Plasma Renin Activity, Reactivity, Concentration and Substrate Following Hypertension During Pregnancy

Effect of Oral Contraceptive Agents

THEODORE A. KOTCHEN, M.D., J. MORLEY KOTCHEN, M.D., GORDON P. GUTHRIE, JR., M.D., AND CAROL M. CotTRILL, M.D.

SUMMARY Plasma renin activity is suppressed in approximately 25% of patients with essential hypertension, and the rate of in vitro angiotensin I production after addition of exogenous renin (renin reactivity) is increased in plasma of hypertensive patients. We have recently observed that blood pressure (116 ± 1.5/68 ± 1.7 mm Hg) of young women who had hypertension during a first pregnancy 3-6 years earlier (n = 63) was higher (p < 0.005) than blood pressure (109 ± 1.4/61 ± 1.7 mm Hg) of women who remained normotensive during pregnancy (n = 52). To determine if alterations of the renin-angiotensin axis observed in patients with established hypertension also occur in young adults with relatively high blood pressure, plasma renin activity (PRA), plasma renin concentration (PRC), plasma renin substrate (PRS) and plasma renin reactivity (PRR) were compared in these two groups of subjects. Overall, PRA and PRC were inversely related to systolic blood pressure (p < 0.02). Excluding women on oral contraceptive agents, the PRA response to standardized treadmill exercise was suppressed (< 1.0 ng/ml/hr) in 19% of women with a history of hypertension during pregnancy and in no women who remained normotensive throughout a previous pregnancy; PRR did not differ (p > 0.8) in the two groups of young mothers (27.1 ng/ml/30 min ± 1.2 SE vs 26.2 ng/ml/30 min ± 0.9 SE). Thus, renin suppression, but not increased PRR, precedes the onset of hypertension. Oral contraceptive usage was associated with higher systolic blood pressures, increased PRS, and low PRC. Highest blood pressures and lowest PRA occurred in women with a history of hypertension during pregnancy who were taking oral contraceptive agents at the time of study. (Hypertension 1: 355-361, 1979)

KEY WORDS • blood pressure • exercise • hypertension • oral contraceptive agents • pregnancy • renin

We have recently reported that arterial blood pressure of women who had elevated pressure during the third trimester of their first pregnancy 3-6 years earlier is higher than that of women who did not have hypertension in pregnancy.1 The purpose of the present study is to compare measurements of the renin-angiotensin system in these two groups of young women to determine if alterations observed in patients with established essential hypertension also occur in young adults with relatively high blood pressure but without clinical hypertension. At the time of study, only 14% of women with a history of hypertension during pregnancy had blood pressure measurements repeatedly above 140/90 mm Hg.

Plasma renin activity (PRA) is suppressed in approximately 25% of patients with essential hypertension,2,* and it is unclear whether renin suppression is related to the pathogenesis of hypertension or is a consequence of elevated arterial pressure. After adding exogenous renin, the in vitro rate of angiotensin I generation in plasma of hypertensive patients is greater than that in plasma of normotensive subjects,7,* possibly due to a deficiency of a normally occurring renin inhibiting factor.7 What relationship this may have to elevated arterial pressure is also unclear. In the present study, these measurements were compared in groups of normotensive young women with relatively high and relatively low blood pressures. Hypertension induced by oral contraceptive agents has been attributed by some investigators to a renin-angiotensin mechanism.10-13 Fortuitously, approximately half of the young mothers were taking oral contraceptives at the time of the study. Conse-
sequently, the effect of oral contraceptives on the ac-
activity of the renin-angiotensin axis was also compared
in these young women with relatively high and rela-
tively low blood pressures.

Methods

The original study population consisted of 409 preg-
nant primiparous adolescent women. Mean age during
pregnancy was 16.9 years ± 1.3 s.d.; 46% of the women
were white and 54% were black. Overall 74 women
(18%) were diagnosed as having hypertension during
pregnancy on the basis of any one of the following
criteria: systolic blood pressure >140 mm Hg;
diastolic blood pressure >90 mm Hg; >30 mm Hg in-
crease of systolic blood pressure during pregnancy;
>15 mm Hg increase of diastolic blood pressure.
Sixty-three of these 74 women and an additional 52
women selected from the same population and who
did not have hypertension during pregnancy, par-
 participated in the current follow-up study 3 to 6 years
after their first pregnancy. Thirty-four of the 63
women (54%) with a history of hypertension during
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women (54%) with a history of hypertension during
pregnancy and 41 of the 52 women (79%) in the nor-

tensive control group were black. Subjects found to
have hypertension at the time of follow-up examina-
tion were not excluded from study.

