Rosiglitazone improves insulin sensitivity and baroreflex gain in rats with diet-induced obesity

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**Short title:** Rosiglitazone improves baroreflex gain in obese rats

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baroreflex gain (BRG)

thiazolidinediones (TZDs)

peroxisome proliferator-activated receptor-γ (PPAR-γ)

Diet-induced obesity (DIO)

High fat diet (HFD)

Obesity prone rat (OP)

Obesity resistant rat (OR)

Control rat (CON)

Mean arterial pressure (MAP)

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ABSTRACT

Obesity decreases baroreflex gain (BRG); however, the mechanisms are unknown. We tested the hypothesis that the impaired BRG is related to the concurrent insulin resistance, and, therefore, BRG would be improved following treatment with the insulin sensitizing drug, rosiglitazone. Male rats fed a high fat diet (HFD) diverged into obesity-prone (OP) and obesity-resistant (OR) groups after 2 weeks. Then, OP and OR rats, as well as control (CON) rats fed a standard diet, were treated daily for 2-3 weeks with rosiglitazone (3 or 6 mg/kg), or its vehicle by gavage. Compared to OR and CON rats, conscious OP rats exhibited reductions in BRG (in bpm/mmHg: OP, 2.9±0.1; OR, 4.0±0.2; CON, 3.9±0.2; P<0.05) and insulin sensitivity (hyperinsulinemic euglycemic clamp in mg/kg·min: OP, 6.8±0.9; OR, 22.2±1.2; CON, 17.7±0.8; P<0.05), which were well correlated (r²=0.49; P<0.01). In OP rats, rosiglitazone dose dependently improved (P<0.05) insulin sensitivity (in mg/kg·min: to 12.8±0.6, 3 mg/kg; 16.0±1.5, 6 mg/kg) and BRG (in bpm/mmHg: to 3.8±0.4, 3 mg/kg; 5.3±0.7, 6 mg/kg). However, 6 mg/kg rosiglitazone also increased BRG in OR rats without increasing insulin sensitivity, disrupted the correlation between BRG and insulin sensitivity (r²=0.08), and, in OP and OR rats, elevated BRG relative to insulin sensitivity (ANCOVA, P<0.05). Moreover, in OP rats, stimulation of the aortic depressor nerve, to activate central baroreflex pathways, elicited markedly reduced decreases in heart rate and arterial pressure, but these responses were not improved by rosiglitazone. In conclusion, diet-induced obesity impairs BRG via a central mechanism that is related to the concurrent insulin resistance. Rosiglitazone normalizes BRG, but not by improving brain baroreflex processing or insulin sensitivity.
INTRODUCTION

Obesity is associated with multiple serious complications. Within the cardiovascular system, one prominent consequence is dysfunction of the baroreceptor reflex; in particular, baroreflex sensitivity or gain (BRG) is decreased (Schreihofer et al., 2007; Barringer and Bunag, 1989; Bunag and Barringer, 1988; Grassi et al., 1998; Emdin et al., 2001). Decreased BRG is a risk factor for the subsequent development of adverse cardiovascular events in patients with type 2 diabetes mellitus (Okada et al., 2010), a common consequence of obesity. Therefore, treatment options are clearly needed.

In obese humans, weight loss improves baroreflex function (Emdin, Gastaldelli, Muscelli, Macerata, Natali, Camasta, and Ferrannini, 2001; Grassi, Seravalle, Colombo, Bolla, Cattaneo, Cavagnini, and Mancia, 1998; Straznicky et al., 2005). However, the success of weight loss as a therapeutic strategy to reverse the pathophysiologic consequences of obesity has been limited (Mark, 2008). As an alternative, pharmaceutical approaches have been investigated. One class of drugs that has been widely prescribed to treat the insulin resistance and hyperglycemia often associated with obesity are thiazolidinediones (TZDs). These drugs activate the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) to increase insulin sensitivity via multiple mechanisms, including improvement of adipocyte energy storage, production of insulin-sensitizing factors, and actions in adipocytes and macrophages to inhibit production of cytokines, which reduce insulin sensitivity (Berger and Moller, 2002; Duan et al., 2008; Tontonoz and Spiegelman, 2008). Several lines of indirect evidence suggest that TZDs may also enhance baroreflex function in obese individuals by improving
insulin sensitivity. First, decreases in BRG and insulin sensitivity are associated in several conditions besides obesity, including metabolic syndrome, type II diabetes, hypertension, heart failure, aging, and pregnancy. Second, the improvements in BRG in obese humans following weight loss are related to increases in insulin sensitivity (Grassi, Seravalle, Colombo, Bolla, Cattaneo, Cavagnini, and Mancia, 1998;Straznicky, Lambert, Lambert, Masuo, Esler, and Nestel, 2005;Emdin, Gastaldelli, Muscelli, Macerata, Natali, Camastra, and Ferrannini, 2001). Third, pioglitazone was shown to improve baroreflex function and to reduce elevated basal sympathetic nerve activity in patients with type 2 diabetes mellitus, soon after myocardial infarction (Yokoe et al., 2012). Finally, our recent studies of pregnancy (Daubert et al., 2007;Brooks et al., 2010) demonstrated that: 1) the development of insulin resistance and baroreflex impairment are temporally correlated in rats and rabbits, and 2) treatment of pregnant rabbits with the TZD, rosiglitazone, normalizes BRG. However, whether TZDs enhance BRG in obese subjects has not been previously investigated.

