

Plasma Dopamine- β -Hydroxylase Activity in Oral Contraceptive Hypertension

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SUMMARY

A prospective study was undertaken to evaluate the relative contribution of changes in sympathetic nervous system activity, as reflected by changes in dopamine- β -hydroxylase (DBH) activity, to the pathogenesis of oral contraceptive-induced hypertension. Precontraceptive and serial postcontraceptive determinations of blood pressure, plasma renin activity (PRA), DBH activity, and changes in body weight were obtained in twelve control patients and forty-one oral contraceptive users. Forty-four percent of oral contraceptive users had increases in blood pressure but remained normotensive and 17% became frankly hypertensive. The precontraceptive and average postcontraceptive levels of mean arterial pressure (MAP), PRA and DBH activity in each patient were compared using paired group analysis. Control patients (group I) exhibited no significant changes in these variables, while the patients with contraceptive-induced increases in MAP (groups III and IV) underwent significant, parallel increases in DBH activity. Finally, the linear regression of changes in MAP on the percent change in DBH activity was examined. The positive slopes in groups III and IV differed significantly from the negative slope of the controls (group I). The data have been interpreted to reflect an inappropriate oral contraceptive-induced stimulus to sympathetic nervous system activity, leading to increases in MAP in susceptible individuals.

Additional Indexing Words:

Plasma renin activity

Angiotensin

Estrogens

IT IS WELL RECOGNIZED that the use of oral contraceptive agents may be associated with an elevation of blood pressure in otherwise healthy individuals, with¹⁻⁵ or without^{6,7} the appearance of overt diastolic hypertension. The reported incidence of hypertension among users of oral contraceptives has varied from a low estimate of less than 2%⁷ to one as high as 18%.² Hypertension is most often mild to moderate in degree; nevertheless, the description of malignant hypertension in two patients^{8,9} attests to the potential severity of the problem.

Although oral contraceptive-induced hypertension has been widely ascribed to a primary influence of

estrogen-mediated alterations of the renin-angiotensin-aldosterone system,¹⁰ such alterations have not provided a completely satisfactory explanation for the variable effects of estrogen-progestagen combinations on arterial blood pressure.¹¹ Previous investigations have centered about the definition of abnormalities in renin metabolism, whereas little or no attention has been focused on alternative mechanisms that might be operative. In view of the possibility that the pathogenesis of individual hypertensive syndromes may well be multifaceted,¹² it was decided to investigate the possible contribution of neurogenic vasomotor changes to the pathogenesis of oral contraceptive hypertension.

The investigation of adrenergic mechanisms in the control of blood pressure has been facilitated by the recent availability of a sensitive assay for the enzymatic activity, in plasma, of circulating dopamine- β -hydroxylase (DBH), the enzyme which catalyzes the conversion of dopamine to norepinephrine in the synaptic vesicles of postganglionic sympathetic neurons.¹³⁻¹⁵ Following neuronal discharge, a portion of the intravesicular enzyme is released into the synaptic cleft and gains entrance into the systemic circulation along with norepinephrine.¹⁶⁻¹⁸ It has been proposed that changes in DBH activity may well serve as a useful index of the activity of the sympathetic ner-

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vous system.¹³ Additional studies in our own laboratories have suggested that the assay of plasma DBH activity may provide a useful clinical tool in the evaluation of primary and secondary forms of hypertension.^{19, 20} In view of these considerations, a prospective study was undertaken in an attempt to evaluate the possible contribution of altered sympathetic nervous system activity, as reflected by changes in plasma DBH activity, to the pathogenesis of oral contraceptive hypertension.

Methods

Patient Population

Forty-one postpartum or nulliparous women from the Family Planning Clinic of the Duke University Medical Center were studied sequentially before and after the initiation of oral contraceptive therapy. In addition, twelve postpartum women who used an alternative form of contraception (intrauterine device or diaphragm) during the same period of study were followed similarly and served as a control population.

