Regulation of Blood Pressure, Appetite, and Glucose by CNS Melanocortin System in Hyperandrogenemic Female SHR

Jussara M. do Carmo, 1 Alexandre A. da Silva, 1,2 Sydney P. Moak, 1 Haley J. Houghton, 1 Andrew Smith, 1,3 and John E. Hall 1

BACKGROUND

Hyperandrogenemia in females may be associated with sympathetic nervous system (SNS) activation and increased blood pressure (BP). However, the importance of hyperandrogenemia in causing hypertension in females and the mechanisms involved are still unclear. We tested whether chronic hyperandrogenemia exacerbates hypertension in young female spontaneously hypertensive rats (SHR) and whether endogenous melanocortin-3/4 receptor (MC3/4R) activation contributes to the elevated BP.

METHODS

Cardiovascular and metabolic effects of chronic MC3/4R antagonism were assessed in female SHR treated with dihydrotestosterone (DHT, beginning at 5 weeks of age) and placebo-treated female SHR. BP and heart rate (HR) were measured by telemetry and an intracerebroventricular (ICV) cannula was placed in the lateral ventricle for infusions. After control measurements, the MC3/4R antagonist (SHU-9119) was infused for 10 days (1 nmol/hour, ICV, at 15 weeks of age) followed by a 5-day recovery period.

RESULTS

MC3/4R antagonism increased food intake and body weight in DHT-treated SHR (14 ± 1 to 35 ± 1 g/day and 244 ± 3 to 298 ± 8 g) and controls (14 ± 1 to 34 ± 2 g/day and 207 ± 4 to 269 ± 8 g). Compared to untreated SHR, DHT-treated SHR had similar BP but lower HR (146 ± 3 vs. 142 ± 4 mm Hg and 316 ± 2 vs. 363 ± 4 bpm). Chronic SHU-9119 infusion reduced BP and HR in DHT-treated SHR (−12 ± 2 mm Hg and −14 ± 4 bpm) and control female SHR (−19 ± 2 mm Hg and −21 ± 6 bpm).

CONCLUSION

These results indicate that hyperandrogenemia does not exacerbate hypertension in female SHR. MC3/4R antagonism reduces BP and HR despite marked increases in food intake and body weight in hyperandrogenic and control female SHR.

Keywords: androgens; blood pressure; hypertension; insulin; leptin; obesity; sympathetic activity.

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Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorders in women of reproductive age and is associated with multiple metabolic abnormalities that may contribute to the development of hypertension and cardiovascular diseases. 1-2 Women with PCOS are 20-40% more likely to have hypertension than age-matched controls even after adjusting for age, body mass index, diabetes, and dyslipidemia. 3-5 Although the mechanisms responsible for hypertension in women with PCOS have not been fully elucidated, hyperandrogenism, insulin resistance, obesity, and increased sympathetic nervous system (SNS) activity have been suggested to be involved. 6-10

Muscle sympathetic nerve activity is increased in women with PCOS compared to weight- and age-matched controls 11 and Schlaich et al. 12 reported that bilateral renal denervation, using a radiofrequency ablation method, lowered blood pressure (BP) in 2 obese hypertensive women with PCOS. 12 These findings suggest that increased SNS activity may contribute to elevated BP in women with PCOS. Although ovarian hyperandrogenism is the most consistent endocrine feature of PCOS, abdominal obesity, insulin resistance, and hyperinsulinemia have also been suggested to contribute to hypertension in women with PCOS. 9 Supporting a role of androgens in PCOS-induced hypertension is the finding that chronic administration of androgens in young female rodents raises BP via sympathetic activation. 13 Increased androgens have also been suggested to contribute to postmenopausal hypertension. 14,15

