Baroreceptor Reflex Impairment and Mild Hypertension in Rats with Dietary-Induced Obesity

Ruben D. Bunag, Lea Eriksson, and Dora Krizsan

Cardiovascular dysfunction associated with obesity was assessed by comparing rats that had been maintained on a regular or high fat diet since weaning. Rats on the high fat diet not only gained weight faster than age-matched controls but also had higher systolic and mean pressures. Development of mild hypertension in obese rats was first detected by indirect tail-cuff measurement and confirmed later by recording intra-arterial pressures directly from indwelling femoral catheters. To assess baroreceptor reflex sensitivity, reflex heart rate responses were elicited by lowering blood pressure with sodium nitroprusside or elevating it with phenylephrine. Initial tests showed that, although reflex tachycardia during depressor responses to sodium nitroprusside did not differ between groups, reflex bradycardia during pressor responses to phenylephrine was weaker in obese than in control rats. Underlying autonomic mechanisms were then examined by repetition of baroreceptor reflex tests after cholinergic blockade with methylatropine or β-adrenergic blockade with propranolol. Reflex tachycardia was equally inhibited in both groups by either antagonist. By contrast, reflex bradycardia was reduced more in obese than in control rats by β-adrenergic blockade but was equally reduced by cholinergic blockade. Because residual responses after β-adrenergic blockade would represent remaining parasympathetic mediation, these results indicate that reflex bradycardia was selectively impaired because of deficient parasympathetic mediation. Considered collectively, our results suggest that impaired parasympathetic mediation of reflex bradycardia could either result from or contribute to the blood pressure elevation in obese rats.

Obese persons tend to be hypertensive, but the reasons for their predisposition to hypertension are poorly understood. Although it would be logical to study underlying mechanisms in animal models for obesity, rat models produced either by genetic inbreeding or by electrolytic or chemical destruction of the ventromedial hypothalamus may not be suitable for studying hypertensive mechanisms. Zucker rats with genetic obesity remain normotensive despite impaired baroreceptor reflexes and many other abnormalities, whereas rats made obese by hypothalamic lesions have equivocal blood pressure changes perhaps in part because normal cardiovascular regulation by the hypothalamus has been irretrievably lost. On the other hand, the unique model derived by inbreeding spontaneously hypertensive Wistar-Kyoto rats with genetic traits for hyperphagia and corpulence seems far removed from reality as all the rats are obese and hypertensive.

Recently, another model was described by Oscai (Oscai and McGarr and Oscai) in which obesity is induced by programming pups to overeat immediately after birth and then maintaining them on a high fat diet. Inasmuch as this model is derived by overfeeding rather than by genetic or neurosurgical manipulation, it probably mimics the natural development of obesity more closely. Accordingly, we aimed to determine cardiovascular status in obese and control rats nurtured as described by Oscai. From 4 to 10 months of age, blood pressures and heart rates were monitored by tail-cuff measurement and, on finding that obese rats had become mildly hypertensive, terminal experiments were done to compare reflex heart rate responses.

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Dietary Induction of Obesity

were fed a high fat diet, whereas those from litters of
and six obese rats were kept two to a cage in a room
weaned 4 weeks later, those raised in litters of four
and 4.11 for dessicated liver. For each sex, six control
for lard, 1.68 for vitamin mix, 3.7 for brewer's yeast,
were given; second, after cholinergic or γ-adrenergic
diet (TD 871d95, Teklad, Madison, Wisconsin) pro-
in pups in small litters, but conversely restricted in
consumption during suckling would be unrestricted
after allowing 1 week for postoperative recovery, reflex responses in each awake rat were
recorded four times: first, before any blocking drugs
were given; second, after cholinergic or β-adrenergic
blockade; third, after cholinergic or β-adrenergic blockade; and fourth, after combined cholinergic and β-adrenergic blockade. The first two tests were done on the same day, and the last tests were done 3 days later. Experiments in seven rats were not completed (i.e., not all four baroreceptor reflex tests were done) because of various technical difficulties so that results finally analyzed for baroreceptor reflex testing were from seven control (two males and five females) and 10 obese (five of each sex) rats.

