Nebivolol Improves Insulin Sensitivity in High-Sucrose Diet and Spontaneously Hypertensive Rats through a Nitric Oxide Independent Mechanism

Maria P. Guarino, PhD; Ana I. Santos, PhD; Pedro F. Costa, MD, PhD; Miguel Mota-Carmo MD, PhD

Abstract
Nebivolol is a β-1 selective adrenergic receptor antagonist which increases nitric oxide (NO) bioavailability. We hypothesized that nebivolol improves insulin sensitivity in pathologies where the NO signaling pathway is compromised through an increase in NO production. The High-Sucrose Diet Rat (HSu) and the Spontaneously Hypertensive Rat (SHR) were used as models of insulin resistance. Control, HSu and SHR rats were treated with either nebivolol (1mg/kg/day) or atenolol (10mg/kg/day). Insulin sensitivity and plasma NO metabolites were determined. Nebivolol reversed insulin resistance both in the HSu and in the SHR animals whereas atenolol did not improve insulin sensitivity. 4 week-treatment with nebivolol significantly increased plasma NO/NO\textsubscript{3} concentration in the HSu group, but not in the SHR animals. We concluded that nebivolol prevents the development of insulin resistance induced by a high-sucrose diet and restores insulin sensitivity in SHR animals through a mechanism that cannot be solely attributed to an increase in endogenous NO production.

Keywords — high-sucrose diet rats, insulin resistance, nebivolol nitric oxide, spontaneously hypertensive rats.

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I. INTRODUCTION

Nebivolol is a β-1 selective adrenergic receptor antagonist with nitric oxide (NO)-mediated vasodilatory properties\textsuperscript{1,2}. The exact mechanism of NO-dependent vasodilation induced by nebivolol remains unknown, although several hypothesis have been raised: via endothelial β2-adrenergic receptor-mediated NO production \textsuperscript{3}, via activation of β3-adrenergic receptors \textsuperscript{4-6}, through stimulation of adenosine triphosphate efflux with consequent P2Y-purinoceptor-mediated NO release \textsuperscript{7} and more recently, by inhibiting a new call of enzymes known as S-nitrosoglutathione reductases responsible by intracellular NO signalling downstream the β-adrenergic receptor \textsuperscript{8}. It has also been reported that nebivolol inhibits NO synthase uncoupling \textsuperscript{9} and produces systemic antioxidant effects through inhibition of NADPH oxidase \textsuperscript{9,10}.

In contrast with other beta-blockers, nebivolol lacks metabolic adverse effects, which is of clinical relevance in the treatment of diabetic hypertensive patients 11,12. Still, although nebivolol has been shown not to impair insulin sensitivity, there are currently no conclusive studies on the effect of nebivolol in preventing or delaying the development of insulin resistance in individuals with high cardiometabolic risk.

Recent reports have shown that insulin sensitivity is mediated by endogenous NO production \textsuperscript{13-15}. Because nebivolol increases NO bioavailability \textsuperscript{16}, we believe this drug may represent a novel therapeutic approach in the prophylaxis of insulin resistance in diseases in which the NO signalling pathway is compromised, such as hypertension, obesity and the metabolic syndrome.

Herein we evaluated the effect of nebivolol on the progression of insulin resistance in two animal models of insulin resistance: the High-Sucrose Diet Rat (HSu rat) \textsuperscript{17} and the Spontaneously Hypertensive Rat (SHR), a genetic pathologic model widely used for the study of the relationship between essential hypertension and insulin resistance, due to the similarity of symptoms to the corresponding human pathology \textsuperscript{18,19}. We also compared the metabolic and hemodynamic protective effects of nebivolol with atenolol, a β-blocker which does not increase NO release \textsuperscript{20}.

