Enovid-Induced Exacerbation of High Blood Pressure in Stroke-Prone Rats (SHR)

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SUMMARY Male and female, normotensive Wistar:Kyoto rats, spontaneously hypertensive rats (SHR), and Stroke-prone SHR (SHR/SP) with severe hypertension were treated with a contraceptive drug (Enovid) from the time of weaning until they became 180 days old. Although the Enovid-treated SHR and SHR/SP had exacerbations of their high blood pressure, none of the animals developed renal or cerebral damage. Chronic treatment with Enovid caused testicular and ovarian atrophy and a significant increase in circulating corticosterone. It is suggested that the potentially deleterious effects, e.g., stroke, hypertension, hyperglycemia, hyperlipidemia, etc., of chronic treatment with contraceptive drugs is largely determined by pre-existing genetically endowed proneness or resistance.

THE CONTRACEPTIVE DRUGS currently available inhibit hypothalamic-pituitary-gonadotrophic hormone release causing inhibition of ovulation. In some women, the chronic use of contraceptive drugs has been reported to be associated with hypertension, hyperlipidemia, hyperglycemia, thromboses, and other untoward cardiovascular changes, e.g., atherosclerosis, myocardial infarction, and "stroke." We have subjected nonarteriosclerotic virgin and arteriosclerotic breeder rats to chronic treatment with Enovid and found that this estrogen-progestogen caused de novo hypertension, hyperlipidemia, hyperglycemia, and vascular damage in virgin rats and exacerbation of the pre-existing degenerative changes in the breeder rats of both sexes. These findings suggested that contraceptive drugs are capable of up-setting the homeostatic equilibrium of the hypothalamic-gonadal axis leading to endogenous hormonal-metabolic alterations which favor the pathogenesis of cardiovascular disease in rats.

The genetically programmed hypertension which occurs in spontaneously hypertensive rats (SHR) could be mediated through the hypothalamic-pituitary-adrenal-gonadal axis. We have found that hypophysectomy, adrenalectomy, early gonadectomy, or early treatment with estrogens will inhibit the development of hypertension in SHR. Therefore, we wished to determine whether early treatment of SHR with a contraceptive drug, e.g., Enovid, would palliate or exacerbate their hypertension. The Japanese have developed a sub-strain of SHR which develop unusually high levels of blood pressure and are concomitantly prone to develop "strokes," i.e., the stroke-prone SHR (SHR/SP), which mimic cerebrovascular accidents as they occur in humans. Because some women have suffered "strokes" while taking a contraceptive drug, we elected to treat SHR/SP with Enovid to determine if this estrogen-progestogen would exacerbate the unusually elevated blood pressure of SHR/SP and condition these animals to develop premature cerebrovascular damage.

Methods

In order to compare sub-strains of rat, male and female, normotensive Wistar:Kyoto rats (from which SHR were originally derived), hypertensive SHR and severely hypertensive stroke-prone SHR (SHR/SP) were used. All of these sub-strains were obtained from brother-sister matings of breeder stock derived from the original SHR strain of Kyoto, Japan and kindly provided by Dr. Carl T. Hansen, Animal Genetics Division, NIH. All of the animals were housed in our air-conditioned, humidity, and light-controlled Animal Research Colony. The animals were fed a commercial rat chow (Purina) which is relatively low in fat (4%) and were given tap water to drink ad libitum.

Blood pressure begins to rise spontaneously in SHR and SHR/SP when these animals are 60 days old, continues to rise reaching levels of 180–190 mm Hg in SHR and 220–240 mm Hg in SHR/SP when they become 120 days old, rising slowly thereafter.

Animals were selected at random to serve as controls (injected with saline) or experiments (injected with Enovid suspended in saline). Tablets of Enovid (Searle) (nor-ethynodrel (2.5 mg) and mestranol (0.1 mg)) were ground into fine powder and suspended in saline at a concentration of 3 mg/Enovid/ml. The experimental animals were given 0.1 mg of Enovid/100 gms bw/sc daily 5 times per week. Animals were weighed twice weekly and their blood pressure recorded weekly.

