Gonadal Hormones Modulate Deoxycorticosterone-Salt Hypertension in Male and Female Rats

Joan T Crofton, Leonard Share

Abstract  We have shown previously that, in rats with deoxycorticosterone (DOC)-salt hypertension, arterial blood pressure rises more rapidly and reaches a higher level in male than in female rats and that the course of the hypertension was ameliorated by gonadectomy in male rats and exacerbated by gonadectomy in female rats. The present investigation was undertaken to examine the role of the gonadal steroid hormones in modulating the course of DOC-salt hypertension in the rat. Our previous findings with respect to the effects of gender and gonadectomy on DOC-salt hypertension were confirmed in this study. Chronic treatment with gonadal steroids was begun 1 week before the start of the DOC-salt protocol. 17β-Estradiol attenuated the course of the hypertension in intact male rats and in gonadectomized females. Testosterone exacerbated the development of the hypertension in gonadectomized male rats but was without effect in intact females. Progesterone alone had no effect on the hypertension in ovariectomized rats but when given to ovariectomized rats in combination with estradiol transiently prevented the ameliorating effect of the estradiol. These effects of the gonadal steroid hormones could not be attributed to effects of saline intake. Thus, these findings demonstrate that the gonadal steroid hormones play an important role in modulating the pathogenesis of DOC-salt hypertension in the rat. It is suggested that the effects of the gonadal hormones on the course of the hypertension may be due to modulation of the cardiovascular and renal actions of vasopressin, since vasopressin is required for this model of hypertension (Hypertension. 1997;29[part 2]:494-499.)

Key Words  gender differences • sex steroids • DOC-salt hypertension

The incidence of hypertension is higher in men than in premenopausal women. Similarly, we have shown that the development of deoxycorticosterone (DOC)-salt hypertension is sexually dimorphic. Thus, the rate of increase in arterial blood pressure and the level to which it rises are greater in male than in female rats. It is likely that these differences are due to the actions of the gonadal steroid hormones, since gonadectomy attenuated the hypertension in male rats and exacerbated it in females. The role of the individual gonadal steroid hormones in the hypertensive processes, however, has not been characterized. The experiments presented here were undertaken to deal with this issue. We have determined in male and female rats the effect of gonadectomy, with and without gonadal steroid hormone treatment, on the development of DOC-salt hypertension. We have also determined the effects of treatment of intact male rats with estradiol and intact female rats with testosterone.

Methods

Male and female Sprague-Dawley rats were gonadectomized or sham gonadectomized by the supplier (Harlan Sprague Dawley, Indianapolis, Ind) when they were 3 weeks old. They were shipped to us at age 4 to 5 weeks and were housed in animal quarters with controlled lighting (10 hours on, 14 hours off) and temperature (23°C to 24°C). The rats were given food (Purina Laboratory Chow) and tap water ad libitum. When the rats were 7 to 8 weeks old, they were unilaterally nephrectomized under ether anesthesia. At that time they were given a subcutaneous implant of gonadal steroid-containing slow-release pellets (Innovative Research of America). Gonadectomized males were given testosterone (10 mg) or a cholesterol placebo (10 mg). Gonadectomized females were given 17β-estradiol (0.5 mg), progesterone (10 mg), 17β-estradiol (0.5 mg) plus progesterone (10 mg), or a cholesterol placebo (10 mg). Intact males were given 17β-estradiol (0.5 mg) or a cholesterol placebo (10 mg), and intact females were given testosterone (10 mg) or a cholesterol placebo (10 mg). These doses of estradiol and progesterone in ovariectomized rats would provide plasma concentrations of estradiol that were approximately 4 times those seen in proestrus and concentrations of progesterone similar to those in proestrus. The dose of testosterone in gonadectomized males would result in plasma testosterone levels similar to those in intact males (communication from Innovative Research of America). One week later, the rats were divided into two groups. One group was given weekly subcutaneous injections of DOC (Percorten Pivalate, Ciba, 30 mg/kg), and 1% saline replaced the drinking water. The rats in the second group continued to drink tap water and were given weekly subcutaneous injections of 0.9% NaCl (1.2 mL/kg) as the vehicle for the DOC. One week after unilateral nephrectomy and at weekly intervals thereafter, systolic blood pressure (SBP) was measured by tail plethysmography, using a Physiograph Six-B (Narco Biosystems). During this procedure, the rats were lightly anesthetized with ether and rested on a slightly warmed heating pad. After the measurement of SBP at week 1, the steroid pellets were removed and replaced with fresh pellets. Fluid intake was measured for the 24-hour period that preceded each weekly measurement of SBP. Body weight was measured just before each measurement of SBP.