Blood pressure was measured in the sitting position
according to a previously described standardized
protocol. Peripheral venous blood was obtained by
separate venepunctures both before and within 30
seconds after standardized treadmill exercise to
measure PRA. Subjects were supine at least 15
minutes before the pre-exercise blood was obtained,
as well as renin substrate. In a separate experiment, substrate but not circulating in-
bhibitors was "selectively" removed by passing plasma
over Sephadex G-50-40. Pharmacia K15 (900 X 15
mm) columns were used. Between 3.5 to 4.0 g of
Sephadex was swollen overnight and columns were
packed by gravity. Five ml of plasma was then placed
on the column, and protein separation was monitored
by reading the absorbance of the eluate at 280 m/n.
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Plasma renin activity (active renin) was assayed in
quadruplicate by radioimmunoassay for angiotensin I
as described by Haber et al. and validated in our
laboratory by bioassay. To maintain a constant pH,
during the 3-hour incubation before assay, tris buffer
(pH 7.4) was added to plasma (100 μl/ml). Total
plasma renin concentration (active plus inactive ren-
in), plasma renin substrate (PRS), and plasma renin
reactivity (PRR) were also measured in plasma ob-
tained before exercise. For measurement of plasma
renin concentration (PRC), plasma was dialyzed
against a glycine buffer to pH 3.3, incubated at 32°C
for 60 minutes and then dialyzed back to pH 7.4
against a phosphate buffer, according to the method of
Skinner. Endogenous renin substrate was totally
denatured by acidification, and a constant concentra-
tion (1000 ng/ml) of exogenous homologous renin
substrate, prepared by the method of Rosenthal et al.,
from plasma of women taking oral contraceptives,
was then added to dialyzed plasma. The concentration
of angiotensin I produced after a 60-minute incuba-
tion at 37°C was measured by radioimmunoassay.
Plasma renin substrate was measured by adding high
concentrations of human renin, (1.6 X 10² GU/ml)
extracted from kidneys by the method of Haas, and
measuring the concentrations of angiotensin I
produced after the reaction between exogenous renin
and endogenous substrate had proceeded to comple-
tion; the concentration of angiotensin I generated
after 3- and 6-hour incubations did not differ.

Plasma renin reactivity is the capacity of a lower
concentration of exogenous renin to generate angio-
tensin I after its addition to plasma. This mea-
surement may be affected by endogenous renin
substrate concentration as well as by normally oc-
curring circulating inhibitors of the renin-renin sub-
strate reaction. The concentration of angiotensin I
produced during a 30-minute incubation after addition
of renin (1.1 X 10⁻³ GU/ml) was measured. At this
eenzyme concentration and incubation time, the rate of
angiotensin generation was linear with time, thus
providing the opportunity to evaluate the kinetics of
the enzymatic activity of added renin. For the com-
putation of PRR, the relatively small concentrations
of angiotensin generated in aliquots of plasma without
added enzyme (1-4% of total angiotensin generated
with added enzyme) were subtracted from the concen-
trations of angiotensin produced after adding renin to
plasma.

To determine if changes in PRR reflect differences
of endogenous renin substrate, PRR was also mea-
sured in plasma in which substrate was denatured by
acidification according to the Skinner PRC procedure;
before addition of enzyme, homologous substrate was
added to these samples (1000 ng/ml). However,
adicion of plasma may denature circulating renin
inhibiting factors as well as renin substrate. In a
separate experiment, substrate but not circulating in-
hibitors was "selectively" removed by passing plasma
over Sephadex G-50-40. Pharmacia K15 (900 X 15
mm) columns were used. Between 3.5 to 4.0 g of
Sephadex was swollen overnight and columns were
packed by gravity. Five ml of plasma was then placed
on the column, and protein separation was monitored
by reading the absorbance of the eluate at 280 m/n.
With this procedure >98% of protein including renin
substrate was separated from plasma. The plasma eluate
without protein contains renin inhibitory activity, but
no measureable renin activity or renin substrate, i.e.,
no detectable angiotensin I was produced after incuba-
tion of the eluate separately with either renin or renin
substrate. The rate of angiotensin production was
measured after addition of this eluate to renin
(8 X 10⁻⁴ GU/ml)-renin substrate (1000 ng/ml).