Furthermore, to determine the correlation between insulin sensitivity and plasma NO levels, we studied the effect of nebivolol on the L-arginine/nitric oxide pathway through measurement of circulating nitrates/nitric oxide in these animals.
II. METHODS

A. Animals and experimental procedures

The experiments were performed in male Wistar rats (200 - 420g) obtained from the animal house of the Faculty of Medical Sciences of Nova University of Lisboa. All the animals were kept in the animal house under temperature and humidity control (21±1°C, 55 +/- 10% humidity) with a 12:12 light-dark cycle and with food and water available ad libitum. The control group was fed a sham diet (7.4% fat + 75% carbohydrate (4% sugar) + 17% protein, SDS diets RM1, Probiológica, Portugal); the Hsu model was obtained by the administration of 35% sucrose (Panlab, Portugal) in drinking water during 28 days.

Rats were divided into six groups, according to the procedure and composition of the water supply, as follows: control - regular animal facility water; Hsu - 35% sucrose solution; N - nebivolol 1 mg/kg/day [21]; HSuN - 35% sucrose plus nebivolol 1 mg/kg/day; A - atenolol 10 mg/kg/day [22]; HSuA - 35% sucrose plus atenolol 10 mg/kg/day. In the first set of experiments treatments lasted for 4 weeks and animals were tested at 9 weeks of age. A second set of experiments was performed to test the effect of nebivolol and atenolol treatment for 12 weeks in the same animal models: control12w - regular animal facility water; Hsu12w - 35% sucrose solution during a period of 4 weeks, followed by tap water for 8 weeks; N12w - nebivolol 1 mg/kg/day for 12 weeks; HSuN12w - 35% sucrose plus nebivolol 1mg/kg/day during 4 weeks followed by nebivolol 1 mg/kg/day during 8 weeks; A12w - atenolol 10 mg/kg/day during 12 weeks; HSuA12w - 35% sucrose plus atenolol 10 mg/kg/day during 4 weeks followed by atenolol 10 mg/kg/day for 8 weeks.

The third set of experiments was performed in Spontaneously Hypertensive Rats (SHR), purchased from Charles River, Spain. After arriving, at 5 weeks of age, SHR animals were maintained in the animal house for one week for acclimation. Rats were divided into 3 groups: the control SHR group (SHR); nebivolol-treated (SHRN; nebivolol 1 mg/kg/day) and atenolol-treated (SHRA; atenolol 10 mg/kg/day). The drugs were administered in drinking water during 4 weeks starting, at 6 weeks of age.

Daily liquid intake was measured to monitor nebivolol and atenolol ingestion. Body weight was assessed twice per week. On the last day of the experimental protocol rats were fasted overnight and allowed free access to water. On the day of the experiment rats were anaesthetized with sodium pentobarbital (65 mg/kg) and transferred to a heating pad to maintain body temperature at 37.5±0.5°C, monitored with a rectal thermometer. The metabolic and hemodynamic effects of nebivolol and atenolol were assessed by measuring insulin sensitivity, mean arterial blood pressure and plasma NO3/NO2.

Principles of laboratory care were followed in accordance with the European Union Directive for Protection of Vertebrates Used for Experimental and Other Scientific Ends (2010/63/EU). Experimental protocols were approved by the Ethics Committee of the Faculty of Medical Sciences. At the end of the experiments the animals were euthanized through an intracardiac overdose of sodium pentobarbital.

B. Measurement of insulin sensitivity

Insulin sensitivity was evaluated by means of the intravenous Insulin Tolerance Test (ITT). The ITT is one of the earliest methods developed to assess insulin sensitivity in vivo and provides an estimate of overall insulin sensitivity, correlating well with the gold-standard hyperinsulinemic-euglycemic clamp [23]. The ITT consists in the administration of an intravenous insulin bolus of 0.1U/kg body weight, after an overnight fast, followed by determination of plasma glucose concentration over 15 minutes at one minute intervals. The constant rate for glucose disappearance (KITT) was calculated using the formula 0.693/t1/2. Glucose half-time (t1/2) was calculated from the slope of the least-square analysis of plasma glucose concentrations during the linear decay phase. Blood samples were collected by tail tipping and glucose levels were measured by the glucose oxidase method (Precision X-Ceed, Abbott Diabetes Care, Portugal) and test strips (Abbott Diabetes Care, Portugal).