Oral contraceptive recipients were given daily estrogen-progestagen combinations containing 0.05–0.10 mg mestranol and norethindrone (Ortho-Novum) or ethynodiol diacetate (Ovulen), either preparation to be taken in 21-day cycles. All participants were specifically instructed not to restrict their dietary sodium intake; none of the patients received diuretic or antihypertensive drug therapy during the period of observation.

All patients underwent an initial clinical and laboratory evaluation prior to the institution of contraceptive therapy; specifically, all postpartum women were first interviewed and examined while hospitalized on the third postpartum day. Nulliparous patients were evaluated initially upon the first clinic visit. All participants were seen thereafter at approximately one month intervals for follow-up evaluations; on the average, each patient was seen on three occasions during the follow-up period (mean interval between follow-up visits: four weeks). Patients were followed until the voluntary discontinuation of contraceptives and/or the termination of the study. The mean duration of follow-up for all patients was twelve weeks (range 10 to 24 weeks).

Methods

At the initial evaluation, each patient was screened for a family or personal history of hypertension. The past medical record was examined for prior evidence of hypertension or pre-eclampsia in preceding pregnancies. Routine biochemical laboratory profiles included urinalysis and measurement of the endogenous creatinine clearance and urinary protein excretion.

Measurements of blood pressure, body weight, peripheral venous plasma renin activity (PRA) and plasma DBH activity were obtained during initial evaluation and thereafter at each return visit.

All blood pressure measurements (arm cuff method) were performed by the same investigator (S.R.) who utilized a mercury manometer in the following manner: after the subject had comfortably maintained a supine position for five minutes, four determinations of systolic and diastolic pressure were obtained at one minute intervals. The recorded blood pressure for a given visit thus represents the average of four separate determinations. Measurements of blood pressure have been arbitrarily expressed as mean

arterial pressure (diastolic pressure + $\frac{1}{3}$ pulse pressure; MAP).

With the patient remaining in the supine position, peripheral venous blood samples were collected in chilled heparinized vacuum tubes for PRA and DBH determinations. After collection, samples were rapidly centrifuged at $20,000 \times g$ for ten minutes; the supernatant was stored at -20°C for subsequent enzymatic assay. All blood pressure recordings and venipunctures were performed in the morning between the hours of 9 a.m. and noon. Body weight was recorded without correction for the weight of indoor clothing.

PRA and DBH assays were performed by different research technicians, neither of whom had prior knowledge of the subjects' clinical status. Peripheral venous PRA was assayed according to the method described by Gunnells et al.²¹ The spectrophotometric assay of Nagatsu and Udenfriend¹⁵ was utilized for the analysis of peripheral plasma DBH activity. The presence of endogenous inhibitors of DBH was excluded as previously described.²⁰

Based on the mode of contraception and level of blood pressure response, patients were placed into the following categories for further analysis (table 1): group I = all control subjects; group II = oral contraceptive users who had no increase in blood pressure throughout the period of observation; group III = oral contraceptive users who exhibited an increase in blood pressure but nevertheless remained normotensive (blood pressure less than 160/90 mm Hg); and group IV = patients who developed overt hypertension while receiving oral contraceptive therapy, as defined by systolic pressure greater than 160 mm Hg and/or diastolic pressure greater than 90 mm Hg.

Chi square, Student's paired *t*-test, and linear regression analysis were performed utilizing standard statistical techniques.

Results

Fifty-three women returned for follow-up evaluation on at least two occasions following the initiation of contraceptive measures. Of these 53 (4 nulliparous and 49 postpartum) women, 12 patients did not receive oral contraceptives and therefore served as controls; 41 were oral contraceptive users whose responses to therapy are herein reported. Throughout the period of this investigation, 189 data determinations were obtained on the participating subjects.