Therefore, the present experiments tested the hypothesis that rosiglitazone treatment improves obesity-induced baroreflex impairment via increases in insulin sensitivity. We used a rat model of diet-induced obesity (DIO), because it exhibits many of the features of human obesity (Dobrian et al., 2000;Levin and Strack, 2008). We determined if the magnitude of insulin resistance and baroreflex dysfunction are correlated in DIO and control rats and if treatment of DIO rats with rosiglitazone resolves the baroreflex dysfunction in association with increases in insulin sensitivity. In addition, since previous studies indicate that the baroreflex impairment observed in DIO rats and Zucker obese rats is due to a depression of the central processing of
baroreceptor afferent information (Huber and Schreihofer, 2010; McCully et al., 2012), we tested the hypothesis that rosiglitazone improves baroreflex function by normalizing brain control of the baroreflex. To test this hypothesis, we determined if the heart rate (HR) and arterial pressure responses to stimulation of the aortic depressor nerve (ADN) are reduced in rats with DIO and if rosiglitazone enhances these responses.

METHODS

Male Sprague-Dawley rats (Charles River Laboratories, Inc.) were housed individually in cages in a temperature-controlled (22±2°C) room with a 12-hr: 12-hr light/dark cycle. Food and water were provided *ad libitum*. All of the procedures were conducted in accordance with the National Institutes of Health Guide for the Health and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University.

**DIO rat model.** Rats (~250 g) were placed on a purified moderately high fat diet [HFD, 33% kcal as fat; LabDiet 571R (5001 with 10% Lard)] or a low fat diet [13.5% kcal as fat, LabDiet 5001, control (CON) diet]. HFD fed rats diverged into populations with high weight gain, obesity prone (OP), or weight gain similar to those fed the low fat control diet, obesity resistant (OR) (Levin and Strack, 2008). Consistent with significant previous work (Boustany et al., 2005; Levin and Strack, 2008; Dobrian et al., 2004), after 2 weeks on the diet, the top tertile of weight gain was defined as OP, and the bottom tertile, OR. The middle 1/3 rats with intermediate weight gain were not used further.
The OP, OR and CON rats were then treated with rosiglitazone (3.0 or 6.0 mg/kg in vehicle; the amount of rosiglitazone was adjusted every 3 days to account for rat weight gain; GlaxoSmithKline) or vehicle (1% carboxymethyl cellulose sodium salt, 0.1mL/100g; Sigma) daily by gavage until experiments were performed after an additional 2-3 weeks. The lower dose of rosiglitazone (3 mg/kg) was chosen, because it is a frequently used efficacious dose in rats [e.g. (Wang et al., 2010; Khan et al., 2005; Toruner et al., 2004; Moore et al., 2008)]. A higher dose (6 mg/kg) was also tested, since 3 mg/kg failed to completely normalize insulin sensitivity. Importantly, rosiglitazone exhibits first order kinetics in rats, such that a doubling of the dose yields a doubling of plasma levels (Wang, Liu, Zhan, Lavallie, Diblasio-Smith, Collins-Racie, Mounts, Rutkowski, Xu, Goltsman, Abassi, Winaver, and Feuerstein, 2010; Gao and Jusko, 2012).

**Experiments in conscious rats: Does DIO impair baroreflex function in association with insulin resistance and are these changes reversed by treatment with rosiglitazone?**

**Surgery.** After ~3.5 weeks on the diets, rats were weighed and anesthetized with 2% isoflurane in 100% oxygen. An arterial catheter (PE 50) was aseptically inserted through a small inguinal incision into the femoral artery and advanced into the distal abdominal aorta. In addition, two venous catheters (PE 10) were inserted into the femoral vein and advanced into the distal inferior vena cava. The catheters were tunneled subcutaneously and exteriorized between the scapulae. Catheter patency was maintained by flushing with heparin saline (100 U/mL) at least 3 times per week. At least 5 days of recovery were allowed before experimentation.
**Experimental Protocols:** Insulin sensitivity and BRG were determined in random order with at least 1 day between experiments using the following protocols.

**Baroreflex curve generation.** Complete baroreflex function curves were produced in conscious rats while they remained in their home cage using well-established, previously published methodology (Xu and Brooks, 1997; Xu et al., 1998). Briefly, arterial pressure was increased and decreased using separate slow IV infusions of increasing doses of either phenylephrine or nitroprusside, with each ramp in pressure completed in ~3-5 min. Blood pressure and HR were allowed to return to basal levels before another ramp was initiated. The data were collected using a Biopac MP100 data acquisition and analysis system sampling at 1000 Hz. The sigmoidal baroreflex relationships between mean arterial pressure (MAP) and HR generated in each experiment were fitted and compared using the Boltzman equation: \( HR = \frac{(A_1 - A_2)}{[1 + e^{(MAP-A_3)/A_4}]} + A_2 \), where \( A_1 \) equals the maximum HR, \( A_2 \) equals the minimum HR, \( A_3 \) equals the MAP at the midpoint between the minimum and maximum HR, and \( A_4 \) is the width or operating range. Maximum baroreflex gain was calculated by dividing the HR range \( (A_1 - A_2) \) by four times the width. Because of technical difficulties (usually catheter failure), it was not possible to assess baroreflex function in all rats.

