Antihypertensive Effects of an Aromatase Inhibitor in Inbred Salt-Sensitive Rats

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Rats susceptible to the hypertensive effect of dietary salt (SS/Jr) have excess urinary 19-nordeoxycorticosterone compared with salt-resistant control rats (SR/Jr). 19-Nordeoxycorticosterone is a hypertensinogenic mineralocorticoid, but whether it contributes to the salt sensitivity of SS/Jr is unknown. This study sought to evaluate the contribution of 19-nordeoxycorticosterone to the salt sensitivity of SS/Jr by lowering its production with an aromatase inhibitor, 10-propargyl-androst-4-ene-3,17-dione (19-acetylenic-androstenedione, 19-AA). This aromatase inhibitor also preferentially inhibits nonaromatizing adrenal 19-hydroxylation, an essential step in the formation of 19-nordeoxycorticosterone. To test this hypothesis, inhibitor (120 mg) or vehicle pellets were implanted into male and female weanling SS/Jr at 42 days of age. A high salt diet (8% NaCl) was started and two additional pellets were implanted at 52 and 62 days of age. Systolic blood pressure was measured in all animals and urinary corticosteroids in males. Compared with vehicle, the inhibitor lowered blood pressure at 50 days of age (when it could first be measured) until 64 days of age in females and 71 days of age in males. Corticosterone and aldosterone levels were not different between 19-AA- and vehicle-treated SS/Jr. 19-Nordeoxycorticosterone levels, however, were mildly reduced with the inhibitor (0.05 < p < 0.10). After 28 days of high salt diet all 23 of the 19-AA-treated SS/Jr were alive, whereas almost one half of the control animals had died. These data demonstrate that 19-AA attenuates the hypertension in SS/Jr; this effect may be through reduction in 19-nordeoxycorticosterone production. This gives support to the contention that 19-nordeoxycorticosterone is involved in the hypertensive effects of dietary salt in SS/Jr. (Hypertension 1991;17:771-775)
These pellets were designed to deliver approximately 10-12 mg/day, and at the end of the experiment, no visible remaining pellet could be found at the injection site. High salt diet (8% NaCl) was begun at the time of the first pellet and continued throughout the remainder of the study. Tail-cuff systolic blood pressures (SBP) of the conscious, unstressed animals were recorded using a physiograph monitor (desk model 4B, Narco BioSystems, Houston, Tex.) in a sound-resistant, constant-temperature room starting at 3 weeks of treatment. Rats were habituated to the procedure during several training sessions. Urine was collected from male animals on days 34 through 41 and 50 through 57. Sodium azide was added as a preservative. The samples were frozen and pooled to complete a week's collection. Aliquots of urine were extracted and purified by thin-layer chromatography before radioimmunoassay for corticosterone, aldosterone, and 19-nor-DOC using procedures that have been previously described.22,23 Dr. Celso Gomez-Sanchez, University of South Florida College of Medicine and James A. Haley, V.A. Hospital, Tampa, Fla., provided the 19-nor-DOC antisera. Statistical inference of control and treatment data was done using a two-way analysis of variance with repetition. Where appropriate, comparison of means was analyzed using Scheffe's multiple-comparison test.

Results

Because of the small size of the young male rats, SBP could not be measured before the first pellets were implanted. Eight days after pellet implantation and high salt diet, SBP was reduced in both male and female 19-AA-treated groups compared with control SS/Jr at 30 days after implantation. Similarly, in female SS/Jr, SBP was lower at 15 and 22 days after implantation, but a steady trend of increasing SBP was observed. SBP in the females could not be measured on day 30 because of technical reasons.

Urinary corticosteroids were measured in male animals before and after the first pellet implant (Figure 2). Urinary free 19-nor-DOC increased with age in both vehicle and 19-AA–treated SS/Jr between 34 and 50 days of age. 19-Nor-DOC levels after pellet implantation were mildly reduced in 19-AA–treated SS/Jr (0.05 < p < 0.10). Urinary free aldosterone levels decreased in both vehicle and 19-AA–treated SS/Jr after the high salt diet was started at 42 days of age. Survival was much better in the 19-AA–treated SS/Jr group. At the end of the study, only 36% of the vehicle-treated SS/Jr were alive whereas 100% of the 19-AA–treated SS/Jr survived.

