Does Obesity Induce Resistance to the Long-Term Cardiovascular and Metabolic Actions of Melanocortin 3/4 Receptor Activation?

Alexandre A. da Silva, Jay J. Kuo, Lakshmi S. Tallam, Jiankang Liu, John E. Hall

Abstract—Previous studies suggest that blockade of melanocortin 3 and 4 receptors (MC3/4-R) markedly attenuates the chronic hypertensive effects of leptin. Although obesity has been reported to be associated with leptin “resistance,” it is unclear whether obesity alters the cardiovascular and metabolic effects of chronic MC3/4-R activation. Therefore, we tested whether the cardiovascular and metabolic actions of MC3/4-R activation are attenuated in Sprague-Dawley rats fed a high-fat diet (HF, n=6) compared with rats fed a standard chow (NF, n=6) for 12 months. A 21G steel cannula was placed in the lateral ventricle for ICV infusion, and arterial and venous catheters were implanted for measurement of mean arterial pressure (MAP) 24 hours/day and IV infusions. After a 5-day control period, rats were infused with MC3/4-R agonist melanotan II (10 ng/h, ICV), for 10 days followed by a 5-day recovery period. HF rats were heavier (558±21 versus 485±13 g) with 140% more visceral fat than NF rats, hyperleptinemic (8.9±0.5 versus 2.7±0.5 ng/mL), and insulin resistant. HF rats also had higher MAP (109±3 versus 100±1 mm Hg). Chronic melanotan II infusion significantly increased MAP in HF and NF (7±2 and 6±1 mm Hg), decreased caloric intake (−32±2 and −25±2 kcal/day), and reduced insulin levels in both groups by ~50%. Thus, the metabolic and cardiovascular actions of chronic MC3/4-R activation are preserved in diet-induced obesity, supporting a potential role for the hypothalamic melanocortin system in obesity hypertension. (Hypertension. 2006;47:259-264.)

Key Words: diet ■ blood pressure ■ insulin resistance ■ heart rate ■ glucose ■ leptin ■ metabolism ■ hypertension

Although obesity is a major cause of human essential hypertension,1,2 the mechanisms responsible for the rise in arterial pressure with excess weight gain are not fully understood. Leptin, a peptide secreted by adipocytes, has been suggested to contribute to obesity hypertension by stimulating the sympathetic nervous system (SNS). Leptin increases SNS activity to the kidneys and adrenal glands,3-5 and previous studies from our laboratory showed that chronic leptin infusion in lean rats raised arterial pressure and heart rate (HR)6 and that these effects are abolished by adrenergic receptor blockade.7

Leptin appears to exert much of its metabolic and cardiovascular actions by stimulating hypothalamic proopiomelanocortin (POMC) neurons. On stimulation, POMC neurons release α-melanocyte-stimulating hormone, which activates the melanocortin 3 and 4 receptors (MC3/4-R) in several hypothalamic centers, especially the paraventricular nuclei.8 Blockade of MC3/4-R abolishes the dietary, metabolic, and cardiovascular effects of chronic hyperleptinemia in Sprague-Dawley rats.9 In addition, activation of MC3/4-R receptors mediates much of the acute effect of leptin to increase renal sympathetic activity.10-12 We have also shown that chronic MC3/4-R activation with the melanocortin receptor agonist melanotan II (MTII) causes sustained elevation in arterial pressure and HR13 and that these effects were abolished by adrenergic receptor blockade.14 These observations are all consistent with the hypothesis that the hypothalamic POMC system may contribute to obesity hypertension by mediating the SNS effects of leptin.

In obesity, however, the actions of leptin to decrease appetite and to increase SNS activity to BAT are impaired,15-17 whereas its effects to increase SNS activity to the kidneys appear to remain intact.15 These findings have led to the concept that obesity is associated with “selective leptin resistance,” although the mechanisms involved are unknown.18 Because a functional melanocortin system appears to be necessary for leptin to exert its metabolic and cardiovascular actions, we tested whether obesity, produced by feeding Sprague-Dawley rats a high-fat diet for 12 months, is associated with selective resistance to the metabolic or cardiovascular effects of chronic MC3/4-R activation.