**Insulin sensitivity measured using the hyperinsulinemic-euglycemic clamp method.** Well established procedures were used (Daubert, Chung, and Brooks, 2007; Brooks, Mulvaney, Azar, Zhao, and Goldman, 2010; Defronzo et al., 1979). Briefly, the rats were fasted overnight (~15 hr). After a 1 hr equilibration period following connection of the catheters to infusion pumps, arterial blood was collected (five 10 µL samples, 5 min apart) for measurement of basal glucose concentration using a
Freestyle Flash Blood Glucose Monitor. An additional blood sample (250 µL) was collected in vehicle and high dose rosiglitazone-treated rats for measurement of plasma insulin concentration by radioimmunoassay, as previously described (Brooks, Mulvaney, Azar, Zhao, and Goldman, 2010). A priming dose of human insulin (Novolin R, Novo Nordisk Pharmaceuticals, Inc.) was then infused over 10 min, followed by a continuous infusion at 3.0 mU/kg/min (5 µL/min in isotonic saline). Beginning 4 min later, an iv glucose (50%) infusion was initiated. Blood samples (10 µL) were collected every 10 min for measurement of glucose concentration, and the glucose infusion rate was adjusted to maintain euglycemia (plasma glucose same as basal). After attaining a constant glucose infusion rate (after ~2 hr), 3 final blood glucose levels were measured, 10 min apart, to document that a steady state had been achieved. The steady-state glucose infusion rate was used as an index of insulin sensitivity; higher infusion rates indicated higher insulin sensitivity. To confirm that similar insulin levels were produced by the infusions between groups, a final blood sample (250 µL) was collected at the end of the experiment and assayed for human insulin, as previously described (Brooks, Mulvaney, Azar, Zhao, and Goldman, 2010).

**Statistical Analysis.** One way ANOVA was used to determine if conscious OP rats differed from OR and CON rats treated with vehicle (e.g to establish features of the DIO model). Two way ANOVA [factors are group (OP, OR, CON) and drug dose (vehicle, 3.0 mg/kg and 6 mg/kg)] evaluated the effects of rosiglitazone treatment. The post hoc Newman Keuls test was used to identify specific within and between group differences. Since baroreflex gain increases exponentially, these data were log
transformed before statistical analysis to normalize variability. Data are expressed as mean ± SEM. $P < 0.05$ was considered statistically significant.

**Experiments in anesthetized rats: does rosiglitazone improve baroreflex function by reversing the effects of obesity to impair central baroreflex processing?** Rats treated with vehicle (2-3 per group) or rosiglitazone (6-9 per group; 6 mg/kg; as described above) were used. In addition, since the responses of vehicle-treated rats were similar to the responses of untreated rats from a previous study (McCully, Brooks, and Andresen, 2012), these data were combined to statistically assess the effects of rosiglitazone.

**Surgery.** Rats were anesthetized with ~2% isoflurane in 100% oxygen, and a femoral arterial catheter and two venous catheters were implanted for the measurement of MAP and HR, and for IV access, respectively. After the completion of the catheter surgery, an IV infusion of inactin (120 mg/ml, Sigma) was administered over 30 min as the isoflurane was slowly withdrawn. A midline neck incision was made and the trachea was cannulated (PE 250) to facilitate spontaneous breathing. Arterial oxygen levels were continuously monitored via use of a pulse oximeter (Starr Life Sciences, Inc), and if necessary adjustments were made in tracheal catheter position to maintain oxygen levels at or above 95%. The left ADN was identified as it joined the superior laryngeal nerve and dissected caudally, and the peripheral end was cut. To remove potential counteracting responses from other arterial baroreceptors inputs, the right ADN was cut and the carotid sinuses were bilaterally denervated as previously described (McCully, Brooks, and Andresen, 2012). After surgery, a 60 min recovery period was allowed...
before the experimental protocol was initiated. Rectal body temperature was maintained at 37±1°C throughout using a heating pad. At the end of the experiment, rats were euthanized with an overdose of pentobarbital sodium (39 mg Euthosol, IV).

**Experimental protocol: baroreflex responses to electrical activation of ADN.** After dissection, the central end of the ADN was placed on bipolar stainless steel electrodes and isolated in warm mineral oil. The electrodes were connected to a programmable stimulator (AMPI Master-8) through a stimulus isolation unit (AMPI ISO-Flex). The frequency and intensity of electrical shocks were varied to activate myelinated A-fibers alone or both A-fibers and non-myelinated C-fibers. More specifically, low intensity shocks (1 V) were used to activate only A-fibers, a moderate stimulation (3 V) was used to maximally activate all A-fibers plus a small contingent of C-fibers, and a high stimulation intensity (20 V) was used to activate both A- and C-fibers (Fan et al., 1999; Fan and Andresen, 1998). At each voltage, 30 sec stimulus trains of short duration (0.1 msec) repeated shocks were administered at 5, 10, 20, 50, and 100 Hz. Reflex responses were measured as peak decreases in MAP and HR (averaged from 5 sec stable recordings) relative to 10 sec of baseline data collected just before each stimulation period. Intensity and frequency combinations of the stimuli were applied in random order, with at least 2 min between stimulations.

