Hypothalamic Melanocortin Receptors and Chronic Regulation of Arterial Pressure and Renal Function

Jay J. Kuo, Alexandre A. Silva, John E. Hall

Abstract—This study examined control of cardiovascular and renal function during chronic melanocortin-3/4 receptor (MC3/4-R) activation or inhibition. Arterial and venous catheters were implanted in Sprague-Dawley rats for measurements of mean arterial pressure (MAP) and heart rate (HR) 24 h/d and for intravenous infusions, and the lateral ventricle was cannulated for chronic intracerebroventricular (ICV) infusions. In experiment 1, after a 5-day control period, rats were administered the MC3/4-R agonist MTII (n=7, 10 ng/h ICV) or 0.9% saline (n=6, ICV) for 14 days, followed by a 5-day recovery period. In experiment 2, after a 5-day control period, rats were administered the MC3/4-R antagonist SHU-9119 (n=7, 1 nmol/h ICV) or 0.9% saline vehicle (n=7, ICV), or pair-fed during SHU-9119 infusion (n=5, 1 nmol/h ICV) for 12 days, followed by a 5-day recovery period. MC4-R activation transiently decreased food intake from 23±1 to 10±2 g/d. Despite the hypophagia, MC3/4-R activation increased MAP by 7±1 mm Hg. MC3/4-R inhibition for 12 days increased food intake from 21±1 to 35±4 g/d, decreased HR by 53±11 bpm, and caused no change in MAP despite the marked weight gain. In rats that were pair-fed to prevent increased food intake, MC3/4-R inhibition further decreased HR (−87±9 bpm), whereas MAP was unchanged. Thus, chronic hypothalamic MC3/4-R activation raises arterial pressure despite decreased food intake, whereas MC3/4-R inhibition causes marked weight gain without raising arterial pressure. These observations are consistent with the hypothesis that an intact hypothalamic MC3/4-R may be necessary for excess weight gain to raise arterial pressure. (Hypertension. 2003;41 [part 2]:686-693.)

Key Words: hypertension, receptors, melanocortin ■ blood pressure ■ heart rate ■ hypertension, experimental ■ obesity ■ insulin

The hypothalamic pro-opiomelanocortin (POMC) system is increasingly recognized as an important regulator of energy balance and body weight. α-Melanocyte stimulating hormone (α-MSH), a bioactive peptide product of the POMC system, activates melanocortin-3 and -4 receptors (MC3/4-R) to cause hypophagia, whereas pharmacological inhibition of the MC3/4-R increases food intake. Targeted disruption of the MC4-R causes hyperphagia and obesity in mice, and mutations of the MC4-R have been suggested to account for 5% of morbid obesity in humans. Moreover, activation of the MC3/4-R may mediate a major component of the satiety effects of leptin, which also plays a major role in regulating energy balance.

The POMC pathway has also been suggested to influence sympathetic activity and blood pressure regulation. Central injections of a MC3/4-R agonist acutely stimulate brown adipose tissue, lumbar, and renal sympathetic activity, and acute injections of N-terminal melanocortin products have been reported to cause small increases in blood pressure. Inhibition of the MC3/4-R has also been shown to prevent the acute effects of leptin to stimulate renal sympathetic activation, suggesting that the POMC system may link leptin and renal sympathoexcitation. However, there have been no studies, to our knowledge, that have examined whether the sympathetic effects of activating or inhibiting the MC3/4-R are sustained and influence long-term regulation of cardiovascular and renal function.

In this study, we examined the cardiovascular, metabolic, dietary, and hormonal responses to chronic activation or inhibition of the central MC3/4-R in normal Sprague-Dawley rats. Since increased food intake and weight gain per se influence arterial pressure regulation, we also examined the chronic cardiovascular and renal actions of MC3/4-R inhibition in rats that were pair-fed the same amount of food as vehicle-treated rats.