C. Measurement of mean arterial pressure

To measure mean arterial pressure (MAP), the animals were placed on a heating pad with the tail positioned under a lamp to increase vasodilation in the tail vessels. MAP was measured five consecutive times in the rats using a tail-cuff non-invasive blood pressure measurement system (LE5001, Panlab, Harvard Apparatus, Madrid, Spain).

D. Measurement of NO/NO2 plasma levels

Plasma NO/NO2 levels were determined at the end of each experiment, in all animals. Blood was collected by cardiac puncture, and immediately centrifuged at 12000 g, for 5 minutes, to obtain plasma samples. Proteins were precipitated from the samples by adding two volumes of ethanol (0°C). After 30 minutes on ice, samples were centrifuged in a microfuge (Eppendorf, Madrid, Spain) at 12000g for 10 minutes. NO/NO2 concentration was determined by using a specific and sensitive NO/ozone chemiluminescence technique (NO-Analyzer 280, Sievers Research Inc., Boulder Colorado).

E. Drugs and chemicals

Atenolol was obtained from Sigma-Aldrich (Madrid, Spain). Sucrose was obtained from PanLab (Lisboa, Portugal). Insulin was commercially available as Humulin® Regular (Lilly, Portugal) in a concentration of 100 UI/ml. Nebivolol was kindly provided by A.Menarini Portugal (Lisboa, Portugal).

F. Data analysis

Data were evaluated using Graph Pad Prism Software, version 4 (GraphPad Software Inc., San Diego, CA, USA) and were presented as mean± SEM. The significance of the differences between the means was calculated by One and Two-Way Analysis of Variance (ANOVA) with Dunnett's and
Bonferroni multiple comparison tests, respectively. Differences were considered significant at $p \leq 0.05$.

### III. RESULTS

A. Effect of nebivolol and atenolol on fasting glyceremia and weight gain

1) **High-Sucrose Diet Rats**

The HSu diet significantly increased fasting glyceremia in comparison with the control groups both in 9-weeks-old ($p<0.001$, table I) and in 17-weeks-old animals ($p<0.01$, table II). Fasting glyceremia was not significantly altered either by nebivolol or atenolol compared to the respective control groups, both after 4 weeks and 12 weeks of treatment (tables I and II). Nebivolol as well as atenolol prevented the hyperglyceremia induced by the HSu diet, independently of the duration of the treatment ($p<0.01; p<0.001$ - tables I and II).

HSu diet did not cause a significant increase in weight gain per day compared with control animals either in the 4-week treated (weight gain control: 5.80±0.25 gr/day; weight gain HSu: 4.6±0.29gr/day, table I) or in the 12 weeks treated animals (weight gain control12w: 4.2±0.19 gr/day; weight gain HSu12w: 4.2±0.22 gr/day, table II). Both nebivolol and atenolol did not modify daily weight gain in the two previously mentioned groups (tables I and II).

2) **Spontaneously Hypertensive Rats**

Liquid intake in SHR, SHR$_N$ and SHR$_\alpha$ was 24.0±1.7 ml/day, 25.2±0.9 ml/day and 27.1±1.1 ml/day respectively, which was not significantly different (table III). Food intake was also not significantly different both in 4-weeks-treated, 12 weeks-treated and SHR groups (data not shown). The SHR group was normoglycemic compared to the control age-matched group and administration of nebivolol and atenolol did not modify fasting glyceremia (table III).

The ingestion of sucrose, nebivolol and atenolol in drinking water was stable throughout the 4-week-duration experiments in all the groups, since there were no significant changes in individual daily liquid intake per weight (table I).

