Effect of gonadectomy on AgRP-induced weight gain in rats

Sean Z. Goodin, Alicia R. Keichler, Marissa Smith, Donna Wendt, and April D. Strader

Department of Physiology, Southern Illinois University Carbondale, School of Medicine, Carbondale, Illinois

Submitted 4 April 2008; accepted in final form 11 October 2008

Goodin SZ, Keichler AR, Smith M, Wendt D, Strader AD. Effect of gonadectomy on AgRP-induced weight gain in rats. Am J Physiol Regul Integr Comp Physiol 295: R1747–R1753, 2008. First published October 15, 2008; doi:10.1152/ajpregu.90345.2008. — Agouti-related peptide (AgRP), the endogenous antagonist to the melanocortin 3 and 4 receptors, elicits robust hyperphagia and weight gain in rodents when administered directly into the central nervous system. The relative influence of AgRP to cause weight gain in rodents partially depends on the activity level of the melanocortin agonist-producing proopiomelanocortin neurons. Both proopiomelanocortin and AgRP neurons within the arcuate nucleus receive energy storage information from circulating peripheral signals such as leptin and insulin. Another modulator of AgRP activity includes the cell surface molecule syndecan-3. Because leptin and insulin affect food intake in a sexually dimorphic way in rodents and syndecan-3-deficient mice regulate adiposity levels through distinct physiological mechanisms, we hypothesized that AgRP-induced weight gain would also be sexually dimorphic in rats. In the present study, the behavioral and physiological effects of centrally-administered AgRP in male and female were investigated. In male rats, AgRP (1 nmol) induced 5 days (P < 0.0001) of significantly elevated feeding compared with vehicle-treated controls, while females displayed 3 days of hyperphagia (P < 0.05). However, 1 wk after the injection, both male and female rats gained the same percent body weight (6%). Interestingly, female rats exhibited a greater reduction in energy expenditure (VO2) following AgRP compared with male rats (P < 0.05). Removal of the gonads did not alter cumulative food intake in male or female rats but did attenuate the dramatic reduction in VO2 exhibited by females. Both intact and gonadectomized rats demonstrated significantly increased respiratory quotient supporting the anabolic action of AgRP (P < 0.01). These findings are novel in that they reveal sex-specific underlying physiology used to achieve weight gain following central AgRP in rats.

AgRP; melanocortin; energy expenditure; sex; food intake

THE HYPOTHALAMUS OF THE CENTRAL nervous system contains multiple circuits that are critical for the regulation of energy balance. Behavioral, genetic, and anatomical data all point to the melanocortin system as being one of the most important of the hypothalamic pathways that regulate body weight (8, 16, 20). Despite the wealth of research on the melanocortin regulatory system, little effort has been dedicated to male and female differences in melanocortin signaling. Nearly all previ-
70°F. All procedures described were approved by the Southern Illinois University IACUC committee. During the entire study, rats were fed Purina rodent Chow ad libitum and had ad libum access to water.

**Intracerebroventricular Cannulation**

Rats were anesthetized using a cocktail of ketamine (60 mg/ml)-xylazine (8 mg/ml) and were prepped for surgery. Fur on the top of the head was shaved and swabbed with Betadine. Following placement of the rat into the stereotaxic apparatus (Stoelting) a small incision was made to expose the sagittal, coronal, and lambdoidal sutures. With the use of the stereotaxic apparatus, the intracerebroventricular cannula was aimed for the third ventricle using coordinates from the Stereotaxic Atlas of the Rat (Paxinos and Watson). A 28-gauge stainless steel cannula (Plastics One, Roanoke, VA) was lowered into the third ventricle using the coordinates (−2.2 mm posterior to bregma and −7.5 mm ventral from the dural meninge). The cannula was fixed to the skull using stainless steel screws and dental acrylic. An obturator cannula was inserted into the guide cannula and secured. All rats recovered on a heating pad until alert and received oral Metacam (1 mg/kg) for postoperative analgesia for 2 days. After a recovery period of 1 wk, rats were tested for cannula placement by administering an angiotensin II (10 ng/ml) intracerebroventricular injection during the light period. Rats that did not respond by immediate drinking (within 5 min) were excluded from the study. Water consumption was measured after angiotensin II administration. Rats that consumed at least 5 ml of water within 1 h were used for the experiments. Rats recovered for an additional week before receiving any other treatments through the intracerebroventricular cannula.