The importance of hyperandrogenism in raising BP and SNS activity in females and the potential mechanisms involved, however, are still unclear. We 16-18 and others 19-21 have provided evidence that the central nervous system (CNS) melanocortin pathway plays a key role in regulating SNS activity and BP as well and food intake and energy balance. Moreover, hyperandrogenism may influence activity of the CNS melanocortin pathway. For instance, testosterone-treated rats have higher levels of proopiomelanocortin (POMC) expression in elevated BP in women with PCOS. Although ovarian hyperandrogenism is the most consistent endocrine feature of PCOS, abdominal obesity, insulin resistance, and hyperinsulinemia have also been suggested to contribute to hypertension in women with PCOS. 9 Supporting a role of androgens in PCOS-induced hypertension is the finding that chronic administration of androgens in young female rodents raises BP via sympathetic activation. 13 Increased androgens have also been suggested to contribute to postmenopausal hypertension. 14,15

The importance of hyperandrogenism in raising BP and SNS activity in females and the potential mechanisms involved, however, are still unclear. We 16-18 and others 19-21 have provided evidence that the central nervous system (CNS) melanocortin pathway plays a key role in regulating SNS activity and BP as well and food intake and energy balance. Moreover, hyperandrogenism may influence activity of the CNS melanocortin pathway. For instance, testosterone-treated rats have higher levels of proopiomelanocortin (POMC) expression in

Correspondence: Jussara M. do Carmo (jdocarmo@umc.edu).

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the arcuate nucleus of the hypothalamus and there is an age-related decrease in POMC expression associated with declining testosterone levels in male rats.

POMC neurons produce and release α-melanocyte stimulating hormone (α-MSH) which then activates melanocortin 3 and 4 receptors (MC3/4R) in second-order neurons, leading to suppressed appetite and increased energy expenditure by stimulating SNS activity. This system also regulates BP and heart rate (HR) by increasing SNS activity to the kidneys and other organs and tissues. Clinical and experimental studies suggest that a functional MC3/4R may be necessary for obesity to increase SNS activity and promote hypertension. For example, MC4R deficient mice do not have elevated BP despite severe obesity, insulin resistance, hyperinsulinemia, and other features of the metabolic syndrome. Humans with mutations of MC4R also exhibit severe obesity and many characteristics of the metabolic syndrome but are not hypertensive and actually have reduced BP and SNS activity, and lower prevalence of hypertension than age-matched control obese subjects. These observations support the concept that MC3/4R activation may be required for excess weight gain to increase BP.

In addition to linking obesity with increased SNS activity and elevations in BP and HR, the CNS melanocortin system may play a more fundamental role in regulating SNS activity and BP than previously appreciated. For example, we showed that chronic MC3/4R blockade in lean male spontaneously hypertensive rats (SHR), a model of hypertension associated with high sympathetic tone, reduced their BP as much as did adrenergic receptor blockade. We also found that MC3/4 antagonism lowered BP after blockade of nitric oxide (NO) synthesis in lean rats. Thus, the CNS MC3/4R may play a key role in regulating SNS activity and BP beyond its role in obesity-induced hypertension. The brain melanocortin system also appears to modulate respiratory function as evidenced by the finding that CNS MC3/4R blockade reduced ventilatory responses to hypercapnia in rats.

Although the CNS melanocortin system appears to be a key regulator of autonomic activity, respiratory function, and BP, its role in hyperandrogenic females with hypertension is still unclear. Previous studies have provided evidence that MC3/4R blockade lowers BP in normotensive female rats treated with dihydrotestosterone (DHT) and in untreated female SHR. However, there have been no studies, to our knowledge, that have determined whether high androgen levels amplify the hypertension in female SHR and if MC3/4R activation contributes to elevated BP in hyperandrogenic hypertensive females. Therefore, we examined whether chronic CNS inhibition of endogenous MC3/4R activity alters regulation of BP and respiratory function in hypertensive female SHR with chronic hyperandrogenemia, as well as in hypertensive female SHR with normal androgen levels. We also investigated the metabolic changes associated with CNS blockade of MC3/4R in hypertensive SHR with chronically elevated or normal testosterone levels.