Just prior to autopsy, blood pressure (systolic) was recorded under light Seconal anesthesia using the Friedman:Freed microphonic manometer and indirect tail-cuff procedure. Equal numbers of males and females of each sub-strain were killed at 30, 60, 90, 120, 150, and 180 days of age to obtain measurements which could be related to intervals of time and maturation. At autopsy, blood samples were collected between 0800–1100 after an all night fast in deference to the diurnal rhythm of adrenal steroids and lipid determinations. In order to determine hormonal levels.
under minimal conditions of stress, blood was withdrawn within 1 min after removing the animal from its cage. Blood samples were centrifuged (refrigerated) and assayed for glucose, free fatty acids, triglycerides, cholesterol, and blood urea nitrogen (BUN), using the automated techniques prescribed for the Auto-Analyzer (Technicon Instruments). Corticosterone was measured by the radioimmunoassay method of Iams, McMurtry, and Wexler.6

The heart and aorta of each animal was examined carefully at autopsy for gross evidence of vascular disease. Pertinent organs from each rat were trimmed and weighed. The hearts were dropped into saline and allowed to pump out residual blood, blotted, and weighed, i.e., as a gravimetric index of cardiac hypertrophy and hypertension. Organs and tissues were embedded in paraffin and sectioned at 3μ. Adjacent sections were stained with hematoxylin-eosin for routine analysis; Verhoef, Van Gieson, and Gomori’s aldehyde fuchsin stains for elastic tissue; Alcian blue and toluidine blue for metachromasia; the Hale stain for glycosaminoglycans; the von Kossa method for calcium. Sudan II and III, oil red O, and Sudan black B were used to demonstrate lipids in both frozen and paraffin sections. Statistical analysis of results was performed using a one-way analysis of variance, chi square test, or Student’s t-test.

Results

All animals appeared healthy and gained weight normally. None showed cerebrovascular damage as described by Okamoto et al.6 There were no differences in the weight of the pituitary, adrenal, thymus, and gonads between controls and Enovid-treated animals. The heart and kidney weights of the Enovid-treated SHR/SP and SHR animals were significantly (p < 0.001) heavier than their non-treated counterparts, commensurate with their more severe hypertension.

The systolic blood pressures of SHR/SP and SHR weanlings were significantly (p < 0.001) elevated above normotensive WKY matched controls (fig. 1). At 35–40 days of age, the spontaneous hypertension of SHR/SP and SHR controls began to rise briskly and by 120 days of age reached 230 ± 10 mm Hg in SHR/SP and 190 ± 5 mm Hg in SHR with a gradual increase thereafter (fig. 1).

The blood pressure levels of the Enovid-treated normotensive WKY were the same as their non-treated WKY partners (fig. 1). Enovid-treated SHR/SP manifested a most dramatic increase in blood pressure, attaining super-elevated levels of 250 ± 5 mm Hg by 120 days of age (fig. 1). Although the blood pressure levels of non-treated and Enovid-treated SHR rose at the same brisk rate, the Enovid-treated SHR eventually attained significantly (p < 0.001) higher levels than their non-treated counterparts (fig. 1).

There were no significant differences in the circulating triglycerides, free fatty acids, cholesterol, glucose, and BUN levels between non-treated WKY, SHR, and SHR/SP animals, as well as in the Enovid-treated vs non-treated animals. The circulating corticosterone levels were significantly (p < 0.001) elevated in all of the Enovid-treated animals except male WKY (fig. 2). Female rats (except Wistar) characteristically manifested higher resting blood corticosterone levels than their male partners and, in general, Enovid-treated female rats showed the greatest increase in corticosterone levels; Enovid-treated, female SHR displayed the greatest increase in corticosterone levels (fig. 2).

As described earlier,6 there were frequent foci of old and new myocardial infarcts in SHR and SHR/SP animals with no apparent difference in the incidence or severity of myocardial damage between treated and non-treated animals. Except for marked cerebral edema in the severely hypertensive Enovid-treated SHR/SP and SHR, there was no evidence of cerebral damage. Both strains of SHR, treated and non-treated, displayed islet hyperplasia and fatty infiltration of the liver. There was no evidence of renal damage in any of the animals. Despite the fact that gonadal weights were the same in treated and non-treated animals, all of the Enovid-treated animals ex-

![Figure 1. Changes in systolic blood pressure of male and female Wistar-Kyoto rats (WKY), spontaneously hypertensive rats (SHR), and stroke-prone, spontaneously hypertensive rats (SHR/SP) given injections of saline (controls) or a contraceptive drug (Enovid) just after weaning and until the animals attained 180 days of age. Animals selected at random were killed at 30 day intervals. Each point depicted is the mean ± se; n = 12.](http://stroke.ahajournals.org/)
Discussion

