Transglutaminase activity is decreased in large arteries from hypertensive rats compared with normotensive controls

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Transglutaminases (TGs) catalyze the formation of covalent cross-links between glutamine residues and amine groups. This cross-linking activity has been implicated in arterial remodeling. Because hypertension is characterized by arterial remodeling, we hypothesized that TG activity, expression, and functionality would be increased in the aorta, but not in the vena cava (which does not undergo remodeling), from hypertensive rats relative to normotensive rats. Spontaneously hypertensive stroke-prone rats (SHRSP) and DOCA-salt rats as well as their respective normotensive Wistar-Kyoto or Sprague-Dawley counterparts were used. Immunohistochemistry and Western blot analysis measured the presence and expression of TG1 and TG2, in situ activity assays quantified active TGs, and isometric contractility was used to measure TG functionality. Contrary to our hypothesis, the activity (52% DOCA-salt vs. control rats and 56% SHRSP vs. control rats, P < 0.05), expression (TG1: 54% DOCA-salt vs. control rats, P > 0.05, and TG2: 77% DOCA-salt vs. control rats, P < 0.05), and functionality of TG1 and TG2 were decreased in the aorta, but not in the vena cava, from hypertensive rats. Mass spectrometry identified proteins uniquely amidated by TGs in the aorta that play roles in cytoskeletal regulation, redox regulation, and DNA/RNA/protein synthesis and regulation and in the vena cava that play roles in cytoskeletal regulation, coagulation regulation, and cell metabolism. Consistent with the idea that growing cells lose TG2 expression, vascular smooth muscle cells placed in culture lost TG2 expression. We conclude that the expression, activity, and functionality of TG1 and TG2 are decreased in the aorta, but not in the vena cava, from hypertensive rats compared with control rats.

Transglutaminase; hypertension; aorta; vena cava; arterial remodeling

Transglutaminases (TGs) are a family of Ca2+-dependent enzymes that are best known for catalyzing the formation of a covalent bond between an amine group and the γ-carboxamide group of a glutamine residue (8). The amine can come from a variety of sources, including polyamines (7), monoamines such as serotonin [5-hydroxytryptamine (5-HT)] (19), or peptide- or protein-bound lysines (14). TG1, TG2, TG4, and factor XIII links between glutamine residues and amine groups. This cross-linking activity has been implicated in arterial remodeling. Because hypertension is characterized by arterial remodeling, we hypothesized that TG activity, expression, and functionality would be increased in the aorta, but not in the vena cava (which does not undergo remodeling), from hypertensive rats relative to normotensive rats. Spontaneously hypertensive stroke-prone rats (SHRSP) and DOCA-salt rats as well as their respective normotensive Wistar-Kyoto or Sprague-Dawley counterparts were used. Immunohistochemistry and Western blot analysis measured the presence and expression of TG1 and TG2, in situ activity assays quantified active TGs, and isometric contractility was used to measure TG functionality. Contrary to our hypothesis, the activity (52% DOCA-salt vs. control rats and 56% SHRSP vs. control rats, P < 0.05), expression (TG1: 54% DOCA-salt vs. control rats, P > 0.05, and TG2: 77% DOCA-salt vs. control rats, P < 0.05), and functionality of TG1 and TG2 were decreased in the aorta, but not in the vena cava, from hypertensive rats. Mass spectrometry identified proteins uniquely amidated by TGs in the aorta that play roles in cytoskeletal regulation, redox regulation, and DNA/RNA/protein synthesis and regulation and in the vena cava that play roles in cytoskeletal regulation, coagulation regulation, and cell metabolism. Consistent with the idea that growing cells lose TG2 expression, vascular smooth muscle cells placed in culture lost TG2 expression. We conclude that the expression, activity, and functionality of TG1 and TG2 are decreased in the aorta, but not in the vena cava, from hypertensive rats compared with control rats.

The thoracic aorta and inferior vena cava were investigated because each has a contrasting structure, function, and remodeling response to hypertension. The aorta is composed of seven layers of smooth muscle, is a conduit vessel, and undergoes extensive remodeling, whereas the vena cava is composed of one layer of smooth muscle, is a capacitance vessel, and undergoes very little remodeling in response to hypertension, at least in DOCA-salt hypertension (20). In this way, the vena cava served as a comparison vessel because it is of similar size and proximity to the aorta.

