A Functional Melanocortin System May Be Required For Chronic CNS-Mediated Antidiabetic And Cardiovascular Actions Of Leptin

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Short Title: MC3/4R mediate the chronic CNS actions of leptin

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**Objective:** We recently showed that leptin has powerful CNS-mediated antidiabetic and cardiovascular actions. This study tested whether the CNS melanocortin system mediates these actions of leptin in diabetic rats.

**Research design and methods:** A cannula was placed in the lateral ventricle of Sprague-Dawley rats for ICV infusions, and arterial and venous catheters were implanted to measure arterial pressure (MAP) and heart rate (HR) 24-h/d and for IV infusions. After recovery from surgery for 8 days, rats were injected with streptozotocin (STZ) and 5 days later either saline or the melanocortin 3 and 4 receptor (MC3/4R) antagonist, SHU-9119 (1 nmol/hr), was infused ICV for 17 days. Seven days after starting the antagonist, leptin (0.62 µg/hr) was added to the ICV infusion for 10 days. Another group of diabetic rats was infused with the MC3/4R agonist, MTII (10 ng/hr, ICV), for 12 days, followed by 7 days at 50 ng/hr.

**Results:** Induction of diabetes caused hyperphagia, hyperglycemia, and decreases in HR (-76 bpm) and MAP (-7 mmHg). Leptin restored appetite, blood glucose (BG), HR and MAP back to pre-diabetic values in vehicle treated rats, whereas it had no effect in SHU-9119 treated rats. MTII infusions transiently reduced BG and raised HR and MAP, which returned to diabetic values 5-7 days after starting the infusion.

**Conclusions:** Although a functional melanocortin system is necessary for the CNS-mediated antidiabetic and cardiovascular actions of leptin, chronic MC3/4R activation is apparently not sufficient to mimic these actions of leptin which may involve interactions of multiple pathways.
Leptin, an adipocyte derived peptide that circulates in proportion to the amount of body fat, is well known for its role in body weight homeostasis (1-3). Leptin informs the brain of the body’s energy storage status and promotes weight loss by reducing appetite and increasing energy expenditure by stimulation of sympathetic nervous system (SNS) activity to various tissues, including brown adipose tissue (4,5). In addition to its role in body weight homeostasis, leptin exerts important cardiovascular actions that are mediated via the central nervous system (CNS). For example, studies from our laboratory and others indicate that leptin may be an important link between excess weight gain and increased arterial pressure (6-9). Chronic hyperleptinemia in lean animals raises arterial pressure, whereas leptin deficiency causes severe obesity and many features of the metabolic syndrome without the accompanying hypertension (7-10).

Leptin also stimulates glucose utilization in peripheral tissues by activating CNS pathways. We recently showed that chronic intracerebroventricular (ICV) infusion of leptin in diabetic rats completely restored blood glucose to normal levels and prevented the hyperphagia and the marked bradycardia associated with streptozotocin (STZ)-induced diabetes (11). These observations indicate that the powerful effects of leptin on glucose regulation and cardiovascular function in insulin-deficient diabetic rats are mediated mainly by leptin’s direct actions on the CNS and are independent of insulin. However, the CNS mechanisms that mediate leptin’s chronic effects on glucose homeostasis and cardiovascular function are still unclear.

Leptin has been shown to suppress several orexigenic pathways including neuropeptide Y (NPY), agouti-related peptide (AGRP), melanin-concentrating hormone (MCH), while activating anorexigenic pathways such as corticotrophin-releasing hormone (CRH), cocaine-amphetamine-related peptide (CART) and the proopiomelanocortin (POMC) system (1-3,12-20). Among these factors, the POMC pathway appears to play a key role in mediating the appetite and cardiovascular CNS actions of leptin. Leptin mediated stimulation of the POMC neurons leads to release of α-melanocyte stimulating hormone (α-MSH) and activation of the melanocortin 3 and 4 receptors (MC3/4R) in several brain nuclei (1,12). Activation of MC3/4R has been demonstrated to contribute importantly to the anorexic actions of leptin (13-15), while absence of functional melanocortin system, either by pharmacological blockade of the MC3/4R (9,16,17) or genetic disruption of the MC4R (18) results in complete unresponsiveness to the chronic blood pressure and heart rate (HR) effects of leptin. Acute and chronic studies also suggest that activation of the MC3/4R improves insulin sensitivity and that blockade of the MC3/4R causes marked insulin resistance (20-22). However, the role of the CNS melanocortin system in mediating the chronic antidiabetic and cardiovascular actions of leptin in insulin-deficient diabetes are still unknown.

