen male Sprague-Dawley rats weighing 300 to 400 g were used in this experiment. They underwent conscious rat preparation as described above. On the experiment day, they were randomly assigned to either the control group (n=7) or DPSPX group (n=7). The DPSPX group received an intravenous bolus injection of DPSPX (Research Biochemicals, 10 mg in 0.5 mL normal saline) and a continuous intravenous infusion of DPSPX (0.15 mg DPSPX in 0.02 mL normal saline per minute) given through one of the two jugular vein catheters. The control group received a 0.5-mL IV bolus injection of normal saline and a 0.02-mL/min IV continuous infusion of normal saline. Thirty minutes after the infusion of either DPSPX or normal saline was started, 2.5 mL of blood was withdrawn from the arterial catheter for measurement of baseline plasma levels of epinephrine and norepinephrine, and 2.5 mL of saline was infused back to both groups to compensate for the blood volume loss. Then all rats received a bolus intravenous infusion of hydralazine (Sigma, 10 mg/kg IV) administered through the jugular catheter immediately before the injection of DPSPX was started. The control group received a normal saline injection. Thirty minutes after the infusion of DPSPX was started, 2.5 mL of blood was withdrawn from the arterial catheter for measurement of baseline plasma levels of epinephrine and norepinephrine, and a bolus intravenous infusion of hydralazine (10 mg/kg dissolved in 0.5 mL of 0.9% saline) was administered. Sixty minutes later, another 2.5 mL of blood was withdrawn from the arterial catheter for measurement of plasma levels of epinephrine and norepinephrine.

In this study, plasma epinephrine and norepinephrine were extracted using the acid-washed alumina method and were measured with a high-performance liquid chromatograph (6000A, Waters Associates Inc) and electrochemical detector system (LC-4B, Bioanalytical Systems).

Data were analyzed using the Statistical Package for Social Sciences (SPSS). Statistical procedures included Student’s t test, two-factor ANOVA, and Newman-Keuls test. All data are presented and graphed as mean ± SEM. Significance was preset at the .05 level.

Results

Effect of DPSPX on Hydralazine-Induced Catecholamine Release

During the study, hydralazine administration significantly decreased mean arterial blood pressure to the same level in both the control group (from 124±6 to 80±6 mm Hg, P<.01 by paired t test) and DPSPX-treated group (from 126±5 to 78±5 mm Hg, P<.01 by paired t test). These blood pressure data were obtained before the baseline epinephrine and norepinephrine samples were withdrawn and before epinephrine and norepinephrine samples were withdrawn at 60 minutes after hydralazine. As published previously, the depressor effect of hydralazine remained at the same level during the entire experimental period.

In the present study using the conscious rat model, basal epinephrine and norepinephrine levels of the control group were 0.34±0.06 and 0.37±0.3 ng/mL, respectively. The rat group receiving DPSPX treatment had higher baseline epinephrine (1.58±0.2 ng/mL) and norepinephrine and the change in blood pressure were measured as the parameters of treatment effect.

Furthermore, to elucidate the underlying mechanism of endogenous adenosine on catecholamine release, we also examined the effect of adenosine antagonist in adrenalectomized rats and in the presence of an angiotensin-converting enzyme inhibitor, captopril. Adrenalectomy was used to determine whether the change in catecholamine levels seen after endogenous adenosine was modulated is from the adrenal medulla. Captopril was used to explore whether endogenous adenosine modulates the catecholamine release from the adrenal medulla through change in the renin-angiotensin system (RAS).
norepinephrine (2.25±0.3 ng/mL) levels. Intravenous injection of hydralazine at 10 mg/kg significantly increased epinephrine and norepinephrine (Fig 1) levels in both groups. In the control group, the epinephrine level increased to 1.77±0.49 ng/mL and norepinephrine level to 3.34±0.74 ng/mL. In the DPSPX group, the epinephrine level increased to 8.66±2.47 ng/mL and norepinephrine level to 10.80±1.84 ng/mL. Rats receiving DPSPX had significantly higher levels of epinephrine and norepinephrine after 60 minutes of hydralazine-induced hypotension. Two-way ANOVA showed that the effects of both hydralazine and DPSPX were significant. The significant ANOVA interaction between hydralazine and DPSPX (P<.01) indicated that the effect of DPSPX was augmented in the state of hydralazine-induced hypotension.