Methods

Animal Surgeries

The experimental procedures and protocols of these studies conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal
Intracerebroventricular Cannulation
Male Sprague-Dawley (Harlan, Indianapolis, Ind) rats (275 to 325 g) were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal), and atropine sulfate (0.37 mg/kg) was administered to attenuate excess airway secretions. During stereotaxic manipulation, anesthesia was maintained with 0.5% isoflurane. A stainless steel cannula (26 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle using the following coordinates, estimated from the rat brain atlas of Paxinos and Watson,\(^1\) relative to the bregma: −0.5 mm anteroposterior, −1.3 mm lateral to the midline, and −4.5 mm dorsosventral from the dura. The guide cannula was secured in place with 3 stainless steel machine screws, a metal cap, and dental acrylic, and a stylet was inserted to seal the cannula until use. Several days after recovery from surgery, accuracy of the cannula placement in the lateral ventricle was tested by measuring the dipsogenic response (immediate drinking of at least 5 mL in 10 minutes) to an acute injection of 100 ng of angiotensin II. Methylene blue staining after the animals were killed was also used as confirmation of cannula placement.

Intra-Arterial and Intravenous Catheterization
Seven to 10 days after the intracerebroventricular (ICV) cannula implantation, arterial and venous catheters were implanted according to procedures previously described.\(^1\) Briefly, under aseptic conditions, a laparotomy was performed and a sterile nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys. Through a left vein incision, a sterile catheter was placed in the vena cava. Both catheters were exteriorized through a subcutaneously implanted stainless steel button.

Metabolic Studies
After recovery, each rat was individually housed in a metabolic cage for determination of daily water and electrolyte balances; a stainless steel spring connected the implanted button to a sterile infusion swivel. Both catheters were connected to the swivel, and a continuous intravenous infusion of saline was maintained. The arterial catheter was filled with heparin (1000 U/mL) and connected to a pressure transducer (Maxim). Pulse-late arterial pressure signals were sent to an AD converter and analyzed by computer with customized software. The analog signal was sampled at 500 samples per second for 4 seconds every minute, 24 h/d, throughout the experiment.

All rats received food and water ad libitum throughout the study, except for 1 group that was used for paired feeding experiments. Total sodium intake was maintained constant at ~3.1 mEq/d by a continuous intravenous infusion of 20 mL/d of 0.9% saline combined with a sodium-deficient rat chow (0.006 mmol sodium/g food, Teklad). Intravenous solutions were infused through a sterile filter (0.22 μm, Millipore), and the saline infusion was started immediately after placement of the rats into the metabolic cages. An acclimation period of 4 to 7 days was allowed before 5 to 7 days of control measurements were recorded.

Experimental Protocols
Experiment 1: Chronic MC4-R Activation
Two groups of rats were used in these experiments. After a 5-day control period, rats received either an ICV infusion of the vehicle (0.9% saline, 0.5 μL/h, n=7) or the MC3/4-R antagonist SHU-9119 (1 nmol/h, n=7, Polypeptide Laboratories) for 12 days through osmotic minipump. This rate of MC3/4-R antagonist was selected because of acute studies demonstrating effective blockade of an MC3/4-R agonist\(^1\) and a dose-response relation showing that 1 nmol yielded a peak hyperphagic response. To control for the increased food intake and weight gain caused by MC3/4-R antagonism, a separate group of rats (n=5) were administered the SHU-9119 ICV for 12 days and were pair-fed the same amount of food the vehicle-treated rats had consumed. The 12-day experimental period was followed by a 5-day recovery period, during which ICV drug infusions were terminated.

MAP, HR, urine volume, urinary sodium and potassium excretion, and food and water intake were recorded daily. Blood samples (1.5 mL) were collected once during the control and experimental periods for measurements of glomerular filtration rate (GFR), plasma insulin, and glucose. The blood samples were replaced with an equal volume of saline.

Statistical Analysis
The data are expressed as mean±SEM and analyzed by using 2-factor ANOVA with repeated measures. The Tukey-Kramer post hoc test was used for comparisons between groups, and the Dunnett test was used for comparisons of experimental and control values within each group, when appropriate. Statistical significance was accepted at a level of P<0.05.

