O-GlcNAcylation Contributes to Augmented Vascular Reactivity Induced by Endothelin 1

Victor V. Lima, Fernanda R. Giachini, Fernando S. Carneiro, Zidonia N. Carneiro, Mohamed A. Saleh, David M. Pollock, Zuleica B. Fortes, Maria Helena C. Carvalho, Adviye Ergul, R. Clinton Webb, Rita C. Tostes

Abstract—O-GlcNAcylation augments vascular contractile responses, and O-GlcNAc-proteins are increased in the vasculature of deoxycorticosterone-acetate salt rats. Because endothelin 1 (ET-1) plays a major role in vascular dysfunction associated with salt-sensitive forms of hypertension, we hypothesized that ET-1–induced changes in vascular contractile responses are mediated by O-GlcNAc modification of proteins. Incubation of rat aortas with ET-1 (0.1 μmol/L) produced a time-dependent increase in O-GlcNAc levels and decreased expression of O-GlcNAc transferase and β-N-acetylglucosaminidase, key enzymes in the O-GlcNAcylation process. Overnight treatment of aortas with ET-1 increased phenylephrine vasoconstriction (maximal effect [in moles]: 19±5 versus 11±2 vehicle). ET-1 effects were not observed when vessels were previously instilled with anti-O-GlcNAc transferase antibody or after incubation with an O-GlcNAc transferase inhibitor (3-[2-adamantanylethyl]-2-[4-chlorophenyl]azamethylene)-4-oxo-1,3-thiazaperhyd roine-6-carboxylic acid; 100 μmol/L). Aortas from deoxycorticosterone-acetate salt rats, which exhibit increased prepro-ET-1, displayed increased contractions to phenylephrine and augmented levels of O-GlcNAc proteins. Treatment of deoxycorticosterone-acetate salt rats with an endothelin A antagonist abrogated augmented vascular levels of O-GlcNAc and prevented increased phenylephrine vasoconstriction. Aortas from rats chronically infused with low doses of ET-1 (2 pmol/kg per minute) exhibited increased O-GlcNAc proteins and enhanced phenylephrine responses (maximal effect [in moles]: 18±2 versus 10±3 control). These changes are similar to those induced by O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate, an inhibitor of β-N-acetylglucosaminidase. Systolic blood pressure (in millimeters of mercury) was similar between control and ET-1–infused rats (117±3 versus 123±4 mm Hg; respectively). We conclude that ET-1 indeed augments O-GlcNAc levels and that this modification contributes to the vascular changes induced by this peptide. Increased vascular O-GlcNAcylation by ET-1 may represent a mechanism for hypertension-associated vascular dysfunction or other pathological conditions associated with increased levels of ET-1. (Hypertension. 2010;55:180-188.)

Key Words: β-N-acetylglucosamine (O-GlcNAc) ■ endothelin 1 ■ vascular reactivity ■ β-N-acetylglucosaminidase ■ hypertension

O-Linked attachment of β-N-acetyl-glucosamine (O-GlcNAc) on serine and threonine residues of nuclear and cytoplasmic proteins is a highly dynamic and ubiquitous posttranslational modification that plays a key role in altering the function, activity, subcellular localization, and stability of target proteins.1–3 The attachment of a single O-GlcNAc is catalyzed by the enzyme O-GlcNAc transferase (OGT; uridine diphospho-N-acetylglucosamine:polypeptide β-N-acetylglucosaminyltransferase; UDP-N-ac transferase), and the hydrolytic cleavage of O-GlcNAc is catalyzed by β-N-acetylglucosaminidase (OGA).3

Although it is clear that O-GlcNAcylation plays a critical role in the regulation of cell function, there is a paucity of information on the vascular effects of O-GlcNAcylation. Preliminary evidence from our laboratory suggests that augmented vascular O-GlcNAc proteins increase reactivity to constrictor stimuli. Accordingly, incubation of arterial segments with O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate (PugNAC), which blocks OGA activity by mimicking the enzyme-stabilized transition state,4,5 leads to increased reactivity to phenylephrine and serotonin.6

