Role of the Adrenal Medullae in Male and Female DOCA-Salt Hypertensive Rats

Darrell L. Lange, Joseph R. Haywood, Carmen Hinojosa-Laborde

Abstract—Female rats treated with deoxycorticosterone acetate (DOCA) and salt do not get as hypertensive as male DOCA-salt treated rats. The adrenal gland contributes to the development and maintenance of DOCA-salt hypertension in male rats. However, little is known about adrenal medullary function in DOCA-salt hypertensive female rats. This study tested the hypothesis that bilateral removal of the adrenal medulla would result in a greater fall in blood pressure in male DOCA-salt compared to female DOCA-salt rats. Five weeks after initiating DOCA-salt treatment, average 24 hour mean arterial pressure (MAP) in female rats was significantly attenuated compared to male rats (142 ± 4 versus 168 ± 6 mm Hg, respectively). Female DOCA-salt rats consumed significantly more saline per day than male DOCA-salt rats (22.3 ± 1.8 versus 33.4 ± 1.8 mL/100 grams body weight, respectively). Following adrenal medullectomy, DOCA-salt males experienced a significant decrease in MAP during the dark period after surgery (182 ± 4 to 154 ± 4 mm Hg) that was not observed in female DOCA-salt rats (150 ± 6 to 135 ± 3 mm Hg). In the following light period, MAP remained significantly decreased when compared to the light period before surgery in DOCA-salt male (171 ± 4 to 156 ± 4 mm Hg), while no effect was observed in DOCA-salt female rats. Adrenal medullectomy significantly increased heart rate (HR) in all groups for 12 days. Male sham and DOCA-salt rats had significantly higher catecholamine content in the adrenal medulla than female sham and DOCA-salt rats, respectively. These data suggest that the adrenal medullae contribute more to the maintenance of blood pressure in DOCA-salt hypertension in male rats than female rats (Hypertension. 1998;31[part 2]:403-408.)

Key Words: sex differences in DOCA-salt hypertension in radiotelemetry in blood pressure in heart rate in adrenal medulla

Several clinical studies have shown that premenopausal women have a lower incidence of cardiovascular disease than men. Similarly, studies in animal models of hypertension have shown that females do not develop hypertension as quickly or as severely as male animals. One such model, DOCA-salt hypertension, has been studied extensively in male rats. Several factors have been shown to contribute to the development of DOCA-salt hypertension, including vasopressin, endothelin, and a hyperactive sympathoadrenal system. Early studies by de Champlain demonstrated that removal of the adrenal gland during established DOCA-salt hypertension decreased blood pressure. In addition, rats that were adrenalectomized and subsequently DOCA-salt treated did not develop hypertension. Other studies have supported a role for the adrenal medulla in DOCA-salt hypertension. Catecholamine synthesis is elevated in the adrenal medulla, likely through increased tyrosine hydroxylase activity. Together, these studies suggest an important role for the adrenal medulla in the development and maintenance of DOCA-salt hypertension in male rats. Only a limited number of studies have examined adrenal medullary function in female rats. Adrenal catecholamine content varies with the stage of the estral cycle. The contents and release of epinephrine (EPI) and norepinephrine (NE) levels are lowest during estrus. Unfortunately, no studies have compared adrenal medullary function in males and females. Considering the variations in adrenal catecholamine content and release during the estral cycle and the contribution of the adrenal medulla to DOCA-salt hypertension, it was speculated that gender differences in sympathoadrenal activation contribute to the gender-dependent development and maintenance of DOCA-salt hypertension. The purpose of this study was to test the hypothesis that bilateral removal of the adrenal medulla would result in a greater fall in blood pressure in male DOCA-salt as compared to female DOCA-salt rats.

Materials and Methods

Animals

All experimental protocols were approved by the University's Institutional Animal Care and Use Committee. Animals were treated in accordance with the American Physiological Society's Guiding Principles for the Use of Animals in Research and Teaching. Age-matched (8–9 weeks) male and female Sprague-Dawley rats were purchased from Harlan Sprague Dawley, Inc (Indianapolis). Rats were kept on a constant light-dark cycle (lights on at 7 AM and off at 7 PM). Vaginal smears were performed daily to monitor phases of the estral cycle in female rats. Under gas anesthesia (Metofane, Mallinckrodt Veterinary), rats were implanted with a CA11PA-C40 radiotelemetry transmitter.