Results
Chronic Melanocortin-3/4 Receptor Activation
Food Intake and Hormonal Responses
Chronic ICV administration of the MC3/4-R agonist MTII transiently decreased food intake for 6 to 8 days. On day 3 of
MTII infusion, food intake averaged 10±1 g/d compared with 23±1 g/d during the control period; thereafter, food intake gradually increased toward control, averaging 20±1 g/d during the last 7 days of MTII infusion (Figure 1). After termination of the MTII infusion, food intake transiently increased to 27±1 g/d before returning to control levels during the recovery period. Food intake was unchanged in vehicle-infused rats, in which body weight increased by 17±1% during the experimental protocol compared with only 12±2% in MTII-treated rats.

Plasma insulin levels declined from 42.0±2.9 to 30.1±2.1 μU/mL during MTII administration. There was no significant change in plasma insulin in vehicle-infused rats (Table 1). Plasma glucose concentration decreased slightly during MTII administration, from 132±2 to 127±2 mg/100 mL. Rats infused with vehicle had no significant change in plasma glucose, which averaged 132±3 mg/100 mL during the control and 134±2 mg/100 mL during vehicle treatment.

**Hemodynamic Responses**

MTII infusion increased MAP from 97±2 to an average of 104±3 mm Hg during the 14-day experimental period; after termination of the MC3/4-R agonist, MAP returned to control levels (Figure 2). MAP remained unchanged in vehicle-infused rats. MTII infusion caused a transient increase in HR from 393±6 to 414±6 bpm by the 2nd day of MC3/4-R activation; afterward, HR slowly declined to an average of 385±7 bpm during the second week of MTII infusion. Although the MTII infusion increased HR by ~10 bpm compared with vehicle-treated rats during the 14-day experimental period, the difference in HR between the two groups was not significant (Figure 2).

**Renal Responses**

Urine volume and sodium excretion were unchanged in both MTII and vehicle-infused rats (Table 1). MTII infusion decreased potassium excretion from a control of 4.1±0.2 to an average value of 3.2±0.2 mmol/d, paralleling the decrease in food intake (Table 1). In vehicle-infused rats, urinary potassium excretion did not change significantly. Cumulative sodium and potassium balances were not significantly different in MTII and vehicle-infused rats. GFR did not change significantly during vehicle or MTII infusion, averaging 4.6±0.7 and 3.9±0.2 mL/min during control and the 8th day of vehicle infusion and 3.9±0.6 and 3.4±0.2 mL/min during control and the 8th day of MTII infusion.

**Chronic Melanocortin-3/4 Receptor Inhibition**

**Food Intake and Hormonal Responses**

Central administration of the MC3/4-R antagonist markedly increased food intake from 21±1 to 35±4 g/d, whereas there were no significant changes in food intake in vehicle-infused rats (Figure 3). Along with the increased food intake, MC3/4-R blocked rats had a 25±4% increase in body weight during the experimental protocol, compared with a 12±3% increase in body weight in vehicle-infused rats. Pair-fed rats infused with the MC3/4-R antagonist had similar increases in body weight as vehicle-infused rats.

![Figure 2. Effect of ICV infusion of vehicle (n=6) or the MC3/4-R agonist MTII (10 ng/hr, n=7) on MAP and HR, measured 24 h/d in conscious Sprague-Dawley rats. *P<0.05 compared with vehicle-treated rats.](http://hyper.ahajournals.org/)

