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Metabolic responses with endothelin antagonism in a model of insulin resistance☆

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Abstract

Atrasentan, an endothelin antagonist, would have beneficial effects on metabolic responses in a model of insulin resistance. Zucker lean or fatty rats were maintained either on regular (lean and fatty control, n = 12) or atrasentan-treated water (5 mg/kg/d, fatty atrasentan, n = 13) for 6 weeks. There was no significant difference in water intake and body weight with the atrasentan-treated group compared with fatty controls. Although atrasentan had no effect on 3-hour fasting glucose levels, it reduced fasting insulin levels between weeks 2 and 4 of treatment by 53% (fatty control vs fatty atrasentan, $P < .01$). Atrasentan decreased the incremental area under the plasma glucose response curve (ΔAUC) after a nutritionally complete meal tolerance test (MTT), by 28% in the atrasentan-treated group compared with fatty controls ($P < .05$), and decreased the MTT-induced insulin ΔAUC by 63% in treated animals compared with the fatty control group ($P < .01$). In addition, atrasentan significantly decreased the MTT-induced glucose-insulin index ΔAUC by 58% in treated rats compared with fatty controls ($P < .01$). In summary, in the Zucker fatty rat, atrasentan significantly reduces (1) 3-hour fasting insulin levels at 4 weeks, (2) glucose and insulin MTT-induced ΔAUCs, and (3) the MTT-induced glucose-insulin index ΔAUC. These results demonstrate an improvement in hyperinsulinemia as well as in glucose tolerance and insulin sensitivity with chronic endothelin antagonism in a model of insulin resistance and suggest that chronic endothelin antagonism may have benefits in the treatment of insulin resistance and/or diabetes.

1. Introduction

Endothelin (ET) is the most potent vasoconstrictor known [1]. In addition to ET's well-known effects on the cardiovascular system [2], this peptide could be involved in pathological conditions such as insulin resistance and diabetes and/or their well-known cardiovascular complications [3,4].

Elevated production of endothelin-1 (ET-1) has been reported in isolated mesenteric arteries from streptozotocin-induced diabetic rats, a well-known model of type 1 diabetes (insulin-dependent diabetes mellitus), compared with controls [5]. Our group demonstrated that, as Zucker fatty rats age and are submitted to meal challenge conditions, their plasma ET-1 levels are elevated compared with their lean littermates [6]. Elevated plasma ET has also been reported in patients with diabetes mellitus as well as in patients with metabolic syndrome [7,8].

Recent studies have demonstrated that ET-1 causes a decrease in insulin-stimulated glucose uptake in isolated rat adipocytes [9-11], and this effect is selectively mediated by ET_A receptors [10]. In addition, it has been shown that chronic ET-1 treatment causes a desensitization of the insulin signaling pathway in 3T3-L1 adipocytes, ultimately leading to a decrease in glucose uptake. [12]. Other studies, however, showed opposite results [13,14]. They demonstrate that combined presence of insulin and ET-1 stimulates glucose uptake as well as GLUT 4 translocation in 3T3-L1 adipocytes [13,14]. One possible explanation for this discrepancy is the difference in the time of exposure to ET-1 (2 or 24 hours vs 30 minutes) [9-14].

In lean type 2 diabetic (non–insulin-dependent diabetes mellitus) individuals, it has been demonstrated that circu-

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lating ET-1 levels increased during a euglycemic hyperinsulinemic clamp [15]. They observed a negative correlation between total glucose uptake and circulating ET-1 levels, suggesting that this peptide might exert negative effects on the insulin sensitivity of target tissues. It has also been demonstrated that exogenous ET-1 infusion causes peripheral insulin resistance in healthy human beings by decreasing insulin-dependent whole-body glucose uptake [16]. Similarly, intravenous infusion of subpressor doses of ET-1 significantly reduced the acute insulin response to glucose, as well as insulin sensitivity, in normal nondiabetic men [17]. Other studies showed that ET-1, administered intraperitoneally in conscious Sprague-Dawley rats, increased plasma glucose and the plasma glucose/insulin ratio [18,19]. These investigators demonstrated, by an oral glucose tolerance test, that glucose levels were significantly higher in rats preinjected with ET-1 than in rats preinjected with saline [18,19].