In addition, the vascular content of O-GlcNAc proteins is augmented in arteries from deoxycorticosterone acetate (DOCA) salt rats, a mineralocorticoid model of hypertension. Interestingly, increased levels of O-GlcNAc (induced by
Infusion of Low Doses of ET-1

Rats were anesthetized with 5% isoflurane in 100% O₂ and maintained during surgery with 1.9% to 2.1% isoflurane in 100% O₂ through a nose cone. The depth of anesthesia was verified by noting the absence of physical responses to firm paw pinch and corneal propping. Osmotic minipumps (ALZET Osmotic Pumps) were implanted SC to deliver ET-1 (2 pmol/kg per minute; Phoenix Pharmaceuticals) or sodium chloride (0.9%) through a catheter in the jugular vein for 14 days. Aortas from ET-1-infused or control rats were freshly removed to evaluate vascular function or protein expression.

DOCA-Salt Hypertension

DOCA-salt hypertension was induced as described previously. Briefly, rats were unilaterally nephrectomized (Uni), and DOCA (200 mg/kg) silastic pellets were implanted SC in the scapular region. DOCA rats received water containing 1.0% NaCl and 0.2% KCl for 5 weeks. Control rats were also uninephrectomized and received silastic pellets without DOCA and tap water. Animals simultaneously received either the ETA antagonist atrasentan (5 mg/d per kilogram of body weight, PO per gavage) or vehicle for 5 weeks. Aortas from DOCA-salt, treated, or those not treated with ETA antagonist, as well as Uni, rats were freshly removed to evaluate vascular function or protein expression.

Systolic Blood Pressure Measurements

Systolic blood pressure was measured in nonanesthetized animals by tail cuff using an RTBP1001 rat-tail blood pressure system (Kent Scientific Corporation).

Euglycemic-Hyperinsulinemic Clamp

Rats were fasted overnight before being anesthetized and evaluated for insulin sensitivity using the euglycemic-hyperinsulinemic clamp method. Catheters were placed in the femoral artery for blood sampling and femoral and jugular veins for infusion of insulin and glucose. Blood samples were obtained at 5-minute intervals (Novolin; 30 mU/kg per minute; Novo Nordisk Pharmaceuticals). Glucose infusion (100 mg/mL of glucose in saline) was adjusted to maintain a plasma glucose level at 125 mg/dL within 60 minutes and maintained for an additional 30 minutes. The final 7 readings were averaged and reported as the glucose infusion rate (in milligrams per kilogram per minute).

Vascular Functional Studies

In 1 set of experiments, aortas were functionally evaluated after incubation with ET-1 for 24 hours, with or without treatments, as described previously. In another set of experiments, vascular function was assessed in aortas freshly harvested from ET-1-infused rats (14 days) or DOCA-salt rats. In this case, after euthanasia, thoracic aortas were removed and cleaned from fat tissue in an ice-cold physiological salt solution. Aortic rings (4 mm in length) were simultaneously received either the ETA antagonist atrasentan (5 mg/d per kilogram of body weight, PO per gavage) or vehicle for 5 weeks. Aortas from DOCA-salt, treated, or those not treated with ETA antagonist, as well as Uni, rats were freshly removed to evaluate vascular function or protein expression.

Methods

Animals

Male Wistar rats (8 to 10 weeks old, 230 to 250 g; Harlan Laboratories, Indianapolis, IN) were used in this study. All of the procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education. The animals were housed on a 12-hour light/dark cycle and fed a standard chow diet with water ad libitum.

