Caffeine Attenuates the Renal Vascular Response to Angiotensin II Infusion

Nancy J. Brown, Diane Ryder, John Nadeau

Non-modulation has been proposed as an intermediate phenotype in human essential hypertension. The trait is characterized by blunted aldosterone and renal plasma flow responses to short-term angiotensin II (Ang II) infusion. Elevated tissue Ang II levels or decreased tissue adenosine levels could account for this decreased sensitivity to Ang II. In support of the latter possibility, endogenous adenosine has been shown to contribute to the renal vasoconstrictive response to Ang II in animals. We therefore tested the hypothesis that endogenous adenosine contributes to modulation of renal plasma flow in sodium-replete humans. We examined the effect of long-term administration of the adenosine receptor antagonist caffeine on baseline renal plasma flow and on the renal plasma flow response to short-term Ang II infusion in six salt-replete normotensive subjects in a single-blind, placebo-controlled study. Para-aminohippurate clearance was used to assess renal plasma flow. Ang II was infused in graded doses (0.3 to 3 ng/kg per minute) in the presence and absence of caffeine (250 mg PO TID for 7 days). Blood pressure, plasma renin activity, Ang II, electrolytes, and para-aminohippurate clearance were measured before and after each dose of Ang II. Caffeine did not alter either baseline blood pressure or the blood pressure response to Ang II but did increase baseline plasma renin activity from 0.72±0.09 to 1.42±0.26 ng angiotensin I/mL per hour (P=.01). Caffeine decreased the baseline renal plasma flow from 553±38 to 476±31 mL/min per 1.73 m$^2$ (P=.004) and attenuated the renal plasma flow response to a 3 ng/kg per minute infusion of Ang II (−106.5±25.2 versus −170.5±18 mL/min per 1.73 m$^2$, P=.006). These data demonstrate that caffeine modulates baseline renal plasma flow and the renal plasma flow response to exogenous Ang II and therefore support the hypothesis that adenosine contributes to modulation of renal plasma flow in salt-replete humans. Thus, non-modulation may be partially acquired, and caffeine consumption must be controlled in studies that define modulation phenotype. (Hypertension. 1993;22:847-852.)

KEY WORDS • caffeine • renal circulation • adenosine • angiotensin II • hypertension, non-modulating

Of patients with normal- or high-renin essential hypertension, 40% to 50% are "non-modulators." Their blood pressure rises when they ingest a high-salt diet, and they fail to increase (modulate) their renal blood flow in response to high-sodium intake. In addition, both their renal plasma flow response to angiotensin II (Ang II) infusion on a high-salt diet and their aldosterone response to Ang II on a low-salt diet are blunted compared with those of normotensive control subjects.

Several lines of evidence suggest that non-modulation is inherited. For example, 84% of non-modulators report a family history of hypertension. There is also a high degree of concordance in the renal vascular response to Ang II infusion among hypertensive sibling pairs. In addition, in patients with normal- to high-renin essential hypertension, the aldosterone response to Ang II infusion appears to be bimodally distributed. This aldosterone response to Ang II correlates well with the renal vascular response to Ang II.

Despite this evidence that non-modulation is inherited, the mechanism underlying the abnormal sensitivity to Ang II in non-modulation is not known. One hypothesis is that tissue levels of Ang II are chronically elevated in non-modulators, resulting in downregulation of Ang II receptors. In support of this hypothesis, administration of angiotensin converting enzyme inhibitors enhances both the aldosterone and renal plasma flow responses to short-term Ang II infusion in non-modulators.

An alternative explanation for the decreased sensitivity to Ang II observed in non-modulators is that tissue levels of adenosine are decreased in these subjects. Adenosine is known to interact with the renin-angiotensin-aldosterone system at a number of levels. In particular, both adenosine and Ang II cause vasoconstriction at the renal afferent arteriole. In dogs, the renal vasoconstrictive effects of adenosine and Ang II have been shown to be synergistic; in rats, adenosine receptor blockade attenuates the renal blood flow response to Ang II.