| Table 1: Effect of Chronic ICV Infusion of Vehicle or MC3/4-R Agonist, MTII, in Rats |
|---------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Group                         | GFR, mL/min | U\textsubscript{NaV}, mmol/d | U\textsubscript{KV}, mmol/d | Urine Volume, mL/d | Water Drinking, mL/d | Insulin, μU/mL | Glucose, mg/100 mL |
| Vehicle                       | Control 4.6±0.7 | 2.7±0.2 | 4.1±0.3 | 32±2 | 5±1 | 57.5±8.5 | 132±3 |
|                              | Vehicle 3.9±0.2 | 2.5±0.2 | 3.7±0.2 | 34±2 | 6±1 | 45.7±4.5 | 134±2 |
|                              | MTII 10 ng/hr | Control 3.9±0.6 | 2.6±0.1 | 4.1±0.2 | 34±2 | 7±1 | 42.0±2.9 | 132±2 |
|                              | MTII 3.4±0.2 | 2.6±0.1 | 3.2±0.2 | 38±2 | 9±1 | 30.1±1* | 127±2 |

GFR indicates glomerular filtration rate; U\textsubscript{NaV}, urinary sodium excretion; U\textsubscript{KV}, urinary potassium excretion. Values for U\textsubscript{NaV}, U\textsubscript{KV}, urine volume, and water drinking are average values for the 5-day control and 14-day experimental periods. GFR and plasma insulin and glucose concentrations were determined on day 4 of control and day 8 of vehicle or MTII infusion.

*P<0.05 vs control.
Plasma insulin levels increased during MC3/4-R blockade compared with vehicle-infused and pair-fed rats infused with MC3/4-R antagonist (198 ± 49 versus 57 ± 11 and 32 ± 9 μU/mL, respectively) (Table 2). Plasma glucose concentrations were not significantly different among the 3 groups during the experimental period despite the changes in plasma insulin. The MC3/4-R antagonist increased PRA by the 8th day of central infusion in ad libitum as well as pair-fed rats (from 2.2 ± 0.5 to 3.6 ± 0.5 and from 3.0 ± 0.7 to 4.2 ± 0.4 ng AI/mL/hr, respectively), whereas ICV vehicle infusion did not significantly alter PRA (Table 2).

**Hemodynamic Responses**

Control rats had a slight decrease in HR of −19 ± 9 bpm, whereas ICV administration of the MC3/4-R antagonist markedly decreased HR by −52 ± 11 bpm during the last 5 days of antagonist infusion (Figure 4). In pair-fed rats infused with MC3/4-R antagonist, HR decreased further to −87 ± 9 bpm during the last 5 days of the experimental period. MAP averaged 91 ± 2 mm Hg during the control period and remained unchanged during MC3/4-R inhibition, averaging 91 ± 2 mm Hg, (Figure 4) despite the weight gain, which usually raises arterial pressure. After termination of the

**TABLE 2. Effect of Chronic ICV Infusion of Vehicle, MC3/4-R Antagonist SHU-9119 With Ad Libitum Food Intake, or MC3/4-R Antagonist SHU-9119 in Pair-Fed Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR, mL/min</th>
<th>U_W, mmol/d</th>
<th>U_V, mmol/d</th>
<th>Urine Volume, mL/d</th>
<th>Water Drinking, mL/d</th>
<th>Insulin, μU/mL</th>
<th>Glucose, mg/100 mL</th>
<th>PRA, ng AI/mL/hr</th>
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<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>3.0 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>3.7 ± 0.3</td>
<td>33 ± 2</td>
<td>7 ± 2</td>
<td>54.7 ± 8.2</td>
<td>123 ± 2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.1 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>34 ± 2</td>
<td>7 ± 2</td>
<td>57.5 ± 11.4</td>
<td>150 ± 4*</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>SHU-9119 (1 nmol/hr)</td>
<td></td>
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<tr>
<td>Control</td>
<td>3.4 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>38 ± 3</td>
<td>12 ± 3</td>
<td>51.2 ± 11.2</td>
<td>147 ± 3</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>SHU-9119</td>
<td>6.0 ± 0.7*</td>
<td>2.7 ± 0.2</td>
<td>5.3 ± 0.7*</td>
<td>41 ± 3</td>
<td>10 ± 2</td>
<td>198.0 ± 49.4*</td>
<td>158 ± 5*</td>
<td>3.6 ± 0.5*</td>
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<tr>
<td>SHU-9119 (1 nmol/hr, pair fed)</td>
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<tr>
<td>Control</td>
<td>3.8 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>35 ± 2</td>
<td>8 ± 1</td>
<td>58.0 ± 17.1</td>
<td>150 ± 4</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>SHU-9119</td>
<td>3.4 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>37 ± 2</td>
<td>8 ± 1</td>
<td>32.1 ± 9.2</td>
<td>151 ± 4</td>
<td>4.2 ± 0.4*</td>
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</tbody>
</table>

