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Parasympathetic Response to Acute Stress is Attenuated in Young Zucker Obese Rats

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Abstract

We compared arterial blood pressure (BP) and heart rate (HR) control in 9 to 11 week old obese Zucker rats (n=10; weight = 452 ± 45 g, average $\pm$ SD) to age-matched, lean Zucker animals (n=13; weight = 280 ± 46 g). BP was measured by indwelling catheter. Baseline pressure was 113.1 ± 7.0 mm Hg in the lean vs. 111.7 ± 5.6 in the obese rats (NS). Baseline HR was 413 ± 43 in the lean vs. 422 ± 22 bpm in the obese animals (NS). Rats were classically conditioned by following a 15-sec. tone (CS+) with a 0.5-sec. tail shock. There were no between group differences in the BP response to CS+. Conversely, heart rate (HR) decreased significantly ($p < 0.05$) more during the last 10 seconds of the tone in the lean group (-46.0 ± 21.5 bpm) vs. the obese (-17.8 ± 21.7 bpm). This bradycardia was blocked by atropine. Finally, the change in HR divided by the change in arterial BP ($\Delta\text{HR}/\Delta\text{BP}$) following an intravenous bolus of phenylephrine (PE; $5 \mu\text{g/kg}$) and following sodium nitroprusside (NP; $5 \mu\text{g/kg}$) was determined. The $\Delta\text{HR}/\Delta\text{BP}$ following PE was smaller in the obese (n=6; -1.36 ± 0.60) vs. lean (n=5; -2.80 ± 0.92); there was no difference in the response following NP. These data indicate that the BP response to a challenge did not differ in the obese rat vs. the lean animal, but that the obese subjects had an attenuated parasympathetic response to the stress, probably secondary to alterations in baroreflex function.

Keywords

autonomic nervous control; classical conditioning; maturation

INTRODUCTION

Obesity is associated with increased incidence of cardiovascular disease, notably including hypertension (e.g., Rexrode, 1996). The role of the sympathetic nervous system in the etiology of the arterial blood pressure (BP) dysregulation in obesity has been rather extensively examined, but any abnormalities in parasympathetic control of cardiovascular function in this

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condition are relatively less well documented. In addressing issues related to regulation of arterial BP and heart rate (HR), we have found that challenging an animal using a sudden, carefully controlled classical aversive (i.e., Pavlovian) conditioning paradigm yields considerable insight into autonomic nervous function (Randall, et al., 1994). The purpose of the present study, accordingly, is to apply this methodology to evaluate the control of the arterial BP and HR conditional response in young (i.e., 9–11 weeks) obese Zucker rats vs. lean animals before and after pharmacologic autonomic blockade. We also particularly explore the role of parasympathetic division in the two strains by evaluating the baroreflex control of the HR response to injection of vasoactive drugs.

We have previously described the hemodynamic response to a sudden-onset behavioral challenge for Sprague-Dawley rat (Randall, et al., 1994). In this animal presentation of a 15 sec. tone (CS+) followed by 0.5 sec. tail shock evokes a sudden, large initial increase in blood pressure (the C₁ component of the response); pressure is then modestly elevated above the resting baseline for the remainder of the tone (C₂ component). C₁ is preceded by an abrupt, short and intense burst of sympathetic nervous activity and thereby has characteristics indicative of an open loop control (Randall, et al., 1994). C₂ is accompanied by a modest, but sustained increase in sympathetic activity (Randall, et al., 1994). In our conditioning paradigm another tone, never followed by shock (i.e., CS–), serves as a discriminative stimulus. In well-trained subjects CS– evokes a C₁ BP increase, but there is no significant increase in pressure corresponding to C₂ (El-Wazir, et al., 2005). We now apply this approach to examine BP and HR regulation in obesity because of the high degree of experimental control it affords, the ability to evoke the response pattern repetitively, the existence of quantitative assessments of the magnitude of the learned response, and the existing insights into the autonomic control of the response. We find no differences in the C₁ and C₂ components of the BP response, but report here that the HR slows less in the obese subject, particularly during C₂. The effects of autonomic antagonists upon the conditional response pattern, and the decreased “gain” of the HR component of the reflex response to a drug-induced pressor event, is consistent with an attenuation of the parasympathetic control of HR in the obese animals. This abnormality could play a role in the etiology of obesity-related hypertension.