**Statistical Analysis.** Three-way ANOVA [factors were group (OP, OR, CON), treatment (rosiglitazone or vehicle/no treatment), and stimulus parameter] was first performed. This analysis revealed highly significant 3-way group by treatment by stimulus interactions for both HR (P<0.001) and MAP (P<0.0001), as well as significant (P<0.05) group, stimulus, and group by stimulus and treatment by stimulus interactions.
for HR and MAP. Specific between and within group changes were determined using two-way ANOVA and the post hoc Newman-Keuls test.
RESULTS

**Diet-induced obesity decreases insulin sensitivity and baroreflex function.**

After almost 4 weeks on the high fat diet, OP rats exhibited significantly higher body weight compared to either OR rats or rats ingesting a normal fat diet (Table 1); however, body weight did not differ between OR and CON rats. Insulin sensitivity was markedly reduced in OP rats compared to both OR and CON animals (Figure 1). Human insulin levels were similar (Table 2), confirming that the insulin clamp was equivalent between groups. MAP, HR, and fasting blood glucose and insulin levels did not vary significantly among OP, OR and control animals (Tables 1, 2). BRG (Figures 2, 3) was attenuated in OP compared to OR and CON rats. Significant differences in other sigmoidal baroreflex parameters were not observed (Table 2); however, there was a tendency for maximum baroreflex HR to be suppressed in the OP rats (P=0.08). Among the 3 groups of vehicle-treated rats, the degree of baroreflex impairment was well correlated to the severity of the insulin resistance (Figure 4, black filled circles; r²=0.49; P<0.005).

**Rosiglitazone improves insulin sensitivity and baroreflex gain in conscious OP rats.** As expected, rosiglitazone treatment dose-dependently increased insulin sensitivity in OP rats to values exhibited by CON rats (higher rosiglitazone dose, Figure 1); however, rosiglitazone did not alter insulin sensitivity in OR or CON rats (Figure 1). Rosiglitazone increased body weight in CON rats, but not OP or OR rats (Table 1). Nevertheless, body weight remained elevated in OP rats treated with rosiglitazone compared to similarly treated OR and CON rats (Table 1), and the body weights of OR and CON rats were not different. Rosiglitazone did not significantly influence MAP, HR or fasting blood glucose and insulin concentrations (Tables 1, 2).
The low dose of rosiglitazone increased BRG in OP rats but not OR or CON rats (Figures 3, 5), such that the differences between OP and OR or CON rats were eliminated. Other baroreflex parameters were not significantly altered (Table 2). The higher dose of rosiglitazone increased BRG further in OP rats; however, the drug also enhanced BRG in OR (but not CON) rats even though insulin sensitivity was not significantly changed (Figure 3). As a result, the relationship between insulin sensitivity and BRG was shifted upward to a higher gain level in OP, OR and CON rats (Figure 4; ANCOVA, P<0.05) such that BRG was significantly elevated for a given level of insulin sensitivity. Moreover, the correlation between these variables was disrupted (Figure 4, r²=0.08, ns). These data suggest that the higher dose of rosiglitazone increases BRG by mechanisms unrelated to its effect to improve insulin sensitivity.

**HR and MAP responses to ADN stimulation are impaired in OP rats, and rosiglitazone does not reverse this effect of obesity.** As in the first experimental series, OP rats weighed more than OR or CON rats, and basal HR and MAP were not different between groups (Table 4). Stimulation of the ADN produced both intensity and frequency dependent decreases in MAP and HR in all groups (Figures 6, 7; P<0.05). At stimulus intensities that activate largely A-fibers (1 and 3 V), the HR and MAP responses of vehicle-treated OP rats were substantially attenuated compared to similarly treated OR and CON animals (Figures 6, 7). At an intensity that activates both A-fibers and C-fibers (20 V), these between group differences largely disappeared, with a significant difference observed only in the HR responses of OP rats compared to OR rats. In agreement with our previous studies (McCully, Brooks, and Andresen, 2012), these data suggest that obesity impairs the responsiveness of the reflex neuronal
pathway mediated by A-fibers at a site beyond the baroreceptors, most likely in the brain.

If rosiglitazone improves baroreflex function by reversing the effects of obesity, then the bradycardic and depressor responses to low/moderate intensity ADN stimulation should improve as well. However, in contrast to this hypothesis, rosiglitazone treatment of OP rats did not enhance responses except at 3V (maximal A-fiber intensity) and the highest frequency; the responses of OR rats fed a HFD were also largely unchanged (Figures 6, 7). On the other hand, at all intensity levels, the HR responses of rosiglitazone-treated CON rats were significantly reduced; the depressor responses of CON rats were also attenuated at the low and moderate stimulus intensity (Figures 6, 7). These results suggest that in rats fed normal chow rosiglitazone impairs baroreflex responses at a level beyond the baroreceptors (brain or efferent pathway); however, this impairment is neutralized by a HFD.
DISCUSSION

The purpose of the present study was to develop a model of DIO in rats that causes baroreflex impairment and to use this model to test the hypothesis that obesity-induced baroreflex dysfunction can be resolved by rosiglitazone treatment, due to improved insulin sensitivity. The major new findings are that (1) among rats fed a high fat diet, those that become obese (OP) exhibit reductions in BRG and insulin sensitivity, and these variables are highly correlated; (2) the low dose of rosiglitazone improves insulin sensitivity and BRG in OP rats, but not in OR or CON rats; (3) the higher dose of rosiglitazone produces further increments in BRG in OP and OR rats relative to insulin sensitivity, and disrupts the relationship between BRG and insulin sensitivity; (4) OP rats exhibit impaired HR and MAP responses to ADN stimulation, and rosiglitazone does not restore these responses; and (5) rosiglitazone attenuates depressor and bradycardic responses to ADN stimulation in CON rats fed normal chow. Collectively, these data suggest that while obesity may impair baroreflex function via a mechanism related to the concurrent insulin resistance, rosiglitazone reverses this impairment largely through distinct mechanisms.

