Oral contraceptive-induced high blood pressure is prevented by renin-angiotensin suppression in female rats but not by sympathetic nervous system blockade

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Received 13 June 2008

The use of oral contraceptive (OC) steroids is associated with high blood pressure, although mechanisms responsible are still unclear. This study sought to investigate the possible roles that renin-angiotensin system (RAS) and sympathetic nervous system (SNS) may play in the development of OC-induced hypertension. Administration of OC led to significant increases in blood pressure, heart weight and significant decrease in urinary output in OC-treated and OC+clonidine-treated groups but not in OC+captopril-treated group. The pressor response to angiotensin II was significantly greater in the OC-treated rats than in the control rats. However, the pressor responses induced by norepinephrine were not significantly affected by OC administration. The results of the present study demonstrate that OC-induced high blood pressure is associated with cardiac hypertrophy, enhanced pressor response to angiotensin II and preserved pressor response to sympathetic activation. The study also suggests that the development of the OC-induced hypertension and cardiac hypertrophy is mediated by RAS, but not by SNS.

Keywords: Cardiac hypertrophy, Hypertension, Oral contraceptive, Renin-angiotensin system, Sympathetic nervous system

Prolonged administration of oral contraceptive (OC) steroids is associated with increased risk of venous and arterial complications in premenopausal women. Pathophysiological basis of venous events has been well documented\(^1,2\), but the mechanisms mediating arterial complications are still debated\(^3,4\). Studies have reported that absolute arterial cardiovascular complications linked to OC use is rather low among OC users without risk factors for cardiovascular disease\(^5\). However, recent studies reported increased risk of arterial events in Western Europeans, Americans, and even more in women from developing countries currently using OC\(^1,3,6\). OCs are the preferred method of contraception because of their ease of use and proven efficacy, and over 100 million women worldwide use OC steroids over a prolonged time\(^7\). Thus, a modest increase in blood pressure associated with OC use would be broad public health significance. However, the possible mechanisms responsible for the development and maintenance of OC-induced high blood pressure are not clearly understood.

Abnormal activation of the renin-angiotensin system (RAS) has been shown to play a significant role in the development and maintenance of hypertension. Angiotensin II (Ang II), the effector substance of the RAS, increases blood pressure, sympathetic nervous system (SNS) activity, aldosterone release, water retention and cardiac hypertrophy\(^8\). Oestrogen status is an important determinant of RAS activity, and the vasopressor responses to Ang II\(^3,4,9\). A promoter region in the angiotensinogen gene is responsive to oestrogen\(^10\). Studies in humans have demonstrated that administration of synthetic oestrogen has the tendency to activate\(^3,11\), exert little or no effect\(^12\) on RAS.

The role of defective cardiovascular automatic regulation in arterial events has been documented both in animal model\(^13\) and patients\(^14\) with hypertension. Moreover, high levels of ovarian hormones, especially oestrogen during luteal phase of the menstrual cycle\(^15\) and in ovarectomized rats following exogenous oestrogen administration\(^16\), have been shown to influence SNS activity on blood pressure regulation. The activation of the SNS could contribute to hypertension via mechanisms, including enhanced vasopressor responsiveness, increased norepinephrine biosynthesis, release and/or through actions of Ang II\(^8,17,18\).

To assess the possible roles of the RAS and SNS in OC-induced hypertension, the effect of chronic administration of captopril (CAP) an angiotensin-
converting enzyme inhibitor and clonidine (CLO) a sympatholytic agent have been examined. Also attempt has been made to investigate whether OC-induced hypertension would be associated with cardiac hypertrophy through the contribution of RAS or SNS in an experimental animal model.

