CHRONIC CAFFEINE CONSUMPTION POTENTIATES THE HYPOTENSIVE ACTION OF CIRCULATING ADENOSINE

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Summary

Mean arterial blood pressure was correlated with arterial plasma adenosine levels during intravenous adenosine infusion in unanesthetized, unrestrained rats. Elevation of plasma adenosine to 5 to 6 μM (normal range 1.6 to 4.6 μM) depressed mean arterial pressure by 20 to 30 percent: this was blocked by a single caffeine injection (15 mg/kg). In contrast, caffeine consumption for 3 weeks, followed by a 1-day washout, markedly potentiated responses to adenosine, plasma levels in the 2 to 4 μM range causing 30 to 40 percent reductions in mean arterial pressure. These observations suggest that chronic occupancy of cardiovascular adenosine receptors by caffeine can enhance tissue responsiveness to adenosine, and that endogenous adenosine might act as a circulating hormone.

Adenosine administration can elicit bradycardia, hypotension, hypothermia, vasodilatation, and sedation; it can also diminish gastrointestinal motility, protect against experimentally induced seizures, and attenuate the responses of the kidney, adipose tissue, and the cardiovascular system to sympathetic stimulation (1-4). In vitro, adenosine can inhibit neurotransmitter release, suppress spontaneous or electrically evoked neuronal firing, relax smooth muscle, and increase (5,6) or decrease (7-9) the activity of adenylate cyclase in various tissues. That some of adenosine's physiological effects are mediated by extracellular receptors is suggested by their potentiation by agents inhibiting adenosine's cellular uptake, and by the presence in membrane preparations from brain, testis, and adipose tissue of macromolecules to which radiolabeled adenosine analogs bind with high affinity. However, similar binding sites have not yet been rigorously demonstrated in other adenosine-sensitive tissues like heart and blood vessels (10-12). Adenosine's pharmacological effects can be blocked by caffeine and theophylline at concentrations (~10 μM) likely to occur in plasma after consumption of coffee or tea (13,14); that this antagonism may mediate the behavioral effects of the methylxanthines is suggested by the fact that the potencies of various alkylxanthines in causing behavioral excitation parallel their potencies in blocking the binding of [3H]cyclohexyl-adenosine to brain membranes (15). However, evaluation of the significance of endogenous adenosine as a physiological regulatory agent, as well as establishment of adenosine-antagonism as a significant mechanism of action for pharmacological effects of methylxanthines, depends on measurement of normal levels of adenosine in tissues as well as determination of the
actual concentrations of adenosine needed to elicit significant physiological responses in vivo.

Determination of actual levels of free adenosine in plasma and tissues in vivo is particularly susceptible to artifactual interference during tissue sampling because 1) adenosine is rapidly taken up and metabolized by most tissues, including blood cells; this can result in extremely low recoveries of adenosine especially in blood samples (16,17) and 2) the autolytic degradation of adenine nucleotides during tissue fixation tends to result in spuriously high estimates of adenosine in most tissues, particularly those with a high metabolic rate such as heart and brain (18). Thus, although a number of estimates of normal tissue and plasma adenosine concentrations have been reported, their reliability is questionable since demonstrably adequate sampling techniques are not generally employed. Moreover, to our knowledge, there are apparently no published data describing the actual levels of arterial plasma adenosine that are needed to elicit significant physiological responses in unanesthetized, intact animals.

We have developed a reliable method for measuring blood adenosine levels, and have used it to assess the plasma adenosine concentrations needed to produce cardiovascular responses in rats receiving adenosine infusions with or without acute or chronic doses of caffeine. Our observations suggest that circulating adenosine can indeed exert important effects in rats when elevated only slightly beyond its basal range, or when animals receive a treatment (such as chronically administered methylxanthines) that enhances the adenosine-sensitivity of the cardiovascular system.

Materials and Methods

Silastic catheters were implanted into the right jugular vein and left carotid artery of 350 to 400 g male Sprague-Dawley rats and externalized at the base of the skull. After 1 week of recovery, the arterial catheter was extended and connected to a Statham blood-pressure transducer interfaced to a Grass Model 70 Polygraph and the venous catheter to a pump that could deliver an adenosine solution (10 mg/ml in 0.9 percent saline) or saline continuously at constant rates varying between 0.01 and 0.1 ml/minute. The extended catheters were led out of the top of the rat's home cage and protected by a light coil spring, such that the rat was essentially unrestrained and undisturbed. Arterial pressure was monitored continuously, and samples of arterial blood were withdrawn at intervals for estimation of plasma adenosine concentration.