Methods

Animals and Surgical Procedures

The experimental procedures and protocols of this study conform to the National Institutes 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.

Three-week-old male Sprague-Dawley (Harlan, Indianapolis, IN) rats averaging 37 ± 1 g body weight were randomly assigned to 1 of 2 dietary groups: normal fat chow (NF; 3.3% fat w/w, n=6) or a high-fat diet (HF, 40.0% fat w/w, n=6) and were maintained on these diets for 12 months (see Reference for detailed dietary composition).

Intracerebroventricular and Intravenous Catheterization

After 12 months on one of the diets, the rats were anesthetized with 50 mg/kg sodium pentobarbital (Nembutal), and atropine sulfate (0.1 mg/kg) was administered to prevent excess airway secretions. Arterial and venous catheters were implanted according to procedures described previously. Briefly, using aseptic techniques, a laparotomy was performed, and a sterile nonocclusive polyvinyl catheter was inserted into the abdominal aorta, distal to the kidneys. Through a left femoral vein incision, a sterile catheter was placed in the vena cava. Both catheters were exteriorized through a subcutaneously implanted stainless steel button.

ICV Cannulation

Immediately after implantation of arterial and venous catheters, a stainless steel cannula (26-gauge, 10-mm long) was implanted into the right lateral cerebral ventricle as described previously. The guide cannula was anchored into place with 3 stainless steel machine screws, a metal cap, and dental acrylic, and a stylet was inserted to seal the cannula until use. During stereotaxic manipulation, anesthesia was maintained with 0.5% isoflurane. Several days after recovery from surgery, accuracy of the cannula placement was tested by measuring the dipsogenic response (immediate drinking of ≥ 5 mL of water in 10 minutes) to an ICV injection of 100 ng of angiotensin II. After the experiments were completed, the rats were killed with an overdose of sodium pentobarbital, and their brains were removed and sectioned to confirm the placement of the ICV cannula.

After recovery from anesthesia, the rats were housed in individual metabolic cages for determination of daily water and electrolyte balances. The arterial and venous catheters were connected to a dual-channel infusion swivel (Instech). The arterial catheter was connected to a pressure transducer (Maxxim) for continuous 24-hour measurement of mean arterial pressure (MAP) and HR using computerized techniques as described previously. All of the rats received food and water ad libitum throughout the study. Total sodium intake was maintained constant at ~ 3.5 mEq/day by a continuous IV infusion 40 mL/day of 0.45% saline combined with the sodium from the diet. 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 ≥ 7 days was allowed before 5 days of control measurements were recorded.

Experimental Protocols

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 period, on day 11 of the experimental period, and once during the recovery period for measurements of glomerular filtration rate (GFR), plasma renin activity (PRA), and plasma concentrations of insulin, glucose, and leptin. The blood was replaced with 1.5 mL of saline 0.9%.

After a 5-day control period, the MC3/4-R agonist MTII (Polypeptide Laboratories) was infused ICV for 10 days at a dose of 10 ng/h via osmotic pump implanted in the scapular region. This dose of MTII was chosen on the basis of previous acute studies examining different doses and on our previous chronic studies showing that this dose raised arterial pressure in lean, young Sprague-Dawley rats. Moreover, this dose of MTII has no effect on arterial pressure when given intravenously (J.J. Kuo, A.A. da Silva, J.E. Hall, unpublished observation, 2003). At the end of day 10 of MTII infusion, the tubing connecting the minipump to the ICV cannula was severed, and measurements were continued during a 5-day recovery period when rats were euthanized and body weight and visceral fat mass (epididymal plus retroperitoneal fat pads) were measured.