Considerable previous research has shown that one of the deleterious cardiovascular consequences of obesity is a decrease in BRG (Barringer and Bunag, 1989; Bunag and Barringer, 1988; Grassi, Seravalle, Colombo, Bolla, Cattaneo, Cavagnini, and Mancia, 1998; Emdin, Gastaldelli, Muscelli, Macerata, Natali, Camasra, and Ferrannini, 2001; Schreihofer, Mandel, Mobley, and Stepp, 2007; Van Vliet et al., 1995). Studies in humans suggest that the baroreflex impairment may be related to the accompanying insulin resistance, since body weight reduction improves both BRG gain
and insulin sensitivity (Grassi, Seravalle, Colombo, Bolla, Cattaneo, Cavagnini, and Mancia, 1998; Emdin, Gastaldelli, Muscelli, Macerata, Natali, Camastra, and Ferrannini, 2001). We sought to more directly test this hypothesis using a rat model of DIO. This obesity model has been used extensively in previous energy balance and cardiovascular investigations, and has been shown to exhibit many of the features of human obesity, including a polygenetic basis, activation of the renin-angiotensin and sympathetic nervous systems, and, after a time delay, mild hypertension (Levin and Strack, 2008; Dobrian, Davies, Prewitt, and Lauterio, 2000). A further advantage of the model is that the comparison of OP and OR rats allows identification of mechanisms related to obesity per se, rather than to the high fat diet. Importantly, the results of the present study indicate that this DIO model demonstrates another characteristic associated with human obesity, that of impaired HR BRG.

In the present study, the degrees of baroreflex impairment and insulin resistance were well correlated among vehicle-treated OP, OR and CON rats. This result supports the hypothesis that the obesity-induced falls in insulin sensitivity and BRG share common mechanisms. The development of obesity (increased adiposity) seems pivotal, since a high fat diet failed to decrease BRG and insulin sensitivity in OR rats; however, the specific mediator was not identified. Nevertheless, in OP rats, stimulation of the ADN at intensities that activate mostly A-fibers, which largely mediate baroreflex responses near or below resting arterial pressure (Thoren et al., 1977), produced severely attenuated bradycardic and depressor responses. These data point toward a central mechanism. Therefore, rather than an action of insulin resistance per se, an adipocyte-derived factor that contributes to obesity-induced insulin resistance may also
impair BRG via a direct or indirect action in the brain. Such factors include cytokines, such as TNF-\(\alpha\) (Guggilam et al., 2008; Rosen and Spiegelman, 2006; Bastard et al., 2006; Yu and Ginsberg, 2005), and angiotensin II (Cassis et al., 2008; Paton et al., 2008; Zucker and Liu, 2000).

The finding that rosiglitazone improved BRG in OP rats in association with increases in insulin sensitivity would appear to support the hypothesis that this TZD enhances baroreflex function by decreasing the levels or actions of a factor that both contributes to obesity-induced insulin resistance and also acts centrally to impair the baroreflex. However, other findings contradict this supposition. First, the higher rosiglitazone dose also increased BRG in OR rats, without altering insulin sensitivity, and elevated BRG relative to insulin sensitivity in OP, OR and CON rats. Second, rosiglitazone disrupted the correlation between insulin sensitivity and BRG. Third, while the bradycardic responses to ADN stimulation were markedly attenuated in vehicle-treated or untreated OP rats, rosiglitazone increased BRG in conscious OP rats without improving the ADN HR responses; thus, this TZD did not reverse the effect of obesity. These results suggest that rosiglitazone likely increases BRG via a mechanism independent of its effect to increase insulin sensitivity, which parallels the previous observation that mice that express a dominant negative mutation of PPAR-\(\gamma\) exhibit hypertension without creating insulin resistance (Tsai et al., 2004).

If not related to improving insulin sensitivity, then how does rosiglitazone enhance baroreflex function? Since PPAR-\(\gamma\) is highly expressed in both endothelial and vascular smooth muscle cells (Marchesi et al., 2008; Duan, Usher, and Mortensen, 2008), one possibility is that rosiglitazone improves the baroreflex by enhancing the
responsiveness of baroreceptor afferents that are imbedded in the vasculature (Borges et al., 2009). This possibility is consistent with the paired findings that rosiglitazone markedly enhanced BRG in conscious OP and OR rats, yet did not improve responses to ADN stimulation. Since ADN stimulation bypasses baroreceptor nerve endings, such an effect would be not be evident in these baroreceptor denervated animals, but would be apparent in intact conscious rats. In support, recent preliminary studies demonstrate that mice that harbor a dominant negative mutation of PPAR-γ in either vascular endothelial cells or vascular smooth muscle exhibit markedly impaired baroreflex sensitivity (Borges, Morgan, Ketsawatsomkron, Rahmouni, and Sigmund, 2009; McCully et al., 2011). However, a direct test of this hypothesis requires measurement of the changes in firing of single A-fiber afferents in response to pressure forcings in rosiglitazone-treated rats, since the present results suggest differences in the effect of rosiglitazone on A- versus C-fiber neuronal populations. Alternatively, PPAR-γ is expressed in brain (Sarruf et al., 2009; Moreno et al., 2004), and systemic rosiglitazone can enter the brain to activate PPAR-γ (Lu et al., 2011; Ryan et al., 2011). Therefore, another possible mechanism is that TZDs, by binding to brain PPAR-γ, increase HR independently of baroreflex circuitry.