These data suggest that adenosine deficiency may contribute to the defect in non-modulation. A second implication of these data is that environmental as well as genetic factors may contribute to the non-modulation phenotype. For example, the animal data suggest that the ingestion of an adenosine receptor antagonist, such as caffeine, might attenuate the renal plasma flow response to Ang II in humans. Because the renal plasma...
flow and aldosterone responses to short-term Ang II infusion are used to classify subjects as modulators or non-modulators; caffeine ingestion may alter the classification of hypertensive and control subjects.

In this placebo-controlled, single-blind study, we examined the effect of long-term caffeine administration on the renal plasma flow dose response to short-term Ang II infusion in modulating normotensive control subjects ingesting a high-salt diet.

**Methods**

**Subjects**

Six normotensive subjects participated in this study. Entrance criteria included a normal physical examination and normal routine laboratory screening, electrocardiogram, and chest x-ray. Subjects with an upright plasma renin activity of less than 2.4 ng/mL per hour on a 10 mEq/d sodium diet were excluded from the study. Women of childbearing potential and subjects taking medicines were also excluded. Smokers were not excluded but were asked to maintain their cigarette consumption constant throughout the study. All subjects gave written, informed consent; the study protocol was approved by the institutional review board of Vanderbilt University.

During the first arm of the study, subjects were classified according to their aldosterone and estimated renal plasma flow responses to short-term Ang II infusion. Subjects whose aldosterone levels increased at least 15 pg/dL in response to a 3 ng/kg per minute infusion of Ang II (in the setting of a low-salt diet) and whose renal plasma flow decreased at least 125 mL/min per 1.73 m² (in the setting of a high-salt diet) were classified as modulators. These criteria have been used previously to classify patients with normal- to high-renin essential hypertension as modulators or non-modulators and appear to yield a bimodal distribution. During the second arm of the study, the effect of caffeine on the renal plasma flow response to Ang II was measured.

**Protocol**

Fig 1 outlines the classification protocol. Subjects first ingested a xanthine-free diet containing 10 mEq sodium, 100 mEq potassium, and 2500 mL fluid per day. Subjects were asked to collect all of their urine each day for measurement of sodium, potassium, creatinine, and volume. On the night of the fifth study day, subjects were admitted to the Clinical Research Center of Vanderbilt University Hospital. On the morning of the eighth study day, blood for plasma renin activity was drawn through an indwelling catheter, first after the subject had remained supine for 120 minutes and then after 120 minutes of upright posture.

On the morning of the 10th study day, each subject's aldosterone and renal plasma flow response to short-term Ang II infusion was measured. Subjects were kept supine and fasting after midnight the night before. At 7:30 AM an intravenous catheter was placed in each arm. One catheter was used for venous blood sampling, and the second was used for drug infusion. An automated blood pressure cuff was placed over the brachial artery of the arm from which samples were drawn. Each subject's blood pressure and pulse were measured every 2 minutes throughout the study period. At 8 AM, each subject was given an 8 mg/kg loading dose of paraminohippurate (PAH) followed by a constant infusion at 12 mg/min. At 9 AM, the subject was given a continuous infusion of Ang II at successive doses of 0.3, 1, and 3 ng/kg per minute for 45 minutes each. Blood samples for PAH levels were drawn at 8:45, 8:50, and 8:55 AM and then after each successive dose of angiotensin. Blood for plasma renin activity, aldosterone, Ang II, catecholamines, and electrolytes was drawn before and after each successive dose of Ang II.

When the infusion study was completed, the sodium content of the subjects' diet was increased to 200 mEq/d. Subjects were also begun on tablets containing placebo three times a day. On the morning of the 14th study day, the short-term Ang II infusion was repeated as before. The placebo tablet was given at 7 AM on the morning of the study day.