In this study we demonstrate that activation of the MC3/4R is required for leptin to exert its chronic antidiabetic and cardiovascular actions. Our results also indicate, however, that chronic stimulation of the MC3/4R alone, using a pharmacological agonist, causes only transient reductions in blood glucose in diabetic rats. These observations suggest that a functional melanocortin system is necessary for the CNS-mediated antidiabetic and cardiovascular actions of leptin, but chronic MC3/4R activation is apparently not sufficient to mimic these actions of leptin which may involve interactions of multiple pathways.
METHODS

Animal Surgeries: The experimental procedures and protocols of this study conformed 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.

Intra-arterial and intravenous catheterization: Male Sprague-Dawley (SD, Harlan, Indianapolis, IN) rats (325-350 g, n=20) 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 previously described (11,22). Briefly, using aseptic techniques, a laparotomy was performed and a sterile non-occlusive 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.

Intracerebroventricular cannulation (ICV). Immediately after arterial and venous catheters implantation, a stainless steel cannula (26 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle using the coordinates previously described (22). 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 to 1.5% isoflurane. After 7 days of recovery from surgery, accuracy of the cannula placement was tested by measuring the dipsogenic response (immediate drinking of at least 5 ml of water in 10 minutes) to an ICV injection of 100 ng of angiotensin II. After the experiment, the animals were killed and the brains removed and sectioned to confirm placement of the cannula.

After recovery from anesthesia, the rats were housed in individual metabolic cages for determination of daily water and food consumption. 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-hr measurement of mean arterial pressure (MAP) and HR using computerized techniques as previously described (7,8). The venous catheter was connected to a syringe pump for continuous infusion of saline (0.45%, 40 ml/day). The rats received food and water ad libitum throughout the study. Total sodium intake was maintained constant at ≈3.1 mEq/day via the continuous saline infusion combined with 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. The rats were allowed to recover for 8-10 days before control measurements were initiated.

Experimental Protocols: MAP, HR, urine volume, food and water intake were measured 24 hours/day and average values were recorded daily.

Induction of diabetes. After 4 to 5 days of stable control measurements, insulin-deficient diabetes was induced by a single intravenous injection of streptozotocin (STZ, 50 mg/kg – Sigma-Aldrich, dissolved in 0.5 ml of 0.05M citrate buffer, pH 4.5).

Chronic ICV leptin infusion in diabetic rats (n=5): Five days after STZ injection, leptin (0.62 µg/hr, 0.5 µl/hr) was infused icv for 10 days via osmotic minipump (model 2002, Durect Corp.) implanted subcutaneously in the scapular region as previously described (20). We have shown that this rate of leptin infusion fully restores euglycemia in STZ-diabetic rats (11).
**Chronic ICV infusion of a MC3/4-R agonist, MTII (n=10):** Five days after STZ injection, the MC3/4R agonist, MTII (10 ng/hr, 0.5 µl/hr, Polypeptide Laboratories), was infused ICV for 10 days via osmotic minipump as described above (n=5). On the 10th day of MTII infusion at 10 ng/hr the rats were lightly anesthetized with isoflurane and the osmotic minipump was replaced by another pump to deliver the agonist at a higher concentration (50 ng/hr).

In a separate experiment, diabetic rats (n=5) were treated with MTII at the dose of 10 ng/hr and were pair-fed the same amount of food consumed by diabetic rats during chronic leptin ICV infusion.