Over the period of observation, 16 of 41 women on oral contraceptives (39%) showed no increase in MAP (group II); 18 women (44%) exhibited an increase in MAP which was sustained for the duration of oral contraceptive use (group III); seven subjects (17%) became, by the stated criteria, overtly hypertensive (group IV), and remained so until oral contraceptives were withdrawn. This observed incidence of hypertension is consistent with earlier reports.² In contrast, only four of the 12 control (group I) subjects (33%) exhibited an increase of MAP above the initial blood pressure value, and none became hypertensive while under our observation. The incidence of blood pressure increase observed in the normotensive oral contraceptive users (groups II and III) was

Table 1

*Initial Mean Arterial Pressure, Plasma Renin Activity and Dopamine- β -Hydroxylase Activity in Controls and Oral Contraceptive Users**

Group	No	Parity	Initial		
			MAP (mm Hg)	PRA (ng A II/100 ml)	DBH (I.U.)
I Controls	12	2 \pm 1.4 (0-4)	84 \pm 6 (72-97)	276 \pm 142 (120-500)	12 \pm 6 (3-27)
II No MAP increase	16	2 \pm 1.2 (1-5)	89 \pm 8 (72-106)	258 \pm 132 (100-533)	12 \pm 8 (1-35)
III MAP increase	18	4 \pm 4.2 (0-6)	78 \pm 17 (60-101)	282 \pm 130 (114-500)	15 \pm 8 (3-25)
IV Hypertensive	7	1 \pm 0.8 (0-3)	93 \pm 11† (73-107)	220 \pm 156 (80-1200)	14 \pm 13 (3-38)

*Values represent mean \pm SD; ranges are in parentheses.

† $P < 0.05$ compared to controls.

significantly greater than that noted in control subjects ($P < 0.05$).

The control (group I) and oral contraceptive populations (groups II, III, IV) were comparable with respect to age, parity, race, and family history of hypertension (table 1). Similarly, the initial values for MAP, PRA and DBH activity were significantly different neither among the control subjects and oral contraceptive users, nor among those users whose arterial pressure became elevated (groups III and IV) and those whose pressures did not increase (group II). However, those who became overtly hypertensive (group IV) had a mean initial MAP which, although normal, was slightly higher than that of control subjects ($P < 0.05$). Thus, in this study population, the initial MAP was the only measured index which was of prognostic value in identifying those ultimately at risk for the subsequent development of hypertension.

Table 2 lists the mean determinations of weight change, MAP, PRA and DBH activity for the four

groups following the initiation of contraceptive measures. Mean changes in body weight were small and did not differ significantly among groups. Similarly, the mean postcontraceptive values for PRA and DBH activity did not differ significantly either between control subjects (group I) and oral contraceptive users (groups II, III, IV) or among the different subcategories of oral contraceptive users.

The precontraceptive and average postcontraceptive MAP, PRA and DBH activity of all patients are illustrated in figures 1 and 2. The levels of each variable were compared within the individual groups utilizing paired group analysis. It is evident that, during the period of observation, the control (group I) participants did not experience a significant change in any of the variables depicted. While group II patients exhibited a small but significant decrease in MAP with oral contraceptive use ($P < 0.01$), this was not associated with a significant change in either PRA or DBH activity. Conversely, in both groups III and IV (fig. 2) the highly significant ($P < 0.005$) post-oral contraceptive increase in MAP was accompanied by a similar increase in plasma DBH activity (group III, $P < 0.001$; group IV, $P < 0.02$). In marked contrast, neither of these latter two patient groups experienced a significant change in PRA.

