ABSTRACT
A variety of estrogen- and progestin-containing oral contraceptive agents reduced renal blood flow (RBF) significantly in 23 healthy, nonhypertensive young women, to a mean of 75 ± 3.3% of the value expected for their age and dietary sodium intake (P < 0.001). There was also significant activation of the renin-angiotensin system: renin substrate was increased approximately 3-fold in association with a striking increase in the circulating renin activity and angiotensin II levels in relation to sodium intake and excretion. Two observations suggested that the RBF reduction was directly mediated by angiotensin II. A correlation was demonstrable between circulating angiotensin II and RBF (P < 0.01), and renal vascular responsiveness to angiotensin II infused into the renal artery was reduced significantly (P < 0.01). Moreover, the oral contraceptive agents modified the basic relationship between sodium balance and renal function in salt-loaded rats despite severe tubular necrosis induced by HgCl₂. These observations provide further evidence for an important role of angiotensin II as a determinant of RBF. Renal vasoconstriction may contribute to the genesis of a number of complications, such as sodium retention and hypertension, associated with oral contraceptive use.
specific action of the sodium ion on renal blood flow (RBF) and renal vascular responsiveness to angiotensin II from a secondary action on the renal vasculature through activation of the renin-angiotensin system. Oral contraceptive agents, which activate the renin-angiotensin system through a mechanism at least partially unrelated to the state of sodium and potassium balance, provide an alternative approach to examining the interaction between the renin-angiotensin system and the renal vasculature.

We recently reported a detailed analysis of the interactions between effects of age and salt intake on renal hemodynamics in normal human subjects. During the survey it became apparent that a number of young women taking oral contraceptive agents had a lower level of renal perfusion than their age and salt intake. Our working hypothesis in the present study was that this was related to activation of the renin-angiotensin system, and that responsiveness of the renal vasculature to angiotensin II provides an index of endogenous angiotensin II concentration in the vicinity of the renal vascular receptor.

Methods
Subjects

Studies were performed in 86 normal female potential kidney donors under the age of 49 years at the time of selective renal arterial catheterization for arteriography. All were normotensive and had never noted edema. Twenty-three of the subjects were taking a birth control pill. Of these, 21 had used contraceptive agents for 6 months to 9 years prior to the study, and two had discontinued the agent 3–4 weeks prior to admission because they assumed that it would interfere with the evaluation. Three additional subjects had discontinued the agent 5–12 months prior to evaluation. The agents were randomly distributed among most of those in common use over the 9 years for which data were available. They included Ortho-Novum (1/50, 1/80, and 2; Ortho Pharmaceutical Corp.), Ovral (Wyeth), Enovid and Enovid E (Seearl), Norinyl (1/50, 1/80, and 1; Syntax, Demuvel and Ovulen (Seearl), and C-Quens (Eli Lilly).

No more than three subjects were taking any one agent or dose, therefore Heinen’s table, which defines the relative potency of estrogens and progesterins in currently available oral contraceptives, was used to calculate dose equivalents. No women were taking the “minipill” which contains only a progestin. An additional 10 subjects were taking estrogens, generally in replacement doses.

Each subject received a careful inpatient evaluation, described earlier, which placed special emphasis on cardiovascular, renal, and adrenal status. Subjects were admitted to a metabolic unit where blood pressure was measured four times daily, with the subject recumbent and standing. For at least 5 days prior to the hemodynamic and endocrine studies they were given a closely supervised diet that included a daily intake of 2000 ml of fluids, 100 mEq of potassium, and a sodium intake that was either unrestricted or fixed at either 10 or 200 mEq/day. Sodium intake was monitored by a daily dietary assessment by an experienced dietitian and by collection of 24-hour urine samples for the measurement of sodium excretion.

Techniques

The techniques for selective renal arterial catheterization, determination of RBF with radioactive xenon, external probe counting, and administration of angiotensin II into the renal artery have been described in detail. In brief, percutaneous selective renal arterial catheterization was achieved with fluoroscopic guidance and with the subjects under local anesthesia. A coaxial catheter system was used: the inner catheter (PE 10) was used for the continuous infusion of 0.9% saline or the vasoactive agent and the outer catheter (red Kifa, USCI Division, C. R. Bard, Inc.) for monitoring arterial blood pressure and injecting radioactive xenon.

Mean RBF was measured from the initial slope of $^{133}$Xe disappearance from the kidney, determined graphically, with a hematocrit-corrected partition coefficient: compartmental analysis was also performed. Curves reanalyzed on a coded basis showed a coefficient of variation of 7%.