**Chronic ICV leptin infusion during MC3/4R blockade (n=5):** Five days after STZ injection, the MC3/4R antagonist, SHU-9119 (1 nmol/hr, 0.5 µl/hr, Polypeptide Laboratories) was infused ICV for 17 days via osmotic minipump as described above. After the first 7 days of ICV SHU-9119 infusion, an additional minipump was implanted 1-2 cm apart from the other minipump and connected to the ICV cannula via a Y connector to deliver leptin ICV for 10 days at 0.62 µg/hr. The rate of SHU-9119 infusion was based on our previous study showing that this dose effectively blocks the MC3/4R and the chronic dietary and cardiovascular effects of leptin in normal SD rats.

Blood glucose concentration was measured each morning between 9:00 and 10:00 am for determination of blood glucose levels using glucose strips (Reli On).

**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 Induction of Diabetes and Chronic ICV Leptin Infusion on Appetite, Blood Glucose and Cardiovascular Function**—Induction of insulin-deficient diabetes with STZ was associated with rapid development of hyperglycemia (433±28 mg/100ml on day 5 post STZ injection, Figure 1B and Table 1), hyperphagia (from 22±1 g/day in the control period to 45±2 g/day on day 5 after STZ injection, Figure 1A) and increased water intake and urine volume (Table 1).

Induction of STZ-diabetes also caused marked bradycardia with HR rapidly falling by as much as -77 bpm five days after STZ injection (Figure 2A). MAP responses following the induction of diabetes were variable among the groups but remained unchanged on average during the first 5 days after induction of diabetes (Figure 2B). We previously showed that it takes approximately 10 to 15 days after the STZ injection for a significant reduction in MAP (11).

Chronic ICV leptin infusion for 10 days in diabetic rats decreased food intake from 45±2 g/day to an average of 16±1 g/day during the last 5 days of leptin infusion (Figure 1A), and reduced blood glucose levels all the way back to pre-diabetic control values (118±19 mg/100ml, Figure 1B and Table 1).

The normalization of blood glucose levels by ICV leptin infusion was accompanied by a marked reduction in urine volume and water intake to values similar to pre-diabetic levels (Table 1). In addition, chronic leptin treatment reversed the bradycardia and raised HR by approximately 40 to 50 bpm above the pre-diabetic control values (Figure 2A). Although leptin tended to raise MAP, the increase was not significant compared to the day before leptin infusion was started (Figure 2B).

**Effects of Chronic ICV Infusion of MC3/4R Agonist on Appetite, Blood Glucose and Cardiovascular Function:** Chronic ICV infusion of the MC3/4R agonist, MTII, at a dose of 10 ng/hr for 10 days in ad
libitum fed diabetic rats caused only a transient reduction in food intake lasting for approximately 3 to 4 days, after which food intake returned to values observed before the MTII infusion was started (Figure 1B). During the course of the 10-day treatment period food intake continued to rise to a level that was more than double the initial control level. A similar pattern was observed for the effects of MTII on blood glucose levels, except for the less pronounced initial reduction in glucose levels (Figure 1A). Chronic MC3/4R activation also resulted in a transient reduction in water intake and urine output, in parallel with the transient changes in blood glucose levels (Table 1).

Chronic MC3/4R activation in ad libitum fed diabetic rats also attenuated the bradycardia associated with induction of diabetes and raised HR by approximately 50 bpm during the initial 5 days of infusion (Figure 2A). However, this effect waned on days 6 to 7 of MTII infusion, and HR gradually fell. We also observed a 10 mmHg initial elevation in MAP during the first 5 to 6 days of MTII infusion that was followed by a return of MAP to the same values observed on the day before MTII treatment was initiated (Figure 2B).

A 5-fold increase in the dose of MTII from 10 ng/hr to 50 ng/hr had no additional effect to prevent the hyperphagia, hyperglycemia and bradycardia associated with STZ-induced diabetes and raised HR by approximately 50 bpm during the initial 5 days of infusion (Figure 2A). However, this effect waned on days 6 to 7 of MTII infusion, and HR gradually fell. We also observed a 10 mmHg initial elevation in MAP during the first 5 to 6 days of MTII infusion that was followed by a return of MAP to the same values observed on the day before MTII treatment was initiated (Figure 2B).