It has also been reported that ET-1 infusion reduces splanchnic glucose production in healthy men [20]. Rodent data, however, are inconsistent with this finding [21,22]. It was found that ET-1 infusion causes an increase in glucose production in the perfused rat liver because of the stimulation of hepatic glycogenolysis. Finally, it has been reported that ET-1 or ET-3 infusion into the portal vein increased glucose output in the perfused rat liver [23]. Discrepancy in these results can be attributed to species differences (human vs rodents). In addition, responses of the splanchnic system (liver and gut) can be different compared with the responses of the liver alone. It is worth noting that ET infusion studies may be difficult to interpret because of the potential of ET to have local or systemic hemodynamic effects which could influence glucose or other carbon source substrate fluxes in tissues.

Taken together, there is evidence to support the idea that endogenous ET could have detrimental effects on glucose homeostasis, possibly leading to insulin resistance and/or diabetes. ET mediates its effects through 2 different receptors, namely, ET_A and ET_B [24,25]. Because ET_A receptors are primarily responsible for the cardiovascular or metabolic effects mediated by ET [2-4,10], the purpose of this investigation was to demonstrate that chronic ET antagonism with the ET_A-selective antagonist, atrasentan, could positively impact metabolic responses in 1 of the most commonly used models of insulin resistance, the Zucker fatty rat.

2. Methods

2.1. Animals used

Animals were treated in conformity with the Abbott Laboratories institutional animal care and use committee guidelines. Five- to 6-week-old genetically obese Zucker fatty (n = 25) and lean rats (n = 12) obtained from Harlan (Madison, Wis) were acclimatized for 1 week before randomization. Rats were housed in standard animal cages on a 12-hour light/12-hour dark cycle and given free access to water and rat chow (Teklad rodent diet 8640; Harlan).

2.2. Treatment with atrasentan

The Zucker fatty rats (n = 13) were treated for 6 weeks with atrasentan at a dose of 5 mg/kg/d in their drinking water. This was adjusted based on water intake and growth weekly. The lean littermates and a control group of Zucker fatty rats (n = 12 per group) were maintained on their regular drinking water.

2.3. Weekly blood sampling

After a 3-hour fast, blood samples, obtained by tail snip (approximately 500 μL), were taken at study initiation, before treatment, and at the end of weeks 2 and 4, for determination of plasma glucose, insulin, ET-1, triglyceride (TG), and free fatty acid (FFA) levels. Three-hour–fasting week-6 results are not available because of the fact that an overnight fast was required in preparation for the meal tolerance test (MTT).

2.4. Nutritionally complete MTT

After the 6-week treatment with atrasentan and after an overnight fast (approximately 8 AM), an MTT was performed in the conscious rats, as previously described by our group [26]. Briefly, the MTT consisted of a complete nutrient challenge (Ensure Plus, Ross Products Division, Abbott Laboratories, Columbus, Ohio) administered by oral gavage at a dose of 1.2 g of carbohydrate per kilogram of body weight (bw). Blood samples were obtained by tail snip at 0 (500 μL), 15, 30, 60 (50 μL), and 120 (500 μL) minutes after the challenge, for determination of plasma glucose, insulin, TG, FFA, and ET-1 levels.

2.5. Processing of blood samples

Plasma glucose was determined immediately on fresh samples by using the Medisense Precision G blood glucose testing system (Medisense Products, Abbott Laboratories, Bedford, Mass). Plasma insulin was measured with a rat insulin enzyme-linked immunosorbent assay kit (Alpco Diagnostics, Winham, NH) using rat insulin standards. The coefficient of variation (interassay) was approximately 4%.

Plasma ET-1 levels were determined with the QuantiGlo human ET-1 chemiluminescent immunoassay (R&D Systems, Minneapolis, Minn). It is noteworthy that the first incubation step was performed overnight at 4°C, instead of a 1.5-hour incubation at room temperature, to increase the sensitivity of the assay. Also, dilutions of 0.8, 4, and 20 pg/mL were included in the standard curve to get a wider range of concentrations.