METHODS

All experimental procedures conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Animals

Female 4-week-old SHR (n = 14) were purchased from Taconic Farms (Hudson, NY). At 5 weeks of age, the rats were implanted subcutaneously with non-aromatizable DHT pellets (7.5 mg/90 days, continuous release, Innovative Research, Sarasota, FL, n = 8) or placebo pellets (control female SHR, n = 6) under isoflurane anesthesia. The 2 groups of rats were age and sex-matched.

Body weight and body composition analysis

A group of DHT-treated female SHR (DHT-SHR, n = 6) and control female SHR (n = 8) were individually housed and fed standard chow for weekly determination of body weight and body composition starting at 4 weeks of age until 13 weeks of age in order to examine the impact of hyperandrogenia on metabolic and cardiovascular regulation. Body composition was assessed weekly using magnetic resonance imaging (EchoMRI-900TM, Echo Medical System, Houston, TX) to quantify lean mass, fat mass, and free water and total water content in conscious rats. When the rats were 12 weeks old we also measured body composition by computed tomography scan as previously described. Briefly, the rats were individually placed in prone position inside of their plastic cages onto the Siemens Sensation 64 computed tomography scanner table. The field of view was made as small as possible in the X–Y direction (~10 cm) and included the whole body (nose through hindfeet) in the Z direction. Abdominal fat was selected using Hounsfield Unit (HU) threshold technique, and the subcutaneous abdominal fat and visceral abdominal fat compartments were separated by a region of interest drawn along the abdominal wall musculature as an anatomic landmark separating the 2 compartments. The paravertebral muscles were selected with a region of interest using anatomic landmarks. Total abdominal fat, subcutaneous abdominal fat, visceral abdominal fat, and paravertebral muscle volumes were measured for each rat.

Animal surgery

A telemetry BP transmitter (Model TA11PAC40, Data Sciences International, MN) was implanted in the abdominal aorta distal to the kidneys under sterile conditions as previously described in DHT-SHR treated (n = 8) and control female SHR (n = 6) at 14 weeks of age. A stainless steel cannula (26 gauge, 10 mm long) was also implanted into the brain right lateral ventricle using coordinates previously described. The rats were allowed to recover from surgery for 10–12 days and housed individually in cages before control measurements were taken, and then we began monitoring food intake, body weight, BP, and HR. The accuracy of implantation of the intracerebroventricular (ICV) cannula was examined by determining the dipsogenic response to an acute injection of 100 ng of Ang II. All rats received water and food ad libitum and total daily sodium.
intake was maintained constant at ~3.2 mEq/day (Harlan Teklad, Madison, WI).

Experimental protocols

Pulmonary ventilation measurements. To examine whether hyperandrogenemia alters the ventilatory responses to hypercapnia, pulmonary ventilation (\(V_T\)) was measured using whole-body plethysmography during control and the last day of SHU-9119 infusion as previously described.\(^{31}\) Briefly, rats were acclimatized to the plethysmography chambers (5 l) at room temperature (~25 °C) for 1 hour before measurements were taken. An internal constant volume was kept by closing the ports for gas entrance or exit. Tidal volume (\(V_T\), mV) and frequency (f\(_T\), breaths/minute) were measured by changes in the pressure inside the plethysmography chamber caused by inspiratory and expiratory fluctuations using a spirometer (model ML141, AD Instruments, Colorado Springs, CO)\(^4\) under normoxia and hypercapnia (7% CO\(_2\)). \(V_E\) was calculated as the product of \(f_T\) and \(V_T\).