The data were analyzed by one-way and two-way analyses of variance. Where appropriate, significant differences were isolated by the Fisher LSD test. Data are presented as mean±SEM

Results

In rats treated with DOC and given a salt supplement, SBP increased more rapidly and to a higher level (P<0.01) in males than in females (Fig 1). Thus, by the end of the
First week of treatment, SBP had increased significantly ($P < 0.01$) in males but was unchanged in females. At the end of weeks 2 and 3, the increases in SBP in male rats was approximately twice those in females ($P < 0.05$ to 0.1).

Castration of males treated with DOC and salt greatly attenuated the course of the hypertension ($P < 0.05$ to 0.1), which remained similar to that seen in intact DOC-salt-treated females (Fig 1). Treatment of gonadectomized males with testosterone restored the development of the hypertension to levels similar to those in intact males (Fig 2), but this effect did not become apparent until the third week of the experiment.

In female rats, ovariectomy greatly enhanced the development of DOC-salt hypertension (Fig 1, $P < 0.05$ to 0.01). Indeed, by the end of 3 weeks of treatment with DOC and salt, the increase in SBP was similar to that in intact DOC-salt males. When DOC-salt-hypertensive ovariectomized rats were treated with estradiol, the increases in SBP were reduced to levels similar to those in intact DOC-salt-hypertensive females, whereas treatment with progesterone did not alter the course of the hypertension in ovariectomized rats (Fig 3). When ovariectomized DOC-salt rats were given both progesterone and estradiol, the progesterone prevented the ameliorating action of estradiol on the development of hypertension for the first 2 weeks, but in the third week of the hypertension, SBP was at a level similar to that in intact DOC-salt-hypertensive females (Fig 3).

Treatment of intact males, given DOC and salt, with estradiol reduced the development of the hypertension to levels similar to those seen in gonadectomized hypertensive males (Fig 2). On the other hand, when intact DOC-salt-hypertensive females were treated with testosterone, there was no significant effect on the course of the hypertension (Fig 3).

All male rats treated with the vehicle for DOC and given tap water (Table 1) remained normotensive, although there was a transient increase in SBP of 17 mm Hg ($P < 0.01$) in the first week of this treatment in the intact females given testosterone.

In intact male and female rats, fluid intake corrected for body weight (Table 2) was increased twofold to threefold by treatment with DOC and substitution of saline for drinking water ($P < 0.01$), and there were no differences between males and females. In rats treated with DOC and drinking saline, gonadectomy in males and females, treatment of gonadectomized males and intact males with testosterone, and treatment of ovariectomized rats with pro-

Fig 1 Systolic blood pressure (SBP) in intact and gonadectomy (GX) male and female rats treated with DOC and drinking 1% saline. Control measurements (C) were made just before the start of treatment. Asterisks above the bars indicate within-group differences from C. Asterisks within the brackets and between the bars indicate differences between groups.

Fig 2 Effect on systolic blood pressure (SBP) of treatment of intact male DOC-salt-hypertensive rats with estradiol (E) and gonadectomized (GX) male hypertensive rats with testosterone (T). The data for untreated intact and gonadectomized hypertensive males are repeated from Fig 1 for comparison purposes. Asterisks above the bars indicate within-group differences from control (C). Asterisks within the brackets and between the bars indicate differences between groups.

Fig 3 Effect on systolic blood pressure (SBP) of treatment of intact female DOC-salt-hypertensive rats with testosterone (T) and gonadectomized (GX) female hypertensive rats with estradiol (E), progesterone (P), and estradiol plus progesterone (EP). The data for untreated intact and gonadectomized hypertensive females are repeated from Fig 1 for comparison purposes. Asterisks above the bars indicate within-group differences from control (C). Asterisks within the brackets and between the bars indicate differences between groups.
The effects of gonadectomy and of testosterone and estradiol on the hypertension in the present experiments could not be due to differences in saline intake. Testosterone increased saline intake, but this would be expected to increase the hypertension rather than decrease it, as observed in these experiments.

The mechanisms by which estrogen and testosterone affect the development of DOC-salt hypertension are, however, uncertain. There are receptors for androgen and estrogen in centers in the brain stem involved in blood pressure regulation. Since there is considerable evidence that the sympathetic nervous system is an important pathogenetic factor in DOC-salt hypertension, the gonadal steroid hormones could act at these centers to modulate the activity of the sympathetic nervous system. Consistent with this possibility is our observation that the sensitivity of the heart rate component of the baroreceptor reflex is reduced to a greater extent in male than in female DOC-salt-hypertensive rats. Receptors for the gonadal steroid hormones are also found in centers in the brain involved in vasopressin release. However, although vasopressin is essential for the production of DOC-salt hy-

Similarly, it has been observed that estrogen treatment attenuated the hypertension in cockerels made hypertensive by treatment with DOC and salt and in the spontaneously hypertensive rat, whereas testosterone treatment increased the hypertension in the spontaneously hypertensive rat. In contrast to these findings, Bunag has reported that pretreatment of female rats with Enovid, a mixture of mestranol and norethynodrel, exacerbated DOC-salt hypertension. It is difficult to explain this report in the light of the findings reported here and by others.