Blood samples (0.3 ml) were rapidly withdrawn, via the arterial catheter, into syringes preloaded with 0.3 ml of ice-cold heparinized (200 U/ml) 0.9 percent saline containing 0.5 mM dipyridamole, an inhibitor of adenosine transport into cells (19). The sample was immediately centrifuged and 450 µl of the resulting supernatant fluid was deproteinized by mixing with 150 µl of 15 percent trichloroacetic acid followed by centrifugation. This final supernatant fluid was neutralized with excess calcium carbonate. To assess the stability of adenosine in, and its recovery from, rat blood, we added [3H]-adenosine (1 µM) (Moravek Biochemicals) to the dipyridamole solution in the blood-sampling syringe described above. Recoveries ranged from 93 to 98 percent, indicating that the adenosine is stable and well-extracted under these sampling conditions.

Adenosine in plasma extracts was assayed by HPLC, using a Waters µBondapak C-18 reversed-phase column with isocratic elution (10 percent methanol in 10 mM potassium phosphate, pH 5.8) at a flow rate of 1.5 ml/minute at 30°C. Adenosine was detected by UV absorbance at 254 nm.
The adenosine peak could be completely eliminated by preincubating plasma extracts with adenosine deaminase; this also caused a corresponding increase in the inosine peak. Gross and visible hemolysis caused elevations in apparent plasma adenosine levels, which could be blocked by addition to the collecting syringe of a 5'-nucleotidase inhibitor, α,β-methylene adenosine diphosphate; however, such hemolysis rarely occurred spontaneously, nor did the inhibitor modify adenosine levels in unhemolyzed samples. The adenosine measured by this method probably does indeed represent free adenosine, not bound to plasma proteins, since deproteination by ultrafiltration gives similar recoveries and values for plasma adenosine; the TCA-calcium carbonate extraction is faster and simpler than ultrafiltration for large numbers of samples.

Caffeine was administered acutely via the arterial catheter or chronically as a 0.1 percent solution replacing the animal's drinking water.

The BMDP3D program was used for all statistical analyses. Data from each treatment group (control, acute caffeine, and chronic caffeine) were subdivided according to adenosine infusion rate for comparisons of adenosine levels vs MAP pressure between the three treatment groups by t-tests and by the Hotelling T² test.

Results

The average basal adenosine concentration in arterial plasma was 3.0 ± 0.8 μM (mean ± S.E.M.; n=9) with a range of 1.6 μM to 4.6 μM. There was no significant correlation between resting plasma adenosine levels and mean arterial pressures (MAP). However, intravenously infused adenosine induced a significant (20 to 30 percent; P < .001) drop in MAP when arterial levels were elevated to 5 to 6 μM, and a maximal (40 to 60 percent; P < .001) fall at adenosine levels of 10 μM or greater (Figs. 1, 2). As expected (20), blood

![Graph](attachment:image.png)

Correlation between MAP and arterial plasma adenosine concentration in control rats (•) and in animals pretreated with caffeine (○) (15 mg/kg intraruminally) 5 minutes before testing. After a basal blood sample (● = control; ○ = caffeine-treated) was withdrawn, animals received continuous adenosine infusions at a constant flow rate for 3 to 5 minutes, by which time blood pressure had stabilized. MAP was then noted (●,○) and another arterial sample drawn. The infusion rate was then increased, and after stabilization, MAP was noted (●,○) and another blood sample was withdrawn via the arterial catheter. Each rat was subjected to 4 or 5 different rates of adenosine infusion. Each treatment group contained 4 rats.
pressure response to exogenous adenosine was markedly attenuated (P < .01) among rats pretreated with caffeine (15 mg/kg intra-arterially 5 minutes before adenosine infusion) (Fig. 1). The caffeine alone caused an insignificant increase in MAP (131 ± 13 vs 113 ± 15 mm Hg).