Analytical Methods

PRA and plasma insulin and leptin concentrations were measured by radioimmunoassay. Plasma glucose concentration was determined using the glucose oxidation method (Beckman glucose analyzer 2). Urine sodium and potassium concentrations were measured using ion-sensitive electrodes (NOVA electrolyte analyzer 1+). GFR was calculated from the 24-hour clearance of [125I]-iothalamate, as described previously.

Statistical Analysis

The data are expressed as mean±SEM and analyzed by using 2-factor ANOVA with repeated measures. The Bonferroni post hoc test was used for comparisons between groups. Dunnett’s 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

Effects of HF Diet on Food Intake, Body Weight, Visceral Fat, and Hormones

Rats fed an HF diet for 12 months gained more weight compared with rats on an NF diet (body weight averaged 558±21 versus 485±13 g, respectively). Rats fed an HF diet also had 140% greater visceral adiposity and almost a 4-fold increase in plasma leptin levels (Table). Fasting plasma insulin concentration was also significantly higher in HF compared with NF rats, although there were no significant differences in fasting plasma glucose levels (Table). PRA was slightly, but not significantly, higher in HF compared with NF rats (Table).

Although food intake was higher in NF compared with HF rats (26.6±2.2 versus 18.7±1.9 g/day), average total caloric intake during the control period was not significantly different (99±6 versus 100±10 kcal/day; Figure 1), because the HF diet contained more calories per gram of food compared with the NF diet (see Reference for detailed diet composition).

Effects of HF Feeding on MAP, HR, and Renal Function

As illustrated in Figures 2 and 3, Sprague-Dawley rats fed an HF diet for 12 months had significantly higher (P < 0.001) MAP compared with NF rats (109±3 versus 100±1 mm Hg, respectively). HR and GFR were not significantly different in the HF compared with the NF rats (Figures 2 and 3 and Table). Urinary sodium excretion also did not differ between the groups, averaging 2.4±0.2 and 2.7±0.2 mmol/day in HF and NF rats, respectively.

Effects of MC3/4R Activation on Food Intake and Hormones

Chronic ICV administration of the MC3/4-R agonist MTII significantly decreased food intake in both NF (from 29±1 to 10±2 g/day) and HF rats (from 19±1 to 8±2 g/day) on day 1 of infusion. Thereafter, food intake gradually returned toward control values, averaging 27±3 g/day in NF rats and 16±3 g/day in HF rats during the last 3 days of MTII infusion. When MTII infusion was stopped, food intake and
caloric intake returned to control levels in both groups (Figure 1).

Long-term ICV MTII administration significantly reduced fasting plasma insulin levels, whereas plasma glucose levels remained unchanged in both NF- and HF-fed rats (Table). Plasma leptin levels also decreased slightly during chronic MTII infusion in both groups, but the changes were not statistically significant in the NF group where leptin levels were already low (Table). PRA did not change significantly during MTII administration in any of the groups (Table).

Effects of MC3/4R Activation on MAP, HR, and Renal Function
MTII administration for 10 days in rats fed an HF diet significantly raised MAP from 109±3 mm Hg to an average of 116±3 mm Hg (P<0.001) during the last 5 days of treatment. In rats fed an NF diet, arterial pressure also significantly increased from 100±1 mm Hg to an average of 106±2 mm Hg (P<0.001) during MC3/4R activation (Figures 2 and 3). Thus, activation of the hypothalamic melanocortin system raised blood pressure by 6 to 7 mm Hg in NF as well as in HF rats. MTII also raised HR in both groups by 14 to 16 bpm (Figures 2 and 3). Chronic MTII administration did not significantly alter GFR in rats fed a HF or NF diet (Table). Also, MC3/4R activation caused no significant changes in urinary sodium excretion, which averaged 2.0±0.2 and 2.4±0.2 mmol/day during the 10 days of MTII infusion in HF or NF rats, respectively.

Figure 1. Response to chronic ICV administration of the MC3/4R agonist MTII (10 ng/h) on food and caloric intakes in Sprague-Dawley rats fed an NF (n=6) or HF (n=6) diet for 12 months.