To provide an estimate of dietary sodium intake,
urine sodium-creatinine ratios were determined in a
timed overnight urine collection. Sodium and
potassium concentration were measured with a flame photometer (Instrumentation Laboratory, Morris Plains, NJ). Serum and urine creatinine concentrations were measured by the method of Kennedy et al.24

Since many variables were not normally distributed, except where noted, it was necessary to utilize non-parametric procedures to test statistical significance. Kendall’s Tau was used to determine if variables were significantly associated, and the Kurskal-Wallis test was used to determine if there were significant differences among groups.25

Results

Based on analysis of variance, at the time of follow-up, in both the supine and sitting positions systolic and diastolic blood pressures of women with a history of hypertension during pregnancy were higher (p < 0.005) than blood pressures of women who had remained normotensive throughout pregnancy, and these differences were maintained after adjusting for an effect of body size on blood pressure (table 1). Blood pressure was not related to race, and blood pressures of blacks and whites did not differ. Systolic blood pressure of women using oral contraceptives was higher (p < 0.004) than respective values of women not using these agents. Excluding women on oral contraceptives and those who were not, the association between PRA and systolic blood pressure was less consistent (table 2). Diastolic blood pressure did not correlate with PRA.

Plasma renin activity increased (p < 0.0001) in response to treadmill exercise; PRA measured after exercise was related to pre-exercise PRA (r = 0.59, p < 0.0001) and duration of exercise (r = 0.21, p < 0.01). Basal and exercise-stimulated PRA were not associated with urinary sodium excretion, estimated by sodium-creatinine ratio in an overnight urine collection. Excluding women on oral contraceptive agents, PRA was lower among blacks than

<table>
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<tr>
<th>TABLE 1. Systolic and Diastolic Blood Pressure, PRA, PRC, PRS, PRR, and Serum Creatinine in Women with a History of Hypertension During Pregnancy and Women who Remained Normotensive During Pregnancy, Compared with Oral Contraceptive (OC) Usage</th>
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<td>Sitting BP (mm Hg)</td>
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<td>PRA (ng/ml/hr)</td>
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*All values are mean ± SE.

Separating subjects into those who were using contraceptives and those who were not, the association between PRA and systolic blood pressure was less consistent (table 2). Diastolic blood pressure did not correlate with PRA.

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<th>TABLE 2. Linear Correlation Coefficients Describing the Association Between Systolic Blood Pressure (SBP) and PRA</th>
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<td>Subjects using oral contraceptives (n = 61)</td>
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<td>Pre-exercise</td>
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<td>Post-exercise</td>
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<td>PRA</td>
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<td>Subjects not using oral contraceptives (n = 53)</td>
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<td>Pre-exercise</td>
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whites, both before and after exercise ($p < 0.03$), although blood pressure did not differ. Both before and after exercise, PRA was significantly lower ($p < 0.02$) in women with a history of hypertension during pregnancy and who were presently taking oral contraceptive agents than in any other group (post-exercise PRA remained significantly lower after adjusting for exercise duration); mean systolic blood pressure was also highest in this group ($p < 0.01$).

Among women not using oral contraceptive agents and excluding women who exercised less than 8 minutes, mean post-exercise PRA of women who were normotensive during pregnancy did not differ from the respective value in women with a history of hypertension during pregnancy. The lowest PRA after exercise of women who were normotensive during pregnancy was 1.0 ng/ml/hr, and this value was included within one standard deviation from the mean (fig. 2). Five of 27, or 19% of women who were hypertensive during pregnancy, had a stimulated PRA below this value, and all five values were below one standard deviation from the mean. Mean exercise duration and mean urinary sodium-creatinine and sodium-potassium ratios of these five women did not differ from respective values in either the remainder of this group or

**FIGURE 1.** Correlation between systolic blood pressure (SBP) and plasma renin activity (PRA) before exercise. O.C. = oral contraceptives.