During the second arm of the study, the renal plasma flow response to short-term Ang II infusion was measured in the presence of caffeine. As before, subjects were given a xanthine-free diet containing 200 mEq sodium, 100 mEq potassium, and 2500 mL fluid per day. They again collected their urine daily. However, during the second study arm, subjects were given tablets containing 250 mg caffeine three times a day. On the night of the fourth study day, subjects were admitted to the Clinical Research Center, and on the morning of the seventh study day the short-term Ang II infusion was repeated.
Analytical Methods

Renal plasma flow was measured by determining the clearance rate of PAH from plasma as previously described.\textsuperscript{13,14} PAH levels were measured using the method of Brun.\textsuperscript{15} Urine and serum electrolytes were measured by flame photometry. Blood samples for plasma renin activity, Ang II, and aldosterone levels were collected on ice in tubes containing 0.3 mL of 10% EDTA, centrifuged at \(-4^\circ C\) for 20 minutes, and separated immediately. Plasma renin activity was measured by radioimmunoassay for angiotensin I.\textsuperscript{16} Aldosterone was measured by radioimmunoassay using the Coat-a-count aldosterone assay kit from Diagnostic Products Corp, Los Angeles, Calif.\textsuperscript{17} Ang II levels were measured by radioimmunoassay.\textsuperscript{18} Blood samples for measurement of norepinephrine and epinephrine were collected in iced heparinized tubes and then measured by high-performance liquid chromatography with electrochemical detection.\textsuperscript{19}

Statistical Analysis

Data are presented as mean±SEM. Statistical analysis was performed by use of the Number Cruncher Statistical System (NCSS, Kaysville, Utah). The angiotensin dose-response curves in the presence and absence of caffeine were compared by two-way analysis of variance in which the variables were Ang II dose and treatment arm. Comparisons between treatment arms at specific Ang II doses were made using a two-tailed, paired Student's \(t\) test or Wilcoxon signed rank test. The criterion for significance was at a value of \(P<.05\).

Because of a malfunctioning Harvard pump, one subject was inadvertently given a bolus of PAH 20 minutes into the 3 ng/kg per minute Ang II infusion during the caffeine arm of his study. This bolus resulted in a large increase in the PAH level measured after the 3 ng/kg per minute Ang II infusion. The estimated renal plasma flow calculated at this time point was therefore falsely low. Despite this error, the change in estimated renal plasma flow in response to 3 ng/kg per minute Ang II was smaller in the caffeine arm than in the placebo arm. This subject's estimated renal plasma flow data are included in the statistical analysis and text. However, the change in PAH clearance after the 3 ng/kg per minute Ang II infusion during either the placebo or caffeine arm of this study is not presented in Fig 2.

Results

Six of seven normotensive subjects screened were classified as modulators. Their upright plasma renin activity in the salt-depleted state was 9.81±2.18 ng angiotensin I/mL per hour. Their baseline estimated renal plasma flow on the 10 mEq diet was 519±41 mL/min per 1.73 m\(^2\); the baseline estimated renal plasma flow was 553±38 mL/min per 1.73 m\(^2\) (\(P=.15\)) on the 200 mEq diet. The mean rise in plasma aldosterone in response to 3 ng/kg per minute Ang II infusion was 28.3±3.9 ng/dL (from 26.7±7.7 to 55±7.8 pg/mL, \(P=.00073\)). The mean decrease in estimated renal plasma flow in response to the 3 ng/kg per minute Ang II infusion was 170.5±18 mL/min per 1.73 m\(^2\) (from 553±38 to 383±23 mL/min per 1.73 m\(^2\), \(P=.00022\)).

The Table lists the baseline characteristics of these normotensive subjects measured in the salt-replete state just before Ang II infusion in the presence and absence of caffeine. Long-term caffeine administration had no effect on baseline blood pressure. Serum sodium, serum potassium, 24-hour urine volume, and 24-hour sodium excretion were similar in the presence and absence of caffeine. Estimated renal plasma flow was significantly lower in the presence of caffeine than in the presence of placebo (476±32 versus 553±38 mL/min per 1.73 m\(^2\), \(P<.005\)). The resting plasma renin activity was also significantly higher in the presence of caffeine (Table). Baseline Ang II and catecholamine levels were similar in the presence and absence of caffeine.