Responses to chronic MC3/4R blockade. Mean arterial pressure (MAP), HR, and food intake were recorded daily. After a 5-day control period, the MC3/4R antagonist, SHU-9119, was infused ICV (1 nmol/hour at 0.5 μl/hour) for 10 consecutive days via osmotic minipump (model 2002, Durect, Cupertino, CA) in DHT-treated and control female SHR. Under isoflurane anesthesia, the osmotic minipump was implanted subcutaneously in the scapular region and connected to the ICV cannula using tygon tubing (Cole Parmer). The rate of SHU-9119 infusion was based on previous studies showing that this dose effectively blocks MC3/4R and increases food intake, promotes weight gain, and reduces BP and HR.\(^{18,34}\) On the last day of SHU-9119 infusion, the cannula connecting the minipump with the ICV cannula was severed to stop the infusion, and the rats were followed for an additional 5-day posttreatment period. All animals were fasted for 5 hours before blood samples (200 μl) were collected via a tail snip once during control, on day 10 of SHU-9119 infusion, and on day 5 of posttreatment period.

Plasma hormones and glucose measurements

Plasma leptin, insulin, and DHT concentrations were measured with ELISA kits (R&D Systems, Minneapolis, MN; Crystal Chem, and Diagnostic System Laboratories, Downers Grove, IL, respectively) and plasma glucose concentrations were determined using the glucose oxidation method (Beckman glucose analyzer 2).

Statistical methods

The results are expressed as means ± SEM. The data were analyzed by 1-way analysis of variance with repeated measures followed by Dunnett’s post hoc test for comparisons between control and experimental values within each group when appropriate. Comparisons between different groups were made by 2-way analysis of variance followed by Dunnett’s post hoc test when appropriate. Statistical significance was accepted at a level of \(P < 0.05\).

RESULTS

Impact of hyperandrogenemia on metabolic phenotypes

Body weight from 8 until 13 weeks of age was significantly higher in DHT-treated SHR compared to control female SHR (Figure 1A). The higher body weight observed in DHT-ShR group was due to increases in lean mass, adiposity, and water (Figure 1B–D). At 12 weeks of age, the total abdominal, subcutaneous, and visceral abdominal fat as well as paravertebral muscle measured by computed tomography scan were significantly higher in DHT-treated SHR compared to control female SHR (Figure 1E). Hyperandrogenemia in female SHR was associated with increased body weight and adiposity, despite similar food intake at 15 weeks of age (Figure 1E,G).

Impact of hyperandrogenemia and MC3/4R antagonism on pulmonary ventilation

Under normocapnia (room air), \(f_T\), \(V_T\), and \(V_E\) were similar in DHT-treated and control female SHR (Figure 2A–C). However, ventilatory responses (\(V_T\) and \(V_E\)) to hypercapnia (7% CO\(_2\)) were significantly higher in DHT-treated compared to control female SHR, despite similar baseline pulmonary ventilation (Figure 2A–C). Baseline \(V_T\) and \(V_E\) in normocapnia were reduced by SHU-9119 treatment in DHT and control female SHR (Figure 2A–C). Treatment with SHU-9119 also significantly reduced \(V_T\) and \(V_E\) responses to 7% CO\(_2\), although \(f_T\) remained elevated in DHT-treated compared to control female SHR group (Figure 2B,C).

Food intake, body weight, plasma glucose, insulin, and leptin responses to chronic MC3/4R antagonism and plasma DHT levels in DHT-treated and control female SHR

As shown in Figure 3A, increased androgen levels in female SHR did not significantly alter baseline food intake, measured at 15 weeks of age, but increased body weight. Chronic central MC3/4R blockade with SHU-9119 caused a significant increase in appetite leading to a doubling of food intake during the last 5–6 days of SHU-9119 infusion in DHT-treated as well as in control rats. The increases in food intake were associated with 22% and 30% increases in body weight in DHT-treated and control female SHR (Figure 3B). After stopping SHU-9119 infusion, food intake remained elevated for an additional 2–3 days and then gradually fell toward baseline values on day 5 post-SHU-9119 infusion.