When the administration of an exogenous agent (e.g., an estrogen-progestagen combination) results in the elevation of mean arterial pressure, it may be proposed that the appropriate physiological response would be a reflexly mediated relaxation of vascular tone. Hence, in this setting, the maintenance of pre-existing adrenergic activity and vascular tone would appear to be inappropriate and might be interpreted to represent a failure of the normal baroreceptor mechanisms. Changes in the plasma activity of DBH have been postulated to reflect alterations in the ac-

Table 2

*Postcontraceptive Maximal Weight Change, Mean Arterial Pressure, Plasma Renin Activity and Dopamine- β -Hydroxylase Activity**

Group	Weight change (kg)	MAP (mm Hg)	PRA (ng A II/100 ml)	DBH (I.U.)
I	0.03 \pm 3.1 (-4.75-6.25)	85 \pm 7 (72-97)	309 \pm 98 (120-511)	18 \pm 10.5 (2-27)
II	1.8 \pm 8.4 (-5.2-5.5)	89 \pm 8 (72-93)	263 \pm 104 (100-500)	17 \pm 8 (1-33)
III	-0.1 \pm 2.9 (-5.5-7.7)	96 \pm 8† (77-108)	319 \pm 113 (120-533)	25 \pm 13 (2-50)
IV	-1.1 \pm 2.9 (-6.0-3.6)	119 \pm 8† (107-127)	399 \pm 361 (150-478)	27 \pm 26 (5-80)

*Values represent mean \pm SD; ranges are in parentheses.

† $P < 0.001$ compared to controls.

tivity of the sympathetic nervous system;¹³ thus, the observed increases in plasma DBH activity in groups III and IV, if they do represent concomitant increases in adrenergic activity, would be consistent with a failure of the normal negative feedback control of arterial pressure via baroreceptor mechanisms.

In order to examine this concept in greater detail, the linear regression of the change in MAP on the percent change from precontraceptive DBH activity within each patient group was examined (fig. 3). In the control patients (group I), this analysis reveals a negative slope of the regression line ($b = -0.08 \pm 0.003$), whereas the slope is distinctly positive in both groups III and IV ($b = +0.03 \pm 0.02$ and $b = +0.07 \pm 0.004$, respectively). The slope of the regression line in group II ($b = +0.005 \pm 0.01$) does not differ significantly from zero. While the slope of group III is not significant, the Spearman rank coefficient yields a significant positive correlation ($R_s = 0.328$, $P < 0.05$). When the slopes of groups III and IV are compared statistically to that of the control group, the differences are found to be highly signifi-

cant ($P < 0.001$ and $P < 0.01$, respectively). In contrast, the slopes derived from the linear regression of change in mean arterial pressure on percent change in PRA (fig. 4) differ significantly neither from zero nor each other.

Follow-up evaluation after the cessation of oral contraceptive use was possible in only three patients (two subjects from group III and one from group IV). However, in all three instances, MAP and DBH activity underwent parallel reductions to precontraceptive values.

Discussion

The administration of exogenous estrogens is known to influence the renin-angiotensin system profoundly. Nevertheless, a consistent relationship between this influence and the occurrence of clinical hypertension has not yet been observed and, for the most part, a causal interrelationship has not been adequately explained.

An estrogen-mediated increase in circulating renin substrate concentration was first described in 1952²²