ET-1 Incubation Procedures

After euthanasia, thoracic aortas were removed and cleaned from fat tissue in an ice-cold physiological salt solution. Arterial segments were incubated in the Eagle minimum essential medium containing L-glutamine (1.0%), FBS (10.0%), penicillin (0.5%), and streptomycin (0.5%). Rings were incubated with either vehicle (H₂O) or ET-1 (1 μM) for 1, 3, 6, 12, or 24 hours to verify the optimal time point of induction of O-GlcNAc proteins, as well as changes in the expression of OGT and OGA. In other experiments, incubations were performed for 24 hours with the following: vehicle (H₂O), PugNAc (100.0 μM), or ET-1 (0.1 μM) for 1, 3, 6, 12, or 24 hours to verify the optimal time point of induction of O-GlcNAc proteins, as well as changes in the expression of OGT and OGA. In some experiments, arteries were pretreated (3 hours) with an OGT inhibitor of antibody in 100 μL of PBS and incubated at room temperature for 30 minutes to allow the complex to form. The aortas were transferred to a sterile 24-well cell culture plate, overlaid with 200 μL of Chariot/antibody complex, and mixed gently. Eagle minimum essential medium (750 μL) was added, and tissues were further incubated for 2 hours at 37°C. After this period, rings were mounted in the myograph, and functional studies were performed.

Antibody Delivery by the Chariot Technique

Antibodies against OGT (Santa Cruz Biotechnology) were intracellularly delivered by the Chariot technique (Chariot Protein Delivery Reagent, Active Motif). This transfection reagent is able to deliver antibodies into cells while preserving their ability to localize to the proper cellular compartment and to recognize antigens within the cell[12] and in our experiments was used to directly inhibit OGT protein. Chariot/antibody complexes were prepared and used accord-
sium chloride (KCl) and, after washing and a new stabilization period, the presence of the endothelium was verified by contracting the segments with phenylephrine (PE; 1 μmol/L) followed by stimulation with acetylcholine (10 μmol/L). Concentration-response curves to PE (1 nM to 100 μM) and serotonin (5-hydroxytryptamine: 1 nM to 100 μM) were performed, and responses are represented as the percentage of KCl-induced contraction.

**Western Blot Analysis**

Proteins (60 μg) extracted from endothelium-intact aortas were separated by electrophoresis, and Western blots were performed as described previously. Antibodies used were anti-O-GlcNAC antibody, CTD 110.6 (1:2000; Pierce Biotechnology), OGT (1:400, Santa Cruz Biotechnology antibodies), and OGA antibody, which was kindly provided by Dr Sidney Whiteheart (1:1000, University of Kentucky). OGA is a 106-kDa heterodimer complex containing a 54-kDa α-subunit and a 51-kDa β-subunit.

Aortas from 3 different experimental conditions (incubated with ET-1, from ET-infused rats, and from DOCA-salt rats) were used to perform Western blots experiments. Analyses of the bands were performed with software (Un-ScanIT Gel 6.1) that evaluates the density profile extraction and band analysis for entire lanes. The result of the sum of the bands was normalized by β-actin and expressed as arbitrary units.

**Real-Time RT-PCR for Prepro–ET-1**

Gene expression of prepro–ET-1 was determined as described previously. Briefly, total RNA was extracted using the RNeasy kit (Qiagen Sciences), and 1 μg of total RNA was reverse transcribed in a final volume of 50 μL using the high-capacity cDNA archive kit (Applied Biosystems). Primers for prepro–ET-1 (No. Rn00561129_m1) mRNA were obtained from Applied Biosystems. Real-time RT-PCR (quantitative PCR) reactions were performed using the 7500 fast Real-Time PCR system (Applied Biosystems) in a total volume of 20-μL reaction mixture following the manufacturer’s protocol, using the TaqMan fast universal PCR master mix (Applied Biosystems) and 0.1 mmol/L of each primer. Relative gene expression for prepro–ET-1 mRNA was normalized to samples from Uni rats (calibrator), and results were calculated with the ΔΔCt method and expressed as n-fold differences in prepro–ET-1 gene expression relative to 18S rRNA and to the calibrator.

**Data Analysis**

The results are shown as mean±SEM, and “n” represents the number of animals used in the experiments. Contractions were recorded as changes in the displacement (in moles) from baseline. Relaxation is expressed as the percentage of change from the PE-contracted levels. Concentration-response curves were fitted using a nonlinear interactive fitting program (GraphPad Prism 4.0; GraphPad Software Inc), and 2 pharmacological parameters were obtained: the maximal effect of the agonist (or E_{max}) and the negative logarithm of the concentration of agonist that produces 50% of the maximum response (−log EC_{50} [or pD_{2}]). Statistical analyses of E_{max} and pD_{2} values were performed using 1-way ANOVA or Student t test. Post hoc comparisons were performed using the Newman-Keuls test. Western blot data were analyzed by 1-sample t test, and the P value was computed from the t ratio and the numbers of degrees of freedom. Values of P<0.05 were considered statistically significant.