### TABLE I

<table>
<thead>
<tr>
<th>4 week treatment</th>
<th>Plasma Glucose (mg/dL)</th>
<th>Daily weight gain (g/day)</th>
<th>Daily liquid intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92.4±2.0</td>
<td>5.8±0.25</td>
<td>28.4±1.2</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>90.2±5.2</td>
<td>6.3±0.21</td>
<td>28.9±3.7</td>
</tr>
<tr>
<td>Atenolol</td>
<td>83.3±5.9</td>
<td>5.5±0.16</td>
<td>28.4±3.5</td>
</tr>
<tr>
<td><strong>HSu Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>133.4±3.4***</td>
<td>4.6±0.29*</td>
<td>26.8±1.1</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>89.7±6.9***</td>
<td>5.0±0.27</td>
<td>26.7±0.8</td>
</tr>
<tr>
<td>Atenolol</td>
<td>92.7±5.3***</td>
<td>4.3±0.24</td>
<td>27.9±0.8</td>
</tr>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>83.3±3.6</td>
<td>4.7±0.12</td>
<td>24.0±1.7</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>97.8±3.5</td>
<td>5.8±0.16*</td>
<td>25.2±0.9</td>
</tr>
<tr>
<td>Atenolol</td>
<td>87.4±5.0</td>
<td>5.5±0.24**</td>
<td>27.1±1.1</td>
</tr>
</tbody>
</table>

The ingestion of sucrose and using sucrose in drinking water was stable throughout the 4-week-duration experiments in all the groups, since there were no significant changes in individual daily liquid intake per weight (table I).

### TABLE II

<table>
<thead>
<tr>
<th>12 weeks treatment</th>
<th>Plasma Glucose (mg/dL)</th>
<th>Daily weight gain (g/day)</th>
<th>Daily liquid intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>82.3±2.0</td>
<td>4.2±0.19</td>
<td>38.9±2.2</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>82.6±1.3</td>
<td>4.4±0.09</td>
<td>39.7±6.1</td>
</tr>
<tr>
<td>Atenolol</td>
<td>90.5±1.0</td>
<td>3.9±0.09</td>
<td>39.9±1.8</td>
</tr>
<tr>
<td><strong>HSu Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>122.0±3.6**</td>
<td>4.2±0.22</td>
<td>39.8±1.1</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>96.6±4.9***</td>
<td>4.2±0.15</td>
<td>34.9±1.5</td>
</tr>
<tr>
<td>Atenolol</td>
<td>95.4±4.0***</td>
<td>3.7±0.15</td>
<td>38.0±1.0</td>
</tr>
</tbody>
</table>

Effect of 12 week-nebivolol (1mg/kg/day) and 12 week-atenolol (10mg/kg/day) treatment on fasting glyceremia, daily weight gain and daily liquid intake in rats fed a standard and a high-sucrose diet. Data represent all the individual samples obtained from 8 rats. ** $p<0.01$ vs control standard diet rats; ## $p<0.05$ vs control High Sucrose diet (HSu) rats. Two-Way ANOVA, with Bonferroni multicomparsion test.

In the 12 weeks treated groups we also did not observe differences in daily liquid intake (table II). Both drugs significantly increased daily weight gain in the SHR$_N$ and SHR$_\alpha$ groups (table III).

### TABLE III

<table>
<thead>
<tr>
<th>Plasma Glucose (mg/dL)</th>
<th>Daily weight gain (g/day)</th>
<th>Daily liquid intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>83.3±3.6</td>
<td>4.7±0.12</td>
</tr>
<tr>
<td>Nebivolol</td>
<td>97.8±3.5</td>
<td>5.8±0.16**</td>
</tr>
<tr>
<td>Atenolol</td>
<td>87.4±5.0</td>
<td>5.5±0.24**</td>
</tr>
</tbody>
</table>

Effect of 4week-nebivolol (1mg/kg/day) and 4 week-atenolol (10mg/kg/day) treatment on fasting glyceremia, daily weight gain and daily liquid intake in SHR rats. Data represent all the individual samples obtained from 7 - 8 rats. ** $p<0.01$ vs control SHR rats. One-Way ANOVA, with Bonferroni multicomparsion test.