**Intracerebroventricular Injections**

Intracerebroventricular injections were made using a 33-gauge internal injector (Plastics One) placed inside the implanted intracerebroventricular 28-gauge cannula. All injections were delivered slowly with the injector left in place for ~60 s before removal. The injection volume for both the vehicle (0.9% saline) and AgRP (dissolved in 0.9% saline) (Phoenix Pharmaceuticals) was 1 ml. AgRP was delivered at a dose of 1 nmol. This dose has previously been used to reliably increase food intake in male rats (13). Following the injections, the rats were immediately placed into their home cage or the indirect calorimeter for energy expenditure measurements.

**Indirect Calorimetry**

Indirect calorimetry was performed to assess energy expenditure (VO2; in ml O2·kg body wt−1·min−1) and substrate utilization respiratory quotient (RQ; VO2exhaled/VO2consumed). The indirect calorimeter by Accuscan Instruments (Columbus, OH) can measure energy expenditure and RQ in eight rats during any single experimental period. The flow rate on the flow controller was set at 2.0 l/min for each experiment. Samples were collected every 4.5 min for each chamber providing ∼12 samples per rat per hour. Samples for each hour were averaged to determine the mean for each rat per hour. The means for each rat per hour were analyzed using a two-way repeated-measures ANOVA to determine statistical significance between AgRP and vehicle. During each calorimeter session, rats were injected immediately prior to being placed in the indirect calorimeter. Once all eight rats were injected, the calorimeter was started and data was collected. All injections were delivered 1 h prior to lights off (6 PM). Rats remained in the indirect calorimeter chambers for a 24-h period (the first day following AgRP or saline treatment) with ad libitum access to food and water. Following the first 24 h, rats were removed and placed in their home cage for the remaining 5–6 days of measurements.

**Gonadectomy**

Male and female rats underwent surgical removal of the gonads 6 wk prior to any experimental treatment. During gonadal removal rats were anesthetized with isoflurane anesthesia. Following experiment 2 uteri weights were collected as a bioassay for proper ovariectomy.

**Experimental Procedures**

**Experiment 1:** comparison of the long-term orexigenic effect of AgRP in intact male and female rats. This experiment aimed to determine gender differences in food intake, energy expenditure, and substrate utilization following a single intracerebroventricular injection of AgRP or vehicle in intact male and female age-matched rats. All rats were cannulated in the third ventricle as described above and allowed to recover for at least 2 wk prior to injections. For this particular study, a within-subjects design was used to compare the vehicle-induced food intake and energy expenditure from each rat with the AgRP-induced food intake and energy expenditure. Because of the long-lasting effects of AgRP on body weight and potential hypothalamic long-term neuronal changes following the injection (5), we first injected rats with vehicle (0.9% saline; 1 μl) and then a week later with AgRP (1 nmol/1 μl). For this study, eleven males and nine females received injections. During the first 24-h period after the injection, each rat was placed with a preweighed food hopper with water bottle in the Accuscan indirect calorimeter (Accuscan Instruments) for energy expenditure data collection and then placed in their home cages for the next 6 days of data collection. Food intake and body weights were measured daily. All intracerebroventricular injections given to female rats were performed during diestrous.