**Results**

The incubation of aortas with ET-1 (0.1 μmol/L) produced a time-dependent increase in vascular O-GlcNAc levels (Figure 1A). No differences on the O-GlcNAc levels were observed between aortas freshly harvested or after 24-hour incubation with vehicle. An inverse temporal correlation was observed for OGT (Figure 1B) and OGA (Figure 1C) expression.

After 24 hours of treatment with ET-1, arteries from Wistar rats displayed increased vascular reactivity to the α1-adrenergic agonist PE, and developed force levels similar to those exhibited by aortas incubated with PugNAc, an inhibitor of OGA (Figure 2A and Table 1).

No differences in KCl-induced contraction were observed among the groups (E_{max} [in moles]: 22.6±1.9 vehicle, 22.8±1.8 ET-1, and 21.67±1.6 PugNAc). In addition, differences in PE-induced contraction, after ET-1 incubation for 24 hours, persisted in arteries without endothelium (E_{max} [in percentage of KCl]: 117.1±4.8 vehicle versus 174.6±11 ET-1).

Seronotonin-induced contraction was increased in aortas after ET-1 incubation (Figure 2B), when compared with control arteries (pD_{2}: 6.3±0.1 vehicle versus 6.9±0.1 ET-1; E_{max} [percentage of KCl]: 79.0±7 vehicle versus 101±1 ET-1; n=5), showing that O-GlcNAcylation does not interfere specifically with α1-adrenergic–mediated responses. In vivo ET-1 infusion in Wistar rats for 14 days enhanced aortic contractile responses to PE in comparison with their respective controls (Figure 2C and Table 1).

No differences in KCl-induced contraction were observed among the groups (E_{max} [in moles]: 22.7±2 control versus 25±1 ET-1). In addition, ET-1 infusion in Wistar rats augmented O-GlcNAc levels of vascular proteins (Figure 3A). ET-1 infusion for 2 weeks did not change vascular OGT expression (Figure 3B) but decreased OGA expression (Figure 3C). Systolic blood pressure was similar between control and ET-1–infused rats (117±3 versus 123±4 mm Hg, respectively; n=5; Table 2). ET-1 had no effect on fasting glucose levels (99.7±2 versus 102±7.4 mg/dL in control and ET-1 infusion, respectively) or body weight (Table 2).

ET-1 had no effect on insulin sensitivity; glucose infusion rate was 6.0±0.6 and 6.8±0.6 mg/kg per minute in control (n=4) and ET-1–infused (n=3) rats, respectively.

The selective inhibition of OGT, with 3-(2-adamantanyl)-2-((4-chlorophenyl)azamethylene)-4-oxo-1,3-thiazaperhydroine-6-carboxylic acid (3-[2-adamantanyl]-2-(4-chlorophenyl)azamethylene)-4-oxo-1,3-thiazaperhydroine-6-carboxylic acid (TimTec LLC), resulted in decreased vascular O-GlcNAc levels (Figure 4A) and also attenuated the effects of ET-1 on vascular reactivity (Figure 4B). As shown in Figure 4, the effects of ET-1 on O-GlcNAc protein levels and vascular reactivity were not observed when vessels were instilled previously with antibodies against OGT (Figure 4C and 4D, respectively), intracellulary delivered by a transfection system (ActiveMotif USA). Incubation with an IgG antirabbit antibody was used as an additional control and did not modify ET-1–induced effects (data not shown).