METHODS

Animals

Ten obese Zucker rats at an age range of 9–11 weeks old, and thirteen age-matched lean subjects from Harlan Industries (Indianapolis, IN) were used in the conditioning study; the responses to vasoactive drug administration were examined in 6 obese and 5 lean subjects. All the animals were housed in a climate-controlled room with a 12-hour light-dark cycle. They were allowed free access to water and regular rat chow except for the time of experimentation. The experimental protocol was approved by the University of Kentucky Animal Care and Use Committee.

Behavioral conditioning to stressful stimuli

Animals were behaviorally trained using a classical (i.e., Pavlovian) conditioning paradigm as previously described (El-Wazir, et al., 2005; Randall, et al., 1994). Briefly, the animals were habituated for 1–2 hours daily for several days to handling and restraint in a comfortable cloth sock. Each rat was then exposed daily to five trials of a 15-second pulsed tone (CS+) and another five trials of a continuous (i.e., steady, non-pulsed; CS–), but otherwise identical tone; the CS+ and CS– tones were presented in pseudorandom pairs (e.g., ...CS+, CS–, CS–, CS+ ...). Only the last pulsed tone was reinforced with the 0.5 sec. shock on this initial training day. The intensity of the shock was adjusted to the lowest value that caused the rat to flinch and squeak which was usually about 0.2 mA and never exceeded 0.3 mA. Training continued for

two additional days with each CS+ shock reinforced. A minimum of five minutes was allowed between presentations of successive trials to allow the studied variables to return to baseline. Each rat was removed from the sock at the end of each daily session and was returned to the animal quarters.

Surgery

All surgical procedures were done under pentobarbital anesthesia (65 mg/kg IP) with aseptic techniques appropriate for rodent survival surgery. A teflon catheter with an internal diameter of 0.012 inch, was introduced into the descending aorta via the femoral artery for measurement of the arterial pressure. Another catheter was introduced into the femoral vein for administration of drugs. Both catheters were tunneled subcutaneously and exited out of the nape of the neck. The animals were allowed a minimum of two days recuperation before further experimentation.

Experimental procedures and protocol

After the recovery period, the rats were reintroduced to the conditioning paradigm and HR and BP conditioning data were collected for two or three consecutive days. Blood pressure was measured using a calibrated transducer (Cobe model CDX-III) connected to a Grass 7-channel polygraph (Model 7H2560). Heart rate was obtained from the pulsatile arterial pressure signal. On the next day the procedure was repeated with autonomic blockade in 6 obese and 8 lean rats. A control recording of blood pressure response to 2 CS+ and 2 CS- trials was performed. A similar set of trials was obtained starting 10 minutes after cholinergic blockade with atropine sulfate (0.1 mg/100 gm iv). Then, another similar recording was made after adding β -adrenergic blockade with propranolol hydrochloride (0.1 mg/100 gm iv) to study the BP response under the effect of both drugs. On the following day, the procedure was repeated except that propranolol was used first to study the effect of isolated β -adrenergic blockade. In an adjunct study in 6 obese and 5 lean rats arterial pressure was recorded in response to administration of an intravenous bolus of phenylephrine (PE; 5 μ g/kg) or sodium nitroprusside (NP; 5 μ g/kg) in the autonomically intact state. The animals were resting undisturbed in the sock restraint. Responses to both agents were recorded in each rat with sufficient time between trials for BP to return to baseline values.

Data collection

Heart rate and blood pressure were sampled at 10,000 Hz using an analog-to-digital converter and a microprocessor; data were acquired, "compressed," and analyzed using a "browser" developed in-house (ViiSoftware, Lexington, KY), as described in more detail elsewhere (El-Wazir, et al., 2005). Sampling began 9 seconds before presentation of either tone and ended 6 seconds after its termination. Data were recorded over at least five trials of each tone. Ten individual CS+ trials were ensemble averaged for each rat to construct a "high resolution" analysis of the conditional response for that animal (Randall, et al., 1994; El-Wazir, et al., 2005); likewise, CS- trials were ensembled for each animal. Data collection for the drug trials commenced before infusion and extended beyond the attainment of maximum BP and HR changes.