**Experiment 2:** comparison of the long-term orexigenic effect of AgRP in gonadectomized male and female rats. We hypothesized that gonadectomy would modulate the effectiveness of AgRP to affect food intake and energy expenditure; therefore, to determine this we gonadectomized age-matched male and female rats and performed the same experiment described in experiment 1. Briefly, male and female rats were gonadectomized and allowed to recover for 4 wk. Following the 4-wk recovery period, all rats were implanted with a stainless steel cannula targeted for the third ventricle. The rats were allowed to recover an additional week and were tested with angiotensin II for cannula placement. On the sixth week after gonadectomy (2 wk after cannulation), rats were injected with vehicle (1 μl, 0.9% saline). Experiment 2 was performed 6 wk following gonadectomy, since it was recently shown that AgRP and neuropeptide Y (NPY) transiently increase immediately following ovariectomy, concurrent with postsurgical hyperphagia and weight gain, and then return to levels comparable to intact animals ~4–5 wk later (5). For the study involving ovariectomized females, the design was a between-subjects study. A between-subjects design was used for the ovariecetomized female study to control for any possible additional weight gain between treatments that would occur with a within-subjects design. Female ovariectomized rats were injected with either vehicle (1 μl, 0.9% saline; n = 9) or AgRP (1 nmol/1 μl; n = 10) and immediately placed into the indirect calorimeter for the first 24 h of the study. Rats were removed after day 1 and then placed into their home cage for the remaining 6 days. Daily food and body weights were measured.

The study involving castrated male rats (n = 7) was a within-subjects design. During the first week of the study (6 wk after castration) male rats received a vehicle (1 μl, 0.9% saline) injection as described in experiment 1, and the following week they received an injection of AgRP (1 nmol/1 μl icv). Again, during the first 24 h following each injection rats were placed in the indirect calorimeter. Food and body weights were measured for the next 5 days (due to poor weather conditions only 6 days of data were collected compared with 7 days in experiment 1). For between-experiment comparisons of cumulative food intake, only 6 days of food intake were used for analysis.

**Statistical Analysis**

Analysis for all experiments was performed using Prism Statistical Software. All data are expressed as means ± SE. Significance was set at P < 0.05 for all tests. Two-way ANOVAs with repeated measures were
performed on the food intake data for both experiments 1 and 2 in between-vehicle and AgRP-treated groups. The change in body weight data in Table 1 was analyzed with a two-way ANOVA with repeated measures. Comparisons of body weight between surgical groups on day 0 were made using a Student’s t-test. Cumulative food intake between intact and gonadectomized male and female rats was analyzed using a paired or unpaired t-test. All of the VO2 and RQ data were analyzed using a two-way ANOVA with repeated measures. Only 20 h of energy expenditure and RQ data are shown due to the time needed to insert, and remove rats from the calorimeter chambers.

RESULTS

A comparison between male and female intact age-matched rats (experiment 1) injected with vehicle and AgRP revealed a 5-day period of elevated hyperphagia in males (Fig. 1A, P < 0.0001). In contrast, female rats injected with AgRP during diestrus displayed significantly elevated food intake compared with vehicle-treated intake for only 3 days (Fig. 1B, P < 0.05).

Table 1. Body weight change following vehicle or AgRP administration in intact and gonadectomized rats