Materials and Methods

Animals and treatment—All experiments were carried out using female Sprague-Dawley rats (110-150g), obtained from the Animal House of the College of Health Sciences, University of Ilorin. The rats were housed two per cage in a well-ventilated room maintained at 25°C±2°C 12:12 hr L:D cycle (lights : 0700-1900 hrs). The rats were provided with standard rat chow (Bendel Feeds and Flourmills Ltd., Benin City, Nigeria) and drinking fluid ad libitum. All experimental procedures involving animals and their care were conducted in accordance with the National Institutes of Health (NIH) USA guidelines on the care and use of laboratory animals. The animals were divided into following 4 groups; control, OC-treated, OC+CAP-treated and OC+CLO-treated rats of similar mean body weight, food intake and water consumption (Table1). The food intake and water consumption were monitored daily while body weight was recorded weekly. Control group received 0.2ml of olive oil per 100g body weight (as vehicle; orally) daily. OC-treated group received a combination of 1.0 µg ethinyl oestradiol with 10.0 µg norgestrel (Wyeth-Ayerst Inc., Montreal, Canada; orally) in 0.2ml of olive oil per 100g body weight daily as previously reported19. OC+CAP-treated group received captopril, an angiotensin converting enzyme inhibitor in drinking water at a concentration calculated to provide at a dose of 25.0mg/kg bw/day (Squibb & Sons, Inc., Princeton, N.J.; USA); a dosing regimen consistent with previous studies in rats 20, in addition to OC treatment. OC+CLO-treated group received clonidine hydrochloride (Sigma Chemicals St Louis, MO, USA), a pharmacological sympatholytic agent added to the feed calculated to provide a dose of 0.4 mg/kg bw/day as previously reported21, in addition to OC treatment. Body weight, food and water intake were monitored before and after experiment. 24-hour urine was collected two days prior to the end of treatment period.

Blood pressure, heart rate and cardiac weight—At the end of treatment, rats were selected from each group for blood pressure and heart rate determinations. The rats were anaesthetized with a mixture of 25% (w/v) urethane and 1% (w/v) α-chloralose (5ml/kg; BDH Chemical Ltd, Poole, England; ip). The femoral artery for blood pressure recording and ipsilateral jugular vein for drug infusion were cannulated with a polyethylene catheter (PE-50), filled with heparinized saline. A tracheostomy was performed to improve ventilation. Pulsatile blood pressure was recorded by connecting artery cannula to a Statham P23ID pressure transducer connected to a Grass Polygraph (model 7D; Grass Instruments, Quincy; MA USA). The mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one-third of the pulse pressure. Heart rate was derived from the pulsatile pressure signals over 60 seconds. The heart was excise, cleared of connective tissue, blotted and weighed. The heart weight was adjusted for body weight by dividing heart weight by the body weight.

Pressor responsiveness to norepinephrine (NE) and angiotensin II (Ang II)—Separate set of animals randomly selected from the control (n=5 in either experiments) and OC-treated (n=5 in either experiments) rats were used for each of these experiments. A period of 30 min was allowed after cannulation as described above for stabilization of blood pressure and heart rate. Pressor responsiveness was measured by recording increases in MAP elicited by intravenous infusion of graded doses of Ang II (30, 60 and 100 ng/kg bw) and of norepinephrine (100, 160 and 260 ng/kg bw) at 5 min intervals. The drugs, supplied by Sigma Chemicals (St Louis, MO, USA) were dissolved in 0.9% saline and the injection volume was maintained at 0.5ml/kg bw, with a flush volume of about 0.1ml 0.9% saline.

Data and statistical analysis—All data are expressed as means ± SE for each group. Mean body weight, food and water intake, urinary output, blood pressure, heart rate and cardiac weight were compared using analysis of variance (ANOVA) followed by a Bonferroni post hoc analysis for multiple comparison. The effects of OC treatment on cardiovascular responses to NE and Ang II were done by unpaired Student’s t test. Significance was set at P<0.05 (95% confidence limit). All statistical comparisons and tests were performed using using Statistical Package for Social Sciences (SPSS Inc., Chicogo, IL. USA) for Windows.

Results

OC-treated, OC+CAP-treated and OC+CLO-treated rats had significantly lower body weight and
food intake than CR rats (Table1). Water consumption in OC+CAP-treated rats was significantly lower than those in CR, OC-treated and OC+CLO-treated rats (Table1). Urine output was significantly lower in OC-treated and OC+CLO-treated rats when compared with OC-treated and OC+CAP-treated rats (Table1). OC treatment led to significant increases in blood pressure and cardiac weight in OC-treated and OC+CLO-treated rats whereas OC treatment did not cause significant increases in blood pressure and cardiac weight in OC+CAP-treated rats (Table1). OC treatment did not cause significant increases in blood pressure and cardiac weight in OC+CAP-treated rats (Fig.1). The heart rate in OC+CLO-treated animals was significantly lower than those observed in the other three experimental groups (Fig.1).

Regardless of the OC treatment, the magnitude of pressor responses elicited by either Ang II or NE increased progressively with increasing doses. Pressor responses to Ang II were consistently larger in OC-treated rats than those observed in CR rats, whereas the responses to NE were consistently similar in both OC-treated and CR rats (Table 2).