All blood samples were collected from recumbent individuals, placed on ice immediately, and centrifuged; the plasma was separated and frozen until the time of assay. Samples for plasma renin activity and angiotensin II levels utilized ethylenediaminetetraacetic acid (EDTA) as the anticoagulant; diisofluorophosphate (DFP) was also added to the samples for angiotensin II assay.

Sodium and potassium in urine and blood were measured by flame photometry with lithium as an internal standard. Angiotensin II values were measured by a double-antibody radioimmunoassay method. This assay is sensitive to a level of 7 pg/ml with a coefficient of variation at the 20 pg/ml level of 6.8%. Recoveries of added angiotensin II at three different levels ranged from 88% to 108%. Plasma renin activity was measured by radioimmunoassay of angiotensin I generated during a 1-hour incubation with endogenous substrate. Renin substrate concentration was measured by allowing the reaction, which was always complete, within 24 hours, to go to completion.

Protocols

Data were available on RBF and urinary sodium excretion for all 86 subjects. Measurements of plasma renin and angiotensin II levels were obtained for 19 subjects taking the birth control pill and 54 normal subjects not taking the pill but studied under identical conditions.

After a control determination of renal blood flow in eight subjects taking the birth control pill, angiotensin amide (Hypertensin, CIBA) was infused into the renal artery in log-dose increments of 1–300 ng/min, calculated as the base. Fresh solutions were prepared by dilution in 0.9% sodium chloride just prior to each study. The concentrations were adjusted so that the infusion would deliver the appropriate dose at a pump flow rate of 0.3–1.9 ml/min. Infusion at each dose level was maintained for 3 minutes before blood flow was determined. Two to four determinations were made at either a single rate of angiotensin administration or with log-dose increments, as described under Results.

Group means are presented with the standard error of the mean as the index of dispersion. Evaluation for statistical probability was carried out, where appropriate, with Stu-
dent's *t*-test. The Wilcoxon rank sum test and Fisher exact test were used for analysis of nonparametric data. The null hypothesis was rejected when the *P* value was less than 0.05.

The protocol was approved by the Human Experimentation Committee at the Peter Bent Brigham Hospital. Permission for the procedure was obtained after careful description of the protocols to the subjects in every case.

**Results**

The relationship between age, sodium intake, and mean RBF is shown in Figure 1. The values have been grouped in cohorts by decade for convenience of presentation. Mean RBF was below the median value for age and sodium intake in 22 of the 23 subjects taking the oral contraceptive agent, a highly significant reduction (chi square = 11.5; *P* < 0.001).

Indeed, the Fisher exact test reveals that such a nonuniform distribution would occur by chance only once in $2.9 \times 10^{20}$ times. The mean value was $75 \pm 3.3\%$ (SEM) of that predicted for age and diet (Fig. 2). The mean blood flow was also strikingly reduced in the two individuals who had stopped taking the agent 3–4 weeks prior to the study. In two of the three subjects who had discontinued the agent 5–12 months earlier, RBF was above the median value, and in the other, just below it. No correlation could be found between the flow reduction and either the amount of progestin or estrogen in individual tablets or the ratio of progestin to estrogen content.

Of the 10 normal subjects who were receiving estrogens at the time of the study, generally in replacement dosage, the RBF value in five exceeded that expected for age and diet; in the other five the value was below expectation. Thus estrogen alone did not influence renal blood flow.

The influence of the oral contraceptive agents on the renal vascular response to angiotensin II infused into the renal artery is shown in Figure 3. For subjects on a high salt diet, there was a striking reduction in responsiveness to angiotensin II (Fisher exact test: *P* = 0.00002). There was virtually no overlap between the renal vascular response to graded doses of angiotensin II in subjects not taking the birth control pill and those taking it. A smaller but statistically significant reduction in responsiveness was also evident in subjects receiving a low salt diet (*P* = 0.004) (Fig. 3).

The relationship between sodium excretion at the time of the study and the renal vascular response to angiotensin II (30 ng/min) infused into the renal artery is shown in Figure 4. In control subjects a significant correlation was found...
The relationship between sodium excretion at the time of study and the renal vascular response to angiotensin II (30 ng/min) infused into the renal artery. Both regression relationships are significant (P < 0.01) and their slopes differ significantly (P < 0.01). A significant correlation between these two variables also was evident for the five subjects taking the birth control pill who received the same dose of angiotensin II (y = -0.25x - 59; r = 0.61; P < 0.01). There was a highly significant difference in the slopes of these two regression relationships (P < 0.01). Thus salt intake did influence responsiveness to angiotensin II in subjects taking the birth control pill, but considerably less than in control subjects.