<table>
<thead>
<tr>
<th>Day</th>
<th>Body Weight, g</th>
<th>Change Body Weight, %</th>
<th>Body Weight, g</th>
<th>Change Body Weight, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact Females Saline</td>
<td>OVX Females Saline</td>
<td>Intact Females AgRP</td>
<td>OVX Females AgRP</td>
</tr>
<tr>
<td>0</td>
<td>238.59 ± 2.96</td>
<td>306.17 ± 9.72</td>
<td>238.59 ± 2.96</td>
<td>306.17 ± 9.72</td>
</tr>
<tr>
<td>1</td>
<td>236.38 ± 7.15</td>
<td>289.08 ± 10.79</td>
<td>236.38 ± 7.15</td>
<td>289.08 ± 10.79</td>
</tr>
<tr>
<td>3</td>
<td>237.80 ± 6.26</td>
<td>300.59 ± 11.44</td>
<td>237.80 ± 6.26</td>
<td>300.59 ± 11.44</td>
</tr>
<tr>
<td>4</td>
<td>241.42 ± 6.90</td>
<td>302.42 ± 12.16</td>
<td>241.42 ± 6.90</td>
<td>302.42 ± 12.16</td>
</tr>
<tr>
<td>5</td>
<td>241.05 ± 7.55</td>
<td>301.00 ± 12.26</td>
<td>241.05 ± 7.55</td>
<td>301.00 ± 12.26</td>
</tr>
<tr>
<td>6</td>
<td>242.03 ± 7.08</td>
<td>301.04 ± 12.04</td>
<td>242.03 ± 7.08</td>
<td>301.04 ± 12.04</td>
</tr>
<tr>
<td>7</td>
<td>243.86 ± 7.58</td>
<td>301.58 ± 12.50</td>
<td>243.86 ± 7.58</td>
<td>301.58 ± 12.50</td>
</tr>
<tr>
<td>8</td>
<td>243.86 ± 7.58</td>
<td>289.08 ± 8.21</td>
<td>243.86 ± 7.58</td>
<td>289.08 ± 8.21</td>
</tr>
<tr>
<td></td>
<td>Intact Males Saline</td>
<td>CAST Males Saline</td>
<td>Intact Males AgRP</td>
<td>CAST Males AgRP</td>
</tr>
<tr>
<td>0</td>
<td>373.77 ± 9.03</td>
<td>367.54 ± 22.27</td>
<td>373.77 ± 9.03</td>
<td>367.54 ± 22.27</td>
</tr>
<tr>
<td>1</td>
<td>372.89 ± 8.26</td>
<td>357.39 ± 23.78</td>
<td>372.89 ± 8.26</td>
<td>357.39 ± 23.78</td>
</tr>
<tr>
<td>2</td>
<td>375.58 ± 8.61</td>
<td>360.36 ± 22.92</td>
<td>375.58 ± 8.61</td>
<td>360.36 ± 22.92</td>
</tr>
<tr>
<td>3</td>
<td>378.61 ± 8.73</td>
<td>366.17 ± 21.05</td>
<td>378.61 ± 8.73</td>
<td>366.17 ± 21.05</td>
</tr>
<tr>
<td>4</td>
<td>381.23 ± 8.35</td>
<td>361.51 ± 21.46</td>
<td>381.23 ± 8.35</td>
<td>361.51 ± 21.46</td>
</tr>
<tr>
<td>5</td>
<td>381.94 ± 7.58</td>
<td>365.41 ± 20.91</td>
<td>381.94 ± 7.58</td>
<td>365.41 ± 20.91</td>
</tr>
<tr>
<td>6</td>
<td>386.66 ± 8.42</td>
<td>373.40 ± 20.08</td>
<td>386.66 ± 8.42</td>
<td>373.40 ± 20.08</td>
</tr>
<tr>
<td>7</td>
<td>386.98 ± 8.73</td>
<td>374.60 ± 20.84</td>
<td>386.98 ± 8.73</td>
<td>374.60 ± 20.84</td>
</tr>
<tr>
<td>8</td>
<td>410.06 ± 7.91</td>
<td>393.72 ± 21.98</td>
<td>410.06 ± 7.91</td>
<td>393.72 ± 21.98</td>
</tr>
<tr>
<td>9</td>
<td>412.61 ± 8.36</td>
<td>398.60 ± 22.42</td>
<td>412.61 ± 8.36</td>
<td>398.60 ± 22.42</td>
</tr>
<tr>
<td>10</td>
<td>416.41 ± 9.01</td>
<td>398.07 ± 22.54</td>
<td>416.41 ± 9.01</td>
<td>398.07 ± 22.54</td>
</tr>
<tr>
<td>11</td>
<td>417.22 ± 8.60</td>
<td>399.90 ± 23.10</td>
<td>417.22 ± 8.60</td>
<td>399.90 ± 23.10</td>
</tr>
<tr>
<td>12</td>
<td>418.00 ± 8.87</td>
<td>396.59 ± 23.03</td>
<td>418.00 ± 8.87</td>
<td>396.59 ± 23.03</td>
</tr>
<tr>
<td>13</td>
<td>417.11 ± 8.84</td>
<td>394.51 ± 23.31</td>
<td>417.11 ± 8.84</td>
<td>394.51 ± 23.31</td>
</tr>
</tbody>
</table>