Methods

Twenty-four Wistar rats were used, half of which (six of each sex) had dietary-induced obesity. Body weights were measured weekly and, starting at 4 months of age, systolic pressures and heart rates were also recorded every 2–4 weeks. When the rats were 10 months old, they were transiently anesthetized for chronic implantation of indwelling vascular catheters to be used for repeated recording of reflex heart rate responses to intravenous infusions of phenylephrine and sodium nitroprusside. After allowing 1 week for postoperative recovery, reflex responses in each awake rat were recorded four times: first, before any blocking drugs were given; second, after cholinergic or β-adrenergic blockade; third, after cholinergic or β-adrenergic blockade; and fourth, after combined cholinergic and β-adrenergic blockade. The first two tests were done on the same day, and the last tests were done 3 days later. Experiments in seven rats were not completed (i.e., not all four baroreceptor reflex tests were done) because of various technical difficulties so that results finally analyzed for baroreceptor reflex testing were from seven control (two males and five females) and 10 obese (five of each sex) rats.

Dietary Induction of Obesity

Following the method described by Oscai,14,15 obesity was induced by initially redistributing pups immediately after birth and then maintaining selected pups on high fat diets after weaning. Litters were redistributed so that access to food and its consumption during suckling would be unrestricted in pups in small litters, but conversely restricted in pups in large litters. Seven pregnant (late-term) females purchased from Charles River Laboratories Inc. (Wilmington, Massachusetts) were allowed to deliver normally and, just after they were born, the pups were redistributed such that four mothers had litters of only four pups, whereas three others had litters of 18–22 pups. As soon as the pups were weaned 4 weeks later, those raised in litters of four were fed a high fat diet, whereas those from litters of 18–22 were fed regular Purina chow. The high fat diet (TD 871d95, Teklad, Madison, Wisconsin) provided 4.87 kcal/g with a caloric density (kcal/g) for each ingredient as follows: 3.61 for casein, 4.0 for DL-methionine, 4.0 for sucrose, 9.0 for corn oil, 9.0 for lard, 1.68 for vitamin mix, 3.7 for brewer's yeast, and 4.11 for dessicated liver. For each sex, six control and six obese rats were kept two to a cage in a room with a 12-hour light/dark cycle and a temperature of 20±2° C until terminal experiments were done at 10.5 months of age.

Tail-Cuff Measurements of Blood Pressure and Heart Rate

Indirect tail-cuff measurements of systolic pressure were recorded using a photoelectric sensor (IITC Inc., Woodland Hills, California) that allows detection of tail pulsations in conscious rats without preheating.16 Because the original photoelectric sensors and rat holders were designed for use on rats weighing less than 400 g, additional sensors and holders for larger rats were purchased from IITC Inc. B60 sensors (cuff diameter ¾ in.) were used routinely and larger B63 sensors (cuff diameter ¾ in.) for rats weighing more than 500 g. Depending on body weight, three sizes of rat holders with the following measurements (i.e., circumference × length in centimeters) were used: 19×14 up to 450 g, 23×17.5 up to 600 g, and 28×17.5 up to 1.2 kg. For each recording, values for mean pressure (for subsequent comparison with mean pressures recorded directly from indwelling femoral catheters) were estimated concurrently by reading the cuff pressure level at peak tail-cuff oscillation. Each systolic and mean pressure measurement was obtained by averaging at least three individual readings. After each blood pressure measurement, heart rate was calculated by multiplying the number of arterial pulsations recorded (with the tail-cuff deflated) for 5 seconds by 12.