The pressor response to Ang II infusion was similar in the presence and absence of caffeine (Fig 2). By con-

\begin{table}
\caption{Effect of Caffeine Administration on Baseline Characteristics of Six Normotensive Control Subjects}
\begin{tabular}{|l|l|l|}
\hline
\textbf{Characteristic} & \textbf{Placebo} & \textbf{Caffeine} \\
\hline
Weight, kg & 76.3±1.9 & 76.6±1.4 \\
Systolic blood pressure, mm Hg & 117±3.26 & 115±3.26 \\
Diastolic blood pressure, mm Hg & 62.2±3.05 & 66.2±2.50 \\
Serum sodium, mEq/L & 141.8±1.14 & 139.1±0.56 \\
Serum potassium, mEq/L & 3.95±0.076 & 3.97±0.088 \\
24-Hour urine volume, mL & 2427±243 & 2072±261 \\
24-Hour sodium excretion, mEq & 224±17 & 187±24 \\
Renal plasma flow, mL/min per 1.73 m\(^2\) & 553±38 & 476±32* \\
Plasma renin activity, ng Ang I/mL per hour & 0.72±0.088 & 1.4±0.26† \\
Ang II, pg/mL & 24.9±4.3 & 33.5±4.7 \\
Norepinephrine, pg/mL & 135.8±24.4 & 161±17.8 \\
Epinephrine, pg/mL & 5.6±3.6 & 9.7±5.2 \\
\hline
\end{tabular}
\end{table}

*Ang indicates angiotensin. Values are mean±SEM. 
†\(P=.0041\), \*\(P=.013\) compared with placebo.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Line graph shows diastolic blood pressure (DBP) response to short-term angiotensin II infusion in the presence (A) and absence (B) of caffeine.}
\end{figure}
Fig 3. Line graph shows change in para-aminohippurate (PAH) clearance in response to short-term angiotensin II infusion in the presence (A) and absence (■) of caffeine. *P<.0001, +P<.01 compared with placebo; *n=5 at this dose (see text).

In contrast, caffeine administration significantly attenuated the renal plasma flow response to Ang II infusion (Fig 3). Because the baseline renal plasma flow was lower in the caffeine arm of the study, the absolute renal plasma flow after the 3 ng/kg per minute infusion of Ang II was similar in the two study arms (383±23 versus 369±18 mL/min per 1.73 m²). Caffeine administration increased the plasma renin activity response (Fig 4) after Ang II infusion. In contrast to baseline levels, Ang II levels after Ang II infusion were higher in the caffeine arm (Fig 5). Catecholamine levels (data not shown) were not significantly higher in the caffeine arm.

Discussion

In this study, long-term caffeine administration significantly decreased both baseline PAH clearance and the change in PAH clearance after a short-term Ang II infusion in salt-replete normotensive volunteers. Caffeine was administered in doses that approximate doses ingested by moderate to heavy coffee drinkers. Thus, a daily dose of 750 mg caffeine is comparable to the daily ingestion of six to eight cups of coffee a day. A single dose of 250 mg caffeine is comparable to the daily ingestion of six to eight cups of coffee a day. A single dose of 250 mg produces peak plasma caffeine concentrations in the range of 10 to 12 µg/mL, comparable to levels measured in coffee drinkers. Moreover, at these concentrations, caffeine is thought to act through adenosine receptor blockade rather than through phosphodiesterase inhibition.

Caffeine was administered for 7 days because earlier studies have shown that tolerance to the humoral and hemodynamic effects of caffeine develops over 1 to 4 days. In contrast to the findings of these earlier studies, however, baseline plasma renin activity was increased after long-term caffeine administration in the current study. Because normotensive subjects with low renin levels were excluded from this study, the study may have been more sensitive to small effects of caffeine on plasma renin activity.