B. Effect of nebivolol and atenolol on insulin sensitivity

1) **High-Sucrose Diet Rats**

$K_{ITT}$ significantly decreased from 5.15±0.39%glucose/min in the control group to 2.72±0.26%glucose/min in the HSu group ($p<0.001$), confirming the insulin resistance induced by the high-sucrose diet (fig. 1A). Nebivolol administration did not modify insulin sensitivity compared to control animals ($K_{ITT}$ N group: 4.21±0.44%glucose/min) whereas atenolol administration significantly decreased insulin sensitivity ($K_{ITT}$ A group: 3.08±0.57%glucose/min, $p<0.05$ compared to control, fig. 1A). Nebivolol administration to sucrose-treated animals prevented the development of insulin resistance after 4 weeks of treatment ($K_{ITT}$ in the HSuN group: 4.29±0.41%glucose/min; $p<0.05$ from control) whereas atenolol did not (HSuA group: 2.67±0.64%glucose/min; fig. 1A). In the 12 week-treatment experiments, the $K_{ITT}$ was 2.99±0.32%glucose/min in the control12w group indicating that the animals were insulin resistant at 17 weeks of age. In the HSu12w, the $K_{ITT}$ was 2.27±0.34%glucose/min which was not significantly different from control12w animals (fig. 1B). Nebivolol administration for a period of 12 weeks prevented the development of insulin resistance induced by sucrose and also by aging ($K_{ITT}$ in the HSuN12w group: [Table IV](#table-iv).

### TABLE IV

<table>
<thead>
<tr>
<th>Plasma Glucose (mg/dL)</th>
<th>Daily weight gain (g/day)</th>
<th>Daily liquid intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
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<tr>
<td>Control</td>
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</tr>
</tbody>
</table>
4.01±0.41%glucose/min, p<0.01 from HSu12w, and KITT in the N12w group: 4.38±0.34%glucose/min, p<0.05 from control12w, fig 1B). 12 week atenolol administration did not modify insulin sensitivity either in control or in HSu animals (A12w group: 3.13±0.30%glucose/min and HSuA12w group: 3.41±0.45%glucose/min, fig. 1B).

2) Spontaneously Hypertensive Rats

The SHR animals were insulin resistant compared to the control group since the KITT was 5.58±0.19%glucose/min in the control group and 3.82±0.48 %glucose/min in the SHR group (p< 0.01) (fig. 2). Nebivolol administration restored insulin sensitivity to control values (KITT SHRN group: 5.93±0.32%glucose/min, p<0.01 compared to SHR group) whereas atenolol administration did not (KITT SHRA group: 4.71±0.39%glucose/min, fig. 2).

C. Effect of nebivolol and atenolol on mean arterial pressure

1) High-Sucrose Diet Rats
Fig. 3 displays the effects of nebivolol and atenolol administration on MAP both after a 4week and 12 week-treatment period. The HSu group showed a significant increase in MAP compared to the control group, independently of the animals age (MAP control = 92.94±4.48mmHg; MAP HSu = 123.3±4.22mmHg, p<0.05; MAP control12w= 90.3±4.87mmHg; MAP HSu12w = 120.9±10.0 mmHg, p<0.05). Both nebivolol and atenolol restored MAP to control values in the sucrose treated animals, regardless of the duration of treatment: (MAP HSuN = 81.0±10.1mmHg, p<0.001 compared to HSu; MAP HSuA = 88.0±3.40mmHg, p<0.001 compared to HSu; MAP HSuN12w = 87.3±6.34mmHg, p<0.01 compared to HSu12w; MAP HSuA12w = 82.4±3.18 mmHg, p<0.001 compared to HSu12w, fig. 3).

2) Spontaneously Hypertensive Rats
The SHR animals were hypertensive compared to the control group (MAP control: 92.7±4.29mmHg; MAP SHR: 122.9±5.36mmHg, p< 0.001) (fig 4). Both nebivolol and atenolol administration restored MAP to control values (MAP SHR N group: 71.8±4.60mmHg; p<0.001 compared to SHR group; MAP SHR A group: 81.4±4.24mmHg; p<0.01 compared to SHR group, fig. 4).