Plasma TG levels were measured with the Lipid Lin-Trol set from Sigma Diagnostics (St Louis, Mo). Plasma

Table 1
Effect of atrasentan on basal TG, FFA, and ET-1 levels in Zucker fatty rats

	Lean	Fatty control	Fatty atrasentan
TG (mg/dL) (3-h fast)			
Wk 0	116 ± 8	385 ± 35[a]	445 ± 17[a]
Wk 2	91 ± 8	612 ± 69[a,b]	478 ± 54[a]
Wk 4	118 ± 9	590 ± 66[a,b]	562 ± 54[a]
FFA (mmol/L) (3-h fast)			
Wk 0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
Wk 2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
Wk 4	0.5 ± 0.1	0.6 ± 0.1[c,d]	0.7 ± 0.1[a]
ET-1 (pg/mL) (3-h fast)			
Wk 0	3.5 ± 0.3	3.9 ± 0.2	3.0 ± 0.2
Wk 2	2.1 ± 0.4[b]	1.9 ± 0.2[d]	12.3 ± 1.4[a,d,e]
Wk 4	1.5 ± 0.2[d]	3.1 ± 0.3[a,f]	14.3 ± 0.9[a,d,e]

[a] $P < .01$, compared with lean.
[b] $P < .05$, week 0 vs 2 or 4.
[c] $P < .05$, compared with lean.
[d] $P < .01$, week 0 vs 2 or 4.
[e] $P < .01$, fatty atrasentan vs fatty control.
[f] $P < .01$, week 2 vs 4.

FFA levels were determined with the NEFA C kit from Wako Chemicals USA, Inc (Richmond, Va). The FFA protocol was modified to accommodate a 96-well plate format.

2.6. Drug administration

Atrasentan (ABT-627, HCl salt, Abbott Laboratories, Abbott Park, Ill) [27] was dissolved with 2 molar-equivalent 1 N NaOH and brought to the desired volume by completing with regular drinking water.

2.7. Calculations and statistical analysis

Incremental area under the plasma glucose, insulin, or glucose-insulin index response curves (ΔAUC) was calculated according to the method of Wolever and Jenkins [28]. Results are given as mean ± SEM. for the indicated number of rats. A 1-way analysis of variance with repeated measures followed by a Tukey-Kramer multiple comparisons test was used. P values of .05 (2-tailed) and lower were considered significant.

Table 2
Effect of atrasentan on meal-challenged TG, FFA, and ET-1 levels in Zucker fatty rats

	Lean	Fatty control	Fatty atrasentan
TG (mg/dL)			
0 min	68 ± 3	979 ± 81[a]	856 ± 68[a]
120 min	89 ± 6	933 ± 65[a]	893 ± 56[a]
FFA (mmol/L)			
0 min	0.84 ± 0.04	1.7 ± 0.1[a]	1.8 ± 0.1[a]
120 min	0.86 ± 0.06	1.13 ± 0.06[b,c]	1.45 ± 0.07[a,c,d]
ET-1 (pg/mL)			
0 min	1.3 ± 0.2	2.3 ± 0.2	6.5 ± 0.3[a,d]
120 min	3.6 ± 0.3[c]	3.9 ± 0.3[c]	9.0 ± 0.6[a,c,d]

[a] $P < .05$, compared with lean.
[b] $P < .01$, compared with lean.
[c] $P < .01$, fatty atrasentan vs fatty control.
[d] $P < .01$, 0 vs 120 minutes.

3. Results

Baseline bw (week 0) were 229.6 ± 2.9 g (lean) vs 309.2 ± 9.5 g (fatty control) vs 318.1 ± 3.7 g (fatty atrasentan). Baseline daily water intake (week 0) was 30 ± 3 mL (lean) vs 45 ± 3 mL (fatty control) vs 43 ± 2 mL (fatty atrasentan). The 6-week treatment with atrasentan did not significantly affect bw (336 ± 4 g [lean] vs 568 ± 10 g [fatty control] vs 563 ± 8 g [fatty atrasentan]) or daily water intake (32 ± 1 mL [lean] vs 45 ± 1 mL [fatty control] vs 44 ± 2 mL [fatty atrasentan]) in the treated rats compared with their control group.