**DISCUSSION**

There are two major findings of this study. First, we demonstrated that the chronic antidiabetic, appetite, and cardiovascular actions of leptin in insulin-deficient STZ-
diabetic rats require a functional CNS melanocortin system, and ultimately activation of MC3/4R; blockade of these receptors completely prevented the chronic effects of leptin on food and water intake, blood glucose, HR, blood pressure, and urine volume. Second, chronic MC3/4R activation alone transiently reduced appetite and blood glucose while raising HR in diabetic rats, but these effects gradually waned and did not mimic the responses observed during chronic hyperleptinemia. These results indicate that activation of the MC3/4R is required for leptin to exert its metabolic and cardiovascular actions, but is apparently not sufficient to mimic these actions of leptin which may involve interactions of multiple pathways.

It is likely that other systems are triggered during prolonged MC3/4R stimulation to offset the reductions in food intake and blood glucose as well as the MAP and HR responses to MC3/4R activation. The identity of these compensatory systems is still uncertain, but may involve activation of orexigenic factors known to be suppressed by leptin (i.e. NPY, MCH, AGRP and others) and/or down-regulation of anorexigenic factors that are stimulated by leptin (i.e. CRH, CART, and BDNF, for example). It is possible that leptin-induced changes in one or more of these factors also contribute to the powerful antidiabetic effect of leptin, and additional studies will be needed to answer these questions.

The observation that MC3/4R antagonism abolished the antidiabetic and cardiovascular actions of leptin is consistent with our previous finding that an intact hypothalamic MC3/4R is necessary for leptin to reduce food intake and fasting insulin levels, and to raise HR and blood pressure in normal non-diabetic rats (9). However, the long-term responses to MC3/4R activation by MTII infusion in STZ-diabetic rats differed from our previous observations in non-diabetic rats where, despite not causing sustained reductions in food intake, the elevations in HR and MAP were maintained through the entire period of MTII treatment (22,23). Increasing the dose of MTII 5-fold did not alter the responses when compared to the lower dosage indicating that the waning responses are not likely to be due to an insufficient level of MC3/4R activation in diabetic rats. One possible explanation for these differences is that activation of compensatory mechanisms during chronic MC3/4R activation is even more pronounced in diabetes than under normal conditions. For instance, in STZ-induced diabetes NPY/AGRP neurons are markedly activated and may play a major role in promoting the hyperphagia in this model of diabetes (24,25).

Another possible explanation for the inability of chronic MC3/4R activation to mimic the antidiabetic effects of leptin is that MC3/4R may have only a short-term anorectic action and that the initial fall in blood glucose is due mainly to a reduction in food intake. It could be hypothesized that the hyperphagia in diabetes and increased intake of glucose and other nutrients (fat and proteins) that can be transformed into glucose overcomes the effects of MC3/4R to lower blood glucose. To test this hypothesis we studied a group of diabetic rats in which food intake was prevented from increasing by pair-feeding them to match the amount of food consumed by the leptin treated group. Preventing the hyperphagia, however, did not improve the effectiveness of MTII to lower blood glucose levels suggesting that the lack of sustained antidiabetic effect during chronic MC3/4R activation was not due to MTII inability to reduce appetite. These results also confirm our previous observation that the reduction of food intake in diabetic rats during ICV leptin infusion does not play a major role in mediating the antidiabetic actions of leptin or in preventing the cardiovascular alterations associated with uncontrolled diabetes (11).

The precise mechanisms by which leptin exerts its powerful effect on peripheral glucose utilization even in insulin-deficient
diabetic animals are still unclear, although our current study clearly indicates that a functional MC3/4R plays a crucial role. Previous studies have implicated a role for the autonomic nervous system in mediating the acute effects of leptin on glucose regulation by showing that the increased insulin sensitivity observed during ICV injection of single doses of leptin can be blocked by adrenergic receptor antagonism (27,28), or that leptin’s ability to suppress liver glucose production can be prevented by denervation of the vagal fibers innervating the liver (29). We recently showed that chronic blockade of the α1, β1, β2 and β3 adrenergic receptors failed to attenuate the leptin’s ability to restore euglycemia in STZ-diabetic rats (11). It is therefore possible that leptin-induced sympathetic stimulation of non-adrenergic receptors may contribute to these effects of leptin and that the suppression of liver glucose output into the systemic circulation of diabetic rats may play an important role in mediating the chronic effects of leptin on glucose homeostasis, but additional studies are needed to test these possibilities.