This investigation demonstrates that the hypertension-prone sub-strains of SHR are susceptible to the hypertension-inducing effects of Enovid, whereas the normotensive WKY strain is resistant. It is remarkable that despite the severely elevated blood pressure in Enovid-treated SHR and SHR/SP, none of these animals had any outward signs of cerebrovascular damage except for cerebral edema. The stroke-prone sub-strain of SHR described by Okamoto et al., and Yamori et al., develop hypertension equal in severity to our sub-strain but the Japanese sub-strains develop strokes as early as 100 to 150 days of age with an average lifespan of only 33 to 41 weeks. The absence of strokes in our sub-strain of SHR/SP despite severe high blood pressure emphasizes the need for caution in comparing sub-strains of SHR. The absence of strokes in our sub-strain of SHR/SP may be due to the nature of the protein content in the diet, the Japanese use fish as a source of protein in their animal diets. This is provocative since the Japanese are particularly prone to develop hypertension and although hypertension is ubiquitous, it appears to eventuate into stroke in those geographic areas where the ingestion of fish is high. The Japanese investigators emphasize the recognized fact that severe hypertension is of prime importance in the pathogenesis of cerebrovascular disease and stroke, experimentally and clinically. Because the strokes in their sub-strain frequently occur in the basal ganglia of the cerebral cortex, they consider their SHR/SP animals to be a good biological model of stroke as it occurs in humans. The resistance of the normotensive WKY to chronic treatment with Enovid vs the susceptibility of the SHR sub-strains isakin to the clinical observation of susceptibility vs resistance of certain women to the hypertension-inducing effects of contraceptive drugs.

In our previous work with Enovid-treated, normo-

FIGURE 2. Circulating corticosterone levels of stroke-prone, spontaneously hypertensive animals (SHR/SP), spontaneously hypertensive (SHR), and normotensive Wistar Kyoto rats (WKY) after having been subjected to injections with saline (white column) or Enovid (black column) from the time of weaning until they were 180 days of age. The height of each column connotes the mean ± SE; ( ) = number of samples.

Hibited severe involution of the testes (fig. 3) and ovaries (fig. 4), histologically.

FIGURE 3. Testes of an Enovid-treated SHR/SP rat at 90 days of age. The tubules are atrophic and spermatogenesis has ceased. The large deeply basophilic, multi-nucleated cells are isolated spermatogonia, i.e., spermatid syncyta composed of clones of imperfect spermatogonia joined by protoplasmic bridges. This condition is indicative of severe hypogonadism. H & E, ×100

FIGURE 4. Involuted ovary of an Enovid-treated SHR/SP rat at 90 days of age. The interstitial ovarian tissue has disappeared with sparse follicles but hyperplastic corpora lutea. The persistent corpus luteum and lack of cyclic follicular development (and ovulation) is indicative of the pseudopregnancy and hypogonadal condition. H & E, ×100
motensive, Sprague-Dawley rats, we encountered marked loss of body weight, adrenal glandular hypertrophy and hemorrhage, thymus gland involution, and testicular and ovarian atrophy. Only the gonadal atrophy was observed in the Enovid-treated WKY, SHR, and SHR/SP which underscores the diverse yet specific genetic nuances of strain and sub-strain differences in rats. The greatly increased heart and kidney weights in SHR/SP and SHR, and particularly in Enovid-treated rats of these sub-strains, is commensurate with their unusually elevated blood pressure.

The Enovid-treated animals did not display the hyperglycemia and hyperlipidemia which we found in Enovid-treated, normotensive Sprague-Dawley rats. This is surprising since our sub-strains of SHR/SP and SHR are characteristically spontaneously hyperlipidemic, hyperglycemic, have a fatty liver and greatly enlarged islets of Langerhans with extensive beta cell degranulation.

Estrogens cause hypoglycemia, increased ACTH release, and steroidogenesis. Estrogens also cause increased protein binding of steroids. It may be that the absence of hyperglycemia in our sub-strains of Enovid-treated SHR was due to the hypoglycemic action of the mestranol (estrogen) component and reduced gluconeogenesis due to the increased protein binding of the elevated adrenal steroid levels.

Particularly noteworthy is the fact that the Enovid-treated rats in this experiment had higher than normal circulating corticosterone levels, whereas normotensive Sprague-Dawley rats have sub-normal levels of corticosterone following the same treatment. Bunag found that Enovid-treated Wistar rats will develop abnormally high blood pressure and suggests that Enovid is capable of stimulating endogenous adrenocortical secretion, e.g., aldosterone. Shibota et al. have described malignant hypertension in SHR/SP kept on 1% saline drinking water. Their animals developed proteinuria, severe fibrinoid necrosis of the renal arteries, and strokes, all of which they ascribe to hyper-reninemia. In direct contrast, our SHR animals had normal BUN levels and no evidence of renovascular damage. Our ancillary investigations suggest that the SHR sub-strains are hyper-responsive to hypothalamic-pituitary-adrenal stimulation, that the morphologic responsiveness of their arterial wall, i.e., the morphology of arterial lesions, may be conditioned by the specific spectrum of adrenal steroids secreted, and that the composition of this spectrum of adrenocorticoids varies from sub-strain to sub-strain. Despite the greatly elevated corticosterone levels in the Enovid-treated rats, it is not likely that the hyper-secretion of corticosterone per se is directly related to the increased blood pressure since we have encountered severely elevated hypertension in SHR with sub-normal levels of corticosterone. It is noteworthy that although chronic treatment with Enovid eventuated in hypothalamic hypogonadism in male and female rats, i.e., atrophic testes and ovaries, these animals had severe exacerbation of their unusual high blood pressure. This is in contrast to our reports that early gonadectomy, i.e., at 30 days of age, or treatment with estrogens would greatly retard the spontaneous hypertension which develops in SHR. Hoeg et al. found that SHR treated with mestranol, the estrogenic component in Enovid, caused attenuation of the development of hypertension in SHR. In the present experiment, although Enovid treatment was begun at 30 days, it is not likely that suppression of hypothalamic release of gonadotrophins became effective until the animals were mature. This delayed gonadal suppression would nullify the hypertension-suppressing effects since our findings indicate that the anti-hypertensive effects of hypogonadism are effective at an early age.

