Antihypertensive Effects of an Aromatase Inhibitor in the Spontaneously Hypertensive Rat

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SUMMARY Recent studies from this laboratory have demonstrated that 19-nor-deoxycorticosterone, a potent mineralocorticoid, has been excreted in excess in the urine of young spontaneously hypertensive rats (SHR). Although urinary 19-nor-deoxycorticosterone levels decline before the onset of hypertension, preliminary evidence suggests that 19-nor-deoxycorticosterone is further oxygenated to other steroid products in older SHR. Since 19-hydroxylation is the essential first step in the formation of 19-nor-deoxycorticosterone from deoxycorticosterone and since the mechanism-based aromatase inhibitor 10-propargyl-androst-4-ene,3,17-dione preferentially inhibits 19-hydroxylation, this agent was administered to weanling SHR to determine whether inhibition of 19-nor-deoxycorticosterone formation could modify or prevent hypertension. Accordingly, either 10 mg of 10-propargyl-androst-4-ene,3,17-dione or vehicle (control) was injected daily for several weeks in 4.5 week-old SHR. Injection of 10-propargyl-androst-4-ene,3,17-dione reduced urinary free 19-nor-deoxycorticosterone and retarded the development of hypertension compared with the effect of vehicle injection (p<0.05). Mean blood pressure levels in SHR receiving 10-propargyl-androst-4-ene,3,17-dione were lower than those in SHR receiving vehicle for each of the first 8 weeks of treatment (p<0.05). These data support the importance of 10-nor-corticosteroids in the pathogenesis of hypertension in SHR.

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KEY WORDS • 19-hydroxylation • mineralocorticoids • 19-nor-deoxycorticosterone

MULTIPLE factors have been implicated in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR), and a large body of evidence exists in the published reports on abnormal activity of the hypothalamo-pituitary-adrenal, hypothalamo-pituitary-gonadal, and hypothalamo-pituitary-thyroidal axes in these genetically preselected animals. The contribution of the adrenal gland to the development of hypertension has been studied extensively. Aoki demonstrated that adrenalectomy could prevent the development of hypertension in the SHR, especially if it is done in young, prehypertensive animals. This finding implies that, if an adrenocorticoid is responsible for hypertension in the SHR, it is probably produced early in life. 19-Nor-deoxycorticosterone (19-nor-DOC) could play a role, since it is a powerful mineralocorticoid that is produced in large excess in prehypertensive, young SHR.

Although 19-nor-DOC levels decline before the onset of hypertension, preliminary evidence suggests that this decline is associated with further oxygenation of 19-nor-DOC precursors to new 19-nor products. Therefore, further investigations of 19-nor-DOC and the 19-nor-corticosteroid pathway in SHR are warranted. We reasoned that since 19-hydroxylation is the obligatory step in 19-nor-DOC biosynthesis as well as in the aromatization of androgens to estrogens, these aromatase inhibitors could inhibit the formation of 19-nor-DOC. Furthermore, if 19-nor-DOC has a pathogenic role in the development of hypertension in SHR, aromatase inhibitors given to weanling rats could alter...
the hypertensive diathesis. To that end, we evaluated the effect of a specific 19-hydroxylase inhibitor on the blood pressure in SHR. We used 10-propargyl-androst-4-ene,3,17-dione (10PA), which is a mechanism-based aromatase inhibitor.

**Materials and Methods**

Male SHR (Okamoto-Aoki strain) were obtained from Taconic Laboratory Animals and Services (Germantown, NY, USA) at the age of 4.5 weeks. The rats were housed in metabolic cages, one rat per cage, and maintained on a diet of regular Purina Rat Chow (St. Louis, MO, USA) and tap water in a constant-temperature environment with 12-hour light/dark cycles. One group of six rats received daily subcutaneous injections of 10PA (MDL 18,962; supplied by Merrell Dow Research Institute, Cincinnati, OH, USA), 10 mg/kg body weight, prepared in 5% ethanol and olive oil and sonicated. Seven control SHR were given injections of vehicle. After 10 weeks of treatment with daily injections of 10PA in the test SHR and of vehicle in control SHR, injections were stopped for 2 weeks and then were resumed on alternate days for an additional 14 days.

Systolic blood pressures (SBPs) of the conscious, unstressed animals were recorded using a physiograph (Desk Model 4B; Narco Bio-Systems, Houston, TX, USA) in a sound-resistant constant-temperature room, starting at 3 weeks of treatment. Rats were habituated to the procedure during several training sessions. Urine collections were performed daily on each animal in the presence of sodium azide as a preservative. The samples were frozen and pooled to complete a week’s collection.

The radioimmunoassay (RIA) of 19-nor-DOC was performed as previously described. Briefly, approximately 10 ml of urine (5% of the weekly collection) was extracted with 5 volumes of dichloromethane after the addition of 5000 dpm 1,2-[3H] 19-nor-DOC to correct for procedural losses. The organic residue was applied to a celite thin-layer plate that had previously been impregnated by developing in 17% propylene glycol in acetone. DOC standard was applied on both edges of the plate. The plate was then developed three times in the thin-layer chromatographic system of toluene/hexane/propylene glycol (1:5:saturation) to a solvent front of 15 cm. 19-Nor-DOC has a migration of 0.75R relative to DOC in this system. The DOC standard was located by spraying with blue tetrazolium, and the area corresponding to 19-nor-DOC was eluted. The eluate was dissolved in acetone, and aliquots (1:10) were removed in duplicate for RIA. The remainder was counted for recovery. The acetone was evaporated to dryness with nitrogen. The RIA was performed using antisera at a dilution of 1:50,000. Interassay and intra-assay coefficients of variation were 9.9 and 10.4%, respectively.