An unexpected finding in the present study was that rosiglitazone treatment of CON rats significantly attenuated both the depressor and bradycardic responses to ADN stimulation. Whether rosiglitazone acted in the brain or the efferent pathway was not investigated. However, this PPAR-γ agonist likely did not impair the response of the heart to efferent autonomic nerves, since 4-8 weeks of treatment of rats fed a normal fat diet with a higher dose of rosiglitazone (8 mg/kg) failed to alter the HR responses to
propranolol or atropine (Hsieh and Hong, 2008). Similarly, knockout of vascular PPAR-γ attenuates vasoconstriction induced by alpha-adrenergic agonists (Halabi et al., 2008; Wang et al., 2009), suggesting that TZDs enhance, rather than inhibit, vascular responses to sympathetic activation. Instead, rosiglitazone may have diminished brainstem processing of arterial baroreceptor afferent inputs. If so, this result may explain why the high dose of rosiglitazone failed to enhance BRG in conscious CON rats; the action of rosiglitazone to improve BRG was presumably counteracted by another central mechanism that impaired this effect. The fact that the diminished ADN responses of CON rats were not observed in either OP or OR rats suggests that a HFD neutralizes this deleterious action. This possibility is supported by recent studies demonstrating that the actions of PPAR-γ agonists or antagonists (or the loss of PPAR-γ in knockout mice) on energy balance are different depending on the presence or absence of a HFD (Ryan, Li, Grayson, Matter, Woods, and Seeley, 2011; Diano et al., 2011; Lu, Sarruf, Talukdar, Sharma, Li, Bandyopadhyay, Nalbandian, Fan, Gayen, Mahata, Webster, Schwartz, and Olefsky, 2011).

It is well established that TZDs induce weight gain in both humans and experimental animals (Vasudevan and Balasubramanyam, 2004; Lu, Sarruf, Talukdar, Sharma, Li, Bandyopadhyay, Nalbandian, Fan, Gayen, Mahata, Webster, Schwartz, and Olefsky, 2011; Diano, Liu, Jeong, Dietrich, Ruan, Kim, Suyama, Kelly, Gyengesi, Arbiser, Belsham, Sarruf, Schwartz, Bennett, Shanabrough, Mobbs, Yang, Gao, and Horvath, 2011; Ryan, Li, Grayson, Matter, Woods, and Seeley, 2011; Lehrke and Lazar, 2005). As previously reported (Toruner, Akbay, Cakir, Sancak, Elbeg, Taneri, Akturk, Karakoc, Ayvaz, and Arslan, 2004), we found that rosiglitazone increased body weight
in CON, but not OR or OP rats. Recently, brain PPAR-γ signaling was shown to mediate this effect of TZDs in part by stimulating food intake (Lu, Sarruf, Talukdar, Sharma, Li, Bandyopadhyay, Nalbandian, Fan, Gayen, Mahata, Webster, Schwartz, and Olefsky, 2011; Ryan, Li, Grayson, Matter, Woods, and Seeley, 2011). In addition, this recent work indicates that a HFD, presumably by increasing the levels of fatty acids that act as endogenous ligands for PPAR-γ, also activates brain PPAR-γ. Collectively, these findings suggest that rosiglitazone stimulated food intake in CON rats via central activation of hypothalamic PPAR-γ, but did not increase body weight further in OP rats fed a HFD, because brain PPAR-γ was already activated by endogenous ligands. On the other hand, OR rats fail to become obese when exposed to a HFD, because (unlike OP rats) these rats retain sensitivity to the anorexic effects of leptin and insulin (Levin and Strack, 2008). Recent studies suggest that PPAR-γ activation increases food intake by reducing reactive oxygen species (ROS) in pro-opiomelanocortin neurons, whereas leptin inhibits food intake by increasing ROS (Diano, Liu, Jeong, Dietrich, Ruan, Kim, Suyama, Kelly, Gyengesi, Arbiser, Belsham, Sarruf, Schwartz, Bennett, Shanabrough, Mobbs, Yang, Gao, and Horvath, 2011). Therefore, rosiglitazone (or a HFD) may not increase food intake in OR rats, because PPAR-γ activation suppresses ROS less, possibly due to a greater sensitivity of POMC neurons to the ROS-increasing actions of leptin.

In conclusion, DIO in rats impairs baroreflex control of HR through central mechanisms related to the concurrent insulin resistance. Insulin resistance is a hallmark of many conditions associated with baroreflex dysfunction besides obesity, such as type II diabetes mellitus, hypertension, congestive heart failure, and pregnancy. Therefore,
these mechanisms are clearly important to identify, given the propensity of this association and the well-established link between decreased BRG and adverse cardiovascular events (Head, 2002; Okada, Takahashi, Yufu, Murozono, Wakisaka, Shinohara, Anan, Nakagawa, Hara, Saikawa, and Yoshimatsu, 2010; Parati, 2005). Potential mechanisms include hormones that cause both insulin resistance and act centrally to impair the baroreflex, such as angiotensin II and TNF-α. In addition, we show that rosiglitazone treatment improves BRG through mechanisms unrelated to increases in insulin sensitivity. Instead, TZDs may increase BRG by sensitizing baroreceptor afferents and increasing their responsiveness to changes in arterial pressure or by central actions to alter HR independently of brain baroreceptor processing. Future experiments are required to test these hypotheses.
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AUTHORSHIP CONTRIBUTIONS:

*Participated in research design:* Zhao, McCully, Brooks

*Conducted experiments:* Zhao, McCully

*Performed data analysis:* Zhao, McCully, Brooks

*Wrote or contributed to the writing of the paper:* Brooks, Zhao, McCully.
REFERENCES


Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, and Feve B (2006) Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur.Cytokine Netw.* **17**:4-12.