Although TG and FFA levels were not affected by atrasentan treatment compared with their control group, these levels were significantly higher in fatty animals (control or atrasentan-treated) compared with their lean littermates (Tables 1 and 2).

We observed that plasma ET-1 levels were significantly higher ($P < .01$) in atrasentan-treated animals compared with their control group or lean littermates throughout the 6 weeks of treatment (Tables 1 and 2).

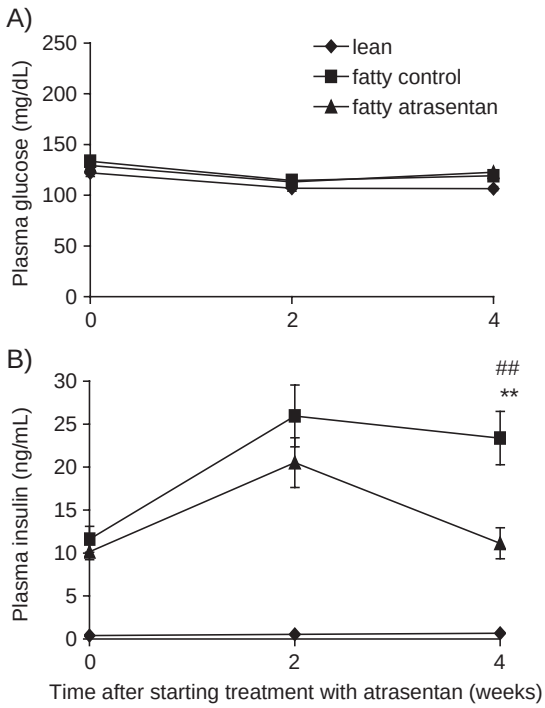

Fig. 1. Plasma glucose (A) and insulin levels (B) in Zucker lean (nontreated) and fatty rats during 6-week treatment with atrasentan (5 mg/kg/d). Measurements were taken at week 0 (before starting treatment) and at weeks 2 and 4 after starting treatment. Each symbol with a bar represents the mean ± SEM of 12 to 13 rats per group. **$P < .01$, fatty control or fatty atrasentan when compared with lean; ##$P < .01$, fatty atrasentan vs fatty control.

Fig. 1A shows that atrasentan treatment did not have any significant effect on basal glucose levels, through week 4, after a 3-hour fast, in the Zucker fatty rat. On the other hand, atrasentan treatment reduced basal insulin levels by 53%, after a 3-hour fast, 4 weeks after the beginning of treatment ($P < .01$) (Fig. 1B). Insulin levels, after an overnight fast, were not significantly different between the atrasentan-treated animals versus their control group (Fig. 2B).

There was no significant difference in absolute plasma glucose levels before and after the MTT, between Zucker fatty control rats and their treated counterparts (Fig. 2A). However, insulin levels after the MTT were significantly reduced in the atrasentan-treated rats in comparison to controls at 30, 60, and 120 minutes (Fig. 2B).

This is further illustrated by a significant decrease in the glucose-insulin index ΔAUC in the atrasentan treated rats compared with the control group ($P < .01$) (Fig. 3C).

Finally, the glucose ΔAUC was decreased by 28% in the atrasentan-treated animals compared with controls (Fig. 3A), and the insulin ΔAUC was greatly decreased (63%), although not to the level of lean animals (Fig. 3B).