Figure 2. Response to chronic ICV administration of the MC3/4R agonist MTII (10 ng/hr) on MAP and HR, measured 24-hours/d, in conscious Sprague-Dawley rats fed an NF (n=6) or HF (n=6) diet for 12 months.
Palatable “cafeteria” diet or in rats that are selected to have reported greater weight gain in rats fed a highly
HF diet, compared with rats fed ad libitum a NF diet, NF rats. Similar modest increases in total body weight in rats
pared with control rats fed a standard diet. Although total
body weight was only 15% greater in HF rats compared with
NF rats, visceral fat mass was 2.4 times as great, and plasma
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Obesity and Increased Arterial Pressure in Rats
Fed an HF Diet

Feeding Sprague-Dawley rats an HF diet for 12 months
classified as obesity prone. The modest increases in body weight ob-
served in HF rats compared with NF rats may be related, in part, to the fact that rats fed an NF diet ad libitum also tend
to gain substantial weight and have large amounts of adipose
tissue. However, the prolonged period of an HF diet in the
present study did cause marked increases in visceral fat
mass and other characteristics of the metabolic syndrome,
including increased blood pressure, insulin resistance, and
hyperleptinemia.

Increased visceral adiposity and circulating leptin levels
may have contributed to the rise in arterial pressure observed
in HF rats. We have shown previously that chronic hyperlept-
inemia raises arterial pressure in rats,6 and humans with
increased abdominal fat have increased muscle sympathetic
activity,22 often associated with hypertension, whereas in-
creased subcutaneous fat does not appear to be associated
with increased sympathetic activity. Other mechanisms,
however, may also contribute to the increased arterial
pressure in this model. Previous studies from our laboratory
suggest that the renin–angiotensin system may participate in
the development of obesity-induced hypertension.24 Al-
though, PRA was only slightly elevated in HF compared with
NF rats, normal PRA, despite increased arterial pressure in
HF rats, may represent an inappropriately elevated PRA in
HF rats.

MC3/4-R Activation Effects on Food Intake and Hormones

Chronic ICV MTII infusion reduced food intake in NF and
HF rat groups during the first 3 to 5 days of infusion, but
appetite gradually returned to control levels despite continued
MTII infusion. We have previously observed this waning
effect of MC3/4-R activation on food intake in lean rats.13,14
Yet, we have also shown that blockade of MC3/4-R com-
pletely prevents leptin from decreasing food intake,9 suggest-
that activation of the melanocortin system is required for
leptin to exert its action. Therefore, one might question why
long-term activation of MC3/4-R fails to cause sustained
suppression of appetite as observed during prolonged leptin
treatment. Although the mechanisms that contribute to the
waning anorectic effect of MC3/4-R activation are still
unclear, adaptive responses of multiple orexigenic pathways
may be responsible for returning food intake to normal and
preventing excessive loss of body weight. Support for this
hypothesis comes from the fact that leptin not only stimulates
the hypothalamic POMC system but also inhibits other neuropeptides
involved in promoting increased food intake, such as neu-
ropetide Y and agouti-related peptide.25 Chronic activation
only of the MC3/4-R with MTII infusion activates rather than
inhibits the neuropeptide Y and agouti-related peptide neu-
rons (see Reference26 for review). However, the importance of
adaptive responses of these orexigenic systems in overdrid-
ing the effects of chronic MC3/4-R activation on food intake
has not been tested.

Another important observation is that the short-term reduc-
tion of food intake and progressive loss of the anorectic
action of chronic MC3/4-R activation was similar in NF and
HF rats. This suggests that prolonged HF feeding did not
attenuate the appetite-suppressing effect of MC3/4-R activa-
tion or the compensatory mechanisms that are involved in returning food intake to baseline levels. Similar observations were made by Mantzoros and Flier,\textsuperscript{17} who tested the chronic dietary effects of MTII in mice fed an HF diet for 10 months. However, Clegg et al\textsuperscript{27} reported attenuated response to central acute injections of MTII after 12 weeks on an HF diet. These apparent discrepancies may be attributed to the length of HF feeding or to the acute versus chronic effects of MTII.