Repeated Recording in Conscious Rats of Cardiovascular Responses to Phenylephrine and Sodium Nitroprusside

Indwelling catheters filled with heparinized (30 units/ml) saline were inserted into a femoral vein and through a femoral artery into the lower abdominal aorta while each rat was anesthetized by intraperitoneal injection of a mixture of ketamine (6 mg/100 g) and xylazine (0.5 mg/100 g). The outer ends of both catheters were exteriorized at the rat's nape, and the rats were then allowed to recover for 1 week.

For recording blood pressure, the aortic catheter was connected through polyethylene tubing to a small-volume-displacement pressure transducer (MP-15, Micron Instrument Inc., Los Angeles, California) placed on the same level as the rat. Analog signals for mean arterial pressure (calculated as diastolic pressure plus one third of the pulse pressure) and heart rate were derived from the pulsatile pressure measurement, heart rate was calculated by multiplying the number of arterial pulsations recorded (with the tail-cuff deflated) for 5 seconds by 12. After leaving the rats untouched for 30–60 minutes to allow them to adapt to the recording conditions, phenylephrine was infused into the femoral vein to elicit reflex bradycardia. A computer (Zenith 100) was used to drive an infusion pump (model 22, Harvard Apparatus, South Natick, Massachusetts) to induce a steadily rising blood pressure. Infusions were repeated two or three times after blood pressure was allowed to return to preinfusion levels between infusions. Subsequently, similar intravenous infusions of sodium nitroprusside were given
to decrease blood pressure and elicit reflex tachycardia. For both drugs, infusion rates were increased from 24 to 117 μl/min/100 g body wt in 20 steps lasting for 2.4 seconds each. With concentrations (μg/ml) of 25 for phenylephrine and 100 for sodium nitroprusside, infused doses increased from 0.6 to 3 and from 2.4 to 12 μg/min/100 g, respectively.

Tests for baroreceptor reflex sensitivity consisted of 1–3 infusions each for phenylephrine and sodium nitroprusside. Of the four tests in each rat, the first was always done before any antagonist drugs were given. The second test was performed on the same day (30 minutes after completion of the first test) by induction of either cholinergic blockade with methyla tropine or β-adrenergic blockade with propranolol (dose of 0.15 mg/100 g used for both antagonists) and, after 15 minutes, repeating intravenous infusions of phenylephrine and sodium nitroprusside. To allow sufficient time for residual effects of the first antagonist to dissipate, the rats were then left untouched for 3 days before the last two tests were done. The third test was done 15 minutes after blockade was induced with the antagonist that had not yet been used (i.e., with methyla tropine if propranolol had been given previously or vice-versa), and the fourth test, 30 minutes later, by adding the other antagonist drug.

As described previously,17,18 a data acquisition system consisting of a Zenith 159 computer, 12 bit A/D board (DT 2801, Data Translation, Marlboro, Massachusetts) and data acquisition and analysis programs (ASYSTANT PLUS, Macmillan Software Co., Rochester, New York), were used to digitize analog outputs from the cardiovascular analyzer. Digitized data was stored in files from which corresponding units in millimeters of mercury for mean pressure and beats per minute for heart rate were determined using the file conversion utility of the ASYSTANT PLUS program. Pressure calibrations were checked and zero levels adjusted by using a mercury manometer.

Drugs, Data Processing, and Statistics

Drugs used were L-phenylephrine hydrochloride, sodium nitroprusside, methylatropine nitrate, and propranolol hydrochloride with all drug doses expressed in terms of the salt given per hundred grams of body weight.

To analyze cumulative dose responses to phenylephrine and sodium nitroprusside, blood pressure and heart rate responses to 1–3 infusions were pooled to allow comparison of larger numbers between sexes, data from both males and females were no consistent differences in blood pressure without showing any definite trends. Because there were wide variations within each group, the difference of pressure thus averaged were significantly higher in