We hypothesized that the activity of vascular TGs is increased in the aorta, but not in the vena cava, from hypertensive rats compared with their normotensive counterparts. Two models of hypertension were used: the DOCA-salt rat model and the spontaneously hypertensive stroke-prone rat (SHRSP) model. A multidisciplinary approach was adopted: immunohistochemistry (IHC) to visualize TG protein, Western blot analysis to measure TG protein expression, a novel in situ activity assay to measure isozyme-specific TG activity, an in situ pentylamine-biotin [5-biotinamidoethylamine (BAP)] incorporation assay to measure general TG activity, isometric contractility to examine TG function, cell culture to investigate...
TG expression in proliferating VSMCs, and mass spectrometry to identify proteins uniquely amidated by TGs in the aorta and vena cava. Contrary to our hypothesis, the expression, activity, and functionality of TG1 and TG2 were decreased in the aorta, but not in the vena cava, from hypertensive rats compared with their normotensive counterparts. Consistent with this idea, TG substrates that were aorta specific were identified, and we found that cultured VSMCs, like growing VSMCs in the artery from hypertensive rats, also lost TG2 expression.

MATERIALS AND METHODS

Animal use/ethics. All protocols involving animals were approved by the Institutional Animal Care and Use Committee and followed guidelines put forth by Michigan State University. Rats were euthanized by an intraperitoneal injection of 60–80 mg/kg pentobarbital; the thoracic aorta and vena cava were removed by dissection for each of the following protocols. Rat systolic blood pressures were measured by standard tail-cuff techniques, as previously described (20).

Animal models. Animals were housed according to Michigan State University Institutional Animal Care and Use Committee standards with a 12:12-h light-dark cycle and had free access to standard rat chow and drinking water.

DOCA-salt rats. Male Sprague-Dawley rats (250–300 g, Charles River Laboratories, Portage, MI) were randomly assigned to DOCA or sham groups. During a uninephrectomy, while under isoflurane anesthesia, a DOCA (150 mg sc, Sigma-Aldrich, St. Louis, MO)-impregnated silicone elastomer (Silastic, Dow Corning, Midland, MI) was implanted subcutaneously in the DOCA group. Rats assigned to the sham group also underwent uninephrectomy but did not receive the DOCA implant. Postoperatively, DOCA-salt rats were given a solution of 1% NaCl and 0.2% KCl (wt/vol) to drink; sham rats drank normal tap water. Rats were on this regimen for 3–4 wk before experimentation. DOCA-salt rats had an average systolic blood pressure of 212 ± 4 mmHg, whereas sham rats had an average systolic blood pressure of 115 ± 7 mmHg (P < 0.05 by t-test). Rats were 13–15 wk old when euthanized.

SHRS rat. Male SHRSP rats were obtained from the breeding colony at the Department of Pharmacology and Toxicology, at Michigan State University (East Lansing, MI) maintained by Dr. Anne M. Dorrance. Age-matched normotensive Wistar-Kyoto (WKY) rats were obtained from Charles River (Wilmington, MA). SHRSP rats had an average blood pressure of 202 ± 9 mmHg, whereas WKY rats had an average blood pressure of 117 ± 1 mmHg. Rats were 12 wk old when euthanized.

Tissues were taken from animals for one of the following protocols.

Bright-field IHC. IHC was performed as previously described (20). After being harvested and cleaned, the rat thoracic aorta and vena cava were formalin fixed, paraffin embedded, and sectioned (8 μm sections were mounted and sealed with Vecta Mount (catalog no. H-5000, Vector Laboratories). Bright-field imaging was performed using a Nikon Eclipse inverted microscope (Nikon Group, Otsuwa, Japan), and images were captured using MMI Cell Tools (Molecular Machines & Industries, Zurich, Switzerland). Human skin (catalog no. 12-701-XA1, ProSci, Loveland, CO) was used as a positive control for TG1, and human breast ductal carcinoma tumor (catalog no. 10-010-XA1, ProSci) was used as a positive control for TG2. In situ TG activity assay. In situ TG1 and TG2 activity was detected using fluorescently labeled isozyme-specific glutamine donor peptides as previously described (9, 10). In addition to active peptides, negative control peptides composed of the same amino acid sequence with reactive glutamine (Q) replaced with nonreactive asparagine (N) were used (QN peptides). After being harvested and cleaned, the rat thoracic aorta and vena cava were fresh frozen, and 8-μm sections were mounted on slides and stored at −80°C until use. Sections were equilibrated to room temperature and blocked for 30 min in PBS with 150 mM NaCl and 1% BSA (catalog no. A7906-10G, Sigma-Aldrich). Next, sections were incubated in substrate reaction solution [5 mM CaCl2, 100 mM Tris·HCl (pH 8.0), and 1 mM DTT] with 0.1 μM FITC-labeled peptide [K5 (YEIQHKLPSSWWF) or K5QN for TG1 activity and T26 (HQSYVDPMWLH) or T26QN for TG2 activity] at 37°C for 90 min. Samples were then incubated in stop solution (25 mM EDTA in PBS) for 5 min at room temperature and washed in PBS (3 × 5 min at room temperature). Coverslips applied using ProLong Gold antifade reagent with 4',6-diamidino-2-phenylindole (catalog no. P36931, Invitrogen, Eugene, OR) and sealed with clear nail polish once dry. Fluorescent imaging was performed using a Nikon Eclipse inverted microscope (Nikon Group), and images were captured using NIS Elements BR 3.0 (Nikon Group).