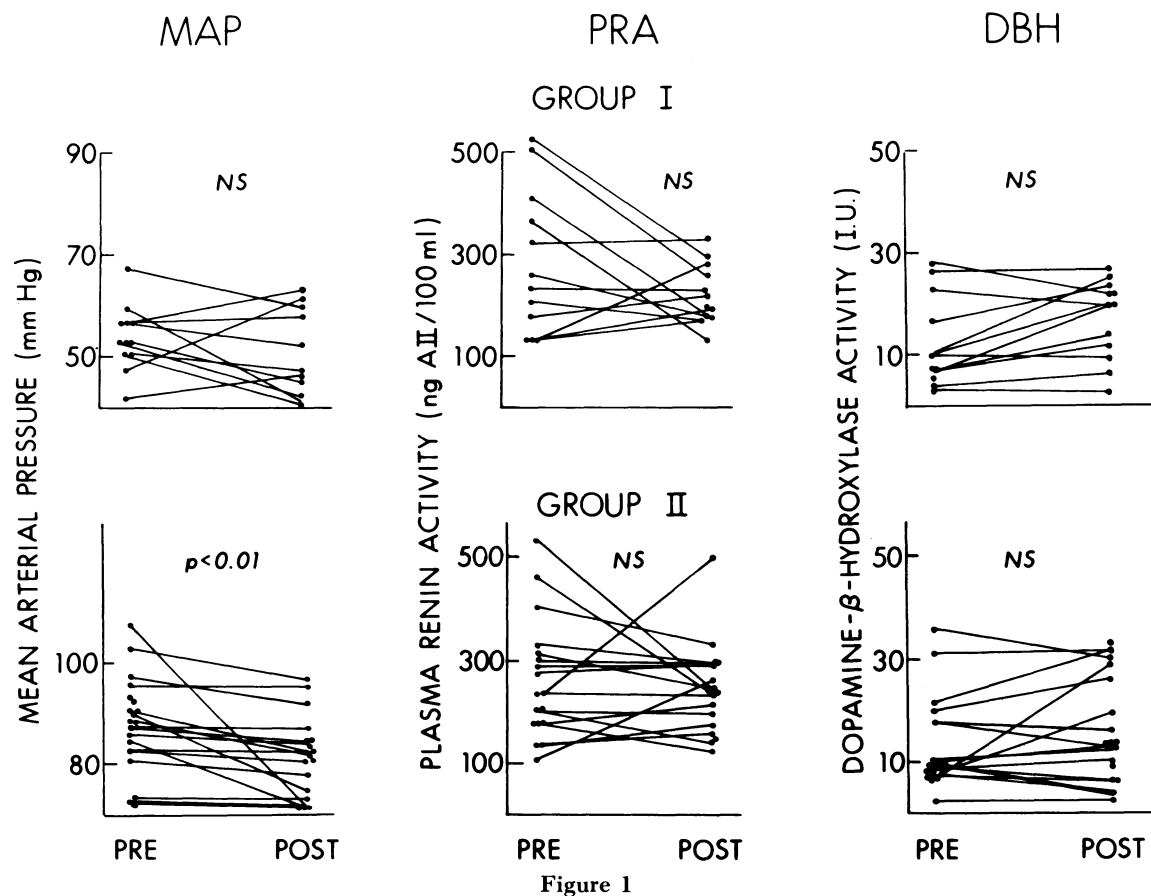


Figure 1
Paired group analysis of precontraceptive and average postcontraceptive mean arterial pressure (MAP), plasma renin activity (PRA) and dopamine- β -hydroxylase activity (DBH) in groups I and II. The three variables did not change significantly in control patients. Group II underwent a small but significant decrease in MAP unassociated with any significant changes in the other variables.