with mean pressure changes of 5, 10, 15, 20, 25, 30, 35, and 40 mm Hg were thereby compiled. All data are expressed as mean±SEM. Three general types of statistical analyses19 were used as follows: 1) a repeated-measures analysis of variance to assess tail-cuff measurements of blood pressure (Table 1), body weight (Figure 1), and cardiovascular drug responses (Figures 2–5); 2) a four-factor analysis of variance for post hoc comparisons of basal blood pressures and heart rates (Table 2); and 3) covariance analysis20 to compare baroreceptor reflex responses before and after antagonist treatment (Tables 3 and 4). Covariance analysis was used to determine whether reflex responsiveness was altered similarly in both control and obese rats by methylatropine or propranolol treatment. Because baselines were altered after treatment with either antagonist drug, ensuing changes in reflex responsiveness were assessed by using data after blockade as the dependent variables with data recorded from the same rats before blockade as the covariate. Whenever F ratios were significant at 5%, Duncan’s multiple range test was applied to determine the significance of differences between pairs of means. Statistical tests (except covariance analysis) were done using a Zenith 386 computer running the Number Cruncher Statistical System.21

Results

Obese Rats are Mildly Hypertensive

As the pups grew older after weaning, those fed the high fat diet, whether male or female, gained weight faster than those maintained on regular chow. Differences in average body weights (g±SEM) began to be statistically significant for males at 7 (125±13 in control and 221±19 in obese) and in females at 9 (173±9 in control and 264±16 in obese) weeks of age. For either sex, obese rats were clearly heavier than the controls by 10 months of age (Figure 1), and by the time terminal experiments were done 2 weeks later (i.e., when the rats were 10.5 months old), body weights averaged 587±8 in control and 935±40 in obese males (p<0.001) and 409±24 in control and 579±77 in obese females (p<0.01). Two of the males weighed more than a kilogram (i.e., 1,035 and 1,054 g) and the largest female weighed 930 g, but because of wide variations within each group, the difference between the two obese groups (i.e., obese males vs. obese females) was not statistically significant.

Tail-cuff measurements taken from the fourth through the 10th month of age showed that in both sexes, averages for systolic and mean pressure were usually higher in obese than in control rats. Corresponding averages for heart rate differed sporadically without showing any definite trends. Because there were no consistent differences in blood pressure between sexes, data from both males and females were pooled to allow comparison of larger numbers of control (n=12) and obese (n=12) rats. Systolic pressures thus averaged were significantly higher in
obese than in control rats from weeks 20 through 38 (Table 1). To determine how frequently hypertensive pressures occurred, arbitrary cut-off points for separating hypertensive from normotensive rats were then established as described previously. By adding 2 SDs to the average pressure for the control group, a cut-off point of 145 mm Hg was obtained (i.e., with a standard deviation of 13 multiplied by 2, the ensuing product of 26 was added to an overall average systolic pressure of 119 mm Hg). On counting the numbers of rats with systolic pressures of 145 or more at different ages (Table 1), the incidence of hypertensive pressures was consistently higher among obese than among control rats. Though not as pronounced, corresponding data for mean pressures recorded with the tail-cuff method also showed a trend for higher pressures in obese rats (Table 1). For mean pressures recorded directly from indwelling femoral catheters during subsequent terminal experiments on seven control and 10 obese rats, the difference was even smaller but still significant (114±3 in control and 123±2 mm Hg in obese rats, p<0.05) (Table 2). Nonetheless, these results as a whole indicate that obese rats tended to be mildly hypertensive.

Reflex Bradycardia Reduced in Obese Rats

Because responses to either phenylephrine or sodium nitroprusside did not differ between sexes, for subsequent comparisons between control and obese rats all results were also pooled. Intravenous infusions of phenylephrine produced a progressive elevation in blood pressure in all rats accompanied by a reflex bradycardia whose magnitude was proportional to that of the pressor response. When average responses were plotted against 5-second increments of infusion time (Figure 2), magnitude of pressor responses seemed larger while that of reflex bradycardia was smaller in obese than in controls rats, but neither of the $F$ ratios comparing the two rat groups for each response was significant (i.e., $F$ ratios of 4.12, $p<0.05$>0.05 for the pressor response and of 1.65, $p>0.1$ for bradycardia). Opposite responses consisting of lowered blood pressure and reflex tachycardia elicited by infusions of sodium nitroprusside also did not differ between groups (Figure 3) ($F$ ratios of 4.21, $p<0.06$>0.05 for the depressor response and of 0.13, $p>0.1$ for tachycardia).