Although our previous study indicates that adrenergic receptor activation does not mediate the chronic antidiabetic effects of leptin, approximately 40 to 50% of the rise in HR and prevention of the bradycardia in STZ-diabetic rats during leptin infusion was dependent of adrenergic stimulation (11). The mechanisms responsible for the remaining 50% to 60% of leptin induced rise in HR in diabetic rats are not well understood. However, the bradycardia caused by induction of STZ-diabetes is associated with marked reductions in the intrinsic HR (30), which is the HR in the absence of sympathetic and parasympathetic inputs to the heart. Moreover, chronic ICV leptin infusion completely restored intrinsic HR back to pre-diabetic values (30). We have also shown that, similar to leptin, MC3/4R activation is associated with sustained SNS activation in non-diabetic rats (31). Whether the lack of sustained increases in HR during MTII in STZ-diabetic is due an inability of chronic MC3/4R activation to cause sustained elevations in cardiac sympathetic activity in a hyperglycemic state or to an inability to increase intrinsic HR remains to be determined.

In summary, leptin has powerful CNS-mediated antidiabetic effects in insulin-deficient diabetic rats that require activation of the CNS melanocortin pathway. Leptin also exerts important cardiovascular effects that require activation of CNS MC3/4R. However, stimulation of the CNS MC3/4R alone does not confer the same long-term metabolic and cardiovascular responses as observed with hyperleptinemia. These observations indicate that although leptin-mediated activation of the melanocortin pathway may be necessary for the antidiabetic and cardiovascular actions of leptin in this model, it is not sufficient to completely mimic leptin’s chronic actions. This suggests that leptin exerts its CNS-mediated effects on appetite, blood glucose, MAP and HR via a complex system that likely involves interaction of multiple pathways. Unraveling these interactions would contribute to a better understanding of the CNS control of appetite, glucose homeostasis and cardiovascular function and could lead to the development of novel therapeutic strategies to treated obesity, metabolic syndrome, diabetes and cardiovascular diseases.

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Table 1 – Blood glucose, water intake and urine output in STZ-diabetic rats treated with leptin alone (0.62 µg/hr, ICV) or during MC3/4R antagonism with SHU-9119 (1 nmol/hr, ICV), and in diabetic rats fed *ad lib* or pair-fed that were infused with the MC3/4R agonist, MTII (10 ng/hr, ICV).

| Experimental Groups | Glucose (mg/100ml) | Water Intake (ml/day) | Urine Output (ml/day) |
|---------------------|--------------------|-----------------------|-----------------------|
| **Leptin Group**    |                    |                       |                       |
| Control             | 99 ± 5             | 8 ± 2                 | 45 ± 2                |
| STZ – day 5         | 433 ± 28*          | 211 ± 20*             | 161 ± 21*             |
| Leptin – day 10     | 118 ± 19           | 6 ± 3                 | 54 ± 4                |
| **MTII ad lib Group** |                   |                       |                       |
| Control             | 112 ± 3            | 11 ± 2                | 39 ± 4                |
| STZ – day 5         | 418 ± 43*          | 139 ± 16*             | 198 ± 18*             |
| MTII (10 ng/hr) – day 2 | 319 ± 53*†       | 42 ± 15*†             | 83 ± 22*†             |
| MTII (10 ng/hr) – day 10 | 401 ± 18*†       | 245 ± 34*†            | 300 ± 43*†            |
| MTII (50 ng/hr) – day 2  | 429 ± 12*          | 159 ± 23*             | 213 ± 30*             |
| MTII (50 ng/hr) – day 7  | 423 ± 13*          | 303 ± 20*†            | 358 ± 19*†            |
| **MTII pair-fed Group** |                   |                       |                       |
| Control             | 97 ± 6             | 12 ± 2                | 45 ± 3                |
| STZ – day 5         | 449 ± 20*          | 193 ± 23*             | 241 ± 18*             |
| MTII (10 ng/hr) – day 2 | 426 ± 27*          | 141 ± 32*             | 177 ± 30*             |
| MTII (10 ng/hr) – day 10 | 401 ± 19*          | 71 ± 21*†             | 103 ± 24*†            |
| **Leptin + SHU-9119 Group** |        |                       |                       |
| Control             | 114 ± 4            | 9 ± 1                 | 41 ± 3                |
| STZ – day 5         | 442 ± 42*          | 144 ± 15*             | 189 ± 18*             |
| SHU-9119 – day 7    | 440 ± 21*          | 268 ± 17*†            | 326 ± 19*†            |
| Leptin + SHU-9119 – day 10 | 436 ± 14*          | 350 ± 34*†            | 419 ± 37*†            |