**FIGURE 2.** Post-exercise plasma renin activity (PRA) in women who were normotensive during pregnancy and in women with a history of hypertension during pregnancy. Subjects on oral contraceptive agents are not included. Shaded area represents 1 standard deviation from the mean.
control women who were normotensive throughout pregnancy. Four of the five women were black, and none of the five was hypertensive.

Plasma renin concentration (PRC) measured before exercise correlated with PRA \( (r = 0.43, p < 0.0001) \) and was also inversely related to systolic blood pressure \( (r = -0.24, p < 0.02) \). Mean PRC of women taking oral contraceptive agents was less than that of women not receiving these drugs \( (p < 0.01) \), although PRC was not related to previous blood pressure history. We found no association between PRC and race or sodium excretion. Overall, PRR was inversely related to PRC \( (r = -0.19, p < 0.05) \). Mean PRR was increased in women taking oral contraceptive agents compared to women not on these agents \( (p < 0.01) \). Among women not using oral contraceptives, mean PRR of women with a history of hypertension during pregnancy was greater than that of women who were normotensive during pregnancy \( (p < 0.04) \).

Overall, PRR was directly related to systolic blood pressure \( (r = 0.31, p < 0.001) \) and to PRS \( (r = 0.69, p < 0.0001) \) and inversely related to PRC \( (r = -0.36, p < 0.001) \). PRR in women with a history of hypertension during pregnancy did not differ from that of women who were normotensive during pregnancy, both unadjusted and adjusted for PRS \( (p > 0.8) \). PRR in plasma of women receiving oral contraceptive agents was greater than that of women not on contraceptive agents \( (p < 0.01) \), presumably reflecting increased PRS.\(^a\)

After acidification of plasma and addition of excess exogenous renin substrate, PRR in women on contraceptive agents \( (36.7 \text{ ng/ml/30 min} \pm 1.1 \text{ se}) \) did not differ \( (p > 0.2) \) from that of women not taking contraceptives \( (38.3 \text{ ng/ml/30 min} \pm 0.9 \text{ se}) \). To further determine if increased PRR associated with oral contraceptive usage is related to high endogenous renin substrate and/or the deficiency of a renin inhibiting factor, endogenous renin substrate was "selectively" removed by passing plasma over Sephadex. "Substrate-free" plasma was then added to renin-renin substrate. Compared to the in vitro rate of angiotensin generation after addition of buffer to renin-renin substrate, less angiotensin \( (p < 0.01) \) was generated after addition of Sephadex fractionated plasma (fig. 3). Slightly although not significantly \( (p > 0.1) \) greater inhibition occurred after addition of Sephadex fractionated plasma of women taking oral contraceptive agents than with plasma of women not on these agents. Thus, substrate-free plasma from women taking oral contraceptive agents inhibited the in vitro renin reaction to at least the same extent as plasma from women not using these drugs, suggesting that increased PRR with contraceptive usage reflects increased renin substrate concentration rather than the deficiency of a renin inhibitor.

Discussion

Several investigators have demonstrated that hypertension during pregnancy is associated with an increased incidence of hypertension at a later date.\(^{27-30}\) In the present follow-up study, blood pressure of women with a history of hypertension during nulliparous adolescent pregnancy was significantly higher than the blood pressure of women who were normotensive during pregnancy. Although mean blood pressures of the two groups of women were within the "normal range," recent evidence suggests that small differences of blood pressure in adolescents and young adults may be important predictors of elevated blood pressure and cardiovascular disease at a later age.\(^{31-35}\)