Data analysis

The magnitude of the mean arterial BP and HR conditional response pattern was assessed relative to the beat-by-beat average BP or HR during the pre-tone baseline period. C_1 was assessed in terms of the peak change during the initial ~2 seconds of the tone (i.e., C_1 peak) as well as the average change during C_1 . C_2 is given as the average change over baseline during the last 10 sec. of the tone. We also assessed the peak BP or HR after shock delivery (i.e., the unconditional response, or UR). Finally, the change in HR divided by the change in arterial

BP ($\Delta\text{HR}/\Delta\text{BP}$) was assessed during a smooth portion of the rising (PE) or falling (NP) BP response to the vasoactive agents; segments that included skipped beats or unusual BP or HR transients were rejected. All data are expressed as mean $\pm$ standard deviation. Comparison between groups of data representing effects of drugs was made using ANOVA with Bonferroni post-hoc t-tests when allowed by significant ANOVA findings or, for the PE and NP data, by t-tests. Statistical significance was accepted for $p < 0.05$.

RESULTS

Weights and Baseline Arterial Pressure and Heart Rate

The average weight of the obese Zucker rats tested in the conditioning paradigm (452 ± 45 g) was significantly greater than their lean counterparts (280 ± 46 g). Pre-tone baseline arterial BP was 113.1 ± 7.0 mm Hg in the lean and 111.7 ± 5.6 in the obese rats (NS). Baseline HR was 413 ± 43 bpm in the lean and 422 ± 22 bpm in the obese animals (NS).

Conditional BP and HR responses in lean and obese rats

Figure 1 shows an illustrative arterial BP and HR responses to CS+ (top) and CS- (bottom) for a lean animal. The dark bar on the time axis indicates the beginning of the tone at 9 seconds and, for CS+, the presentation of the unconditional stimulus (US) shock 15 seconds later. The shock-reinforced CS+ trial shows the expected BP response pattern (Randall, et al., 1994) consisting of the initial C_1 pressor event followed by the C_2 phase that is sustained during the remainder of the tone (dark bar, time axis). There was only a very modest change in HR coincident with C_1 , but a definite bradycardia throughout C_2 . There was essentially no change in either variable during the CS- tone (i.e., that was never followed by shock), demonstrating that the animal discriminated between the two behavioral situations.

Figure 2 shows a “high resolution analysis” of the conditional response to CS+ for a lean animal (left panel; same animal as in Figure 1) and for the obese rat (right panel) run concomitantly with the lean animal. Each panel was constructed by ensemble averaging two days of mean arterial BP (top) and HR (bottom) responses for CS+ trials ($n=10$); as such, they faithfully represent the form and amplitudes of the two responses. For ease of comparison across animals the data for each rat are plotted using the same scales. The dark bars on the time axis demarcate the presentation of the tone. The HR tracing during delivery of the US shock immediately following the tone is unreliable because of poor triggering from the phasic BP signal. Comparison of the two responses suggests a similarity in the pattern and amplitude of the BP conditional responses, but documents a clearly smaller bradycardia during the tone in the obese as opposed to the lean animal.

Figure 3 shows the average changes in BP and HR in both obese (dark bar) and lean (open bar) subjects, in response to the different phases of CS+ trials. There were no between group differences in any of the arterial pressure components of the conditional (i.e., peak C_1 , avg. C_1 , C_2) or unconditional responses. The bradycardia relative to pre-tone baseline for the HR conditional response was statistically significant for the lean ($F_{4,48} = 12.9$) and for the obese ($F_{4,36} = 7.3$) rats. Particularly note that there were significant between group differences in the HR response (CR) pattern and that the Group $\times$ CR interaction was significant ($F_{2,42} = 4.62$). In fact, Figure 3 confirms that the average HR slowing was significantly smaller (*) in the obese vs. lean rats for the average and peak C_1 and, especially, during the last ten seconds of the tone (i.e., C_2) where HR in the lean animals slowed by 46.0 ± 21.5 bpm while the obese rats slowed (only) 17.8 ± 21.5 bpm. This difference was seen easily in the illustrative data shown in Figure 2. Finally, the figure shows that the lean rats discriminated between CS+ and CS- (indicated by #) in both the BP and HR components of the response while the obese showed discrimination only in the C_2 BP response.