D. Effect of nebivolol and atenolol on plasma NO/NO3- concentration

1) High-Sucrose Diet Rats
Plasma NO/NO3- concentrations determined in the groups submitted to 4 weeks and 12 weeks drug treatment are depicted in Figure 5. At 9 weeks of age, plasma NO/NO3- levels were significantly lower in the HSu treated group compared to control animals (Control: 30.7±3.2µM, HSu: 12.7±1.4µM, p<0.01). Nebivolol increased plasma NO/NO3- concentration in the both in the N (58.5±11.8µM, p<0.001 compared to control) and in the HSuN (34.5±7.8µM, p<0.05 compared to HSu) groups. Atenolol did not change plasma NO/NO3- levels either compared to the control group (A group: 31.8±3.5µM) or to the HSu group (HSuA group: 22.5±1.7µM), fig. 5A.

At 17 weeks of age, there were no significant differences in plasma NO/NO3- concentrations between control12w and HSu12w groups: control12w= 15.8±1.5µM; HSu12w = 11.9±1.1µM, fig 5B). Surprisingly, after 12 weeks of treatment with nebivolol, plasma NO/NO3- levels did not increase either in control (N12w: 18.4±1.4µM) or in sucrose-treated animals (HSuN12w: 15.7±1.7µM). Atenolol did not change plasma NO/NO3- levels after 12 weeks of treatment: A12w: 17.0±2.3µM; HSuA12w: 17.6±1.9µM, fig. 5B.

2) Spontaneously Hypertensive Rats
Plasma NO/NO3- levels were significantly lower in all the SHR groups, independently of the treatment with nebivolol or atenolol, compared to control age-matched Wistar animals (fig. 6): (Control: 21.1±1.0µM; SHR: 12.2±1.2µM, SHRN: 11.2±1.8µM; SHR A: 10.9±1.2µM).