Chronic MC3/4R antagonism did not significantly alter plasma glucose levels in DHT-treated or control female SHR (Figure 3C). However, chronic MC3/4R antagonism increased plasma insulin and leptin levels in both groups (Figure 3D,E). These results suggest that inhibition of MC3/4R was associated with insulin resistance in DHT-treated and control female SHR. The 5-fold increase in plasma leptin levels observed in both groups was likely caused by the marked weight gain and increased adiposity during chronic MC3/4R blockade. Plasma DHT levels were significantly increased in DHT-treated compared to control female SHR (Figure 3D).
Chronic MC3/4R antagonism attenuated hypertension and reduced HR in DHT-treated and control female SHR

Baseline MAP was similar in DHT-treated and control SHR (Figure 4A), but HR was significantly lower in DHT-treated compared to controls (Figure 4B). Chronic MC3/4R antagonism reduced MAP in both groups, but the reduction in BP was attenuated in DHT-treated SHR. The attenuated chronic effect of MC3/4R antagonism to reduce
BP in DHT-treated compared to control SHR is more evident when analyzing the area under curve of the BP response to SHU-9119 infusion (Figure 4C). Baseline HR was lower in DHT-treated than in control rats (Figure 4B), and the reduction in HR during SHU-9119 infusion was similar in DHT-treated SHR (Figure 4B). After the infusion of MC3/4 antagonist was stopped, HR remained below baseline values in both groups.

**DISCUSSION**

In the present study, we demonstrated that chronic antagonism of endogenous MC3/4R in the CNS reduced BP and HR in DHT-treated and control female SHR despite causing marked increases in food intake and body weight which would normally tend to increase BP. Thus, in DHT-treated as well as untreated female SHR, tonic activation of MC3/4R appears to play a significant role in maintaining elevated BP. However, the impact of central MC3/4R blockade on BP regulation was slightly attenuated in DHT-treated compared to untreated female SHR. Our observations therefore provide no evidence that hyperandrogenemia further increases BP by activation of MC3/4R above the level already present in female SHR.

Although the compound used in our studies to investigate the role of CNS melanocortins (SHU-9119) is an antagonist of both MC3R and MC4R, its effects on BP and body weight appear to be mediated largely by blockade of MC4R. In fact, there is evidence that blockade of MC3R tends to increase rather than decrease BP. For example, deficiency of MC3R produces salt-sensitive hypertension by impairing the kidneys’ ability to excrete sodium. The MC3R also appears to play only a modest role in body weight regulation as evident by the fact that genetic disruption of MC3R signaling does not substantially reduce appetite or body weight, although it does result in increased adiposity due to more efficient fat storage.

Our results together with previous studies highlight an important role of the CNS melanocortin system in the development and/or maintenance of increased BP in SHR, which are known to have increased SNS activity. We also found that MC4R activation contributes to other forms of hypertension associated with increased SNS activity, including hypertension caused by obesity or by nitric oxide synthase inhibition. In contrast, MC4R activation does not appear to contribute to those forms of hypertension associated with normal or reduced SNS activity, such as hypertension caused by chronic intravenous angiotensin II infusion.

Although the mechanisms by which MC4R activation occurs in various models of hypertension have not been fully elucidated, it is clear that MC4R activation raises BP by sympathetic activation. For instance, acute central injections of MC4R agonists increase SNS activity to several tissues, including the kidneys, and combined α- and β-adrenergic receptors blockade completely prevented hypertension caused by chronic central MC3/4R activation.

In obesity, increased levels of leptin may contribute to activation of POMC neurons and subsequent activation of MC4R. However, in non-obese forms of hypertension, such as the SHR strain, which has increased SNS activity, the factors that make BP more dependent on MC4R activation are still unclear. Some studies suggest that sex steroids may stimulate the POMC–MC4R pathways. For example, neonatal testosterone appears to program a sex-dependent
differentiation of POMC neurons and predisposes female mice to develop leptin resistance during adulthood by regulating POMC expression in neurons of the arcuate nucleus. In addition, there is an age-related decrease in POMC expression in male rats.