In this study, PAH clearance was used as a measure of renal blood flow. The possibility that caffeine decreased baseline PAH clearance and by decreasing renal blood flow cannot be excluded. Gouyon and Guignard have shown that doses of 10 mg/kg caffeine do not alter the renal PAH extraction ratio in newborn rabbits. In addition, had caffeine blocked the tubular secretion of PAH, this would falsely lower not only the baseline renal blood flow but also the renal blood flow after Ang II. Instead, the decrease in PAH clearance was less than...
expected after Ang II in the presence of caffeine. Therefore, the data suggest that long-term caffeine administration lowered both baseline renal plasma flow and the renal plasma flow response to short-term Ang II in these salt-replete normotensive volunteers.

The finding that long-term caffeine administration causes renal vasoconstriction contrasts the finding of Beutler et al.\(^5\) that short-term theophylline administration does not affect renal plasma flow. Several methodological differences may explain this discrepancy. These include the drug used (caffeine versus theophylline), the time course of administration (long-term versus short-term), the subjects studied (modulating normotensive versus random normotensive subjects), and sodium intake (200 mEq versus 100 mEq). In addition, Beutler et al did not examine the interaction between methylxanthine administration and Ang II.

The mechanism through which caffeine lowered renal plasma flow and attenuated the renal plasma flow response to short-term Ang II infusion in this study is not known. Administered acutely, caffeine is a diuretic.\(^5\) One possibility is that caffeine-induced sodium depletion caused renal vasoconstriction and increased responsiveness to Ang II. The similar serum sodium levels, urinary sodium excretion, and weight in the presence and absence of caffeine do not support this mechanism.

Alternatively, caffeine may alter the renal plasma flow response to Ang II by acting directly on renal vascular adenosine receptors. In dogs, intrarenal administration of adenosine causes transient renal vasoconstriction followed by vasodilatation.\(^9\) Vasoconstriction appears to be angiotensin dependent, in that administration of an Ang II antagonist blocks the vasoconstrictive response to adenosine in a dose-dependent fashion.\(^11\) Conversely, administration of the adenosine receptor blocker 1,3-dipropyl-8-(p-sulfophenyl)xanthine attenuates the renal vascular response to Ang II infusion in rats.\(^12\)

Caffeine administration increases both sympathetic activity and plasma renin activity.\(^20,22\) Thus, caffeine may cause renal vasoconstriction by increasing renin release and endogenous tissue Ang II levels. Increased tissue Ang II levels would lead to downregulation of vascular Ang II receptors and desensitization to the effects of exogenous Ang II. Baseline plasma renin activity was significantly elevated during the caffeine arm of the present study. Baseline Ang II levels were not significantly higher during the caffeine arm, but this may reflect a type II error. Ang II levels were higher during the angiotensin infusion. Catecholamines were not higher in the caffeine arm of the study, but we did not measure sympathetic nerve activity. The fact that the absolute renal plasma flow after the 3 ng/kg per minute infusion of Ang II was similar in the presence and absence of caffeine would also suggest that increased Ang II levels rather than altered vascular responsiveness underlies the caffeine effect.

If the effects of caffeine on renal plasma flow observed in this study are mediated through the effects of caffeine on renin release and Ang II production, these data would support the hypothesis that tissue Ang II levels are elevated in non-modulators. We have observed that caffeine attenuates the renal vascular response to short-term Ang II infusion in non-modulators pretreated with angiotensin converting enzyme inhibitors but not in untreated non-modulators (unpublished data). This would suggest that both increased tissue Ang II levels and decreased tissue adenosine levels may play a role in non-modulation.

Finally, regardless of the mechanism, the present study demonstrates that caffeine alters the renal plasma flow dose response to short-term Ang II infusion in salt-replete subjects. Because both the renal plasma flow and aldosterone responses to short-term Ang II infusion are used to classify subjects as modulators or non-modulators,\(^3\)\(^-\)\(^5\) ingestion of caffeine may alter the classification of hypertensive subjects. Thus, caffeine consumption must be carefully controlled in studies in which patients with normal- to high-renin essential hypertension are phenotyped. Further studies are needed to examine the effect of caffeine on the aldosterone response to Ang II and to elucidate the mechanism through which caffeine administration alters renal plasma flow.

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