On plotting reflex heart rate responses against corresponding changes in mean pressure, the magnitude of reflex bradycardia with every 5 mm Hg...
increase in mean pressure produced by phenylephrine, was significantly smaller in obese than in control rats. Comparison of the two rat groups using a repeated-measures analysis of variance gave an overall $F$ ratio of only 1.34 ($p>0.1$), but the correspond-

ing $F$ ratio of 3.63 for the interaction between pressure levels and rat groups was significant ($p<0.002$). Further analyses with Duncan's test showed that for pressure increments of 15 mm Hg or more, all differences between rat groups were signif-

### TABLE 2. Effects of Cholinergic or β-Adrenergic Blockade on Mean Pressure and Heart Rate in Conscious Control and Obese Rats

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Measurements relative to blockade</th>
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<tr>
<td></td>
<td>Without</td>
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<tr>
<td>Mean blood pressure (mm Hg)</td>
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</tr>
<tr>
<td>Control</td>
<td>114±3</td>
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<tr>
<td>Obese</td>
<td>123±2$^+$</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>356±6</td>
</tr>
<tr>
<td>Obese</td>
<td>374±7</td>
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$p<0.05$ as compared with average for the same group without blockade using a four-factor ANOVA and Duncan’s multiple range test.

$tp<0.05$ as compared with average for control rats using a four-factor analysis of variance (ANOVA) and Duncan’s multiple range test.

**FIGURE 2.** Line graphs showing average (±SEM) cardiovascular responses to intravenously infused phenylephrine in two groups of conscious control and obese rats. $F$ ratios comparing rat groups were 4.12 ($p<0.06$) for pressor responses and 1.65 ($p>0.2$) for reflex bradycardia; corresponding $F$ ratios for group interactions at different times were 1.59, ($p>0.1$) and 3.53 ($p<0.0001$), respectively. * Indicate $p$ values $<0.05$ on comparing control and obese groups by using Duncan’s multiple range test.

**FIGURE 3.** Line graphs showing average (±SEM) cardiovascular responses to intravenously infused sodium nitroprusside from same rat groups presented in Figure 2. $F$ ratios comparing rat groups were 4.21 ($p<0.06$) for depressor responses and 0.13 ($p>0.7$) for reflex tachycardia; corresponding $F$ ratios for group interactions at different times were 3.14 ($p<0.001$) and 1.46 ($p>0.1$), respectively. * Indicate $p$ values $<0.05$ on comparing control and obese groups by using Duncan’s multiple range test.
significant (Figure 4). By contrast, for opposite responses produced by sodium nitroprusside the magnitude of reflex tachycardia did not differ significantly between control and obese rats (F ratios of 1.67, p>0.1 for comparing the two rat groups and of 1.13, p>0.1 for the interaction between pressure levels and rat groups). These analyses indicate, therefore, that although reflex tachycardia did not differ between rat groups, reflex bradycardia was weaker in obese rats.

Cholinergic, β-Adrenergic, or Combined Blockade Alters Basal Heart Rate But Not Mean Pressure

Because heart rate reflexes normally depend on neural mediation through efferent parasympathetic and sympathetic pathways, the weakened reflex bradycardia in obese rats could reflect an imbalance in parasympathetic and sympathetic tone. This possibility was tested by recording reflex responses in the same rats after appropriate pharmacological blockade: cholinergic blockade with methylatropine to reveal sympathetic mechanisms, or β-adrenergic blockade with propranolol to reveal parasympathetic mechanisms.