Statistical inference of control and treatment data was done by a two-way analysis of variance with replication. Multiple comparison of means was done by a Dunnett’s two-way t test. The null hypothesis was rejected at a p level below 0.05.

**Results**

The first reliable SBP measurements were made at the age of 7.5 weeks. The animals were relatively unstressed since they were gradually habituated to the blood pressure apparatus. SBP is difficult to obtain at early time points. The SBP in SHR receiving 10PA was substantially reduced during the first 8 weeks of treatment (p<0.05; Figure 1). The SBP was lower at each time point in 10PA-treated SHR, with a mean reduction of 60 mm Hg in 10PA-treated versus the vehicle-treated animals for these 8 weeks. This is an approximate 70% reduction of hypertension in the 10PA-treated SHR.

After 8 weeks of treatment, 10PA was discontinued and the SBP difference with vehicle decreased to 30 mm Hg. After 2 weeks, the 10PA was restarted at half the dosing frequency, and the SBP difference again

**Figure 1.** SBP in male SHR treated with 10-propargyl-androst-4-ene,3,17-dione (10PA; n = 6) or vehicle alone (n = 7). The injection of 10PA (10 mg/kg/day) was stopped after Week 8 and resumed at an alternate-day dosing frequency after Week 10. Asterisk indicates significant difference between groups (p<0.05).
increased to 39 mm Hg. The SBP in 10PA-treated SHR at 12 weeks was not significantly different because one animal did not respond to the half dosage and became extremely hypertensive (SBP, 240 mm Hg). The other animals at 12 weeks all had either similar or lower SBP on the half dose of 10PA when compared with levels in vehicle-injected controls.

Urinary free 19-nor-DOC was also reduced substantially in SHR receiving 10PA (p < 0.05; Figure 2). Peak levels of 19-nor-DOC occur at 4 to 6 weeks of age in SHR, and this peak coincided with Week 1 of treatment, in which urinary excretion of free 19-nor-DOC was markedly increased in control SHR, in excess of 250 ng/wk/rat, as compared with 19-nor-DOC excretion in 10PA-treated SHR, which amounted to slightly more than 25 ng/wk/rat. At Week 2, the differential rates of 19-nor-DOC in control SHR declined markedly, as we previously reported. Compared with vehicle-treated SHR, the 10PA-treated rats excreted significantly less 19-nor-DOC in urine at this time also.

Discussion

The principal findings in this study are that the aromatase inhibitor 10PA retards and modifies hypertension in SHR and that 10PA reduces the excretion of 19-nor-DOC in young SHR. Since treatment with 10PA began in the prehypertensive phase of SHR (4.5 weeks), it would be of interest to know whether earlier treatment with 10PA might further retard or prevent development of hypertension, since Aoki was able to prevent hypertension by performing bilateral adrenalectomy earlier in the prehypertensive phase. In any event, 10PA treatment significantly interfered with the hypertensive diathesis in SHR.

It cannot be established at this time whether inhibition of the 19-nor-corticosteroid pathway by the aromatase inhibitor is the principal reason for attenuation of the time-course development of hypertension in SHR. It is likely that 10PA inhibits 19-hydroxylation of DOC, which is probably the obligatory first step in the formation of 19-nor-DOC. Studies by Griffing et al. (unpublished observations, 1987) have shown that 10PA treatment preferentially inhibits 19-OH-DOC production from both rat and human adrenal mitochondria. In the present study, 10PA treatment clearly reduced secretion of 19-nor-DOC in SHR. Inhibition of aromatase activity preferentially affects 19-hydroxylation, but not 18,19-dihydroxylation or 11-dehydroxylation. In vitro incubations used to evaluate the conversion of 1,2-[3H]DOC to corticosterone and 18-deoxycorticosterone showed no significant inhibition of any of these enzyme systems on addition of 1 or 10 \( \mu \)M 10PA. During the administration of 10PA, these other mitochondrial enzymes continued to be active without a disturbance in product formation, although selective inhibition of 19-hydroxylation could not completely mimic total adrenarche. Inhibition of 19-hydroxylation might result in a reduction of the synthesis of other 19-nor-corticosteroids. Based on previous studies by Melby et al., 19-nor-corticosterone excess production other than 19-nor-DOC may occur in SHR following the initial markedly elevated production of 19-nor-DOC. Nevertheless, although the interruption of 19-nor-corticosteroid formation by aromatase inhibitors is an appealing hypothesis to explain the observed antihypertensive effect, it remains circumstantial.

References

9. Bartsh G, Baumgartner U, Rohr HP. A stereological study of
Antihypertensive effects of an aromatase inhibitor in the spontaneously hypertensive rat.
J C Melby, M Holbrook, G T Griffing and J O Johnston

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