IV. DISCUSSION
Our study aimed to characterize the effects of nebivolol on metabolic and hemodynamic parameters in two animal models of insulin resistance, the high-sucrose diet rat (HSu) and the spontaneously hypertensive rat (SHR). We observed that nebivolol prevented the development of insulin resistance in HSu rats and also restored insulin sensitivity in SHR animals, whereas atenolol, a beta-blocker without vasodilatory properties, not only did not restore insulin action in the pathological models but also decreased insulin sensitivity in healthy Wistar rats. We concluded that nebivolol increases endogenous nitric oxide production in young Wistar rats submitted to a standard-diet or to a high-sucrose diet, but not in aged animals or in Spontaneously Hypertensive rats. Concomitantly, the effect of the drug in enhancing insulin action did not correlate with changes in circulating NO/NO3- levels, suggesting that nebivolol increases insulin sensitivity through a mechanism that is not exclusively related to an increase in endogenous NO production. Four week nebivolol administration was sufficient to restore insulin sensitivity in the HSuN group to control values. In contrast, atenolol administration not only did not prevent sucrose-induced insulin resistance, but it also induced insulin resistance in control healthy animals. Such detrimental effect of atenolol on insulin sensitivity is consistent with previous reports [24, 25], although the mechanism remains to be clarified. Despite the adverse effect of atenolol on insulin sensitivity, rats submitted to atenolol administration presented lower fasting plasma glucose values both after 4 and 12 weeks of treatment, suggesting an improvement in whole-body glucose homeostasis. One plausible explanation for the return of fasting glucose levels to control values in atenolol-treated animals is a compensatory increase in insulin production by the pancreatic beta-cell, in attempt to compensate for the insulin resistance caused by the drug. One of the limitations of the study resides is that we did not quantify plasma insulin, to evaluate the effect of nebivolol and atenolol on endogenous insulin secretion. However, others have observed that plasma insulin concentrations increase in association with atenolol treatment [24]. In our experimental setting the control standard-diet fed animals were insulin resistant at 17 weeks of age. These results are in agreement with
previous results, obtained by our group, in which a gradual decrease of insulin sensitivity was observed with aging, starting at 9 weeks of age [26]. We observed that the protective role of nebivolol on glucose homeostasis is sustained throughout time: after 12 weeks of administration the drug was able to prevent the development of insulin resistance associated both with aging and with exposure to a high-sucrose hypercaloric diet. Treatment with nebivolol restored insulin action to control values in the Spontaneously Hypertensive Rat, a genetic pathologic model of hypertension and insulin resistance[27] after 4 weeks of treatment. Once again, atenolol did not modify insulin sensitivity in SHR animals. Overall the results obtained in this study imply that nebivolol, in contrast with atenolol, has the ability to reverse physiological, genetic as well as pharmacologically-induced insulin resistance in rats. Others have reported that nebivolol improves insulin sensitivity in a transgenic rodent model of hypertension, insulin resistance, and enhanced tissue renin-angiotensin-system expression, the TGR(Ren2)27 rat, when administered in a dose of 10mg/kg/day during 21 days [27]. In contrast, Renna et al. described that chronic treatment with nebivolol 1mg/kg/day during 4 weeks did not reversed diet-induced insulin resistance [21]. In the latter study, the authors used an indirect measure of insulin sensitivity, the glucose tolerance test (GTT), which may be altered due to changes in pancreatic insulin secretion without changes in insulin action, making it difficult to conclude on direct effects of nebivolol on whole-body insulin action. Also, the diet-induced insulin resistant model used by Renna’s group was the fructose-fed rat submitted to chronic administration of 10% fructose in drinking water during 8 weeks, which was different from the HSu model used in our work and may justify the differences observed in nebivolol metabolic effect. In this work, we also tested the hypothesis that nebivolol increases plasma NO/NO$_3^-$ levels. We expected that the increment observed in plasma NO/NO$_3^-$ would be higher in Wistar rats than in sucrose-fed and SHR animals since these two animal models have altered NO bioavailability [28-30]. It was also expected that the increase in circulating NO/NO$_3^-$ would correlate with increased insulin sensitivity in the nebivolol treated groups, since several authors have described the insulin-sensitizing effects of endogenous nitric oxide [13, 31, 32]. HSu rats showed insulin resistance and significantly lower plasma NO/NO$_3^-$ levels at 9 weeks of age, however, at 17 weeks of age, there were no statistically significant differences between NO/NO$_3^-$ levels in standard-diet and HSu-fed animals. Whereas 4 weeks nebivolol treatment significantly increased plasma nitrate levels in both standard-diet fed animals and high-sucrose diet fed animals, 4 week-treatment with atenolol did not change NO/NO$_3^-$ concentration either in the standard diet or in the HSu fed animals. Notwithstanding, after a 12week-duration treatment neither nebivolol nor atenolol increased plasma NO/NO$_3^-$ levels. We also did not observe an increase in endogenous NO production induced by nebivolol in the SHRN rats, as measured by circulating NO/NO$_3^-$ levels, despite the improvement in insulin sensitivity induced by the drug. There are several possible mechanisms to explain the results obtained: either nebivolol action on insulin sensitivity has an NO-independent mechanism that assures increased insulin sensitivity, even when the drug does not cause a significant increase in endogenous NO production; or the endogenous NO produced in the N$_{12ew}$, HSuN$_{12ew}$ and SHR$_N$ groups was rapidly inactivated to other metabolites, that not NO$_3^-$, after exerting its positive effect on insulin action. One of the end-product formed may be S-nitrosoglutathione (GSNO), since nebivolol has been described as being a inhibitor of S-nitrosoglutathione reductases. According to this hypothesis, long-term treatment with nebivolol would favor the catabolism of NO to GSNO in detriment of NO$_3^-$, making NO production undetectable using the experimental design described herein. Finally, another option would be that in aging rodent model [33] and SHR [34] increased oxidative stress decreases NO bioavailability, preventing a significant enhancement in plasma NO/NO$_3^-$ [35, 56]. The latter hypothesis is in disagreement with Fratta Pasini’s results that nebivolol decreases oxidative stress in essential hypertensive patients and increases nitric oxide by reducing its oxidative inactivation [37]. Therefore, the hypothesis that nebivolol increases insulin-sensitivity through an NO independent mechanism is also noteworthy, due to the drugs pleiotropic effects which include activation of β2 and β3 adrenoreceptors, antagonism of α1-adrenoreceptors [58] and anti-oxidant effects [9, 10, 39]. We have previously reported that physiological insulin action requires both NO and glutathione (GSH), an endogenous anti-oxidant [15, 40]. Nebivolol’s intrinsic anti-oxidant actions may increase endogenous GSH levels, leading to a synergistic action on insulin sensitivity, via both NO and GSH pathways. Hence, an alternative mechanism of action to nebivolol-induced insulin sensitization may be through reestablishment of anti-oxidant defenses in biological system exposed to increased oxidative stress as in aging-models or diet-induced insulin resistant states. Therefore, further studies are necessary to conclude on the role of NO production on nebivolol’s positive effects on insulin sensitivity and glucose homeostasis. Our work contributed to strengthen the paradigm that vasodilating β-blockers are neutral on glycemic and metabolic factors [25], taking it one step forward by showing that nebivolol both prevents the development of diet-induced insulin resistance and restores insulin sensitivity in animal models of impaired insulin action. These effects appear to be partially independent of nebivolol ability of increasing endogenous NO production since nebivolol did not increase the major nitric oxide metabolite, NO$_3^-$, in all test groups, but it enhanced insulin action in animal models of diet-induced, aged-induced and genetically-induced insulin resistance.