Effect of autonomic blockade on conditional response

Table 1 summarizes pre-tone baseline HR and BP for both groups of animals before and after the pharmacologic antagonist administration. There were no statistically significant drug effects on resting (i.e., pre-tone) arterial BP. In contrast, β -blockade decreased HR while atropine increased HR in both groups. There were no between group differences in the drug effects upon the BP components of the behavioral response. Figure 4 summarizes the effects of autonomic blockade upon the HR response for the CS+ tone in the lean and obese rats; data are average change $\pm$ SD relative to pre-tone baseline. In the lean group, ANOVA within this group revealed significance across the components (pre-tone, peak C_1 , avg. C_1 , C_2) of the conditional response ($F_{4,28} = 25.72$), and across treatments (no drug, propranolol, atropine, propranolol + atropine; $F_{3,21} = 51.17$) with a significant interaction ($F_{12,84} = 7.95$). The conditional decrease in HR during C_2 in these animals was significant relative to pre-tone baseline in the no-drug condition, and following propranolol. Conversely, atropine alone, or in combination with propranolol, essentially eliminated the conditional bradycardia. For a between groups ANOVA the group $\times$ drug $\times$ conditional response interaction was significant ($F_{6,66} = 5.64$). In the obese group, ANOVA within this group revealed significance across CR components ($F_{3,12} = 12.94$) and across treatments ($F_{3,12} = 26.12$), again with a significant interaction ($F_{9,36} = 4.98$). Atropine administration to the obese animals eliminated the C_2 HR slowing ($F_{2,8} = 4.33$; $p=0.053$).

Response to Vasoactive Drug Administration

The 6 obese animals tested with NP and PE weighed 421 ± 49 g, while the 5 lean animals weighed 223 ± 24 g. Figure 5 summarizes the Δ HR/ Δ BP findings in the two groups. The index of the gain of baroreflex function was significantly larger in response to PE injection in the lean as compared to the obese rats. There was no significant difference in the HR acceleration for a given drop in BP following NP infusion.

DISCUSSION

The obese Zucker fat rat (fa/fa) simulates many of the characteristic features of human obesity such as hyperinsulinemia, hyperlipidemia and insulin resistance (Jeanrenaud, 1988; Ruggeri, et al., 2006; Schreihof, et al., 2007). We now report that both the obese and lean strains exhibited similar arterial pressure increases during a controlled, acute stress, but the obese animals demonstrate significantly attenuated HR slowing compared to age-matched lean animals. These findings are consistent with a diminished cardiac parasympathetic nervous reactivity in young, obese Zucker rats.

Baseline HR and BP

Pre-tone baseline arterial BP was not significantly different between lean and obese subjects at 9–11 weeks of age. This is consistent with previous findings of Pawloski et al. (1992) in Zucker rats and also in some studies in established human obesity (Stoddard, et al., 1992). Yakubu-Madus and colleagues (1999) report average 24-hour arterial pressures in male Zucker rats which were 8–12 weeks old of 111 ± 1 (SE) mm Hg, which is the same as we report here during the pre-tone interval. Conversely, in a recent study D'Angelo et al. (2006), using 24-hour telemetry, reported elevated arterial BP in obese Zucker rats (122 ± 5 mm Hg, mean $\pm$ SE vs. 100 ± 2 mm Hg in lean littermates) that were ~3–4 weeks older than ours. The older age of these latter animals may explain the differences between our findings since arterial pressure in the Zucker animal is age dependent (Khan, et al., 2005). Finally, Khan, et al. (2005) reported radiotelemetry pressures from ~10 week old Zucker obese rats (~104 mm Hg; read by present authors from graph) that modestly, but significantly, exceeded those in the lean strain (~95 mm Hg); pressure increased progressively with age in the obese strain.

Elevated renal sympathetic activity, leading to a decreased renal sodium excretion, has been thought to link obesity and hypertension (Hall, 1994; Kassab, et al., 1995). Although direct recording of the renal sympathetic nerve activity in the obese Zucker rats has shown increased activity compared to the lean rats, the resting HR and BP were not elevated except when the obese animals were kept on a high sodium intake (Morgan, et al., 1995). Our animals were kept on a regular salt intake. This suggests that the proposed role of renal sympathetic nerve activity in sodium retention and genesis of hypertension needs to be reinforced by a high salt intake.

Cardiovascular response to an acute behavioral challenge in the obese vs. lean rats

The effects of obesity upon the BP “stress response” are not well-resolved, but D’Angelo, et al. (2006) recently reported a greater “area under the curve” (AUC, or “integrated pressor response”) of the BP recording from Zucker obese (13–16 weeks of age) during three minutes of air jet stress. A physiological basis for such an enhanced pressor response, in fact, may well be provided by the observation that the increase in renal sympathetic nerve activity evoked by electrical stimulation of the sciatic nerve (i.e., a somatosympathetic reflex) was larger in seven week old, anesthetized Zucker obese vs. lean controls, despite the fact that the two strain’s arterial pressures were similar (Ruggeri, et al., 2006). The apparent differences between our findings (i.e., similar BP response in obese vs. lean subjects) and those of D’Angelo, et al. (2006) are probably attributable to (a) the different stressors (e.g., the novel air jet vs. the learned conditional response) and (b) the age differences of the obese rats in the two studies. We believe this demonstrates the importance of carefully specifying the nature of the behavioral “stress” in experiments of this nature.