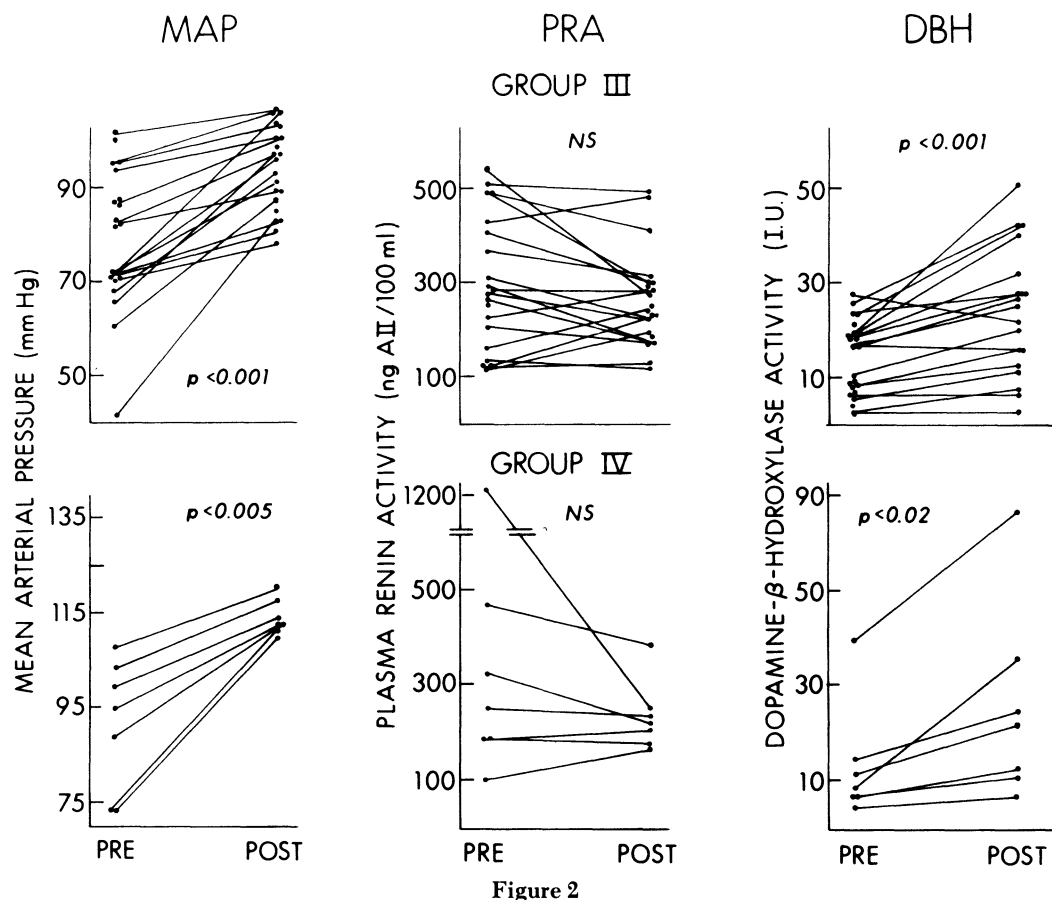


Figure 2
Paired group analysis of pre and mean postcontraceptive MAP, PRA, and DBH in groups III and IV. Groups III and IV experienced significant parallel increases in MAP and DBH.

and its presence subsequently has been confirmed in virtually every clinical investigation in which it has been sought.^{1, 4, 23-26} The occurrence of this phenomenon is almost universal in oral contraceptive users, irrespective of the presence or absence of altered blood pressure; its occurrence therefore bears no consistent relationship to the development of hypertension. However, a quantitatively greater increase of renin substrate, along with a failure of suppression of renin concentration, has been reported by one investigator in patients who subsequently became hypertensive.⁵

A spectrum of changes in other components of this system has been reported subsequently. For example, plasma renin activity has been observed to be variably increased by estrogen administration,^{7, 25-27} although as many as 50% of women have been found to exhibit normal plasma renin activity despite oral contraceptive-induced hypertension.^{1, 25} Furthermore, an identical increase in plasma renin activity has been observed in women who remain normotensive.^{1, 4, 28} Urinary aldosterone excretion has been found to be normal or variably increased in both normotensive

and hypertensive women,^{1, 4, 24} often in circumstances where an associated correlation with changes of plasma renin activity has been lacking.¹ Vander and Geelhoed²⁹ have described an inhibitory effect of angiotensin II upon renin secretion which is independent of changes in the mean arterial pressure. This, combined with the frequent, although not universal, observation of decreases in plasma renin concentration, has led several investigators to postulate that oral contraceptive hypertension may be related to an inadequate suppression of renin release.^{4, 25, 26}

It is clear that an appreciation of the kinetics of the renin reaction is fundamental to an understanding of the pathophysiology of this hypertensive disorder, and at present, opinion is divided as to the substrate dependence^{1, 4, 23, 26, 30} or independence^{31, 32} of the reaction. Hence, for the purposes of this investigation, it was decided to monitor serial changes in the activity of peripheral plasma renin alone, as a reflection of the physiological impact of exogenous estrogens on the renin-angiotensin system.