Average body weights are shown during the entire 6- to 7-day experimental period. The %change in body weight on each day of the experiment was calculated by dividing the daily body weight by the starting (day 0) body weight. AgRP, agouti-related peptide; OVX, ovariectomized; CAST, castrated. Absolute and %body weight change. †P < 0.05 vs. saline; ‡P < 0.05 vs. intact (same treatment).

However, cumulative food intake (expressed as g consumed/g body wt*100) between female AgRP-treated and male AgRP-treated rats was not different (Fig. 2A and B). Both intact male and female rats injected with AgRP showed significant increases in percent body weight (expressed as %change from baseline) throughout the duration of the experiment. Rats were ~6% heavier after the initial 24 h after AgRP treatment (Table 1, P < 0.05). At the end of the 6- to 7-day measuring period, intact male and female rats treated with AgRP gained ~6–8% of their initial body weight (Table 1, P < 0.05).

To determine the effects that gonadal hormones play in modulating the effectiveness of AgRP to stimulate food intake, gonads were removed and the same dose of AgRP (1 nmol) and vehicle (0.9% saline) were given (experiment 2). In contrast to the 5-day period of hyperphagia seen when intact males were given a single intracerebroventricular injection of AgRP, castrated males displayed a slightly shorter (4-day) period of hyperphagia (Fig. 1C, P < 0.05) compared with vehicle-treated controls. However, when female rats were ovarietomized, the period of AgRP-induced hyperphagia did not differ from AgRP-treated intact females. Females that were ovarietomized displayed a 3-day period of elevated food intake when injected with AgRP compared with vehicle-treated females (Fig. 1D, P < 0.0001). Despite what appeared to be a slightly altered time course of daily hyperphagia by castrated males compared with intact males, when cumulative food intake normalized for body weight differences was compared, there was no significant difference (Fig. 2A and B; AgRP vs. saline, P < 0.05; AgRP-intact vs. AgRP-castrated = not significant, AgRP-intact vs. AgRP-ovariectomized = not significant). Cumulative food intake was significantly lower (when normalized for body weight) for vehicle-treated castrated and ovarietomized rats compared with intact vehicle-treated rats (Fig. 2A and B, P < 0.05). In addition, administration of both vehicle and AgRP to gonadectomized animals yielded a lower-than-average food intake during the first 24 h following the injection (Fig. 1, C and D). Because of this, the %change in body weight was significantly reduced for ovarietomized females treated with vehicle compared with intact vehicle-treated females during the first few treatment days (Table 1; P < 0.05).

Following ovarietomy, female rats gained weight and were significantly heavier than the intact females (Table 1, P < 0.05). The body weights of castrated rats were not significantly different from intact males. As in experiment 1 with intact rats, the %increase in body weight following the first 24 h was ~4–6% for gonadectomized AgRP-treated rats (Table 1, P < 0.05). At the end of the 6- to 7-day period, the %change in body weight of intact AgRP-treated rats was significantly elevated compared with that of vehicle-treated rats (Table 1, P < 0.05). Gonadectomy appeared to prevent the sustained increase in body weight seen in the intact rats.

Intact female rats lowered their VO2 to a greater extent than intact males immediately following AgRP administration (Fig. 3, A and B, P < 0.01, P < 0.05). Following gonadectomy, differences in energy expenditure (VO2) between AgRP and vehicle-treated females were no longer present (Fig. 3, C and D).