We sought to determine whether ET-1 activation is a key element for increased vascular O-GlcNAc protein levels and, consequently, increased vascular reactivity in mineralocorticoid hypertension. To address this question, we used a pharmacological approach: treatment of DOCA–salt rats with an ETA receptor antagonist (atrasentan; 5 mg·kg⁻¹·d⁻¹). At 5 weeks of treatment, systolic blood pressure was higher in DOCA–salt rats in comparison with Uni rats (Uni: 124.9±3.6 mm Hg versus DOCA: 163.6±6.4 mm Hg; n=6; Table 2). DOCA–salt rats exhibited decreased body weight in comparison with Uni (Table 2). Prepro–ET-1 gene expression was augmented in aortas from DOCA–salt rats (fold of...
change: 2.1±0.4 versus 1 control), and ET<sub>A</sub> blockade with atrasentan did not prevent increased prepro–ET-1 mRNA expression (fold of change: 1.8±0.1), as determined by quantitative PCR. Treatment with atrasentan attenuated, but did not normalize, blood pressure in DOCA-salt rats (137.5±5.74 mm Hg; n=6; Table 2) and did not change body weight in DOCA-salt animals (Table 2). On the other hand, the ET<sub>A</sub> antagonist abrogated augmented vascular levels of O-GlcNAc in DOCA-salt rats (Figure 5A) and also prevented increased contractile responses to PE in aorta from these animals (Figure 5B). These results suggest that ET<sub>A</sub> receptor activation plays a role in ET-1–induced vascular effects. They
are further reinforced by in vitro experiments, where atrazen-
tan (1 μmol/L) attenuated the effects of ET-1 incubation on
O-GlcNAc protein levels and vascular reactivity (Figure 5C
and 5D, respectively).

Discussion

O-GlcNAc has important implications for the regulation of
protein structure and function and the interplay with other
posttranslational modifications, such as phosphorylation.4,20,21
However, with respect to vascular function, O-GlcNAc is a
relatively unexplored area. Our preliminary studies showed that
O-GlcNAc is increased in the vasculature from DOCA-salt
hypertensive rats.8 Considering that ET-1, which is increased in
this model of salt-dependent hypertension,9,10 contributes to
vascular dysfunction in arteries from DOCA-salt rats, we sought
to investigate whether O-GlcNAc underlies effects of ET-1 on
vascular function.

Our data show for the first time that ET-1 augments
vascular O-GlcNAcylation and that this posttranslational
modification contributes to the vascular changes produced by
this peptide. O-GlcNAcylation of nuclear and cytoplasmic
proteins is regulated by OGT and OGA. We demonstrated
that ET-1 produces a time-dependent and transient decrease
in OGT vascular expression. Interestingly, PugNAc incuba-
tion for 24 hours, which increases vascular content of
O-GlcNAc proteins in arteries from control animals,6,22 also
decreases OGT expression.6 Decreased OGT may represent a
compensatory mechanism for the augmented vascular levels
of O-GlcNAc proteins. On the other hand, aortas from rats
chronically infused with low doses of ET-1, which exhibit
increased O-GlcNAc proteins, did not exhibit changes in
vascular OGT expression at 14 days. This may be related to
the transient changes in OGT expression produced by ET-1.
A possible explanation is that OGT expression was measured
at a time point (after 14 days) where expression has already
returned to basal levels (as is the case for OGT expression
after 24-hour incubation with ET-1 in vitro).

OGA expression was also decreased after treatment with
ET-1, similarly to what has been demonstrated in aortas
incubated with PugNAc.6 In addition, ET-1 infusion for 14
days decreased OGA expression in thoracic aortas. Increased
vascular O-GlcNAcylation on in vivo and in vitro treatment
with ET-1 may be mediated by decreased OGA expression or
activity.