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(Data Sciences) Rats were placed in individual cages containing an RLA-3000 radiotelemetry receiver. Every 10 minutes, a 10-second sample of systolic, diastolic, and mean blood pressure and HR was taken, and the data were averaged to obtain a single data point for each parameter.

Two weeks later, all rats underwent a right nephrectomy and were implanted with a sham silastic pellet or a silastic pellet containing 200 mg/kg DOCA (Sigma). Rats treated with DOCA drank a 0.9%NaCl/0.2% KCl solution while rats with a sham pellet drank tap water. All rats were given normal rat chow (Teklad). Five weeks later, all rats underwent bilateral adrenal medullctomy during the afternoon. Under methoxyflurane anesthesia, the adrenal gland was exposed and a small slit made in the cortex, permitting extrusion of the adrenal medulla with the application of gentle pressure. Adrenal medullae were immediately frozen on dry ice and stored at -80°C until assayed for catecholamine content. Rats were allowed to recover from surgery and returned to their cage by 7 PM.

Catecholamine Assay
Adrenal medullae were homogenized in ice-cold 0.1N perchloric acid and centrifuged at 4°C for 15 minutes at 15000 rpm in a DuPont SM24 rotor. A 500 PL aliquot of supernatant was removed. On the day of assay, the supernatant was diluted 1:20 in perchloric acid. Catecholamines were measured using a radioenzymatic assay as described by Peuler and Johnson. Results are expressed as pg catecholamine per gram of adrenal medullary tissue.

Statistics
The statistical program SuperANOVA (Abacus Concepts) was used to generate three-, two-, and one-way repeated measures ANOVA tables, as appropriate. Nonrepeated one- and two-way ANOVA were used as appropriate. Bonferroni/Dunn post-hoc tests were used as determined by the ANOVA results. Differences were considered significant when P<0.05. All data are expressed as mean±SEM.

Results
Development of DOCA-Salt Hypertension
Body weight was measured daily before the induction of hypertension through the following 5 weeks. Female rats had regular 4 to 5 day estral cycles, as determined by daily vaginal lavage. During the course of the study, body weight changes were male sham 344±4 to 442±6 grams, male DOCA 354±4 to 435±7 grams, female sham 227±5 to 260±6 grams, and female DOCA 229±5 to 273±5 grams. All groups of rats significantly increased body weight and there were no differences in weight gain by sham and DOCA-salt treated rats. Male rats had significantly higher body weights than female rats.

Due to the difference in body weight between male and female rats, fluid intake (tap water or saline, as appropriate) was expressed as milliliters per 100 grams body weight. Male DOCA-salt rats consumed significantly more saline per day than their respective sham controls that drank tap water. Female DOCA-salt rats consumed significantly more saline than male DOCA-salt rats. Female sham rats, after nephrectomy and pel-
Figure 3. Twenty-four hour averages of MAP, HR, and fluid intake before and after adrenal medullectomy are shown. The 24-hour period in which the adrenal demedullation (MDX) was performed has been omitted, as indicated by the stippled bar. For MAP, the overall ANOVA indicated differences before and after adrenal demedullation between the normotensive and hypertensive animals as well as gender differences. Importantly, there was a significant interaction between the level of MAP and time. Similar interactions occurred for fluid intake. There was an overall increase in HR in all groups that was sustained through the post-surgical period. * indicates a significant difference from baseline value. $ indicates a significant difference from control value in each group.

DOCA-salt treated rats. MAP in male DOCA-salt treated rats was significantly greater than female DOCA-salt treated rats after day 17 of treatment. MAP did not change in male and female sham rats (115±3 to 113±2 mm Hg and 105±2 to 100±1 mm Hg, respectively), but female sham rats maintained significantly lower MAP than male sham rats.

HR was significantly higher in female as compared to male rats in normotensive and hypertensive groups (Fig 2). There was an initial post-surgical increase in HR in all groups that subsided within 1 to 2 days. Male and female sham rats experienced significant decreases in HR, from 395±7 to 346±5 beats/min in male rats, and from 410±4 to 381±3 beats/min in female rats. Similarly, HR in male DOCA-salt rats fell from 380±4 to 330±9, while in female DOCA-salt rats HR fell from 408±5 to 381±3 beats/min.