In rats (n = 7) allowed to drink a caffeine solution (0.1 percent) for 3 weeks, followed by an abrupt return to tap water 24 hours before testing, basal MAP was not significantly different from that of control animals (117 ± 12 vs 113 ± 15 mm Hg) and resting arterial plasma adenosine levels were slightly reduced to 1.5 ± 0.6 μM (P < .02). Cardiovascular responses to infused adenosine were now observed at arterial plasma concentrations which had been ineffective in control animals, and which fell within the control range for basal adenosine concentrations (Fig. 2). A significant fall (P < .01) in blood pressure was observed at 2 to 4 μM adenosine, and the fall at 5 to 6 μM was significantly greater than that seen in control animals (P < .01).

![FIG. 2](image)

Correlation between MAP and arterial plasma adenosine concentrations during adenosine infusion in control rats (○) and in animals that had consumed a caffeine solution (■) (0.1 percent) for 3 weeks followed by a return to tap water 24 hours before testing. Adenosine was administered and samples collected (+ = basal conditions; ● = adenosine infusion) as described in the legend to Fig. 1. The control group contained 5 rats and the caffeine-withdrawal group 7 rats.

Rats consumed 26 ± 4 ml of the caffeine solution per day, providing approximately 65 mg/kg caffeine per day. Control animals consumed 25 ± 5 ml of tap water per day. Blood pressures and plasma adenosine levels were unaffected by infusing saline at the same rates as the adenosine solution, or by the withdrawal of blood samples for chemical assays.

**Discussion**

The values for adenosine concentration in normal rat arterial plasma reported here are substantially higher than most other reported measurements in rats, or other species including man, which typically are in the range of 0.1 μM. This may reflect the fact that careful sampling procedures, such as direct withdrawal of blood into dipyridamole and rapid sample processing, are not generally used (21-23); in the absence of such precautions, up to 90 to 95 percent of plasma adenosine can be taken up into blood cells during normal sample processing (16,17). Blood adenosine levels in the range of 1 to 12 μM have been reported in dogs, rats and rabbits in cases in which samples were
withdrawn into a dipyridamole solution (16,24). The major sources of circulating adenosine are not yet defined; tissues such as skeletal muscle, kidney, heart, brain, adipose tissue, and vascular endothelium can produce adenosine, particularly when energy demands exceed tissue capacity for regeneration of ATP, resulting in net degradation of adenine nucleotides. The liver, however, normally salvages circulating waste purines (especially hypoxanthine), converts them to adenosine and then releases this adenosine into the circulation; adenosine concentrations as high as 32 nM have been found in the hepatic venous effluent of rabbits (16). Lung tissue is reported to avidly take up adenosine (25), but the possibility that it also releases adenosine into the circulation has not been assessed; the liver for instance, in addition to producing adenosine can also take up and metabolize the nucleoside (26).

The observations that micromolar adenosine concentrations can depress blood pressure in vivo, and that this effect is antagonized by a relatively low caffeine dose are consistent with the hypothesis that the hypotension is mediated by adenosine receptors associated with the cardiovascular system.

The reduction of basal plasma adenosine levels after chronic caffeine consumption suggests that this treatment might modify the physiologic disposition of adenosine; however our technique of correlating actual plasma adenosine concentrations with blood pressure changes noted during adenosine infusions should minimize the influence of any such modifications upon our estimations of cardiovascular sensitivity to adenosine. At any given infusion rate, the increase in steady-state arterial adenosine levels tended to be similar in caffeine-treated and control animals, and infusion of inosine and hypoxanthine, the immediate catabolites of adenosine, had no effect upon blood pressure in our system (P < .01). The enhancement by chronic caffeine of the blood pressure response to adenosine at the lower plasma adenosine concentration examined most likely reflects a change in sensitivity to receptor-mediated actions of adenosine. Possibly the prolonged blockade of adenosine receptors in blood vessels or heart by caffeine induces a compensatory increase in the density or sensitivity of the receptors, thereby potentiating adenosine's hypotensive action once the caffeine is removed. Alternatively, the increase in tissue sensitivity to adenosine may be a compensation secondary to the reduction in arterial plasma adenosine levels seen after chronic caffeine exposure.

It has been reported that daily caffeine administration for one week results in a small (20 percent) increase in brain adenosine receptor density (27). Although there have been several reported attempts to identify cardiovascular adenosine receptors with radiolabeled ligands (28-30), the adenosine-binding sites described in these studies have not been adequately demonstrated to possess the pharmacological and kinetic properties expected of an adenosine receptor, according to the rigorous standards set in the identification of brain adenosine receptors; thus it is not yet possible to determine whether chronic caffeine administration produces alterations in cardiovascular adenosine receptors. Moreover, physiologic studies (31) dissociating the chronotropic and hypotensive effects of adenosine derivatives suggest that more than one receptor subtype could contribute to adenosine's hypotensive effect.

