Contraceptive Steroids and Hypertension: An Experimental Model[1,2] (37366)

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Clinical observations suggest the possibility of a cause and effect relationship between the use of oral contraceptives (OC) and either the development or enhancement of high blood pressure (BP) (1). It is established that OC increase plasma levels of angiotensinogen (2) and angiotensin II (3), as well as aldosterone secretion (4). In addition, estrogens may cause sodium retention (5). Which, if any, of these alterations is responsible for the BP effect is not known.

In previous attempts by our laboratory to develop an experimental model, norethynodrel and mestranol were administered to uninephrectomized female rats drinking 1% saline, to rats with experimental renal hypertension, and to spontaneously hypertensive rats of the Okamato strain (6). There was no effect on blood pressure.

The present study was designed to determine the effect of gonadal steroids on a strain of rats genetically predisposed to develop hypertension from a variety of noxious stimuli including a chronic excess of NaCl (7–9).

Methods. Fifty-two female weanling rats of the salt-sensitive strain were divided into 4 groups of 13 each in such a way that average weights (85 g) of the groups were the same. Animals were caged individually in an air-conditioned room and allowed tap water *ad libitum*. They were maintained on 0.38% NaCl chow from the third to fifth week of life while control BP measurements were obtained. The groups were then assigned to the treatment regimens shown in Table I.

Systolic blood pressures were measured according to the method of Friedman and Freed (10) except that the animals were neither anesthetized nor immobilized with curare. They were restrained in wire tubes and placed in a constant temperature box at 40° for 5 min prior to BP measurement. The BP value recorded was the average of 4 successive measurements. Values obtained by this modified technique earlier were found to range from 20 to 40 mm Hg higher than those obtained on anesthetized animals. Readings were obtained weekly for the first 22 weeks of the experiment and biweekly thereafter. Weights were taken routinely at the time BP was measured.

The rats receiving contraceptive steroids (CS) were given norethynodrel (600 μg/kg) and mestranol (9.1 μg/kg) subcutaneously daily except Sunday. These doses of norethynodrel are those required to uniformly prevent pregnancy in rats (11). These steroids were suspended in absolute ethanol (5% by volume), Tween-80 (5% by volume), and distilled water. Control groups (B and D) received the vehicle only.

The experiment was terminated after 38 weeks on the treatment regimen at which point it was possible to calculate mean survival of the animals.

Statistical analysis of the blood pressures

[1] Supported by a grant-in-aid from the North Carolina Heart Association. L. K. D. is supported primarily by the U.S. Atomic Energy Commission with partial support from Public Health Service Grants HL 13408-02, HL 14913-01, and a grant-in-aid from the American Heart Association (AHA 70-772).

[2] A preliminary report of this investigation was presented at the 45th Scientific Sessions of the American Heart Association. (Circulation 46: II-83, 1972).

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TABLE I. Experimental Design.

Group	Regimen	No. of rats
A	0.38% NaCl + CS	13
B	0.38% NaCl	13
C	4% NaCl + CS	13
D	4% NaCl	13

and weights for each group was done by means of a multivariant growth curve analysis (12). This allowed the entire course of a group to be evaluated by analysis of a fitted curve of the weekly values. Each point in the group weight curves represents the average weights of the surviving rats whereas blood pressure curves for the groups are cumulative, *i.e.*, the last measurement before death is carried to the end of the experiment for the nonsurvivors. Survival data for the groups were analyzed by means of a nonparametric test appropriate for censored samples (13).

Results. Three animals died before the sixth week of the study (2 from group C and 1 from group D) and were not included in the analysis of data.

Body weight. As is shown in Fig. 1, rats receiving CS grew more slowly and consistently had a lower weight ($p = 0.0001$) than those not receiving CS. It is well known

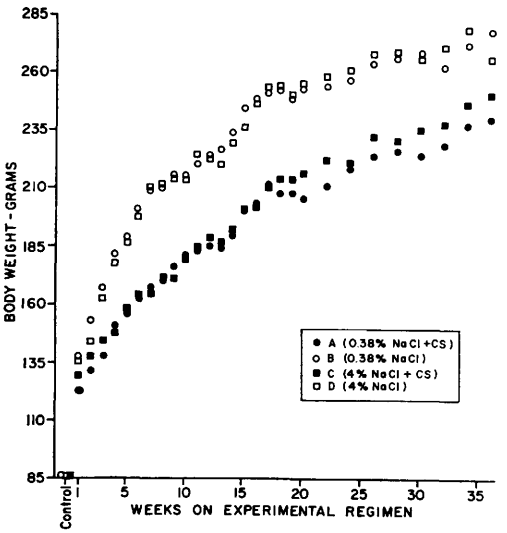

FIG. 1. Average weights of groups A, B, C, and D. CS = contraceptive steroids.