Effect of Adrenal Demedullation
Adrenal demedullation caused a significant decrease in fluid intake in male and female DOCA-salt rats (Fig 3). Initially, both male and female DOCA-salt rats decreased saline consumption by 10

Figure 4. The hour-by-hour recording of MAP, before and after adrenal demedullation, is shown in all 4 groups (noted at the right of the figure). Standard error bars have been omitted for clarity. The light period (14 hours) is indicated on the x-axis by the white band and the dark period (10 hours) by the black band. Adrenal demedullation was performed during the light period as noted by the vertical gray bar. No data was collected at this time to allow for recovery from surgery.

nL/100 grams body weight. Male DOCA-salt rats returned to baseline levels within 4 days of surgery, while female DOCA-salt rats returned to baseline levels within 3 days.

In response to adrenal demedullation, the 24-hour average measurement of blood pressure did not change in any group (Fig 3). However, the three-way ANOVA performed on MAP data revealed a significant interaction of time and hypertension, indicating that adrenal medullectomy affected blood pressure differently in the sham and DOCA groups. In addition, adrenal demedullation caused significant increases in HR that were sustained above baseline levels for 12 days in all groups of rats (Fig 3). These sustained elevations in HR suggest the activation of compensatory mechanisms in response to decreases in blood pressure. Thus, the depressor responses in blood pressure shown in Figure 3 most likely represented buffered responses integrated over 24 hours to adrenal medullectomy. Therefore, to identify the maximal effect of adrenal demedullation on blood pressure, data within 24 hours of adrenal demedullation was analyzed.

The average hourly measurements of MAP 3 days before and 7 days after adrenal demedullation for all animals are shown in Fig 4. MAP demonstrated a typical circadian rhythm of higher MAP during dark periods and lower MAP during the light periods. The immediate fall in MAP associated with adrenal medullectomy is clearly demonstrated during the dark and light periods after surgery as is the return of MAP to presurgical levels.

To evaluate the effect of adrenal demedullation within 24 hours of surgery, blood pressure measurements were averaged during the dark period (10 hours) and subsequent light period (14 hours) following surgery. These measurements were compared to those made before surgery (Fig 5). The assessment of blood pressure during the dark period began 4 to 6 hours after surgery at which time the rats had recovered from anesthesia. During the dark periods there was no difference in blood pressure before and after adrenal demedullation in either sham
Figure 5. MAP’s before (black bar) and after (white bar) adrenal medullectomy are shown in sham rats (top panels) and in DOCA-salt rats (bottom panels). The left side of each panel denotes the MAP during the dark period and the right side denotes MAP during the light period. The overall ANOVA indicated an effect of adrenal demedullation on MAP during the dark period. In addition, there is a significant interaction suggesting that males and females respond to adrenal demedullation differently. * indicates a significant difference in MAP before and after adrenal demedullation.

Figure 6. Adrenal medullary catecholamine tissue content is shown. Data are expressed as μg of catecholamine per gram of medullary tissue weight. * indicates significant difference from respective sham or DOCA-salt male group.

Discussion
Sex Differences in the Development of DOCA-Salt Hypertension
The present study confirmed earlier findings that the level of hypertension in female DOCA-salt rats is lower than in male treated male (116 ± 2 to 118 ± 3 mm Hg) or female (101 ± 21 to 103 ± 2 mm Hg) rats. In the DOCA-salt treated groups, significant differences in blood pressure were observed before and after surgery. In DOCA-salt rats (150 ± 4 to 135 ± 3 mm Hg), the decrease in blood pressure in female DOCA-salt rats did not reach statistical significance. These responses represent a statistically greater decrease in male DOCA-salt rats (-15.2 ± 1.4%) compared to female DOCA-salt rats (-9 ± 2.2%).

No significant blood pressure differences were observed between light periods before and after adrenal demedullation in sham treated male (117 ± 3 to 110 ± 2 mm Hg) and female (99 ± 1 to 97 ± 2 mm Hg) rats. In DOCA-salt treated rats, blood pressure decreased significantly after adrenal medullectomy in male (171 ± 4 to 156 ± 4 mm Hg) but not female (139 ± 5 to 134 ± 4 mm Hg). These data represent respective changes of -8.5 ± 1.7% and 3.8 ± 1.8%, which are not statistically different.