Clearly, the small and statistically insignificant changes in plasma renin activity that were observed in

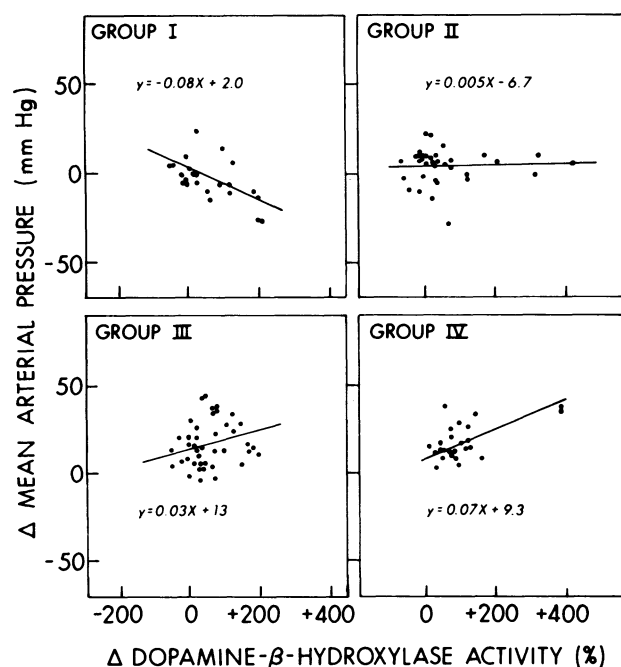


Figure 3

Linear regression of mean arterial pressure (MAP) upon percent change in dopamine- β -hydroxylase (DBH) activity. The positive slopes of group III and IV differ significantly from the negative slope of the control group ($P < 0.001$ and $P < 0.01$, respectively).

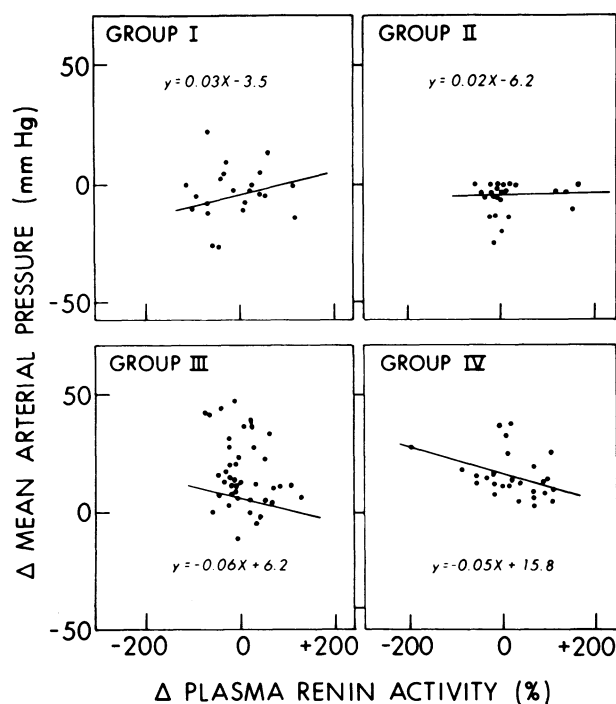


Figure 4

Linear regression of mean arterial pressure (MAP) upon percent change in plasma renin activity (PRA). The slopes of the four groups differ significantly neither from zero nor from each other.

the present study stand in sharp contrast to the striking increases that have been reported previously, although, as reported by others, the nonspecificity of these increases (as a reflection of changes in blood pressure) has again been documented. This disparity is not likely to be explained solely on the basis of differences in methodology since in this study, as in those previously reported, the observed changes in PRA were calculated from the precontraceptive values. Furthermore, the small changes of body weight that were observed in each subject category would mitigate against causative changes in sodium balance, and hence circulating plasma volume, as an explanation for the appearance of hypertension. However, no definite or firm conclusions can be drawn from these observations in the absence of measured changes in sodium balance.