Respiratory quotients reveal the relative amount of substrate being utilized for energy, with lower respiratory quotients favoring fatty acid oxidation and higher respiratory quotients indicative of carbohydrate utilization and fat storage (12). Intact male (Fig. 4A, P < 0.01) and female (Fig. 4B, P <
0.001) rats showed an elevation in RQ following central AgRP treatment compared with vehicle. Gonadectomy did not affect the elevation in RQ following AgRP-treatment in either males (Fig. 4C, P < 0.05) or females, (Fig. 4D, P < 0.05) although VO2 was not lowered as in intact animals. Castration removed some difference between AgRP and vehicle-treated rats (with the exception of a single time point) (Fig. 4C). The significance of these findings may be questionable due to the fewer number of rats in the castrated group and the increased variability in the data.

**DISCUSSION**

The prolonged behavioral effect of centrally-delivered AgRP on food intake has been well described in male rats by others (10, 11). A single intracerebroventricular injection of AgRP has been shown to induce overconsumption of rodent chow lasting up to 7 days in male rats (13, 16). In our hands, male rats displayed increased food intake following AgRP-administration for 5 days. Furthermore, we demonstrated that an equivalent dose (1 nmol) of AgRP administered centrally to female rats in diestrus resulted in an abbreviated response that lasted only 3 days compared with vehicle-treated controls. While the absolute grams of food intake consumed between males and females may be different, when intake was normalized for body weight, cumulative food intake following AgRP-treatment between male and female intact rats was similar. Body weight gain as a percentage of original body weight was also similar between male and female rats immediately following treatment with AgRP. The gain in body weight persisted throughout a 7-day measuring period. Because energy balance is the result of food intake and energy expenditure, VO2 was analyzed during the first 24 h following AgRP and vehicle treatment. Surprisingly, AgRP treatment resulted in a disproportionate decrease in energy expenditure in intact female rats compared with intact males.

It is well known that circulating gonadal steroids affect appetite in rodents (1). In females, circulating estrogens exert inhibitory effects on food intake while stimulating activity levels. In contrast, male rats tend to exhibit a reduction in food intake in the absence of testosterone. To determine whether the shorter duration of feeding induced by AgRP in females was...
due to changes in circulating estrogen, we performed ovariec-
tomies and repeated the study. Castrated males were also
compared with intact males as a comparison. AgRP treatment
was withheld until 6 wk following gonadectomy because
endogenous levels of hypothalamic AgRP and NPY mRNA are
significantly elevated in concert with robust hyperphagia in the
weeks immediately following ovarieectomy (5). Interestingly,
despite slightly different daily consumption patterns, gonadec-
tomized male and female rats consumed similar cumulative
amounts of food (normalized for body weight) during the 6

![Graph A](image1)

![Graph B](image2)

*Fig. 2. Cumulative food intake for intact vs. gonadectomized rats (A and B). Food intake for all groups of rats were added for experimental days 1-6. Because body weight for the intact and ovariectomized females (B) were significantly different, food intake was normalized for body weight by dividing cumulative food intake by grams of body weight for all groups. CAST, castrated; OVX, ovariectomized.*

![Graph C](image3)

![Graph D](image4)

*Fig. 3. Energy expenditure (VO2) during the first 20 h following AgRP or vehicle administration in intact male (A) and female (B) and gonadectomized male (C) and female rats (D). Hours 0–12 represent the initial dark phase while hours 12–20 represent the light phase.*
days following AgRP treatment, with or without gonads. In addition, the previously identified reduction in \( V_{O_2} \) following AgRP in intact female rats was absent following ovariectomy. One interpretation of these collective findings is that the interaction of the melanocortin system with gonadal steroids may be related to energy expenditure only. However, data from Gao et al. (9) has demonstrated that estrogen directly stimulates proopiomelanocortin neuronal activity and that this is one mechanism for estrogen’s anorectic effects. Alternatively, it is possible that the data are illustrative of a “floor effect,” meaning that AgRP could not further reduce \( V_{O_2} \) below the already reduced level following ovariectomy. Body weight is significantly increased in ovariectomized females, and energy expenditure appears reduced, even before any AgRP treatment. In contrast to intact rats, the %change in body weight of AgRP-treated male and female gonadectomized rats was not significantly different than vehicle-treated gonadectomized rats at the end of the measuring period. Although speculative, it is possible that the body weight of ovariectomized female rats approached a level of adiposity that could not be further increased by AgRP treatment.