STZ indicates streptozotocin. Values expressed are for day 5 of control period, 5 days after injection of STZ, and during the experimental periods as indicated in the table. Note: total daily fluid intake equals the water plus a fixed continuous IV saline infusion (40 ml/day, 0.45% saline) throughout the study. * p<0.05 compared with control; † p<0.05 compared with STZ – day 5.
FIGURE LEGENDS

**Figure 1.** Food intake (A) and blood glucose (B) responses to chronic ICV infusion of leptin (dark circles, n=5) or the MC3/4R agonist, MTII, in *ad libitum* fed (white squares, n=5) and pair-fed (dark triangles, n=5) STZ-diabetic rats. Data expressed as mean ± SEM.

**Figure 2.** Heart rate (A) and mean arterial pressure (B) responses to chronic ICV infusion of leptin (dark circles, n=5) or the MC3/4R agonist, MTII, in *ad libitum* fed (white squares, n=5) and pair-fed (dark triangles, n=5) STZ-diabetic rats. Baseline HR and MAP values for leptin, MTII *ad lib* and MTII pair-fed groups were, respectively: 378±5 bpm and 100±3 mmHg, 366±11 bpm and 90±1 mmHg, 398±8 bpm and 101±4 mmHg. Data expressed as mean ± SEM.

**Figure 3.** Food intake (A) and blood glucose (B) responses to chronic ICV infusion of MC3/4R antagonist (SHU-9119) and leptin during SHU-9119 infusion in STZ-diabetic rats (n=5). Data expressed as mean ± SEM.

**Figure 4.** Heart Rate (A) and mean arterial pressure (B) responses to chronic ICV infusion of MC3/4R antagonist (SHU-9119) and leptin during SHU-9119 infusion in STZ-diabetic rats (n=5). Data expressed as mean ± SEM.
FIGURE 1

**A)**

- Induction of Diabetes (STZ - 50 mg/kg)
- Leptin (0.62 µg/hr, ICV) or MTII (10 ng/hr, ICV)
- MTII (50 ng/hr, ICV)

**B)**

- Induction of Diabetes (STZ - 50 mg/kg)
- Leptin (0.62 µg/hr, ICV) or MTII (10 ng/hr, ICV)
- MTII (50 ng/hr, ICV)
FIGURE 2

A) 

Induction of Diabetes (STZ - 50 mg/kg) or MTII (10 ng/hr, ICV) or MTII (50 ng/hr, ICV)

Leptin (0.62 µg/hr, ICV)

MTII - Ad Lib

MTII - Pair Fed

B) 

Induction of Diabetes (STZ - 50 mg/kg) or MTII (10 ng/hr, ICV) or MTII (50 ng/hr, ICV)

Leptin (0.62 µg/hr, ICV)

MTII - Ad Lib

MTII - Pair Fed
FIGURE 3

A) Induction of Diabetes (STZ - 50 mg/kg)
Leptin (0.62 µg/hr, ICV)
MC3/4R Antagonist (SHU-9119, 1 nmol/hr, ICV)

B) Induction of Diabetes (STZ - 50 mg/kg)
Leptin (0.62 µg/hr, ICV)
MC3/4R Antagonist (SHU-9119, 1 nmol/hr, ICV)
FIGURE 4

A) heart rate

B) mean arterial pressure