In our study the conditional bradycardia was attenuated in obese compared to the lean subjects. This bradycardia was abolished by atropine, but not by propranolol, indicating that it is mediated by an increased vagal discharge rather than by withdrawal of sympathetic outflow to the heart. Attenuated HR slowing was also reported in diet-induced obesity in Wistar rats after pharmacologic elevation of BP (Buñag, 1990) and in aged, genetically obese Zucker animals as a response to an inhibitory avoidance situation (Nyakas, et al., 1995). HR slowing during stress, in fact, is reportedly attenuated at 14–15 months of age and disappears in older rats (Nyakas, et al., 1990). Conversely, chronically elevated parasympathetic drive purportedly contributes to the pathogenesis of congenital obesity in the pre-obese stage (Rohner-Jeanrenaud, et al., 1983; Zucker, et al., 1972). Our observations suggest that any such age-dependent changes initially appear when the parasympathetic system is challenged.

This vagal cardiac dysfunction in obesity may be related to the insulin resistance that is associated with obesity. Insulin resistance, without the confounding factors of obesity, diabetes, and significant hypertension, reportedly is associated with a large reduction in vagal activity, which occurs via attenuation in reflex activity (Miller, et al., 1999). Leptin is another hormonal factor that can affect the cardiac autonomic activity. It was shown that elevated plasma leptin levels were associated with higher LF/HF ratio of heart rate variability suggesting a shift towards a sympathetic predominance (Paolisso, et al., 2000). Finally, HR response to infused phenylephrine in the Zucker strain (~3 months of age) is reportedly blunted compared to lean animals (Barringer, et al., 1989; Pamidimukkala, et al., 1996), suggesting impaired baroreflex function. Our $\Delta\text{HR}/\Delta\text{BP}$ data for PE infusion support this conclusion for our rats of a similar age, though we find no difference following NP infusion. A recent study of juvenile (7 wk old) and adult (13 wk old), anesthetized Zucker obese and lean rats clearly showed blunted HR and SNA responses to baroreflex activation in the adult obese animals compared to the age-matched lean controls, but not in the juvenile subjects (Schreihöfer, et al., 2007). Finally, weight loss (5–10%) in overweight younger (~33 years) and older (~60 years) men increased baroreflex sensitivity (Alvarez, et al., 2005).

The possibility that a strain difference, rather than obesity *per se*, explains the different conditional HR response cannot be rejected out-of-hand. One immediate possibility, for example, is that the obese rat's perception of pain is dulled. This seems unlikely, however, given that the unconditional increase in BP was virtually identical in the two groups. Likewise, the observation that the BP responses were almost identical in amplitude and form argues against there being a difference in "learning" across groups, or even a difference in auditory acuity.

Clinical implications of altered autonomic function in obesity

Our results suggest that the syndrome of genetic obesity is associated with an attenuated vagal cardiac control as early as 9–11 weeks-of-age in the Zucker rats. Although this attenuation may not be manifest during the resting state, it became uncovered during our behavioral challenge. Deterioration of visceral function regulation in obese subjects probably is not confined to only one of the autonomic nervous system divisions, but results from the combined dysfunction of both sympathetic and parasympathetic controls. Multiple factors may contribute to inappropriate function in each division. In particular, "learned" autonomic response patterns to repeated stressful situations, such as occurs in our conditioning paradigm, might mitigate a tendency for sympathetic over-reaction to novel stressors, and, at the same time, exacerbate parasympathetic dysfunction in repetitive stress situations. Conversely, the effects of sympathetic arousal, even if mitigated by learning, may be exaggerated by the reported delay in recovery of cardiovascular pressor response to behavioral stress in obese Zucker rats due to an impaired β -adrenergic activity (D'Angelo, et al., 2006). Under such circumstances, any progressive deterioration of the vagal "break" would be expected to yield an abnormal hemodynamic response to repeated, daily stresses.

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Figure 1.