There are a number of serious questions for investigators utilizing the SH rat. The ubiquitous disbursement of the original Okamoto:Aoki spontaneously hypertensive rat has resulted in confusion. The SH rat which investigators allege are descendants of the original Okamoto:Aoki stock and therefore “pure” is a statement no longer tenable. It is apparent that because of differences in breeding techniques and other considerations, investigators around the world are dealing with several substrains of the original SHR. To pronounce the normotensive Wistar:Kyoto rat the only suitable control for SHR rats is to overlook the more important consideration that many workers are dealing with substrains of the original SHR and results may not be readily comparable. For example, although many investigators complain of progressive reduction of the elevated blood pressure in their strain of SHR, the blood pressure in our strain of SHR has remained consistently elevated; many investigators complain of the great susceptibility of SHR to respiratory disease, our strain is exceptionally resistant and free of lung disease; most strains of SHR are short-lived, our strain is unusually long-lived, i.e., 2½ yrs. Most investigators report comparatively low aldosterone and corticosterone levels and decreased responsiveness to stress in their strain of SHR, we have found high levels of aldosterone and corticosterone and hyperresponsiveness to stress in our strain. Okamoto and Aoki assert that early gonadectomy does not inhibit the pathogenesis of hypertension in SHR, in our strain of SHR early gonadectomy is an effective retardant of the spontaneous hypertension. Okamoto et al. describe a stroke-prone sub-strain of SHR, our descendants of the same stock of stroke-prone SHR (courtesy of NIH) develop exceptionally severe hypertension but they do not have spontaneous “strokes” (this discrepancy between the Japanese and U.S. substrains may be due to differences in the protein composition of animal diets in U.S. vs Japan). The obese/SHR described by Kolesky have lipid-rich atheromatous lesions, our obese/SHR/NIH are devoid of atheromatous lesions. Yamori et al. describe ring-like fatty deposits in their fat-fed strain of SHR; the same high fat diet caused complete inhibition of hypertension, severe hyperlipidemia, but no atherosclerosis in our strain of SHR.
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References


Cerebral Ischemia in Gerbils: Polyribosomal Function During Progression and Recovery

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SUMMARY Cerebral ischemia was produced by clipping the right common carotid artery in Mongolian gerbils. Polyribosomal function in cerebral ischemia during progression and recovery was studied by investigation of morphology (electronmicroscopy), physical property (size distribution profiles) and biochemical property (polypeptide synthesis). Polyribosomes and their function were relatively well preserved during progression of ischemia. The most striking finding was an extensive disaggregation of polyribosomes and suppression of polypeptide synthesis immediately after re-establishment of cerebral circulation. These phenomena occurred not only with irreversible ischemia at 3 h but also with reversible ischemia at 30 min. In the latter, disaggregation of polyribosomes gradually recovered, but no tendency for recovery was observed after an ischemic period of 3 h. The disaggregation and delay in reaggregation of ribosomes after re-establishment of cerebral circulation may be a significant factor in the irreversibility of cerebral ischemia. The observed deterioration of cellular function during the recovery process may have an important implication not only for medical management of stroke but also for surgical recirculation during acute stroke.

ENDOVI-DUCED HYPERTENSION/Wexler

EVEN THOUGH the investigation of the biochemical aspects of cerebral ischemia or stroke is difficult in human beings, our understanding of the biochemical events that lead to cerebral infarction has advanced considerably by the study of animal models. Mongolian gerbils (Meriones unguiculatus) have been used as a convenient model because of an incidence of cerebral ischemia in approximately 30% of animals after occlusion of one common carotid artery.1-3 With this model, depletion of energy source and decline of energy state and other biochemical aspects have been extensively investigated both during progression and recovery period from ischemia.4-8 The effort of this laboratory in the past has been focused on the alteration of nuclear regulatory mechanism and protein synthesis.8-11 Although this series of investigations demonstrated a prompt impairment of the mechanism for protein synthesis and its reversibility within a certain period after ischemia, the molecular mechanism

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