The relationship between daily urinary sodium excretion and the circulating renin and angiotensin II levels in normal subjects and subjects taking the birth control pill is shown in Figure 5. In 54 normal subjects a correlation between urinary sodium excretion (x) and the angiotensin II level (y) was demonstrable (y = 0.18x + 58; r = 0.54; F = 19.7; P < 0.001). No difference in either the slope or the intercept was demonstrable between males (y = 0.18x + 55; r = 0.58; F = 13.25; n = 32) and females (y = 0.20x + 66; r = 0.54; F = 8.1; n = 22), therefore the normal data were pooled for the analysis. Subjects taking the birth control pill showed an asymmetrical distribution around the normal subjects' regression line: 18 of 20 points fell above the line and created highly significant asymmetry (chi square = 16.1; P < 0.0005). A similar correlation was shown between urinary sodium excretion and plasma renin activity for normal subjects. Again a significant difference was not demonstrated between males and females. A striking asymmetry of renin
levels around the regression was associated with use of the birth control pill (chisquare = 18.1; P < 0.0005). Plasma renin substrate levels were also increased in the subjects taking the pill (1173 ± 45 vs. 423 ± 43 ngA1/ml; P = 0.014 by Fisher exact test).

It was not possible to demonstrate any relationship between estrogen content, progestin content, or their ratio in the individual agents used and either RBF or the elevation of renin or angiotensin II levels.

In six subjects taking the pill, blood flow and the circulating angiotensin II concentrations were measured simultaneously. These were matched for age and salt intake with values for six healthy females for whom the same measurements were available and who were not taking the pill. In this subset a significant reduction in renal flow (Wilcoxon rank sum test: P = 0.0076) was associated with a significant increase in angiotensin II (P = 0.0076). RBF (y) showed a significant regression relationship with the logA1 of the angiotensin II concentration in these 12 subjects (y = 587 - 162 x; n = 12; r = 0.65; F = 7.27; P < 0.01).

Discussion

This investigation has revealed an unequivocal association between consumption of oral contraceptive agents and a reduction in RBF. Subjectivity in the analysis of the xenon washout data did not play a role because the association was recognized retrospectively in most of the subjects. The possibility that reduced RBF led to consumption of the oral contraceptive agents is equally unlikely. Thus the oral contraceptive agents must have reduced blood flow either through a direct action of either their estrogen or progestin on the renal vasculature or through an indirect action.

Data are available on the acute direct action of these agents in animals and in man. Neither bilateral oophorectomy nor the acute administration of estrogens alters renal perfusion in humans.10-12 Large doses administered to dogs for several days either failed to influence renal perfusion13-14 or increased flow.15 A decrease was not documented. Similarly, estrogens administered in replacement doses, which generally exceed the estrogen content of the contraceptive agents, did not alter renal perfusion in this study. Moreover, estrogens did not influence renal vascular responsiveness to angiotensin II either in this study or in an earlier report.12 Progesterone administered to humans increased renal plasma flow12 but had no direct effect on the dog.15 Thus the direct effect of each hormone either increases or does not change renal perfusion. The explanation for reduction in blood flow must be sought in some indirect action: their profound influence on the renin-angiotensin system provides a likely candidate.

In the decade since oral contraceptives have come into general use a prodigious number of reports on their metabolic effects have appeared and have been summarized in recent reviews.6-11 Their effect on the renin-angiotensin system has been explored primarily because a small number of patients exposed to these agents develop significant hypertension which is apparently mediated through an influence on this system.16-19

Unanimity has not been reached concerning the influence of oral contraceptive agents on the elements of the renin-angiotensin system. In a recent review Oparil and Haber4 pointed out that it was not yet clear whether the oral contraceptive agents increased the angiotensin II concentration in vivo. There does seem to be universal accord that circulating renin substrate is increased, approximately 3-fold, as in our study.18-21,23-26 Plasma renin activity, that is, enzyme activity determined with endogenous plasma substrate, is generally increased19-23,23-26 although exceptions have been reported.4 However, when renin concentration is measured using sufficient exogenous substrate to mask differences in endogenous substrate, reports indicate a reduction.19,21,23-26 Thus it is not surprising that the circulating angiotensin II concentration has been reported to be either normal23 or increased.31,22 In this study an unequivocal increase in both plasma renin and angiotensin II levels was evident when posture and sodium intake were controlled. The plasma angiotensin II concentration was adequate to account for renal vasoconstriction if one considers the exquisite sensitivity of the renal vasculature to angiotensin II.2,5,8