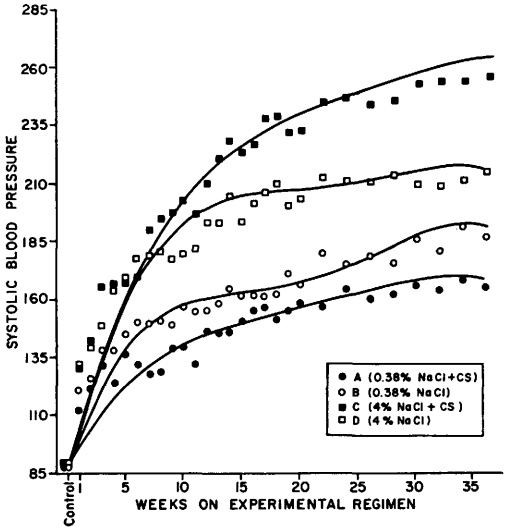

FIG. 2. Average cumulative systolic blood pressure with fitted curves. CS = contraceptive steroids.

that exhibition of estrogens prior to skeletal maturity results in a smaller than normal adult skeletal size (14). Dietary sodium intake had no significant effect on weight.

Blood pressure. Appropriate curves were fitted to the cumulative blood pressures (Fig. 2). Those animals on a high-salt diet (C and D) had a significantly higher blood pressure than those on a low-salt diet (A and B) with a p value equal to 0.0001. The animals of group C, who ate chow containing 4% NaCl and who received CS, had a significantly higher BP than their controls (group D) with $p = 0.0001$. Rats of group A had a lower BP than their controls, group B ($p = 0.006$).

Survival. At the termination of the Expt 3 animals had died in group A (Weeks 17, 21, and 21), 2 in group B (Weeks 34 and 36), 7 in group C (Weeks 3, 6, 14, 17, 18, 35, and 38) and 9 in group D (Weeks 1, 12, 14, 17, 17, 23, 25, 30, and 32).

Although the groups on a low-salt diet (A and B) lived longer than those on a high-salt diet (C and D) there was no significant difference in longevity in group A as compared to group B or in group C as compared to group D.

Since survival was being evaluated, most rats died unattended, tissues were autolyzed at autopsy, and cause of death could not

be determined. For this reason, it is not possible to define the effect of hypertension *per se* on mortality.

Discussion. Dahl and colleagues in previous studies (7–9) have proposed that the salt-sensitive inbred strain of rats has a genetic make-up which significantly promotes the effect of nongenetic factors that have been shown to play etiological roles in the induction of experimental hypertension. These include not only an ingestion of a sustained excess of NaCl but also unilateral renal artery compression without NaCl, cortisone without NaCl, adrenal regeneration with NaCl, uninephrectomy without NaCl, and the combined use of NaCl and desoxycorticosterone. The present study supports this proposition. Contraceptive steroids enhanced the hypertension in this strain of rats while chronically ingesting a 4% NaCl diet. As a clinical corollary, recent observations (J. W. Woods, W. A. Algary, and F. M. Stier—submitted for publication) suggest that a genetic predisposition to hypertension may be involved in women who appear to develop hypertension *de novo* when exposed to OC. In this selected group of women among whom there was a 64% prevalence of high blood pressure in the parents, 50% manifested essential hypertension at a later date.

Why the animals of group C, with significantly higher systolic blood pressures than those of their controls (group D), did not have an increased mortality is not clear. Determination of the exact cause of death might have been helpful as regards this question but was not possible in a survival study. It is conceivable that the estrogen exerted a protective effect on the arterial system. Wolinsky (15) has recently demonstrated that estrogen treatment has a distinctly inhibitory effect and progestogen treatment a slightly stimulating effect on aortic wall morphological and chemical responses to hypertension.

The mechanism involved in enhancement of hypertension in this experimental model is unidentified. Increased circulating angiotensin II and/or increased sodium content of critical tissues or compartments are the most obvious possibilities. Studies in which exchangeable sodium is measured, and a new highly specific synthetic peptide competitive inhibitor of angiotensin II (16) is administered are planned.

Summary. The effect of contraceptive steroids on a strain of rats genetically predisposed to develop hypertension from ingestion of an excess of NaCl has been examined. Rats given CS grew more slowly and had a lower weight regardless of salt intake. CS enhanced the hypertension of rats receiving the high-salt diet but not of those eating a low-salt diet. CS had no effect on survival but salt intake did. This model offers the opportunity of studying the mechanism by which gonadal steroids enhance hypertension.

We thank Dr. J. E. Grizzle and Mr. R. F. Woolson for help with statistical analysis, and G. D. Searle and Co. for supplies of norethynodrel and mestranol.

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Received Feb. 13, 1973. P.S.E.B.M., 1973, Vol. 143.