PRA indicates plasma renin activity. Values for U_W, U_V, urine volume, and water drinking are average values for the 5-day control, 12-day experimental, and 5-day recovery periods. GFR, plasma insulin and glucose concentration, and PRA were measured on day 4 of control, day 8 of vehicle or SHU-9119 infusion, and day 4 of recovery.

*P<0.05 vs control.
MC3/4-R antagonist, MAP increased to 98±2 mm Hg by the end of the 5-day recovery period. MAP was unchanged during vehicle infusion and in pair-fed rats infused with the MC3/4-R-antagonist.

Renal Responses
Urine volume, sodium excretion, and cumulative sodium balance were not significantly changed during vehicle infusion, MC3/4-R inhibition, or in pair-fed rats infused with the MC3/4-R antagonist (Table 2). Potassium excretion, however, increased significantly from 3.9±0.2 to 5.3±0.7 mmol/d during MC3/4-R antagonist infusion, paralleling the increased food intake (Table 2). This resulted in a positive cumulative potassium balance of +4.6±1.0 mEq during MC3/4-R antagonism. There were no significant changes in potassium excretion in vehicle-infused or in pair-fed rats infused with MC3/4-R antagonist. In addition, no significant changes in GFR were observed during vehicle treatment or in pair-fed rats given the MC3/4-R antagonist (Table 2). However, in rats infused with MC3/4-R antagonist and permitted to eat ad libitum, GFR increased from 3.4±0.4 mL/min to 6.0±0.7 mL/min on the 8th day of the antagonist infusion.

Discussion
The present study suggests that the MC3/4-R may have important influences on chronic cardiovascular regulation as well as on control of energy balance and body weight. MC3/4-R activation for 14 days caused small but significant increases in arterial pressure despite decreases in food intake and body weight, which usually tend to reduce blood pressure. Conversely, chronic inhibition of the MC3/4-R caused dramatic increases in food intake and body weight but prevented the increases in arterial pressure and heart rate that usually accompany excess weight gain. These observations suggest that an intact hypothalamic melanocortin system may be necessary for excess weight gain to raise arterial pressure and heart rate.

Metabolic and Hormonal Responses to Chronic MC3/4-R Activation or Inhibition
Chronic ICV infusion of the MC3/4-R antagonist SHU-9119 caused a 66% increase in food intake that was sustained after 12 days of infusion, resulting in rapid weight gain. The increased body weight was due primarily to higher food intake since rats that were pair-fed the same amount as control rats maintained a relatively constant weight during chronic ICV infusion of the MC3/4-R antagonist. These observations suggest that activation of the MC3/4-R by endogenous ligands, primarily α-MSH,1,2 have a tonic effect to suppress appetite in normal rats.

Chronic activation of the MC3/4-R, however, caused only a transient decrease in food intake, lasting 6 to 8 days, and a relatively small decrease in body weight compared with vehicle-infused rats. These results are consistent with previous studies demonstrating that although acute injections of MC3/4-R agonist caused hypophagia,1,2 chronic administration of the agonist decreased food intake for only a few days.15,16 The mechanisms responsible for the return of food intake toward normal are not entirely clear but may be due to activation of orexigenic mechanisms, such as neuropeptide Y (NPY) or agouti-related peptide (AGRP), as a compensatory response to insufficient caloric intake.17 However, we cannot rule out the possibility that higher doses of the MC3/4-R agonist may have caused a more sustained decrease in food intake.