Discussion

The present study has demonstrated that prolonged administration of OC steroids, ethinyl oestradiol in combination with norgestrel caused a modest increase in blood pressure that is associated with cardiac hypertrophy and enhanced pressor response to Ang II in female rats. The administration of OC did not alter pressor responses to NE. The study also showed that OC-induced high blood pressure and the associated cardiac hypertrophy were normalized by chronic treatment with angiotensin-converting enzyme inhibition but not affected by sympathetic nervous system blockage.

A high dose of OC steroids have been employed in a rat model of OC-induced hypertension though, a modest increase in blood pressure that was observed in these animals is consistently comparable in magnitude to that found in OC users. Weight loss appears to be a common feature of female Sprague-Dawley rats made hypertensive with OC treatment and could be attributed to reduced food intake in these animals. The reduction in food intake observed in these animals corroborates previous reports that ovarian hormones, especially oestrogen can suppress hypothalamic feeding central.

The role of the RAS in the development and maintenance of OC-induced hypertension has been investigated in human and in experimental animal model; however, a prominent contribution of RAS seems to be inconclusive. Kang et al. demonstrated that increases in blood pressure and renal vascular resistance were reduced by an Ang II receptor blocker, losartan. Another study in human failed to demonstrate a clear contribution of RAS in OC-associated high blood pressure, despite the finding of a profound increased plasma renin activity in OC users. Contradictory results have also been reported.

Table1—Body weight, food intake, water consumption and 24-hr urine output in the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Control (12)</th>
<th>OC-treated (12)</th>
<th>OC+CAP (8)</th>
<th>OC+CLO (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>125.5±6.0a</td>
<td>128.5±8.7a</td>
<td>123.8±6.7a</td>
<td>125.8±6.5a</td>
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<tr>
<td>Final</td>
<td>205.6±8.3a</td>
<td>158.3±11.9b</td>
<td>176.5±8.2b</td>
<td>164.2±6.7b</td>
</tr>
<tr>
<td><strong>Food intake, (g/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>20.0±0.9a</td>
<td>19.2±0.5a</td>
<td>20.2±1.1a</td>
<td>20.3±0.8a</td>
</tr>
<tr>
<td>Final</td>
<td>18.1±1.3a</td>
<td>13.1±0.5b</td>
<td>11.8±0.8b</td>
<td>12.1±0.6b</td>
</tr>
<tr>
<td><strong>Water intake, (mL/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>21.9±1.7a</td>
<td>23.1±0.9a</td>
<td>22.9±0.7a</td>
<td>22.5±0.8a</td>
</tr>
<tr>
<td>Final</td>
<td>23.1±1.2a</td>
<td>19.8±1.6a</td>
<td>15.7±0.9b</td>
<td>21.4±2.3a</td>
</tr>
<tr>
<td><strong>Urine output, (mL/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>7.2±0.5a</td>
<td>7.0±0.6a</td>
<td>7.1±0.8a</td>
<td>7.3±0.8a</td>
</tr>
<tr>
<td>Final</td>
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<td>6.6±0.2b</td>
<td>10.3±0.4a</td>
<td>7.4±0.5b</td>
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</table>

Means in rows not sharing common superscript letters are significantly different, P<0.05.
in an animal model of hypertension induced by a 26-week period of feeding animals on mestranol, a synthetic oestrogen and norethinodrel, a progestogen. Infusion of the Ang II receptor blocker saralasin, also failed to reduce increased blood pressure in the animals, despite the observed markedly elevated concentration of plasma renin substrate. Byrne et al. made female Sprague-Dawley rats hypertensive with ethinyl oestradiol and contrary to earlier animal study, increased blood pressure was abolished by enalapril, an angiotensin converting enzyme inhibitor. OC-induced high blood pressure and associated cardiac hypertrophy were abrogated by chronic treatment with captopril and pressor responsiveness to Ang II was enhanced by OC administration in the present study. Because captopril is an inhibitor of the angiotensin-converting enzyme, the results implies that the RAS is involved in OC-induced hypertension.

Previous studies have shown that increased cardiac weight is an adaptive response to hemodynamic overload, which is independently related to increased risk of premature cardiovascular morbidity and mortality. Studies have suggested that RAS plays significant role in cardiac hypertrophy in response to pressure or volume overload. OC administration in this study significantly increased cardiac weight while this effect was abrogated by captopril. This finding further implicates activation of RAS in OC-induced hypertension.