In the adrenalectomy study, there was no difference in arterial blood pressure before the first 2.5 mL of blood was taken (92±4 versus 89±3 mm Hg, adrenalectomy versus sham operation). Up to this point there was no difference in protocol between the adrenalectomy and control groups. Immediately after the first 2.5 mL of blood was withdrawn, adrenalectomy or sham operation was conducted, and the hydralazine injection was given. Hydralazine caused the same pattern and extent of blood pressure drop in the two rat groups. At 30 and 60 minutes after hydralazine, there was no difference in blood pressure between the two groups (adrenalectomy group: 62±3 versus 65±3 mm Hg, 30 versus 60 minutes; sham operation group: 60±4 versus 66±5 mm Hg, 30 versus 60 minutes). The norepinephrine and epinephrine data in this part of the study showed that the sham-operated animals had lower epinephrine (3.8±0.9 ng/mL) and norepinephrine (2.6±0.7 ng/mL) responses (Fig 2) to hydralazine-induced hypotension when the data were compared with the conscious rat model. Adrenalectomy eliminated the epinephrine response; the level dropped below the lowest detection limit of our assay. There was no significant difference in norepinephrine levels caused by adrenalectomy as examined by Student's t test and a nonparametric test.

Effects of DPSPX on Hydralazine-Induced Catecholamine Secretion in Rats Pretreated With Captopril

In the captopril pretreatment study, there was no difference in blood pressure between the two groups before captopril and DPSPX administration (128±6 versus 124±5 mm Hg, captopril versus control). Thirty minutes after the administration of captopril or normal saline, there was no difference in blood pressure between the two groups (124±6 versus 122±6 mm Hg, captopril versus control). In this experiment, hydralazine caused the same degree and pattern of hypotension as...
observed in the experiment without captopril pretreatment. The animals' blood pressure decreased to a steady hypotensive state by 15 minutes after hydralazine injection (86±4 versus 83±3 mm Hg, captopril versus control). There was no statistical difference (P>.05). Blood pressure stayed at this hypotensive level throughout the protocol. Sixty minutes later, after hydralazine was given, at the time before the second epinephrine and norepinephrine samples were taken, the animals' blood pressure values were 80±4 mm Hg (captopril group) and 77±4 mm Hg (control group).

Rats pretreated with normal saline or captopril followed by DPSPX treatment had similar prehydralazine epinephrine (1.63±.19 versus 1.50±.60 ng/mL) and norepinephrine (2.36±.26 versus 2.14±.21 ng/mL) levels (Fig 3). However, pretreatment with captopril significantly attenuated the plasma epinephrine (from 8.92±2.37 to 5.17±1.21 ng/mL, n=9, P<.01) and norepinephrine (from 11.22±1.73 to 6.53±1.46 ng/mL, n=9, P<.05) responses to hydralazine-induced hypotension (Fig 3).

**Discussion**

In this study, we tested the hypothesis that endogenous adenosine inhibits catecholamine release from the adrenal medulla. The stimuli of catecholamine release used in this study were a combination effect of blood withdrawal and hydralazine-induced hypotension. To avoid the influence of anesthesia and surgical procedures, we used a conscious rat model. We used the surface adenosine receptor antagonist DPSPX to explore the effect of endogenous adenosine. DPSPX does not penetrate into the intracellular space, and this characteristic should prevent it from affecting intracellular cyclic nucleotide and calcium levels. Also, with its sulfonate group, DPSPX does not pass across the blood-brain barrier into the central nervous system.

In the present study, intravenous infusion of DPSPX significantly elevated baseline epinephrine and norepinephrine concentrations in the conscious rat model. When hydralazine decreased blood pressure, the plasma catecholamine levels of both the control and DPSPX groups rose in response to hypotension. Most interesting was the fact that the DPSPX-treated group had significantly higher levels of epinephrine and norepinephrine responses to hydralazine-induced hypotension. This finding suggests that endogenous adenosine suppresses baseline plasma catecholamine release. When the sympathetic system was activated by physiological or pharmacological stimuli, this inhibitory effect of endogenous adenosine on plasma catecholamine level became even more prominent. Possible adenosine receptors responsible for this modulatory function include those located on the peripheral sympathetic endings and those regulating the RAS. Despite our prior study demonstrating the direct effect of adenosine receptors of the central nervous system in the control of autonomic outflow, DPSPX is unlikely to be able to cross the blood-brain barrier to block the central adenosine receptors because of its chemical characteristics. However, this does not preclude the possibility of a secondary mechanism such as an augmented angiotensin level causing a higher level of central sympathetic outflow, which results from blockade of the inhibitory effect of adenosine on the RAS. We designed the second part of our study to assess how much the effect of DPSPX on serum epinephrine and norepinephrine levels was from the alteration of the RAS.