Levin BE and Strack AM (2008) Diet-induced obesity in animal models and what they
tell us about human obesity, in *Neurobiology and Obesity* (Harvey J and Withers DJ

Lu M, Sarruf DA, Talukdar S, Sharma S, Li P, Bandyopadhyay G, Nalbandian S, Fan W,
Gayen JR, Mahata SK, Webster NJ, Schwartz MW, and Olefsky JM (2011) Brain
PPAR-gamma promotes obesity and is required for the insulin-sensitizing effect of

Marchesi C, Paradis P, and Schiffrin EL (2008) Role of the renin-angiotensin system in

Mark AL (2008) Dietary therapy for obesity: an emperor with no clothes. *Hypertension*
51:1426-1434.

McCully BH, Brooks VL, and Andresen MC (2012) Diet-induced obesity severely
impairs myelinated aortic baroreceptor reflex responses. *Am.J.Physiol Heart

McCully BH, Norton BC, Sigmund CD, and Brooks VL. (2011) Dominant-negative
mutation of endothelial peroxisome proliferator-activated receptor gamma (PPAR-


activator rosiglitazone in rodents: a translational medicine investigation.


FOOTNOTES.

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FIGURE LEGENDS

Figure 1. Insulin sensitivity (IS) in OP, OR and CON rats treated with vehicle, 3 mg/kg and 6 mg/kg rosiglitazone. Two way ANOVA revealed significant group (P<0.001) and rosiglitazone treatment (P<0.005) effects, as well as a significant group by treatment interaction (P<0.05). *, P<0.01 compared to OP-vehicle; †, P<0.01 compared to vehicle-group. Numbers in bars are n.

Figure 2. Sigmoidal baroreflex curves constructed from the means of Boltzman parameters (Table 2) derived from fits of relationships between arterial pressure and heart rate obtained in CON (closed circles), OR (gray-filled circles) and OP rats (open circles). n=5 per group.

Figure 3. Baroreflex gain in OP, OR and CON rats treated with vehicle, 3 mg/kg and 6 mg/kg rosiglitazone. Two way ANOVA revealed significant group (P<0.05) and rosiglitazone treatment (P<0.0001) effects, as well as a group by treatment interaction (P<0.05). *, P<0.05 compared to OP-vehicle; †, P<0.05 compared to vehicle-group respectively. Numbers in bars are n.

Figure 4. The degree of baroreflex impairment was well correlated to the severity of the insulin resistance among the vehicle-treated OP, OR and CON rats (r²=0.49, P<0.005). Rosiglitazone (3 mg/kg or 6 mg/kg) did not alter the slope of this relationship, but the higher rosiglitazone increased baroreflex gain relative to the degree of insulin sensitivity (significant increase in intercept, ANCOVA, P<0.05). Solid black circles, vehicle treated rats; solid gray circles, rosiglitazone (3 mg/kg); open black circles, rosiglitazone (6 mg/kg).
Figure 5. Top: Sigmoidal baroreflex curves constructed from the means of Boltzman parameters (Table 2) derived from fits of relationships between arterial pressure and heart rate obtained in OP (n=5), OP+3 mg/kg (n=7) and OP+6 mg/kg (n=5) rosiglitazone treated rats. Bottom: Representative experiments showing that treatment of OP rats with 6 mg/kg rosiglitazone increases baroreflex gain. Open circles, vehicle-treated OP rats; gray-filled circles, rosiglitazone treated (3 mg/kg) OP rats; closed circles, rosiglitazone treated (6 mg/kg) OP rats.

Figure 6. Bradycardic responses to ADN stimulation are severely attenuated in OP rats, and rosiglitazone treatment does not restore these responses. *, P<0.05 compared to CON; †, P<0.05 compared to OR; ‡, P<0.05 rosiglitazone-treated is different from vehicle-treated or untreated rats within groups of OP, OR and CON animals. Closed circles are CON rats (n=6, rosiglitazone-treated; n=7, vehicle- or untreated), gray-filled circles are OR rats (n=6, rosiglitazone-treated; n=7, vehicle- or untreated), and open circles are OP rats (n=8, rosiglitazone-treated; n=9, vehicle- or untreated).

Figure 7. Depressor responses to ADN stimulation are severely attenuated in OP rats, and rosiglitazone treatment does not restore these responses. *, P<0.05 compared to CON; †, P<0.05 compared to OR; ‡, P<0.05 rosiglitazone-treated is different from vehicle-treated or untreated rats within groups of OP, OR and CON animals. Closed circles are CON rats (n=6, rosiglitazone-treated; n=7, vehicle- or untreated), gray-filled circles are OR rats (n=6, rosiglitazone-treated; n=7, vehicle- or untreated), and open circles are OP rats (n=8, rosiglitazone-treated; n=9, vehicle- or untreated).
Table 1. Basal values (conscious rats).