A major indication that circulating, and perhaps intrarenal, angiotensin II contribute to the control of renal vascular tone in normal man came from the assessment of the responsiveness of the renal vasculature to angiotensin II and the influence of dietary sodium intake on that response.7 A change in the inflow concentration of 3-10 pg/ml of angiotensin II resulted in an unequivocal reduction in blood flow and larger doses induced a dose-related progressive reduction in flow. Moreover, a reduction of sodium intake reduced RBF and renal vascular responsiveness to angiotensin II in parallel.3 A similar reduction in sensitivity of vascular smooth muscle to angiotensin II by reduced sodium intake is evident for a number of systems.27-30 Agents that interfere with the action of angiotensin on vascular smooth muscle, either through competitive antagonism at the receptor site31,39 or through blockade of the conversion of the inactive precursor and angiotensin I to angiotensin II,33 increase RBF in the dog and rabbit only when sodium intake is restricted. This finding suggests that the reduced RBF induced by sodium restriction is attributable to occupation and activation of angiotensin II receptors on vascular smooth muscle. On this basis, the reduced response to exogenous angiotensin II found in this study also may reflect occupation of receptors by endogenous angiotensin II the formation of which was stimulated by the oral contraceptive agents. Thus this study provides further strong support for the concept that circulating or intrarenal angiotensin II exerts an important influence on the state of the renal vasculature. Activation of the renin-angiotensin system, in this case by a nondietary means, resulted both in a highly significant reduction in RBF and in its response to angiotensin II. Thus the influence of reduced sodium2-5 and potassium32 intake on the renal blood supply shown in earlier studies seems unlikely to be specific for the individual ion, but rather to reflect the state of the renin-angiotensin system.

The altered relationship between urinary sodium excretion (and, by inference, the state of sodium balance) and renal vascular responsiveness to angiotensin II in the subjects taking the birth control pill makes it unlikely that the contraceptive agent acted through an influence on total body sodium. If all of the influence were expressed in this
way, a parallel shift in the line relating vascular responsiveness to urine sodium would have been anticipated. In fact the relationship was significantly flatter in subjects taking the oral contraceptives: although increased sodium intake and excretion did enhance the renal vascular response to angiotensin II in subjects taking the pill, it did so to a much lesser degree than in subjects who were not taking the pill. An additional, nondietary factor, presumably the influence of estrogen on renal substrate, complicated the relationship.

Progestosterone, and progestin in the oral contraceptive agents, also might exert an indirect influence on the renin-angiotensin system. These agents competitively antagonize the action of aldosterone on the distal tubule of the kidney and thus promote sodium excretion. The sodium loss also tends to stimulate renin secretion. It is possible that this secondary effect of the progestins, acting in concert with the primary effect of estrogen on renin substrate, combine to produce their profound influence.

In this survey, 23 of 86 women under the age of 49, or 27%, were taking the birth control pill. This was significantly greater (P < 0.01) than the 68 of 450 (15%) recently reported from 12 urban U.S. centers. In our study the prevalence of women under 40 years of age who were taking oral contraceptives was 19 of 46, or 47%. In part the higher rate of use in our study reflects the fact that many potential donors were young mothers of sick children, and therefore were extremely anxious to avoid an unplanned pregnancy. Another contributing factor, however, was the detailed history. In this special setting of donor evaluation, where possible gonadal exposure to diagnostic radiation made an aggressive history necessary, oral contraceptive use was revealed on many occasions after initial denial for one of a number of personal reasons. Surveys may well underestimate the use of these agents in the community.

This study confirms earlier reports which indicated that activation of the renin-angiotensin system occurs in even the healthy, normotensive young woman who is free of symptoms. There is an unequivocal increase in the circulating angiotensin II concentration which is inappropriate in relation to the state of sodium balance. Moreover, the activation is biologically significant: active renal vasoconstriction occurs. The renal response may well contribute to the edema experienced by many ingesting oral contraceptives and to the pathogenesis of hypertension in the small group which suffers from this complication. Insight into the pathogenesis of hypertension in this subgroup of patients, indeed, may illuminate pathogenesis in the larger population of individuals with essential hypertension.

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Renal blood flow and its response to angiotensin II. An interaction between oral contraceptive agents, sodium intake, and the renin-angiotensin system in healthy young women.

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