In our adrenalectomy study, there was no difference in blood pressure between the adrenalectomy and sham operation groups. The catecholamine response to hydralazine was significantly lower than that obtained with the conscious animal model. This result indicates that the conscious animal model is more physiologically relevant and responsive. As predicted, anesthesia suppresses catecholamine responses. The epinephrine level after adrenalectomy dropped to an undetectable level (our detection limit for epinephrine was 0.2 ng/mL), suggesting that the augmented epinephrine response to hydralazine in DPSPX-treated rats was from the adrenal medulla. This supports our hypothesis that endogenous adenosine modulates epinephrine release from the adrenal medulla. The mean value of norepinephrine after adrenalectomy demonstrated a tendency to decrease, but without statistical significance. This result suggested that the difference in norepinephrine level produced by DPSPX treatment is mainly from sympathetic nerve ending spillover. Adrenalectomy successfully eliminated the epinephrine response but did not cause a similar effect on norepinephrine level. In our earlier data, DPSPX did not alter norepinephrine spillover of the mesenteric sympathetic nerve ending under a continuous fixed frequency firing. However, it is conceivable that the augmented RAS by DPSPX treatment could either increase sympathetic outflow or enhance norepinephrine release from the nerve endings. As shown in this part of the data, DPSPX treatment increased the...
serum norepinephrine level under hypotension, but the major source of this elevated norepinephrine level was not from the adrenal medulla. We could not assess whether the norepinephrine release from the adrenal medulla was regulated by endogenous adenosine receptors, as in the case of epinephrine release. In this discussion, because there was little literature or theoretical evidence to suggest that adenosine blockade would alter catecholamine clearance, we interpreted an increase in serum catecholamine level to be a result of an increase in release.

Regarding the interaction between adenosine receptors and the RAS, previous studies have shown that the inhibitory effect of adenosine on the RAS is augmented whenever the sympathetic system is stimulated, e.g., during sodium restriction, and during hemorrhagic shock. There is also evidence that the inhibitory effect of endogenous adenosine on epinephrine release from the adrenal medulla and its suppressing effect on plasma norepinephrine levels are partially attributable to endogenous adenosine-adenosine receptors in response to hydralazine-induced hypotension. Based on our prior published data, we suggest that the effect of adenosine on RAS is through the inhibitory effect of endogenous adenosine on renin release. DPSPX, an adenosine antagonist, causes an increase in renin level, which leads to a higher angiotensin II level. Angiotensin II then facilitates the neural/adrenal release of catecholamines. Plasma renin activity was not measured in this study because of the limitation of how much blood we could take from the experimental rats. Each measurement of plasma renin activity would have needed an additional 1 mL of blood from the animal, which we did not feel was reliable or worthwhile after the first 2.5-mL blood sample for epinephrine and norepinephrine.

In the present experiment, short-term captopril injection with the concurrent administration of DPSPX did not change the anesthetized animal's blood pressure. It is not surprising that captopril does not work as a short-term hypotensive agent, as has been observed in clinical experience. The captopril effect on epinephrine and norepinephrine with DPSPX administration was probably not secondary to its effect on blood pressure changes. Even if captopril did decrease the animal's blood pressure within minutes to an hour, the captopril-treated animals would have a lower blood pressure, which would theoretically contribute to higher epinephrine and norepinephrine levels. This would be exactly opposite to what we observed.

In summary, we propose that endogenous adenosine restrains epinephrine release from the adrenal medulla and suppresses plasma norepinephrine levels in conscious rats under physiological conditions. This modulatory function of endogenous adenosine is achieved partially by an effect of adenosine on the RAS, and this effect is augmented whenever the sympathetic system is stimulated.

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