The activity of the renin-angiotensin system was compared in these young women with relatively low and relatively high blood pressures. Exercise was selected as a standardized stimulus for renin because it is brief, well tolerated, and requires no drug administration. We have previously observed that the magnitude of the renin response is related to the intensity of exercise.\(^a\) Plasma renin concentration, basal PRA, and PRA stimulated by standardized treadmill exercise were inversely related to systolic but not to diastolic blood pressure. Blood pressure was highest and PRA was lowest among women with a history of hypertension during pregnancy who were taking oral contraceptive agents at the time of study. In response to exercise, excluding women on oral contraceptive agents, we found that PRA was suppressed in 19% of women with a history of hypertension during pregnancy, despite a somewhat lower percentage of black subjects in this group. It is well recognized that PRA is suppressed in approximately 25% of patients with essential hypertension, generally measured in response.
to dietary sodium deprivation or to acute furosemide administration.\textsuperscript{14} Fasciola et al.\textsuperscript{37, 48} reported that patients with essential hypertension and their first degree relatives also have a suppressed renin response to exercise. The current demonstration that renin is suppressed in young women with relatively high, but “normal” blood pressure and normal renal function, suggests that low renin is an appropriate physiologic response to relatively higher arterial pressures. An inverse correlation between blood pressure and unstimulated PRA has previously been observed in normotensive subjects\textsuperscript{49} and between blood pressure and “total renin” in hypertensive patients.\textsuperscript{50, 51}

A number of investigators have reported that the enzymatic activity of added renin is increased in plasma of hypertensive patients.\textsuperscript{52} Increased renin reactivity has been attributed to the deficiency of a renin inhibiting factor\textsuperscript{7} or to the presence of a renin activator.\textsuperscript{8} In the present study, overall, PRR was directly related to systolic blood pressure. However, excluding women on oral contraceptive agents, PRR was not related to blood pressure, suggesting that in the absence of oral contraceptive usage increased PRR is specifically related to the hypertensive state.

Similar to earlier reports,\textsuperscript{11-13, 42-45} we observed that PRS was increased and PRC was decreased by oral contraceptive therapy. Because the velocity of the in vitro renin reaction is related to PRS, and because of an apparent negative feedback between PRS and PRC, several investigators have suggested that increased substrate results in maintenance of constant PRA via suppression of PRC.\textsuperscript{11, 12} Failure of this feedback mechanism, resulting in increased PRA, has been proposed as a possible cause of contraceptive induced hypertension; however, Beckerhoff et al.\textsuperscript{44} observed that PRC was lower in hypertensive than normotensive women taking oral contraceptives. Although it may not be appropriate to extrapolate to patients with clinical hypertension, we observed that higher blood pressures related to contraceptive usage were associated with suppressed rather than increased PRA. Confirming earlier reports, we also observed that PRR is increased in women using oral contraceptive agents.\textsuperscript{46, 47} Although elevated PRR has generally been attributed to high substrate concentrations, McDonald et al.\textsuperscript{47} have recently reported that alterations of modifiers of the renin reaction by estrogen therapy also contribute to the increased enzymatic activity of added renin. To evaluate the potential contribution of renin activators or inhibitors in the present study, renin substrate was denatured by prior acidification of plasma and the reactivity of added renin was measured after replacing denatured endogenous substrate with a relatively crude preparation of homologous substrate. Under these circumstances, PRR in plasma of women taking contraceptives did not differ from that of women not using these agents, suggesting that cofactors present in the preparation of added substrate may have masked renin inhibitors or acceleration associated with contraceptive usage. We have recently reported that dialysis of plasma against an acid buffer also denatures a circulating renin inhibitor.\textsuperscript{48} To “selectively” extract substrate, without loss of inhibitory activity, plasma was passed over a Sephadex column. Substrate-free plasma from women using oral contraceptives inhibited the in vitro renin reaction to at least the same extent as plasma from women not on contraceptives. Thus, it is unlikely that increased PRR is related to loss of a renin inhibiting factor.

In summary, current blood pressures of young women with a history of hypertension during pregnancy were higher than blood pressures of women who remained normotensive throughout pregnancy. Overall, among these women, PRA and PRC were inversely related to blood pressure, and the PRA response to exercise was suppressed in 19% of normotensive subjects with relatively high blood pressure. However, PRR did not differ in women with relatively high and relatively low blood pressures. Thus, renin suppression but not increased PRR appears to precede the hypertensive state. Oral contraceptive usage was associated with higher systolic blood pressures, high PRS and PRR, and low PRC. Highest blood pressures and lowest PRA occurred in those women with a history of hypertension during pregnancy who were taking oral contraceptive agents at the time of study.

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