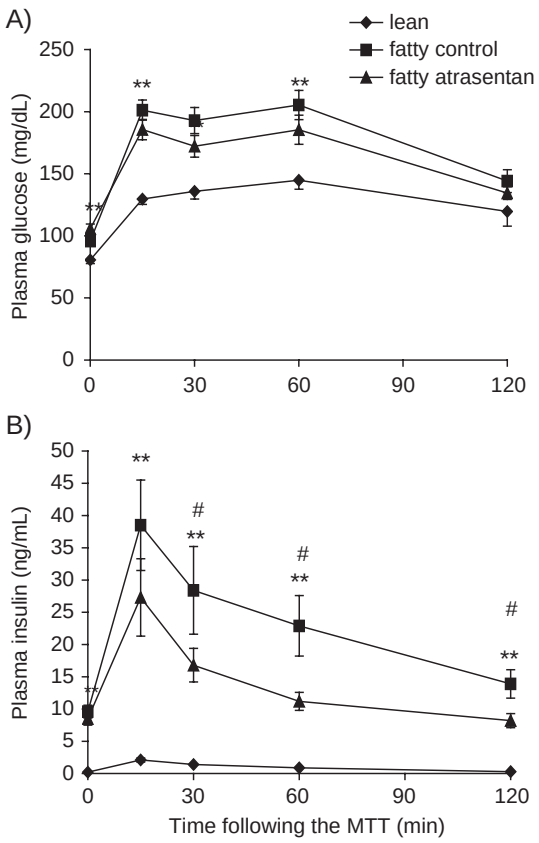

Fig. 2. Plasma glucose (A) and insulin levels (B) in Zucker lean (nontreated) and fatty rats before and after a MTT at the end of the 6-week treatment with atrasentan (5 mg/kg/d). Each symbol with a bar represents the mean ± SEM of 12 to 13 rats per group. $^{**}P < .01$, fatty control or fatty atrasentan when compared with lean; $\#P < .05$, fatty atrasentan vs fatty control.

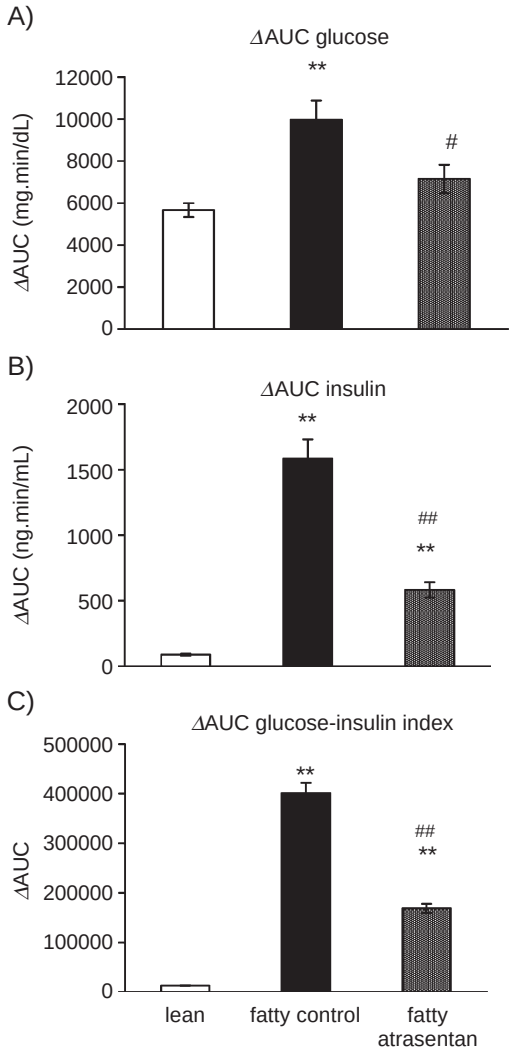

Fig. 3. Glucose (A), insulin (B), or glucose-insulin index (C) ΔAUC in Zucker lean (nontreated) and fatty rats at the end of the 6-week treatment with atrasentan (5 mg/kg/d). Each column with a bar represents the mean ± SEM of 12 to 13 rats per group. $^{**}P < .01$, fatty control or fatty atrasentan when compared with lean; $\#P < .05$, fatty atrasentan versus fatty control; $\#\#P < .01$, fatty atrasentan vs fatty control.

Furthermore, we observed a significant decrease in the glucose-insulin index ΔAUC in the atrasentan-treated rats compared with the control group ($P < .01$) (Fig. 3C).

4. Discussion

In this study, we investigated the effects of chronic treatment with an ET_A-selective antagonist, atrasentan, on metabolic responses in 1 of the most commonly used models of insulin resistance, the Zucker fatty rat. Atrasentan is an orally active and highly potent ET_A receptor antagonist with approximately 1000-fold more selectivity for the ET_A compared with the ET_B receptor [27].