Activation of the melanocortin system has been suggested to mediate a major component of leptin’s satiety effects. Leptin receptor mRNA is colocalized with POMC mRNA in neurons of the arcuate nucleus, and leptin-deficient obese mice have reduced POMC mRNA that can be normalized by leptin replacement.18 Moreover, the effects of leptin to decrease food intake can be markedly attenuated by inhibiting the MC3/4-R.6 These observations suggest that the POMC pathway may play an important role in mediating at least part of the effects of leptin on energy balance. However, chronic activation of the MC3/4-R caused only a transient decrease in food intake in the present study, whereas leptin infusions caused sustained reductions in food intake.11–13 Although a higher dose of MTII may have caused a more prolonged reduction in food intake, it is likely that leptin’s chronic effect to inhibit appetite cannot be explained entirely by activation of the MC3/4-R. Additional mechanisms, including inhibition of the NPY system,19 may also be involved in mediating leptin’s satiety effects.

Activation and inhibition of the MC3/4-R also caused significant changes in plasma insulin concentration, in parallel with the changes in food intake and body weight. Previous studies have suggested that activation of the MC3/4-R may increase insulin sensitivity, whereas inhibition of the MC3/4-R decreases insulin sensitivity and glucose uptake in peripheral tissues.20–22 Although our studies were not designed to test the mechanisms by which chronic activation or inhibition of the MC3/4-R alters plasma insulin concentration, it is interesting to note that in pair-fed rats administered the MC3/4-R antagonist, there were no major changes in plasma insulin concentration. This suggests that most of the increase in plasma insulin concentration observed after inhibition of the MC3/4-R was due to hyperphagia and weight gain rather than direct effects of MC3/4-R inhibition on insulin sensitivity or insulin secretion.

PRA increased significantly during administration of the MC3/4-R antagonist. We have previously shown that PRA is elevated during excess weight gain.19 However, in the present study, PRA increased in rats that were pair-fed to prevent hyperphagia and weight gain during administration of the MC3/4-R antagonist, suggesting that additional factors besides weight gain may be involved in raising PRA.

Cardiovascular Responses to Chronic MC3/4-R Activation or Inhibition
Chronic infusion of the MC3/4-R agonist in the present study caused significant increases in arterial pressure that were sustained for the entire 12 days, despite reductions in food intake and body weight. This is, to our knowledge, the first evidence that chronic activation of the MC3/4-R can significantly alter long-term regulation of arterial pressure.
Previous studies have shown that acute activation of the POMC pathway increases sympathetic activity, although it is not clear whether this effect can be sustained with chronic activation of the MC3/4-R. In the present study, there was a transient increase in heart rate during the infusion of the MC3/4-R agonist, but this effect was not sustained, although blood pressure remained elevated for 14 days of MTII infusion. Chronic inhibition of the MC3/4-R caused no significant effects on arterial blood pressure, although there was a sustained reduction in HR. Moreover, the decrease in heart rate occurred in spite of marked increases in food intake and weight gain that normally elevate HR and blood pressure. In pair-fed rats in which food intake was maintained at the same level as in vehicle-infused rats, there was an even further reduction in HR with chronic inhibition of the MC3/4-R. This observation suggests that endogenous activity of the MC3/4-R has a tonic effect to reduce heart rate in normal rats. Whether this effect is due to inhibition of sympathetic activity or activation of parasympathetic activity is unclear.

In the agouti yellow mouse, overexpression of agouti protein, which acts as an antagonist of melanocortin receptors, is associated with obesity and increased blood pressure. However, it is unclear whether there may be additional factors raising blood pressure in agouti mice besides changes in activity of the melanocortin receptors. Our observations and previous studies using pharmacological inhibitors of melanocortin receptors suggest that activation of the MC3/4-R raises HR and blood pressure, whereas inhibition of the MC3/4-R lowers HR and prevents increases in blood pressure normally associated with weight gain.