To clarify whether ET-1-induced augmented vascular
O-GlcNAc protein content plays a role in the vascular
functional changes induced by this peptide, we determined
the vascular contraction to the
1-adrenergic agonist PE in
various conditions. Treatment with ET-1 for 24 hours en-
hanced contractile responses of rat aortas to PE. Developed
force levels were similar to those exhibited by aortas incu-
bated with PugNAc. Furthermore, ET-1 infusion in Wistar
rats enhanced aortic contractile responses to PE in compar-
ison with their respective controls, as has been shown in other
studies.9,23 Of importance, chronic ET-1 infusion did not
produce consistent increases in blood pressure (in agreement

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Table 1. E_{max} and pD_{2} Values for Phenylephrine in Arteries
From Rats Incubated With ET-1 or From Rats Infused With ET-1

<table>
<thead>
<tr>
<th>Incubation (24 h)</th>
<th>E_{max}</th>
<th>pD_{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>69±6</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>PugNAc</td>
<td>115±5*</td>
<td>6.7±0.06</td>
</tr>
<tr>
<td>ET-1</td>
<td>107±5*</td>
<td>7.1±0.1*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infusion (14 d)</th>
<th>E_{max}</th>
<th>pD_{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77±5</td>
<td>7.4±0.06</td>
</tr>
<tr>
<td>ET-1 infusion</td>
<td>103±3*</td>
<td>6.9±0.06*</td>
</tr>
</tbody>
</table>

Values are mean±SEM for N=6 experiments in each group. Contractile
response to PE are represented as the percentage of KCl-induced contraction.
*P<0.05 vs vehicle or control.
with a recent report by Wang and Wang\(^{24}\), glucose levels, or insulin sensitivity.

Recent work by Clarke et al.\(^{14}\) has demonstrated that antibodies can be targeted to selective signaling cascades in smooth muscle cells of isolated ring segments of arteries. We have performed experiments with arteries incubated for 24 hours using this innovative approach to target signaling cascades as an additional method to complement our pharmacological studies. In our experiments, Chariot protein transfection reagent was used to deliver anti-OGT antibodies to intact aortic rings. The effects of ET-1 are not observed when vessels are transfected previously with antibodies against OGT. Empty Chariot did not significantly change actions of ET-1. This result is further supported by experiments where arteries were incubated with a selective OGT inhibitor: 3-(2-adamantanylethyl)-2-[(4-chlorophenyl)azamethylene]-4-oxo-1,3-thiazaperhyd roine-6-carboxylic acid. This pharmacological inhibitor attenuated both the effects of ET-1 in O-GlcNAc proteins levels and vascular reactivity. On the basis of these observations, we conclude that elevated levels of O-GlcNAc by ET-1 represent a common mechanism underlying the adverse effects of the peptide on vascular function.

We have demonstrated previously that increased levels of O-GlcNAc augments responses to contractile stimuli\(^6\) and

![Image](http://hyper.ahajournals.org/)

**Figure 3.** ET-1 infusion in vivo for 14 days augments O-GlcNAc levels in aortas and decreases vascular expression of OGA. Top, Representative Western blot images of (A) O-GlcNAc-proteins, (B) OGT, and (C) OGA. Bottom, Corresponding bar graphs showing the relative expression of O-GlcNAc, OGT, and OGA after normalization to β-actin expression. Results are presented as mean±SEM for n=4 in each experimental group. *\(P<0.05\) vs control (rats infused with sodium chloride [0.9%]).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ET-1 Infused</th>
<th>Uni</th>
<th>DOCA</th>
<th>DOCA + Atrasentan</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>117.5±3</td>
<td>123.4±4</td>
<td>124.9±4</td>
<td>163.6±6(*)</td>
<td>137.5±6(*)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>385±10</td>
<td>380±9</td>
<td>391±13(*)</td>
<td>320±7</td>
<td>309±12(*)</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure. Values are mean±SEM for N=6 in each group.

*\(P<0.05\) vs respective control.
that O-GlcNAcylation is associated with the development of increased vascular contractility in DOCA-salt hypertension. Interestingly, ET-1, for which expression is increased in DOCA-salt hypertension, is associated with vascular dysfunction. Here, we showed that ET-1 exposition in vitro and in vivo not only augments vasoconstriction to PE but also increases O-GlcNAc protein levels and modulates OGT and OGA expression. Accordingly, we demonstrated that treatment with an ETA receptor antagonist prevents changes in O-GlcNAc levels acutely and chronically induced by ET-1. In addition, treatment with atrasentan was able to normalize O-GlcNAc levels and to prevent an increased contractile response to PE stimulation in arteries from DOCA-salt rats.