In all rats, baselines for heart rate were consistently elevated after cholinergic blockade and lowered after β-adrenergic blockade, but those for mean pressure were usually unaltered so that average pressures remained higher in obese than in control rats (except for an unexplained elevation of mean pressure produced by propranolol in control rats; Table 2). None of the differences between rat groups in heart rate after blockade with methylatropine or propranolol, either alone or combined, were significant (Table 2).

Baroreceptor Reflex Alterations Produced by Cholinergic, β-Adrenergic, or Combined Blockade

All reflex heart rate responses, whether consisting of bradycardia or tachycardia (Figure 5), were consistently reduced after either cholinergic blockade with methylatropine or β-adrenergic blockade with propranolol. The magnitude of ensuing reductions seemed generally smaller in obese than in control rats, but because baselines for heart rate were markedly altered after either type of blockade, direct quantitative comparisons could not be made. Accordingly, an analysis of covariance was performed using heart rate responses before blockade as covariants to determine whether the reductions produced by methylatropine or propranolol occurred equally in both rat groups. Responses after blockade were then expressed not only as absolute values, but also as adjusted means.

After cholinergic blockade with methylatropine, neither the absolute values nor the adjusted means for reflex tachycardia differed between groups (Table 3) thereby indicating that residual sympathetic mediation (i.e., remaining after parasympathetic mediation was abolished by methylatropine) of reflex chronotropic responses was the same regardless of body weight. Similarly, after β-adrenergic blockade with propranolol, absolute values and adjusted means for reflex tachycardia did not differ between groups, but reflex bradycardia elicited by pressor responses of 25 mm Hg or more was consistently smaller in obese than in control rats (Table 4). Although the overall F ratio (i.e., comparison of rat groups in Table 4 with an F ratio of 0.26, p>0.5) was not significant, the F ratio of 4.13 (p<0.001) for the interaction between pressure lev-
Baroreceptor Reflex Impairment in Obese Rats

Figure 5. Plots showing effects of drug-induced autonomic blockade on reflex bradycardia and tachycardia elicited in conscious control and obese rats.

els and rat groups indicates that some of the group differences at higher pressure levels were significant. Using Duncan's multiple range test, further comparison of paired averages during phenylephrine infusion showed that, at pressure increases of 35 and 40 mm Hg, bradycardia was significantly weaker in obese than in control rats (Table 4). These results imply that residual parasympathetic mediation (i.e., remaining after sympathetic mediation was abolished by propranolol) had already started to diminish in obese rats.

Finally, when baroreceptor reflex tests were repeated after pretreatment with both methylatropine and propranolol, arterial pressure still rose or

<table>
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<tr>
<th>Rat group</th>
<th>Change in mean pressure (mm Hg)</th>
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<tr>
<td>Reflex bradycardia to phenylephrine</td>
<td></td>
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<tr>
<td>Control</td>
<td>0±4</td>
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<td></td>
<td>(2.7)</td>
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<tr>
<td>Obese</td>
<td>1±1</td>
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<td>(3.8)</td>
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<tr>
<td>F ratio comparing rat groups=0.10, p&gt;0.5</td>
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<td>F ratio for interactions=0.50, p&gt;0.5</td>
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<th>Reflex tachycardia to sodium nitroprusside</th>
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<tr>
<td>Control</td>
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<tr>
<td></td>
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<tr>
<td>Obese</td>
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<tr>
<td>F ratio comparing rat groups=1.15, p&gt;0.1</td>
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<td>F ratio for interactions=0.44, p&gt;0.5</td>
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Values are averages±SEM with numbers in parentheses below each average representing adjusted means obtained by covariant analysis; interactions assessed by the second F ratio are between pressure levels and rat groups.
overestimation of the prevalence of hypertension,23-24 conjectural. Were beginning to rise. Whether the impairment in ratio for interactions=1.57, p>0.1

F ratio comparing rat groups=0.26, p>0.5  
F ratio for interactions=4.13, p<0.001