The adrenal medullary tissue content of NE and EPI, expressed as micrograms catecholamine/gram tissue, is shown in Fig. 6. There was significantly more NE (630 ± 64 versus 379 ± 27 μg/gram adrenal tissue) and EPI (2396 ± 211 versus 1692 ± 153 μg/gram adrenal tissue) in the adrenal medulla from male sham rats compared to female sham rats, respectively. In addition, male DOCA rats have significantly more NE (526 ± 40 versus 401 ± 44 μg/gram adrenal tissue) and EPI (2254 ± 144 versus 1782 ± 234 μg/gram adrenal tissue) in the adrenal medulla than female DOCA rats. There were no differences between normotensive and hypertensive groups.

DOCA-salt rats were observed that the blood pressure difference between male and female DOCA-salt rats did not appear until 17 days after beginning the DOCA-salt treatment. Others have seen this difference within one week of DOCA-salt initiation. These differences may be due to different sources of rats, different doses and routes of DOCA administration, or different methods of measuring blood pressure.

This study also showed that females consume more saline than male rats when treated with DOCA. Sodium and water balance were not measured during the development of hypertension in the present study. However, others have examined the role of sodium in the gender-dependent development of hypertension. In a study by Ouchi et al., female DOCA-salt rats had higher saline intake and urine sodium concentrations than male DOCA-salt rats, suggesting that females are able to effectively excrete the increased sodium intake. The phenomenon of a diminished salt sensitivity in female rats has also been documented in Dahl salt-sensitive rats, spontaneously hypertensive rats, and renal-wrap hypertensive rats. The mechanism of the attenuated development of salt sensitive hypertension in females is apparently not due to reduced sensitivity to sodium centrally. Crofton and Sharpe demonstrated that females had a lower threshold to the effects of sodium-stimulated vasopressin release, especially during estrus. Rather, evidence suggests that females excrete more sodium over a range of renal perfusion pressures and the fractional excretion of sodium is greater than in males. These observations would suggest that females are more effective in excreting a sodium load resulting in a diminished sodium stimulus.

The sexual dimorphism in the development of DOCA-salt hypertension appears to depend on gonadal hormones. Crofton et
al have demonstrated that testosterone enhances while estrogen attenuates the development of DOCA-salt hypertension. The mechanism(s) by which these gonadal hormones contribute to gender differences in the development of hypertension is uncertain, but may be related to gender differences in vascular reactivity or sympathetic nervous system regulation. Isolated vascular smooth muscle preparations from normotensive female rats are less sensitive to the effects of catecholamines as compared to vascular smooth muscle preparations from normotensive males. Other studies using vascular smooth muscle preparations have shown that estrogen enhances the vasodilator effect of nitric oxide. Crofton et al have demonstrated in vivo that females are less sensitive to the vasoconstrictor effects of vasopressin, which contributes a significant role to the development of DOCA-salt hypertension.

Gender differences in sympathetic nervous system regulation may contribute to the attenuated level of DOCA-salt hypertension in female rats. While this concept has not been extensively studied, there is evidence that autonomic reflex mechanisms in females are more effective in buffering changes in blood pressure. In DOCA-salt hypertension, baroreflex sensitivity is impaired, but the impairment is attenuated in female DOCA-salt rats. Sciscio and DiCarlo demonstrated that normotensive female rats had greater inhibition of lumbar sympathetic nerve activity than male normotensive rats to mechanical and chemical stimuli of the cardiopulmonary receptors. In response to vagal stimulation in an isolated rat heart preparation, there was a greater slowing of HR in female rats compared to male rats. In the present study, we observed an initial increase in blood pressure and fall in HR, that were similar in both male and female DOCA rats. After 17 days, male rats continued to show increases in pressure, while further decreases in HR. In contrast, female rats did not show any further increases in blood pressure, while HR continued to fall. Although neuronal mechanisms were not assessed in the present study, these results suggest that neural regulation of autonomic nervous system may be different in male and female rats.