The observed changes of plasma DBH activity stand in sharp contrast to those for PRA. In the control population (group I), the negative slope of the linear relationship between changes in MAP and DBH is consistent with a normally functioning negative feedback system: that is, increases in arterial pressure ostensibly result in a reflex reduction in adrenergic activity. The observation of an apparently random relationship between these variables in group II is unexplained, but may simply reflect the small changes in MAP observed in that group of patients. In contrast, the positively sloping linear relationship observed in groups III and IV is inconsistent with intact negative feedback control and may in fact be interpreted to reflect a stimulus to adrenergic activation. Thus, in those patients in whom the use of oral contraceptives is associated with a rise in MAP, the data seem to be consistent with an inappropriate increase in sympathetic nervous system activity, at least as reflected by the changes in plasma DBH activity.

The possible pathogenetic significance of our observations relies heavily upon the acceptance of the mechanism outlined above. However, alternative explanations for the observed elevations in DBH activity must be considered as well. First, one might postulate the existence of an estrogen-mediated alteration in the plasma clearance of enzyme protein. While there are no human data presently available which define the exact determinants of DBH clearance, the available animal data would suggest that the metabolic clearance of the protein is reasonably constant.¹⁴ An alteration, within the synaptic vesicle, of the ratio between soluble DBH and catecholamine is theoretically feasible, although the available experimental data in animals would suggest that such an alteration is not likely.^{13, 16, 18} Differences in the ratio between enzymatic activity and enzyme protein concentration in plasma are also possible; however, while

currently available data have suggested that such a dichotomy may exist in certain circumstances,³³ more recent observations would suggest that the immunological cross-reactivity of animal DBH with the human enzyme is poor (unpublished observations), and that radioimmunoassay performed with antihuman DBH antibody yields good correlation between immunologically reactive protein and enzymatic activity.³⁴ In addition, data are available which support the use of DBH activity as an index of sympathetic nervous system activity.¹⁶⁻¹⁸

There is some evidence which suggests that an increase in sympathetic nervous system activity accompanies the administration of exogenous estrogen. In direct support of this concept, Castren et al. have reported an oral contraceptive-induced increase of neurogenic vascular reactivity, as measured during Valsalva maneuver and passive upright tilting.³⁵ Furthermore, the changes in carbohydrate and lipid metabolism that are commonly seen in oral contraceptive users have been noted to be exaggerated in those patients who develop hypertension.³⁶ Although these changes have been attributed to primary alterations in cortisol metabolism,³⁷ their occurrence is not incompatible with catecholamine-induced lipolysis, glycogenolysis, and insulin antagonism.

The present findings provide no insight into the mechanism by which the administration of oral contraceptives might exert a stimulus to sympathetic nervous system activation in susceptible individuals. It is possible that its appearance is an independent phenomenon, and that the inadequate renin suppression postulated by others relates to unsuppressible β -adrenergic stimulation of renin release. A more intriguing, unitary hypothesis relates to the recently appreciated central nervous system effects of angiotensin II.³⁸ It has been shown that components of the renin-angiotension system are endogenous to brain tissue³⁹ and that there is a central, hypertensive response to angiotensin II which consists of a sympathetically mediated rise in peripheral vascular resistance.⁴⁰ Thus, it may be envisioned that, in susceptible individuals, oral contraceptives effect a rise in circulating brain substrate which, with access to the centrally active renin enzyme, results in a simultaneous increase of central angiotensin II generation, and hence increased peripheral arterial pressure and circulating DBH activity. The final common pathway is therefore the adrenergic system, with variable effects on peripherally detectable plasma renin activity.

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