Reflex bradycardia to phenylephrine  
Control 5±3 0±4 -3±5 -9±6 -16±6 -26±9 -42±10 -48±9  
(-5.1) (-4.7) (-4.7) (-7.9) (-13.6) (-22.7) (-36.6) (-41.0)  
Obese 0±1 -2±1 -6±2 -9±2 -14±3 -18±3 -23±3* -32±5*  
(-5.6) (-7.1) (-8.6) (-9.8) (-12.9) (-16.0) (-20.5) (-28.2)  

F ratio comparing rat groups=0.24, p>0.5  
F ratio for interactions=1.57, p>0.1

Reflex tachycardia to sodium nitroprusside  
Control 5±4 10±6 15±5 19±5 20±5 18±6 15±7 11±7  
(12.2) (14.6) (15.3) (16.6) (15.6) (11.7) (7.1) (2.6)  
Obese 1±1 4±2 7±2 10±3 12±3 14±4 14±5 13±5  
(9.2) (9.3) (10.3) (11.5) (11.8) (11.8) (10.6) (9.8)  

Values are averages±SEM with numbers in parentheses below each average representing adjusted means obtained by covariant analysis;  
interactions assessed by the second F ratio are between pressure levels and rat groups.  
*p<0.05 as compared with corresponding average for control group using Duncan's multiple range test.

t fell as phenylephrine or sodium nitroprusside was infused, but reflex heart rate changes were no longer elicited (Figure 5). Abolition of reflex heart rate responses by combined blockade indicates that they were elicited mainly through efferent parasympathetic and sympathetic pathways.

Discussion

Dietary induction of obesity with the Oscai method14,15 obviously affects not only body weight (Figure 1) but also cardiovascular homeostasis. Obese rats compared with age-matched controls at 10 months of age had the following cardiovascular characteristics: 1) mild or borderline hypertension detectable with either indirect tail-cuff (Table 1) or direct intra-arterial measurement (Table 2); 2) selective impairment of reflex bradycardia during pressor responses to intravenously infused phenylephrine (Figures 2 and 4) but not of reflex tachycardia during depressor responses to sodium nitroprusside (Figures 3 and 4); and 3) parasympathetic mediation of reflex bradycardia may have been impaired (Table 4). These findings suggest that after obese rats had been maintained on a high fat diet for 10 months they had a selective impairment of parasympathetic mediation during reflex bradycardia and their blood pressures were beginning to rise. Whether the impairment in reflex bradycardia contributed to or resulted from the development of mild hypertension remains conjectural.

As in obese hypertensive patients, attempts to diagnose the presence or absence of borderline hypertension in obese rats are also fraught with technical difficulties. Because the use of standard sphygmomanometer cuffs on obese patients results in overestimation of the prevalence of hypertension,23,24 we used larger cuff sensors for tail-cuff measurement to minimize similar errors whenever the rats exceeded 500 g in body weight. Despite this precaution, however, average systolic and mean pressures still became consistently elevated in obese rats beginning at the fifth month of age (Table I). When cut-off points for separating normotensive from hypertensive pressures were then established (by using average values plus 2 SDs from the control group), we further found hypertensive pressures more frequently in obese than in control rats (Table 1). Moreover, although pressure differences recorded later from indwelling femoral catheters in the same rats were no longer as large as those detected indirectly by tail-cuff measurement, average mean pressures still remained significantly higher in obese rats (Table 2) thereby confirming the conclusion that they had become mildly hypertensive.