**Discussion**

We had found previously that in rats treated with DOC and given saline to drink, arterial pressure rises faster and to a higher level in male than in female rats and that gonadectomy attenuated the development of hypertension in male rats and exacerbated it in female rats. This suggested that the gonadal steroid hormones can affect the course of this model of hypertension. The findings in the present report indicate that this is indeed the case. Thus, in DOC-salt-hypertensive rats, the effects of gonadectomy were reversed by chronically treating males with testosterone and females with estradiol. Additionally, chronic treatment of intact male DOC-salt-hypertensive rats with estradiol retarded the development of the hypertension.

<table>
<thead>
<tr>
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<td>117±3</td>
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<tr>
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<td>136±2</td>
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<tr>
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<td>128±2</td>
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**Table 2. Fluid Consumption in DOC-Salt Hypertensive Rats**

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<tr>
<th>Time, weeks</th>
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<th></th>
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<th>Females</th>
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<td>Group</td>
<td>C</td>
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<td>+2</td>
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<td>72±6</td>
<td>GX+P (n=17)</td>
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Values are expressed as mean±SEM. Regardless of sex or treatment, saline intake (mL/100 g body weight), weeks 1 through 3 was significantly greater (P<0.01) than water intake (mL/100 g body weight), week C (control).

*P<0.01 vs intact and gonadectomized rats with appropriate sex. Abbreviations for groups and treatments were defined in Table 1.*
pertension, \textsuperscript{19,20} and although plasma levels of vasopressin are elevated in the hypertensive animals, \textsuperscript{2-4} differences in plasma levels of vasopressin are not responsible for the gender-dependent difference in the development of the hypertension or the effects of gonadectomy on that development, since plasma vasopressin concentrations in DOC-salt-hypertensive rats were unaffected by either gender or gonadectomy. \textsuperscript{2-4}

DOC-salt hypertension is dependent on both the vasoconstrictor and antidiuretic actions of vasopressin, mediated by V\textsubscript{1} and V\textsubscript{2} receptors, respectively. Thus, DOC-salt hypertension is reduced by treatment with either V\textsubscript{1} or V\textsubscript{2} receptor antagonists. \textsuperscript{16,19,21} Indeed, chronic combined treatment with V\textsubscript{1} and V\textsubscript{2} antagonists almost completely prevented the development of the hypertension. \textsuperscript{21} It is likely that estrogen attenuates this form of hypertension by reducing the pressor and antidiuretic actions of vasopressin. Both of these actions of vasopressin are greater in male than in female rats in the nonestrous phases of the estrous cycle, \textsuperscript{2,24} and both of these gender differences are dependent on estradiol. \textsuperscript{25,26}

The mechanisms by which estrogen attenuates the pressor response to vasopressin are uncertain. One possibility is that estrogen, by means of its actions on centers in the brain involved in cardiovascular regulation, can decrease the gain of the baroreceptor reflex. Indeed, we have found (Y.-X. Wang, unpublished observations, 1996) that the sensitivity of the heart rate component of the baroreceptor reflex is greater in nonestrous female rats than in males. There is also considerable evidence that estrogen can act directly on the vascular smooth muscle to modulate the response to vasoactive agents, but the nature of this response is controversial. On the one hand, acute treatment with 17β-estradiol decreased the contractile response of rat tail artery strips to vasopressin. \textsuperscript{27} On the other hand, treatment of intact female rats with estradiol, compared with untreated ovariectomized rats, resulted in an increase in the vasoonstensive action of vasopressin in the isolated perfused mesenteric vascular bed. \textsuperscript{24}

It is likely that estrogen attenuates the antidiuretic action of vasopressin by a direct action on the collecting duct V\textsubscript{2} receptor density and the ability of vasopressin to stimulate cAMP synthesis are lower in renal collecting duct cells obtained from female than from male rats, \textsuperscript{29} and in cultured renal medullary cells, estradiol decreased the ability of vasopressin to stimulate cAMP production. \textsuperscript{30}

To the extent that increased activity of the sympathetic nervous system contributes to DOC-salt hypertension, modulation by estrogen of the vasoconstrictor action of catecholamines could contribute to the sexual dimorphism in DOC-salt hypertension, but there too the reports are controversial. The vasoconstrictor action of norepinephrine or phenylephrine was decreased by estradiol in rat aortic rings \textsuperscript{30} and rat tail artery strips \textsuperscript{27} and was increased by estradiol in preparations of the rat mesenteric vascular bed. \textsuperscript{31,32} In intact male rats, acute treatment with estradiol reduced the pressor response to norepinephrine.