The hypothesis that gonadal steroids regulate the behavioral response to melanocortin ligands has been previously examined by others. Clegg et al. (6) examined food intake following a wide range of centrally-administered melanotan-II in male and female rats. In their study, male and female rats exhibited similar reductions in food intake following the MC3R and MC4R agonists. Similarly, Polidori and Geary (17) studied the role of estradiol replacement on food intake induced by the MC3R and MC4R antagonists AgRP and SHU9119. Although only females were directly examined in the study by Polidori, the feeding effects following central administration of these antagonists lead to the conclusion that male and female rats respond identically (in duration of feeding) to melanocortin ligands. Also reported in their study was that female rats consumed significantly more food following AgRP treatment compared with artificial CSF for 5 days (although data is shown for only 3 days). The interpretation of these previous findings are limited. In both the Clegg and Polidori and Geary studies changes in body weight or energy expenditure were not shown or reported following melanotan-II, AgRP, or SHU9119 treatment. Since body weight is coordinated by both food

---

**Fig. 4.** Respiratory quotient (RQ) during the first 20 h following AgRP or vehicle administration in intact male (A) and female (B) and gonadectomized male (C) or female (D) rats. AgRP treatment caused an immediate increase in RQ in intact male rats compared to females, although both were significantly elevated compared with vehicle-treated controls. Gonadectomy removed the dramatic difference in RQ seen in male rats immediately after AgRP injection. However; ovariectomized female rats exhibited a pattern similar to intact females.
intake and energy expenditure, sex differences in energy expenditure cannot be excluded.

Whereas energy expenditure was not reduced by AgRP in male and female gonadectomized rats, RQ exhibited similar increases in all treatment groups. These data show that removal of gonadal steroids are sufficient in reducing AgRP’s effectiveness to lower energy expenditure; however, significant elevations in food intake and substrate utilization may still persist. Further studies utilizing hormone replacement are needed to definitively determine the involvement of gonadal steroids in this response. Genetic ablation of the melanocortin receptors have revealed that both MC3R and MC4R contribute to the regulation of body adiposity and substrate utilization (3). Here we report that AgRP rapidly (within the first hour) increased RQ in male rats, indicating that the preferred energy substrate following AgRP was carbohydrate. The onset of this increase appeared sooner in intact male rats compared with female rats, but the magnitude of the increase was greater in females. Calorimetry performed throughout a second day (24–48 h) following AgRP-treatment (performed only in males) demonstrated a persistently elevated RQ (data not shown). A recent study by Nogueiras et al. (15) examined the specific sites and genes responsible for direct melanocortin regulation of peripheral lipid metabolism. Most importantly, the authors showed that central blockade of melanocortin tone with SHU9119 in pair-fed rats resulted in a net effect of lipogenesis. Together, these findings support a direct effect of central melanocortin antagonism on lipid biosynthesis and storage in rats.

**Perspectives and Significance**

In sum, the present data describe similar feeding responses (when normalized for body weight) by intact and gonadectomized rats that have received AgRP centrally. These findings suggest that the neuronal substrates of AgRP action that regulate food intake may not be sexually dimorphic in rats. While it is known that AgRP and proopiomelanocortin neurons possess receptors for the hormones leptin and insulin, which when centrally delivered elicit sexually dimorphic feeding responses, these cells may not be the primary mediators of these responses. However, when AgRP-induced changes in energy expenditure were examined, significant effects of gonadal removal were seen, particularly with females. The present data suggest that sex differences in the melanocortin regulation of energy balance may be limited to energy expenditure. Further investigation into the mechanisms underlying these findings are needed to fully understand the coordination of food intake and the sexually dimorphic energy expenditure regulation by the melanocortin system.

**REFERENCES**