Discussion

The data from this study demonstrate that the aromatase inhibitor 19-AA attenuates the hypertensive effects of a high salt intake in male and female SS/Jr and modestly reduces 19-nor-DOC excretion.
Beginning 10 days after implantation, the antihypertensive effects of 19-AA gradually decreased in the female rats; this took 23 days in male rats. The reason for loss of antihypertensive effect was probably dissipation of 19-AA, which may have been accelerated in the female animal. Clearly, 19-AA is antihypertensive in this animal in both sexes, but whether it is related to 19-nor-DOC inhibition is not certain.

19-AA is a mechanism-based inhibitor that has been designed to selectively inactivate enzymes that oxygenate steroids at the C19 position.24,25 19-AA was first used as an inhibitor of aromatase, an enzyme that catalyzes oxidative removal of the C19-methyl from androgenic steroids.26,27 Inhibition of aromatase decreases the conversion of androgens to estrogens resulting in lower estrogen levels. Lowered estrogen production, however, is unlikely to explain the antihypertensive effect since this was observed in male as well as female SS/Jr.

The biosynthesis of 19-nor-DOC is analogous to the aromatization of androgens to estrogens and therefore may be susceptible to 19-AA inhibition. Both reactions have an initial C19-hydroxylation, followed by multiple C19-oxygenations, resulting in loss of the C19-methyl group. Adrenal 19-hydroxylase differs from aromatase since the A-ring is not aromatized. Instead, three C19-oxygenations result in the formation of the C19-alcohol, C19-aldehyde, and C19-acid intermediates.28,29 Ultimately, the C19-substituent is lost, resulting in a 19-norcorticoid, 19-nor-DOC.

19-AA inhibits the adrenal 19-hydroxylation of DOC in both isolated mitochondria and reconstituted purified enzyme preparations.30 19-AA decreases 19-nor-DOC excretion and blood pressure in the spontaneously hypertensive rat (SHR) compared with vehicle-treated SHR.31 This antihypertensive effect is similar to the present report in the SS/Jr, but the decrease in 19-nor-DOC was less in the SS/Jr. The difference in inhibition by 19-AA of 19-nor-DOC could be attributed to differences in delivery systems of 19-AA. In the SHR study, 19-AA (10 mg) was given by daily injection, whereas in the present SS/Jr study, 19-AA (120 mg) was given as a 10-day subcutaneous pellet. Although at the end of the study all of the pellet appeared to have been absorbed, it was not possible to tell whether the pellet had been completely absorbed before the end of the 10-day study period.

Mechanism-based inhibitors possess a latent reactive function that becomes unmasked at the enzyme's active site. Such an inhibitor is extremely specific because it inactivates only enzymes for which it is a substrate.32,33 19-AA has a highly reactive C19-acetylenic group that
will theoretically only inactivate enzymes that oxygenate steroids at the C19 position.25,30 Other closely related reactions, including the adrenal 11β- and 18-hydroxylases, are not inhibited to the same extent by 19-AA. This is based on the finding that 11β- and 18-hydroxylation of DOC is not inhibited by 19-AA in vitro and that urinary corticosterone and aldosterone levels are not decreased in rats receiving 19-AA in vivo.25,30,31

It is possible that antihypertensive effects of 19-AA in the SS/Jr and SHR may be through mechanisms other than inhibition of 19-nor-DOC production. Future studies should perhaps include restoration of 19-nor-DOC levels in these 19-AA-treated animals to ascertain whether this will nullify the antihypertensive effects. It is also possible that 19-AA may be inhibiting other known and unknown hypertensinogenic 19-oxysteroids and 19-norsteroids, including 19-norprogesterone, 19-noraldosterone, and 19-OH-androstenedione.34-41 Only 19-nor-DOC, however, has been found to be elevated in the SS/Jr. Whatever the mechanism, 19-AA is clearly antihypertensive in the SS/Jr and further studies are warranted.

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