Another possibility to be considered is that estradiol, by increasing hepatic synthesis of plasma proteins that could bind DOC, could ameliorate DOC-salt hypertension by decreasing the bioavailability of DOC. However, since the dose of DOC used in these experiments was greatly in excess, it seems highly unlikely that any increase in plasma binding protein concentration could have been sufficient large to have had an impact on the course of the hypertension.

Our observation that gonadectomy in male rats attenuated DOC-salt hypertension and that this effect was reversed by treatment with testosterone indicates that testosterone in male rats exacerbates this form of hypertension. This effect cannot be due to modulation of the actions of vasopressin by testosterone, since gonadectomy in males was without effect on either the pressor \textsuperscript{25} or antidiuretic \textsuperscript{24} responses to vasopressin. However, since increased activity of the sympathetic nervous system is a contributing factor to DOC-salt hypertension, testosterone may exci-
erbate the hypertension by increasing the pressor response to catecholamines.23-25

It is perhaps not surprising that chronic treatment of intact male rats with estradiol attenuated the development of DOC-salt hypertension. Many of the experiments demonstrating a vascular action of estradiol were carried out in male rats,27,32,36 indicating that there are vascular estrogen receptors in male rats. Similarly, estrogen binding was demonstrated in catecholamine-containing neurons of the brain stem in male as well as in female rats.11-12 If estradiol increased the synthesis of plasma proteins that bind testosterone, the availability of testosterone could have been decreased, and this could have attenuated the hypertension.

The failure of testosterone to affect the development of DOC-salt hypertension in female rats suggests that they lack testosterone receptors in relevant sites in the vasculature or central nervous system.

Body weight was lower in intact males and gonadectomized females chronically treated with estradiol than in untreated intact males and gonadectomized females, respectively. Although the hypertension was attenuated in the estradiol-treated rats, we are unaware of data that support an influence of growth rate or body mass on the development or severity of hypertension in rats. Indeed, the body weight was higher in intact hypertensive females chronically treated with testosterone, but the development and severity of the hypertension was similar to that in untreated intact females.

Testosterone can be converted to estrogen in both male and female rats by the enzyme aromatase. It is unlikely that this was a significant factor in the responses to testosterone treatment in the hypertensive gonadectomized males. Body weight did not fall, whereas treatment with estradiol lowered body weight, and more importantly SBP was increased to levels seen in intact hypertensive males.

On the other hand, conversion of testosterone to estrogen could have been a factor in the failure of testosterone to exacerbate the hypertension in intact females. Indeed, SBP was lower in the second week of the DOC-salt regimen in intact females treated with testosterone than in untreated females.

Progesterone alone had no effect on the development of DOC-salt hypertension in ovariectomized rats. However, when progesterone was given to ovariectomized rats in combination with estradiol, the ameliorating action of estradiol on the hypertension was prevented for the first 2 weeks of the experiment. The fall in SBP in the third week of the experiment to a level similar to that in intact hypertensive females was associated with a fall in salivary intakce, in the preceding 2 weeks, saline intake was elevated due to the influence of the estradiol. There does not, however, appear to be a direct relationship between salivary intake and the magnitude of DOC-salt hypertension. Thus, for example, treatment of gonadectomized female and intact male DOC-salt-hypertensive rats with estradiol attenuated the hypertension and increased salivary intake. One can only speculate that the ability of progesterone to prevent transiently the ameliorating action of estradiol on the hypertension may be due to an effect of progesterone on estrogen receptors or postreceptor events at sites involved in the development of DOC-salt hypertension.

In conclusion, the gender difference in the development of DOC-salt hypertension in the rat can be attributed to the gonadal steroid hormones. Estrogen attenuates the hypertension, whereas, in male rats, testosterone exacerbates the hypertension. It seems likely that the effects of estrogen result at least in part from modulation of the antidiuretic and pressor actions of vasopressin, both of which contribute importantly to DOC-salt hypertension. It is possible that the effects of testosterone on the hypertension, as well as, in part, the effects of estrogen, result from modulation of the sympathetic nervous system, at the level of the central nervous system or the vasculature.

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