The role of SNS activity on OC-induced hypertension has not been well documented in this experimental model. Ang II has been shown to cause activation of SNS. OC administration did not affect pressor response to NE. This finding appears to rule out the involvement of overactivity of SNS in the maintenance of OC-induced hypertension in these animals model, and also imply that prolonged use of OC may not lead to exaggerated vasopressor response when SNS is stimulated. The present finding is consistent with previous finding that OC use may not affect circulating NE levels and vasoconstrictor responses to NE. This observation contrasts the reported effect of endogenous oestrogen, which has been shown to reduce SNS activity in premenopausal women.

![Graphs showing blood pressure, heart rate, and heart weight](image)

Fig. 1—Blood pressure (a) heat rate (b) and heart weight (c) of control, OC-treated, OC+CAP-treated and OC+CLO-treated female rats. [Values are expressed as mean±SE of 6-9 rats/group, *P<0.05 compared with the other groups]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (ng/kg)</th>
<th>Ang 60</th>
<th>100</th>
<th>120</th>
<th>NE 260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.4±1.2a</td>
<td>38.4±1.6a</td>
<td>43.2±2.0a</td>
<td>30.9±1.1a</td>
<td>35.1±2.4a</td>
</tr>
<tr>
<td>OC-treated</td>
<td>34.2±1.6a</td>
<td>42.8±1.5b</td>
<td>49.8±1.9b</td>
<td>29.8±1.2a</td>
<td>33.1±2.1a</td>
</tr>
</tbody>
</table>

Means in columns not sharing common superscript letters are significantly different, *P<0.05.

Table 2—Pressor responses (mmHg) by injecting graded doses of angiotensin II (Ang II) or norepinephrine (NE) in the control and OC-treated rats.

[Values are expressed as mean±SE of 6 rats/group]
women during the high oestrogen phase of the menstrual cycle\textsuperscript{29}, in ovariectomized rats\textsuperscript{16} and in postmenopausal woman after oestrogen therapy\textsuperscript{30,31}.

The discrepancy in the effect of exogenous steroids in OC and endogenous steroids may be attributed to difference in physiological effectiveness of exogenous compared with endogenous form of oestrogen and progesterone. Ethinyl oestradiol, exogenous oestrogen in combination with progestogen has been shown to deliver pharmacological concentrations of oestrogen that exhibit 6-10 times the oestrogenic activity\textsuperscript{32}. Another possible explanation could be due to the fact that reduced SNS activity by oestrogenic component of OC could be negated by progestogen\textsuperscript{32,33}. The finding that OC use is associated with unaltered sympathetic activity could imply that OC administration may exert some form of protection against adverse effects of overactivity of RAS on the progression of arterial cardiovascular complications in OC-induced hypertension. This may provide explanation for the small increase in blood pressure during OC use despite the significant contribution of the RAS observed.

The findings in the present study that blockade of SNS activity by clonidine did not prevent OC-induced increase in blood pressure and associated cardiac hypertrophy, lend no support to the hypothesis that activation of SNS is involved in the development and/or maintenance of OC-induced high blood pressure and associated cardiac hypertrophy. Taken together, these findings suggest that activation of RAS during OC use in young healthy women may be minimized by preserved sympathetic activity. Hence, premenopausal women taking OC may be protected from the progression of arterial cardiovascular events in conditions that are associated with stimulation of sympathoadrenal system. OC use may not also increase the susceptibility of women to orthostatic intolerance. Thus, the effect of OC use on neurogenic mechanisms of blood pressure regulation and/or orthostatic tolerance in women, especially during prolonged cold exposure that has been associated with activation of RAS and SNS\textsuperscript{34} deserves investigations.

OC-treated rats in this study exhibited significant reduction in urine excretion and unaffected water intake compared with control rats (Table 1). This reflects fluid retention, which has been previously reported in premenopausal women treated with a combination of oestrogen and progesterone\textsuperscript{32,35}. Fluid retention is expected to suppress rather than to activate renin-angiotensin system. The result that suppression of RAS by captopril treatment abated the reduced water excretion during OC administration indicates that contraction of extracellular fluid volume is unlikely to cause activation of renin-angiotensin system during OC administration.

In conclusion, the study in rats demonstrated that administration of OC steroids (ethinyl oestradiol plus norgestrel) causes increased blood pressure that is associated with cardiac hypertrophy and enhanced pressor responsiveness to Ang II. The study also suggests that RAS but not SNS is involved in the development of OC-induced high blood pressure.

Acknowledgement
Thanks are due to Mr. T. Shehu-Tijani, Department of Physiology, University of Ilorin for secretarial assistance.

References