Since leptin activates the POMC system and increases α-MSH formation, stimulation of the MC3/4-R in the arcuate and paraventricular nucleus by increased levels of α-MSH may be a link between hyperleptinemia, sympathetic activation, and increased blood pressure. Hayes et al. have also shown that blocking the MC3/4-R completely abolished the acute effects of leptin to stimulate renal sympathetic activity without altering the increase in sympathetic activity in brown adipose tissues. These results suggest that the sympathoexcitatory effects of leptin are heterogeneous and only partly mediated through the melanocortin system. In addition, the chronic cardiovascular responses to MC3/4-R activation are not precisely the same as those observed with leptin infusion. For example, hyperleptinemia caused slow, gradual increases in arterial blood pressure and HR that were sustained. In contrast, activation of the MC3/4-R caused a more rapid increase in blood pressure and the initial tachycardia was not sustained. One possible explanation for these differences is that hyperleptinemia not only activates the POMC system but also inhibits NPY, whereas chronic activation of the MC3/4-R may lead to compensatory increases in NPY, which tends to reduce HR. However, the importance of NPY in modulating the chronic effects of leptin and MC3/4-R activation are unclear and deserve further investigation.

Renal Responses to Chronic MC3/4-R Activation or Inhibition
In the present study, chronic activation or inhibition of the MC3/4-R had no major effects on urine volume, sodium excretion, or water drinking. This does not imply, however, that the melanocortin pathway is unimportant in long-term regulation of renal function. In fact, our observation that chronic activation of the MC3/4-R raised arterial blood pressure without increasing urinary sodium excretion suggests that the renal-pressure natriuresis relation was shifted to higher blood pressures. Whether this effect of the MC3/4-R agonist to alter pressure natriuresis is due to sympathetic stimulation or to other actions is unclear. As discussed above, previous acute studies suggest that activation of the MC3/4-R raises renal sympathetic activity, which could contribute to increased renal tubular reabsorption of sodium and altered pressure natriuresis. However, there have been no previous studies, to our knowledge, which have determined whether chronic activation of the MC3/4-R causes sustained increases in renal sympathetic activity.

Activation of the MC3/4-R decreased potassium excretion, whereas inhibition of this receptor increased potassium excretion. It is likely, however, that changes in potassium excretion observed with altered activity of the melanocortin system were due mainly to changes in food intake and therefore intake of potassium. In the present study, the changes in potassium excretion closely paralleled the changes in dietary potassium intake, although we cannot exclude the possibility that altered activity of the melanocortin system may have other influences on urinary potassium excretion.

Perspectives
Our results suggest that chronic activation or inhibition of the MC3/4-R has significant effects on control of cardiovascular and renal function as well as food intake and body weight regulation. Some of the changes in renal function, such as increased GFR and increased potassium excretion observed with blocking the MC3/4-R, are likely to be related to hyperphagia and weight gain. However, other effects, such as the bradycardia observed with inhibition of the MC3/4-R or increased blood pressure during chronic activation of the MC3/4-R, are obviously not due to changes in food intake or body weight. In fact, the increase in blood pressure observed with chronic activation of the MC3/4-R occurred in spite of decreased food intake and weight loss that would tend to lower blood pressure. Our observation that chronic inhibition of the MC3/4-R caused marked hyperphagia and weight gain without elevating blood pressure is also consistent with the possibility that a functional melanocortin system may be necessary for obesity to cause hypertension. If this hypothesis is correct, obese humans with MC3/4-R mutations may have normal or even reduced blood pressure as the result of inhibition of sympathetic activity as long as there are no major pathological changes in the kidneys caused by long-term obesity. Likewise, if the MC3/4-R plays a major role in mediating the effect of leptin on sympathetic activation, chronic hyperleptinemia should not cause sustained increases in sympathetic activity or blood pressure in animals or humans with a deficient melanocortin system due to mutations or pharmacologic blockade. Although there are still many unanswered questions, the role of the POMC pathway and MC3/4-R in mediating the chronic effects of obesity on

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sympathetic activity and blood pressure remains an important area for further investigation.

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**References**


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