Adenosine is known to have at least three actions that could contribute to its hypotensive effect in vivo (32): it directly depresses cardiac and vascular smooth muscle; it attenuates the responsiveness of myocardial and smooth muscle cells to catecholamines; and it acts presynaptically to inhibit catecholamine release from sympathetic terminals. The relative contributions of these three actions to the hypotension observed in intact animals is unresolved: in isolated, perfused vascular preparations, adenosine is most potent as a presynaptic inhibitor of norepinephrine release (33). In vivo, direct relaxation of
smooth muscle may be more significant, since the hypotensive potency of adenosine is not reduced in rats pretreated with reserpine (2 mg/kg/day) for the two days before testing (unpublished results). However, during the mild hypotension induced by infusion of adenosine at relatively low rates, we observe no obvious evidence for a compensatory increase in sympathetic outflow (e.g., tachycardia), and the reduced blood pressure remains remarkably constant for at least 30 minutes at plasma adenosine concentrations of about 6 μM. This observation is compatible with the view that adenosine’s hypotensive effect in vivo is mediated in part by inhibition of tonic sympathetic transmission. We did not see significant bradycardia at arterial adenosine levels below 8 to 10 μM, suggesting that vascular effects are of primary importance in producing the hypotension.

The effects of endogenous adenosine on cardiovascular tissues have generally been thought to be restricted to the particular tissue in which the adenosine was formed and released (33). Indeed, adenosine is produced in the heart and vasculature in response to hypoxia or sympathetic stimulation, and may act locally to attenuate effects of these stimuli by inducing vasodilation and by inhibiting further norepinephrine release (34). However, the cardiovascular sensitivity to adenosine observed in the present studies (particularly in the case of rats withdrawn from chronic caffeine exposure) suggests that circulating adenosine might also be able to function in a hormone-like role, perhaps affecting vascular dynamics in tissues throughout the body.

These data, however, are not sufficient to establish a primary causative role for endogenous circulating adenosine in cardiovascular regulation, as circulating adenosine might merely reflect higher local concentrations in tissue extracellular spaces; however the levels of circulating adenosine needed to affect blood pressure do fall within the normal range for circulating adenosine, particularly in the case of the rats withdrawn from chronic caffeine exposure.

This strategy for determining the actual adenosine levels needed to produce cardiovascular effects in unanesthetized, unrestrained rats, might be useful for evaluating the possible physiological relevance of other easily monitored functions that peripherally administered exogenous adenosine is known to modulate, such as lipolysis (35), renal function (36), sympathetic nervous activity (32), and behavior (1). This might be particularly significant since anesthetized animals (or isolated tissues) are generally used for such studies and we have observed that the hypotensive potency of adenosine is increased approximately 5-fold in urethane-anesthetized rats (unpublished observations); whether other adenosine actions are similarly affected remains to be determined. In addition, such studies could be used to test for alterations in tissue sensitivity to adenosine following various hormonal, nutritional or pharmacological manipulations.

The presence of significant adenosine concentrations in tissue or blood is usually attributed to a disruption in energy metabolism causing a net degradation of adenine nucleotides (18). However, since conversion of circulating hypoxanthine to adenosine by the liver is not a result of impaired hepatic energy metabolism (16), and since hepatic purine metabolism (and thus perhaps adenosine production) can be modified by nutritional manipulations (37), circulating adenosine levels (which we found to vary over almost a 3-fold range in otherwise-untreated rats) might also reflect normal variations in nutritional state. Since circulating adenosine is transported across the blood-brain barrier by a transport system largely unsaturated at normal plasma adenosine levels (38), variations in its levels might also affect brain composition and function.
Our observation that the cardiovascular system responds to long-term adenosine-receptor blockade (by caffeine) by becoming more sensitive to adenosine raises the possibility that some of the common sequelae of caffeine withdrawal such as headache and fatigue (39), might reflect enhanced tissue sensitivity to endogenous adenosine. Caffeine itself is used to treat several types of headache, where its beneficial effect is believed to result from its ability to constrict cerebral arteries (40); adenosine is a potent dilator of the cerebral vasculature (41).

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References

34. R.M. BERNE, Circ. Res. 47 807-813 (1980)