Basal glucose levels in Zucker fatty rats were not affected by chronic atrasentan because these animals are

not hyperglycemic [29]. This was an expected response. However, we observed a change from baseline glucose and ΔAUC for glucose after an MTT in atrasentan-treated Zucker fatty rats compared with the Zucker fatty control group. These changes resulted in levels similar to those observed in lean animals.

Basal insulin levels were elevated in fatty animals compared with their lean littermates, as anticipated in this model [29]. These levels were significantly reduced after 4 weeks of atrasentan treatment. In addition, the insulin change from baseline and ΔAUC was significantly reduced in atrasentan treated rats compared with controls in response to a MTT, indicating an improvement in glucose tolerance and possibly in insulin sensitivity. The glucose-insulin index and the glucose-insulin index ΔAUC were significantly reduced in the atrasentan treated rats compared with the control group, suggesting enhanced insulin sensitivity. This index has been reported as a robust indicator of insulin sensitivity [30].

The results from the present study are consistent with the hypothesis that endogenous ET-1 can have detrimental effects on glucose homeostasis that could be improved by ET antagonism. Our results show a reduction in hyperinsulinemia as well as in glucose and insulin rise after a meal challenge with ET antagonism. This is consistent with studies showing a desensitization of insulin signaling with ET-1 treatment in rat isolated and 3T3-L1 adipocytes [9-12]. Our results are in agreement with previous studies reporting that ET-1 induces insulin resistance in rats and in human beings [16-18] and with studies reporting an increase in hepatic glucose production in the perfused rat liver [21-23]. In addition, our results are consistent with studies showing elevated ET-1 levels in insulin-resistant human beings: diabetic patients and obese subjects with metabolic syndrome [7,8]. On the other hand, 2 studies demonstrated increased glucose uptake in 3T3-L1 adipocytes with ET-1 treatment [13,14], and 1 study showed decreased splanchnic glucose production in human beings [20]. The reason for this discrepancy is not clear. However, time of exposure to ET-1 (glucose uptake) and species differences (hepatic glucose production) might explain the difference in results.

Consistent with the present study, ET_A receptor antagonism with atrasentan and other ET receptor antagonists has been reported to increase plasma ET-1 levels in rats as well as in human beings [31]. It has been shown that ET_A-selective blockade can increase plasma ET-1 levels, although not nearly to the same extent as ET_B selective blockade does [31]. This observation is consistent with the finding that ET_B receptors are primarily responsible for ET-1 plasma clearance [31,32]. It has also been suggested that prolonged inhibition of ET_A receptors could induce a feedback mechanism, resulting in an up-regulation of expression of the ET-1 gene, and in an increase in ET-1 levels [31]. It is worth noting that atrasentan does not affect blood pressure in Zucker fatty rats in comparison to their control group, despite an increase in plasma ET-1 levels (N. Berthiaume et al, unpublished observations). The pressor effect induced by elevated ET-1 levels might be counterbalanced by atrasentan blood pressure–lowering effect [27], thereby maintaining blood pressure at control levels. However, although ET_A receptors are selectively blocked by atrasentan, we cannot rule out that ET activity at the ET_B receptors might contribute to the results observed.

Finally, atrasentan has no effect on bw or on plasma TG and FFA levels in the Zucker fatty rat. However, TG and FFA levels are significantly higher in fatty rats compared with their lean littermates, as anticipated in this model [29]. Because atrasentan reduces plasma insulin levels, a correction of the dyslipidemia, in this model, would be expected. The length of exposure to atrasentan may not have been sufficient to engender lipid-lowering effects. Other explanations for this could be that atrasentan targets glucose transport by blocking ET-1 actions on specific tissues (eg, skeletal muscle) [15-19] or glucose metabolism at the level of the liver [21-23] or insulin secretion from the pancreas [33,34] rather than targeting bw regulation and/or lipid metabolism directly. In conclusion, ET antagonism improves glucose tolerance, hyperinsulinemia, and insulin sensitivity in 1 of the most commonly used models of insulin resistance, the Zucker fatty rat. It can be postulated that ET antagonism might possibly have beneficial effects in the treatment of insulin resistance and/or diabetes.

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