One may suggest that atrasentan reduced protein modification via a decrease in blood pressure. However, we found that atrasentan attenuated, but did not normalize, blood pressure in DOCA-salt rats. In addition, ET-1–infusion for 14 days did not result in changes in blood pressure. It seems that, in this case, O-GlcNAc protein modification does not correlate directly with blood pressure but does correlate with PE reactivity. The use of other antihypertensive agent that does not affect the ET-1 system may help to further elucidate this suggestion.

In this sense, it is well established that increased contractile responsiveness of the vasculature, as a result of increase contraction or decreased relaxation, is a hallmark of hypertension. We speculate that ET-1 modulates signaling proteins, via O-GlcNAc modification, that are important for vascular tone control, such as NO synthase, protein kinase C, members of the mitogen-activated protein kinase family, and

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Figure 4. ET-1 effects on O-GlcNAc protein levels and vascular reactivity are not observed when vessels are transfected previously with antibodies against OGT or incubated with OGT inhibitor. Treatment with (A and B) the OGT inhibitor, as well as (C and D) neutralizing antibodies for anti-OGT (Chariot [OGT]) decrease vascular O-GlcNAc levels. OGT inhibition (A and C) reduced vascular contraction and (B and D) decreased O-GlcNAc proteins levels on ET-1 incubation for 24 hours. B and D, Top, Western blot image of O-GlcNAc proteins. Bottom, Corresponding bar graphs showing the relative O-GlcNAc proteins after normalization to β-actin expression. Experimental values of contraction were calculated relative to the contractile response produced by 120 mmol/L of KCl, which was taken as 100%. Results are presented as mean ± SEM in each experimental group. *P < 0.05 vs vehicle (dimethyl sulfoxide).
small G proteins, and that are targets for O-GlcNAcylation. Therefore, O-GlcNAc modification of proteins induced by ET-1 may contribute to an augmented vascular response to constrictor stimuli.

In addition, increasing evidence suggests that O-GlcNAcylation can modulate protein function by interfering with protein phosphorylation. This suggestion takes into consideration that both modifications occur on serine and threonine residues, are dynamically added and removed from proteins in response to cellular signals, and alter the function and association of the modified protein. The relationship between O-GlcNAc and phosphorylation is obviously complex but may be a key to understanding the function of O-GlcNAc addition. The reciprocal interplay between O-GlcNAc and phosphorylation may represent an additional link between ET-1–activated signal transduction mechanisms and the effects of O-GlcNAc on the vasculature. Conversely, phosphorylation has been shown to directly affect OGT expression and activity.26

In conclusion, our data provide evidence that ET-1 augments O-GlcNAc levels, and this modification contributes to the vascular effects of ET-1. We propose that modulation of increased vascular O-GlcNAc by ET-1 may represent a novel mechanism contributing to the vasoactive properties of this potent peptide. Definition of O-GlcNAc–modified vascular proteins will contribute to a better understanding of how this posttranslational modification affects vascular reactivity in physiological and pathophysiological conditions.
Perspectives
Arterial hypertension often coexists with diabetes mellitus, and >80% of patients with type 2 diabetes mellitus develop hypertension. Most of the deleterious effects associated with abnormal O-GlcNAcylation have been described in diabetic or hyperglycemic conditions, but we have shown recently that O-GlcNAcylation, which augments vascular contractile responses, is increased in the vasculature of hypertensive animals. The present study showing that O-GlcNAcylation plays a role on ET-1–induced vascular responses further suggests that this posttranslational modification is a key regulator of vascular function. Important insight into the pathological processes leading to vascular dysfunction can be gained from elucidating the mechanisms by which O-GlcNAcylation disrupts vascular function.

Sources of Funding
This study was supported by grants from the National Institutes of Health (HL-74167), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (Brazil).

Disclosures
None.

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Hypertension. 2010;55:180-188; originally published online November 30, 2009; doi: 10.1161/HYPERTENSIONAHA.109.143818

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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