We also observed a marked reduction in plasma insulin levels, whereas plasma leptin levels remained unaltered in NF and HF rats during MC3/4-R activation. This result is consistent with previous results obtained by our laboratory and by others in lean\textsuperscript{14,28} and obese\textsuperscript{19} rats. The results of the present study in rats fed an HF diet and results by Pierroz et al\textsuperscript{29} in obese mice suggest that marked resistance to the metabolic actions of melanocortin receptor activation does not occur in dietary-induced obesity.

**Arterial Pressure and HR Responses to MC3/4-R Activation**

We have previously provided evidence for an important role of the hypothalamic melanocortin system in regulating arterial pressure and HR in normal lean rats.\textsuperscript{13} The present study, however, also demonstrates that chronic MC3/4-R activation caused sustained increases in arterial pressure and HR, measured 24-hours per day, beat-by-beat, in obese rats. This indicates that the downstream signaling pathways after activation of the MC3/4-R are intact and functional in dietary-induced obesity and that resistance to the cardiovascular actions of chronic MC3/4-R activation does not appear to develop in this model of visceral obesity. Furthermore, our data also suggest that reduced cardiovascular responses to MC3/4-R activation do not develop with aging, because the rise in arterial pressure and HR observed in the present study in 13- to 14-month-old rats was similar to the responses to MTII infusion that we observed previously in young 3- to 4-month-old rats.\textsuperscript{13,14}

Whether MC3/4-R activation contributes to increased arterial pressure in HF rats compared with NF was not tested in the present study, although high levels of leptin (which in the present study were ≈4 times greater in HF than in NF rats) are known to activate the melanocortin system.\textsuperscript{8} Although the cardiovascular responses to chronic leptin infusion were not tested in the present study, Rahmouni et al\textsuperscript{30} reported that chronic leptin injections in obese mice fed an NF diet for 10 weeks raised renal sympathetic activity and increased arterial pressure. These observations suggest that the cardiovascular actions of leptin may also be preserved in dietary-induced obesity.

Results from the present study showing that the melanocortin system appears to be fully functional in obese rats and results from our previous study suggesting that MC4-R knockout mice are markedly obese and hyperleptinemic but normotensive compared with wild-type mice\textsuperscript{31} may help explain why the renal SNS effects of leptin are not impaired in obese rodents\textsuperscript{15,30} and are consistent with an important role for the melanocortin system in obesity hypertension. If chronic activation of the hypothalamic melanocortin system does indeed raise blood pressure in obese humans, this may complicate the overall effectiveness of targeted activation of the melanocortin system as a treatment for obesity. However, almost all of the available data on the chronic cardiovascular effects of MC3/4-R activation have been obtained in rodents, and studies in humans are badly needed.

**Effects of MC3/4-R Activation on Renal Function**

In the present study and in previous studies from our laboratory,\textsuperscript{12,13} we found no significant changes in sodium excretion or urine volume during chronic MC3/4-R activation. The absence of significant changes in sodium excretion despite a rise in arterial pressure during chronic MTII infusion suggests that melanocortin activation may have caused a shift in the renal–pressure natriuresis relationship to higher arterial pressures.

**Perspectives**

Our results indicate that obesity is not associated with substantial resistance to the metabolic or cardiovascular actions of chronic MC3/4-R activation. Because the hypothalamic melanocortin system appears to play a major role in mediating the chronic hypertensive effects of leptin, our results also imply that intact MC3/4-R activation and signaling may be important in preserving the cardiovascular effects of leptin in obesity. Our observation that chronic MC3/4-R activation has important effects to enhance glucose use in obese as well as in lean rats also has important implications for understanding the role of the central nervous system in regulating peripheral glucose homeostasis, although the specific effenter pathways involved are still unclear and deserve additional study. Better understanding of the hypothalamic melanocortin system could result in novel strategies for management of the cardiovascular and metabolic disorders associated with obesity and diabetes.

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