Role of the Adrenal Medulla in Hypertension
The role of the sympathoadrenal system has been extensively explored in the development and maintenance of hypertension. In the spontaneously hypertensive and renal-wrapped models of hypertension, the sympathetic nervous system and adrenal gland are essential for hypertension to develop. Studies in DOCA-salt hypertensive rats have shown that removal of the entire adrenal gland resulted in the attenuation or prevention of DOCA-salt hypertension. In support of these data, catecholamine synthesis is increased in the adrenal medulla of DOCA-salt hypertensive rats. These data suggested an important role of the adrenal gland in the development and maintenance of DOCA-salt hypertension. Other studies have shown that the reactivity of the sympathoadrenal system is hyper-reactive to carotid artery occlusion. However, all of these studies were carried out in male rats. The role of the adrenal gland in hypertension in female rats has not been explored.

Studies of the adrenal medullae from normotensive female rats by Ramos et al have shown that the estral cycle may alter catecholamine concentration and release from the adrenal medulla. EPI content is higher in diestrus than in the other stages of the cycle and correlated with increased PNMT activity. In addition, in vitro basal secretion of both NE and EPI was lowest during estrus. Considering the evidence that the adrenal gland plays a significant role in DOCA-salt hypertension and that adrenal medullary function is affected by the estral cycle, we proposed that the adrenal medulla contributes to the difference in blood pressure between male and female DOCA-salt rats.

As discussed above, previous studies of DOCA-salt hypertension have examined the contribution of the entire adrenal gland to the development and maintenance of DOCA-salt hypertension. In the present study, we sought to evaluate the contribution of the adrenal medulla specifically by removing only the medulla and leaving the cortex intact. When the medullae were removed, there was a significant decrease in blood pressure only in the male DOCA-salt group and only within 24 hours of adrenal demedullation. The decrease in blood pressure was not reduced to the level of normotension, indicating that other mechanisms were involved in the maintenance of hypertension. This effect of adrenal medullectomy was also transient, and blood pressure returned to presurgical levels within 3 days. The return of blood pressure to presurgical levels suggests that other mechanisms are able to compensate for the decrease in blood pressure. Activation of the sympathetic nervous system after adrenalectomy has clearly been demonstrated. Other compensatory mechanisms may include increased production of vasoactive factors such as vasopressin, angiotensin, and possibly endothelin.

Measurement of adrenal medullary catecholamine content revealed that male rats had higher levels of NE and EPI as compared to female rats. These levels were not affected by DOCA-salt hypertension. Although plasma catecholamines were not measured in this study, normotensive female rats have been reported to have higher circulating levels of NE and EPI, consistent with unpublished observations from our laboratory. It is difficult to speculate how these gender differences in catecholamine levels may be related to the differential effect of adrenal medullectomy. Catecholamine levels could be affected by factors such as the level of sympathetic activation, rate of synthesis, release, uptake, and metabolism. Future studies of catecholamine turnover in the adrenal medulla will be necessary to determine specific alteration in adrenal medullary function between male and female rats.

The differential response to adrenal medullectomy could occur in the post-junctional response to catecholamines or to differences in receptor number. Studies on vascular smooth muscle preparations from normotensive male and female rats suggest that female rats have an attenuated response to phenylephrine. In DOCA-salt hypertension, one study has shown that responses to phenylephrine in mesenteric vascular smooth muscle preparations were not different in male and female DOCA-salt rats. Thus, the gender-dependent response to alpha-receptor stimulation observed in normotensive rats may be altered in hypertension.

Vasodilation in response to beta-receptor stimulation may be different in male and female rats. Preliminary studies in our laboratory have shown, that in response to restraint stress, normotensive female rats do not increase blood pressure as much as males unless beta-receptors are blocked (unpublished data). Thus, EPI may attenuate blood pressure increases in...
DOCA-salt female rats by acting on vasodilator β-receptors. This hypothesis has yet to be directly tested.

In summary, this study has demonstrated the gender-dependent development of DOCA-salt hypertension, as reported by others. The novel finding of this study is that male DOCA-salt rats have a significant decrease in blood pressure in response to adrenal demedullation that is not observed in female DOCA-salt rats. These data suggest that the adrenal medulla plays a greater role in the maintenance of DOCA-salt hypertension in male than female rats.

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