Previous studies also suggest that increased testosterone may contribute to SNS activation and hypertension in women with PCOS. However, the mechanisms linking increased testosterone, PCOS, SNS activation, and hypertension are still unclear. Based on our observations suggesting that MC4R activation may play a more fundamental role in SNS activation than previously recognized, we postulated that blockade of MC3/4R activation would reduce BP in young female SHR treated with DHT, a model of hypertension and hyperandrogenemia. Our results indicate that MC3/4R antagonism significantly reduced BP and HR despite increasing food intake and body weight in DHT-treated and untreated female SHR. However,
contrary to our initial hypothesis that DHT treatment would exacerbate hypertension due to additional activation of MC4R, we found that hyperandrogenemia did not further increase BP in young female SHR and that the fall in BP after MC3/4R blockade was actually attenuated in DHT-treated compared to untreated female SHR.

Thus, our findings do not support the concept that chronic hyperandrogenemia exacerbates hypertension in female SHR by activation of MC3/4R. We do not know why chronic hyperandrogenemia attenuated the impact of MC3/4R blockade on BP in female SHR. It seems unlikely that DHT treatment would decrease POMC expression and therefore release of α-MSH, the main agonist of MC4R, since testosterone-treated rats have higher levels of hypothalamic POMC expression.22 Further studies, beyond the scope of our current aims, would be required to address this issue by determining the chronic impact of androgens on POMC neuronal activity, MC4R sensitivity to α-MSH, MC4R expression, and post-receptor signaling.

Antagonism of endogenous MC3/4R also reduced HR in DHT-treated and control untreated rats. Although this effect is consistent with reduced cardiac SNS activity, increased parasympathetic activity could also have contributed to this effect. The brain areas where MC3/4R activation regulates autonomic nervous system activity and cardiovascular function have not been fully elucidated. However, MC4R are abundant in the brainstem and intermediolateral medulla as well as in the paraventricular nucleus of the hypothalamus areas involved in autonomic regulation.42,43 Acute activation of MC4R in the paraventricular nucleus or intermediolateral medulla raises renal sympathetic nerve activity and HR, respectively,43 while MC4R located on cholinergic preganglionic parasympathetic and sympathetic neurons appear to contribute, at least in part, to obesity hypertension.44 Additional studies are needed to determine the brain regions where the melanocortin system is most important for modulating cardiovascular function and the role of sex hormones in regulating MC4R activity.

The brain melanocortin system also modulates respiratory function as evidenced by the finding that MC3/4R blockade in rats and agouti yellow mice attenuates the ventilatory responses to hypercapnia.31,45 Although the present study was not designed specifically to investigate the role of MC3/4R in respiratory regulation, we found that MC3/4R antagonism attenuated baseline and ventilatory responses to CO₂ in young female SHR. We also observed increased ventilatory responses to hypercapnia, despite similar baseline pulmonary ventilation, in DHT-treated compared to control SHR. This finding suggests that testosterone may enhance the respiratory responses to hypercapnia, consistent with the observation that men have higher ventilatory response to CO₂ than women.46 Therefore, our observations provide further evidence for a role of testosterone as well as for MC3/4R activation in respiratory control.

In summary, our observations suggest that hyperandrogenemia does not exacerbate hypertension in young female SHR. MC4R activation, however, appears to contribute to increases in BP and HR in female SHR. Chronic hyperandrogenemia in young female SHR appears to slightly decrease the tonic influence of endogenous MC4R on regulation of BP. However, blockade of MC3/4R had similar effects on food intake in untreated and DHT-treated SHR, suggesting that control of appetite by endogenous MC3/4R activity was not altered by chronic hyperandrogenemia. Unraveling the mechanisms responsible for differential control of appetite, HR, and BP by the
CNS melanocortin system will significantly improve our understanding of how the brain regulates metabolic and cardiovascular functions under different physiological and pathological conditions, including conditions associated with altered sex hormones.

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DISCLOSURE

The authors declared no conflict of interest.

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