V. CONCLUSIONS

THE RESULTS PRESENTED HEREIN DEMONSTRATE THAT NEBIVOLOL INCREASES INSULIN SENSITIVITY IN INSULIN RESISTANT ANIMAL MODELS SUGGESTING IT SHOULD BE PREFERRED OVER ATENOLOL AS AN ANTI-HYPERTENSIVE IN THE PRESENCE OF RISK FACTORS FOR THE METABOLIC SYNDROME.

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Fig. 1. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on insulin sensitivity in standard-diet and high-sucrose diet rats (HSu). A) the effect of nebivolol and atenolol administration during 4 weeks on insulin sensitivity determined by the insulin tolerance test, expressed as KITT. B) the effect of nebivolol and atenolol administration during 12 weeks on insulin sensitivity determined by the insulin tolerance test, expressed as KITT. Values represent mean±SEM. *p<0.05, **p<0.01 compared with respective 4 week and 12 week control standard diet groups; #p<0.05, ##p<0.01, compared with respective control HSu groups. Two-Way ANOVA with Bonferroni multicomparison test.

Fig. 2. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on insulin sensitivity in spontaneously hypertensive rats (SHR) determined by the insulin tolerance test, expressed as KITT. Values represent mean±SEM. **p<0.01 compared with control group; ##p<0.01, compared with SHR group. One-Way ANOVA, with Bonferroni multicomparison test.
Fig. 3. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on mean arterial pressure (MAP) in standard-diet and high-sucrose diet rats. A) effect of nebivolol and atenolol administration during 4 weeks on MAP. B) effect of nebivolol and atenolol administration during 12 weeks on MAP. Values represent mean±SEM. #p<0.05 compared with respective 4 week and 12 week control standard diet groups. ***p<0.001 compared with respective control high-sucrose diet groups groups. Two-Way ANOVA with Bonferroni multicomparison test.

Fig. 4. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on mean arterial pressure in spontaneously hypertensive rats (SHR). Values represent mean±SEM. **p<0.01 compared with control group; ###p<0.001, ##p<0.01 compared with SHR group. One-Way ANOVA with Bonferroni , multicomparison test.
Fig. 5. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on plasma NO/NO$_3$ levels in standard-diet and high-sucrose diet rats (HSu). A) effect of nebivolol and atenolol administration during 4 weeks on plasma NO/NO$_3$. B) effect of nebivolol and atenolol administration during 12 weeks on plasma NO/NO$_3$. Values represent mean±SEM. **p<0.01, ***p<0.001 compared with control standard diet group in the 4 week treatment protocol. #p<0.05 compared with control HSu group in the 4 week treatment protocol. Two-Way ANOVA, with Bonferroni multicomparison test.

Fig. 6. Effect of nebivolol (1mg/kg/day) and atenolol (10mg/kg/day) on plasma NO/NO$_3$ levels in spontaneously hypertensive rats (SHR). Values represent mean±SEM. ***p<0.001 compared with control group. One-Way ANOVA with Bonferroni multicomparison test.
References