<table>
<thead>
<tr>
<th>Value</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>484±15</td>
<td>402±13*</td>
<td>380±9*</td>
<td>491±8</td>
<td>432±4*</td>
<td>451±14†</td>
<td>488±8</td>
<td>416±8*</td>
<td>436±11†</td>
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<tr>
<td>(g)</td>
<td>(9)</td>
<td>(8)</td>
<td>(8)</td>
<td>(9)</td>
<td>(8)</td>
<td>(3)</td>
<td>(6)</td>
<td>(5)</td>
<td>(6)</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>101±6</td>
<td>103±6</td>
<td>99±3</td>
<td>106±3</td>
<td>100±3</td>
<td>97±2</td>
<td>107±3</td>
<td>102±3</td>
<td>110±5</td>
</tr>
<tr>
<td>(bpm)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(7)</td>
<td>(5)</td>
<td>(3)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>Blood glucose (mg/dl)</strong></td>
<td>94±3</td>
<td>92±3</td>
<td>89±2</td>
<td>99±4</td>
<td>93±3</td>
<td>89±6</td>
<td>91±3</td>
<td>90±2</td>
<td>93±2</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(9)</td>
<td>(8)</td>
<td>(3)</td>
<td>(6)</td>
<td>(5)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

*, p<0.05 compared to similarly treated OP rats; †, P<0.05 significant effect of rosiglitazone within group. OP, obesity prone; OR, obesity resistant; CON, control diet; MAP, mean arterial pressure; HR, heart rate; 3-R, 3 mg/kg rosiglitazone; 6-R, 6 mg/kg rosiglitazone. Group n within parentheses.
Table 2. Plasma insulin levels in fasted rats before (rat insulin) and after (human insulin) the hyperinsulinemic-euglycemic clamp.

<table>
<thead>
<tr>
<th>Value</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+6-R</td>
<td>+6-R</td>
<td>+6-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat insulin</td>
<td>1.84±0.48</td>
<td>1.05±0.37</td>
<td>1.02±0.31</td>
<td>1.13±0.15</td>
<td>0.71±0.09</td>
<td>2.09±0.60</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>(7)</td>
<td>(5)</td>
<td>(7)</td>
<td>(5)</td>
<td>(4)</td>
<td>(6)</td>
</tr>
<tr>
<td>Human insulin</td>
<td>57.8±11.6</td>
<td>66.5±11.0</td>
<td>57.1±2.9</td>
<td>55.8±5.5</td>
<td>60.7±5.6</td>
<td>64.7±5.0</td>
</tr>
<tr>
<td>(µU/ml)</td>
<td>(7)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
<td>(5)</td>
<td>(6)</td>
</tr>
</tbody>
</table>
Table 3. Baroreflex parameters in OP, OR, and CON rats, either treated or not treated with rosiglitazone.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OP (5)</th>
<th>OR (5)</th>
<th>CON (5)</th>
<th>OP +3-R (7)</th>
<th>OR +3-R (5)</th>
<th>CON +3-R (3)</th>
<th>OP +6-R (5)</th>
<th>OR +6-R (5)</th>
<th>CON +6-R (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum (bpm)</td>
<td>439 ±19</td>
<td>494 ±22</td>
<td>508 ±21</td>
<td>443 ±20</td>
<td>469 ±17</td>
<td>455 ±14</td>
<td>468 ±26</td>
<td>481 ±14</td>
<td>434 ±17</td>
</tr>
<tr>
<td>Minimum (bpm)</td>
<td>271 ±13</td>
<td>267 ±16</td>
<td>268 ±15</td>
<td>261 ±12</td>
<td>258 ±9</td>
<td>255 ±16</td>
<td>259 ±7</td>
<td>259 ±7</td>
<td>240 ±18</td>
</tr>
<tr>
<td>Range (bpm)</td>
<td>167 ±20</td>
<td>227 ±26</td>
<td>240 ±19</td>
<td>182 ±21</td>
<td>211 ±19</td>
<td>200 ±20</td>
<td>210 ±32</td>
<td>222 ±16</td>
<td>194 ±22</td>
</tr>
<tr>
<td>BP&lt;sub&gt;50&lt;/sub&gt; (mmHg)</td>
<td>100 ±4</td>
<td>98 ±8</td>
<td>93 ±3</td>
<td>104 ±3</td>
<td>94 ±4</td>
<td>102 ±6</td>
<td>102 ±3</td>
<td>97 ±4</td>
<td>102 ±4</td>
</tr>
<tr>
<td>Width (mmHg)</td>
<td>14.6 ±2.3</td>
<td>14.3 ±1.4</td>
<td>15.3 ±1.2</td>
<td>12.3 ±1.5</td>
<td>12.5 ±1.4</td>
<td>12.0 ±1.1</td>
<td>10.3 ±2.0</td>
<td>10.3 ±0.8</td>
<td>12.1 ±2.0</td>
</tr>
</tbody>
</table>

OP, obesity prone; OR, obesity resistant; CON, control diet; MAP, mean arterial pressure; HR, heart rate; 3-R, 3 mg/kg rosiglitazone; 6-R, 6 mg/kg rosiglitazone. Group n within parentheses.
Table 4. Basal values (anesthetized rats).

<table>
<thead>
<tr>
<th>Value</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
<th>OP</th>
<th>OR</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(9)</td>
<td>(7)</td>
<td>(7)</td>
<td>(8)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>532±12</td>
<td>440±10*</td>
<td>437±11*</td>
<td>536±11</td>
<td>444±12*</td>
<td>464±8*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>117±6</td>
<td>110±5</td>
<td>117±5</td>
<td>120±3</td>
<td>122±5</td>
<td>128±4</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>377±12</td>
<td>387±17</td>
<td>395±8</td>
<td>382±12</td>
<td>376±7</td>
<td>368±4</td>
</tr>
</tbody>
</table>

*, p<0.05 compared to similarly treated OP rats; OP, obesity prone; OR, obesity resistant; CON, control diet; MAP, mean arterial pressure; HR, heart rate; 6-R, 6 mg/kg rosiglitazone. Group n within parentheses.
Figure 2

![Graph showing heart rate vs. mean arterial pressure]

- **Heart Rate (bpm)**
- **Mean Arterial Pressure (mmHg)**

Legend:
- **CON**
- **OR**
- **OP**
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.