Illustrative arterial blood pressure and heart rate response to CS+ (top) and CS- (bottom) in a lean rat. Conditional stimulus, CS+ pulsed tone (dark bar on time axis) began at 9 seconds, lasted for 15 seconds, and was followed immediately by a 0.5 sec. unconditional stimulus (US) tail shock. BP panels are digital reconstructions of original analog recording. BP response to CS+ consisted of an initial, but transient, pressor component (i.e., C₁) followed by a more sustained second component (i.e., C₂). The BP unconditional response (UR) was a sharp, transient pressor event. The behaviorally conditioned (i.e., learned) HR response in this lean subject was dominated by a prominent bradycardia concomitant with C₂. The lack of response to the steady CS- tone, which was never followed by shock, demonstrates that the subject discriminated between the two situations.

Figure 2.

“High resolution analysis” of the conditional mean arterial pressure (top) and heart rate (bottom) response in a lean rat (left) and a paired obese rat (right). Each was constructed by ensemble averaging 10 trials conducted during the first two days of the experiment. Calibrations on BP and HR panels are identical in both panels. Dark bars on time axis demarcate duration of CS+ tone. Data for lean animal are from the same rat as Figure 1. The pattern and amplitude of the mean BP response are similar in both strains. The progressive, robust bradycardia during the latter seconds of CS+ in the lean animal exceeded the HR slowing in the obese subject.

Figure 3.

Summary of average change (Δ) in arterial blood pressure (top) and heart rate (bottom) relative to pre-tone baseline for the indicated components of the conditional response in lean (open bars; $n=13$) and obese (solid bars; $n=10$) rats. A “hat” on the SD bar indicates that the given change was statistically significant relative to pre-tone baseline. A “*” indicates that the indicated response component differed ($p<0.05$) between the lean and obese animals. Note the absence of between-group differences in the BP components of the response. Conversely, the conditioned HR slowing in the lean animals exceeded that in the obese subjects. A “#” shows a significant difference for the indicated component between CS+ and CS-. The BP components showed discrimination for both strains, but only the lean animals showed HR changes that differed across tones.

Figure 4.

Average change (Δ) $\pm$ SD relative to pre-tone baseline in HR for lean ($n=8$; top panel) and obese ($n=6$; bottom panel) rats during CS+ during C₁ (left) and during C₂ (right) without autonomic blockade (C; solid bar), following atropine (A; vertically hatched), following beta adrenergic blockade (β B, cross hatch) and following combined blockade (A+ β B, open bar). Average C₁ HR response in obese rats following A+ β B was too small (-0.2 ± 1.3) to depict at scale used. Muscarinic blockade in both groups essentially eliminated the C₂ conditional bradycardia; the significantly smaller bradycardia in unblocked, obese rats is consistent with there being a lesser increase in parasympathetic activity during acute stress in these subjects. A “hat” on the SD bar indicates that the given change was statistically significant relative to pre-tone baseline. # = $p < 0.05$ atropine vs. control and vs. β B; + = $p < 0.05$, atropine vs. control; * = $p < 0.05$ vs control and β B; ^ = $pp < 0.05$, atropine plus β B vs β B alone.

Figure 5.

Average change (Δ) in HR divided by average change (Δ) in BP $\pm$ SD during pressor response evoked by intravenous bolus infusion of phenylephrine (PE; left) and during depressor response evoked by intravenous bolus infusion of nitroprusside (NP; right) in lean (n= 5; open bar) and obese (n=6; solid bar) rats. The index of baroreflex gain was significantly smaller in obese rats in response to PE. Conversely, there was no detectable difference in baroreflex function in response to the fall in arterial BP.

Table 1
Average $\pm$ SD Baseline Arterial BP and HR before and after β -adrenergic and/or Muscarinic Receptor Antagonist Administration

	Baseline	Lean		Obese		Baseline	β B	β B+MB	MB	β B+MB
		β B	MB	β B	MB					
aBP	117 $\pm$ 7	117 $\pm$ 8	116 $\pm$ 7	105 $\pm$ 7	107 $\pm$ 12	109 $\pm$ 10	105 $\pm$ 7	115 $\pm$ 4	107 $\pm$ 12	104 $\pm$ 8
HR	427 $\pm$ 32	384 $\pm$ 36 *	470 $\pm$ 52 *,†	311 $\pm$ 40 *	454 $\pm$ 44 *,†	399 $\pm$ 58	311 $\pm$ 40 *	405 $\pm$ 38	454 $\pm$ 44 *,†	374 $\pm$ 38

aBP = arterial blood pressure; β B = propranolol; MB = atropine; β B+MB = propranolol + atropine;

* = $p < 0.05$ vs. pre-tone baseline;

† = $p < 0.05$ vs. β B alone and vs. β B+MB