Whether other rat models for obesity also develop hypertension is debatable. Although hypertension develops consistently in spontaneously hypertensive rats with genetic traits for obesity,12,13 the model seems somewhat unnatural because all the obese rats are hypertensive. In genetically obese male Zucker rats, Kasikse et al25 found elevations in tail-cuff systolic pressure averaging 14, 11, and 25 mm Hg at ages of 12, 24, and 60 weeks, respectively, but there was considerable overlap between lean and obese rats, and their tail-cuff measurements were never verified by direct measurement. By contrast, Barringer and Buñag9 recently obtained almost identical mean pressures (recorded from indwelling femoral catheters) in conscious 3-month-old lean (92±2 mm Hg) and obese (91±2 mm Hg) female Zucker rats. Likewise equivocal are the blood pressure data from obese rats with ventromedial hypothalamic
(VMH) lesions. Bernardis and Skefton showed that although VMH lesions prevented the usual increase in tail-cuff systolic pressure occurring in 27-day-old rats, similar lesions made at later ages were ineffective. Mean carotid pressures recorded by Reisin et al were 10 mm Hg higher in VMH-lesioned than in sham-operated (with averages of 128 ± 3 and 118 ± 3 mm Hg, respectively) rats anesthetized with pentobarbital, but then Reisin and Wilson, using either tail-cuff or intra-arterial measurements, were later unable to find any significant differences between lesioned and sham-operated rats. Hence, the existence of hypertension in rats with obesity induced either genetically or by VMH destruction remains unresolved. In both instances, some discrepancies may have resulted from inaccuracies in tail-cuff measurement that should have been authenticated by recording intra-arterial pressures from indwelling catheters in the same rats.

Assuming that the obese rats we studied here were indeed hypertensive, we next examined baroreceptor reflex sensitivity to determine if they could still regulate blood pressure normally. Unlike obese but normotensive Zucker rats in whom reflex bradycardia and tachycardia were both blunted, the baroreceptor reflex impairment we found was limited only to reflex bradycardia. It can be argued that because reflex bradycardia would tend to lower blood pressure, its inhibition favors development of hypertension, but whether reflex bradycardia became reduced before or after hypertension developed is unknown. Possible cardiovascular effects in our obese rats of the high fat diet or of behavioral stresses due to differences in litter size cannot be completely ruled out. However, because behavioral stresses would have occurred long before blood pressure became elevated, their participation in the development of hypertension is unlikely. Whereas behavioral stresses would start as soon as litters were redistributed (i.e., immediately after birth), the first significant elevation in tail-cuff systolic pressure occurred only at 20 weeks of age (Table 1).

Abolition of all heart rate responses after combined autonomic blockade using both methylnitropine and propranolol indicates that these responses were mediated through efferent parasympathetic and sympathetic pathways. Cholinergic blockade with methylnitropine would eliminate parasympathetic mediation so that only sympathetic activity would remain to increase during reflex tachycardia and decrease during reflex bradycardia. Conversely, β-adrenergic blockade with propranolol would remove sympathetic mediation to leave only parasympathetic activity to increase during reflex tachycardia and pressure during reflex bradycardia. Based on this scheme, therefore, mediation of residual reflex responses remaining after cholinergic blockade would be solely by sympathetic activity, and those remaining after β-adrenergic blockade only by parasympathetic activity. Thus, the finding that reflex tachycardia was equally reduced in both rat groups during either cholinergic impairment (Table 3) or β-adrenergic (Table 4) blockade indicates that mediation of reflex tachycardia, whether sympathetic or parasympathetic, was unaltered by obesity. On the other hand, the more pronounced antagonism of reflex bradycardia during β-adrenergic (but not during cholinergic) blockade (Table 5) indicates that residual parasympathetic mediation of reflex bradycardia was already declining in obese rats. Aside from alterations in reflex pathways, the impairment in reflex bradycardia could also be explained by alterations in efferent receptor responsiveness.

Exactly how obesity causes cardiovascular dysfunction is still undetermined. Possibly related are the extensive studies by Landsberg and Young who showed that in rats and mice fasting suppresses, and overfeeding (with sucrose) stimulates, sympathetic activity. If their findings mean that obesity resulting from overfeeding would induce sympathetic overactivity, then diminished parasympathetic mediation of reflex bradycardia in obese rats could represent yet another indication of a beginning autonomic imbalance inclined toward sympathetic predominance. This interpretation may, however, be oversimplified as it would not account for our failure to find significant differences in residual sympathetic mediation (Table 3) after cholinergic blockade.

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