In situ BAP assay and BAP/RECA-1 colocalization assay. After being harvested and cleaned, the rat thoracic aorta and vena cava were fresh frozen, and 8-μm sections were mounted on slides and stored at −80°C until use. Sections were equilibrated to room temperature and blocked for 30 min in PBS with 1% BSA (catalog no. A7906-10G, Sigma-Aldrich). Sections were incubated with substrate reaction solution [100 mM Tris·HCl (pH 8.0) and 1 mM DTT] with or without the amine donor substrate BAP (4 mM, catalog no. 21345, Pierce Biotechnology, Rockford, IL) at 37°C for 90 min. Stop solution (25 mM EDTA in PBS) was added for 5 min at room temperature. Sections were washed in PBS (3 × 5 min at room temperature), incubated in Alexa fluor 555-conjugated streptavidin (1:1000 in PBS, catalog no. S32355, Invitrogen) at 37°C for 1 h, and washed in PBS again (3 × 5 min at room temperature). Coverslips were mounted using ProLong Gold antifade reagent with 4’,6-diamidino-2-phenylindole (catalog no. P36931, Invitrogen) and sealed with clear nail polish once dry. Fluorescent imaging was performed using a Nikon Eclipse inverted microscope (Nikon Group), and images were captured using NIS Elements BR 3.0 (Nikon Group). The colocalization assay was performed in the same manner but with the following additions: 1.5% goat serum in the blocking solution and reaction solution, RECA-1 primary antibody (1:50, catalog no. ab22492, Abcam) in the reaction solution, and Alexa fluor 488 goat anti-mouse secondary antibody (1:1,000, catalog no. A11029, Invitrogen) in the secondary antibody solution.

Cultured VSMC assay. Aortic and vena cava smooth muscle cells were derived from explants of the thoracic aorta or inferior vena cava and fed with DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and 1% glutamate. Cells were verified as smooth muscle cells by staining with a smooth muscle cell-specific α-actin antibody. Both aortic and vena cava cells were used between passages 1 and 2. Cells were then prepared for Western blot analysis as described below.

Western blot analysis. Protein isolation, protein concentration determination, and Western blot analysis were performed as previously described.
DECREASED TG1 AND TG2 IN AORTAS FROM HYPERTENSIVE RATS

described (20). Briefly, 50 μg of homogenized total protein from rat aorta and vena cava or smooth muscle cells cultured from these vessels were separated by gel electrophoresis, blotted onto a polyvinylidene difluoride membrane (TG1) or a nitrocellulose membrane (TG2), blocked for 3 h at 4°C in 4% (wt/vol) egg chick ovalbumin, and incubated overnight in primary antibody [mouse anti-TG1 (1:100, catalog no. sc-166467, Santa Cruz Biotechnology) or rat anti-TG2 (1:2,000, Hybridoma Facility, University of Alabama, Birmingham, AL)] diluted in blocker. Samples were incubated in secondary antibody [horseradish peroxidase-linked anti-mouse IgG (TG1, catalog no. NA931V, GE Healthcare, Buckinghamshire, UK) or horseradish peroxidase-linked anti-rat (TG2, Abcam)] at 1 h for 4°C. The positive control for TG1 was rat skin lysate (catalog no. 1480, ProSci). Blots were developed with ECL. Western blotting detection reagents (catalog no. RPN2209, GE Healthcare) on film (catalog no. F-9023-8X10, GeneMate, BioExpress, Kaysville, UT) using a Kodak X-OMAT film developer (Eastman Kodak, Rochester, NY). ImageJ [National Institutes of Health (NIH), Bethesda, MD] was used for densitometric analysis and quantification.

Isometric contraction. Cleaned rings (length: ~5 mm) of endothelial cell-intact thoracic aortas from sham and DOCA-salt rats were mounted in tissue baths for isometric tension recordings using Grass (Gilford Instruments, Rockford, IL) was used for densitometric analysis and quantification.

SDS-PAGE and in-gel digestion for mass spectrometric analysis. Bead-bound protein samples were incubated with 40 μl of 2× SDS-PAGE sample buffer at 60°C for 10 min in an Eppendorf Thermomixer R (Eppendorf, Hauppauge, NY). Solutions were then cooled to room temperature and spun at 21,000 g to pellet particulates. The supernatant was loaded onto a Criterion 12.5% Tris-HCl precast gel (Bio-Rad, Hercules, CA) and electrophoresed at 50 V constant for ~15 min or until the dye front migrated 2–3 mm below the well. Electrophoresis was then stopped, and the gel was fixed in 40% methanol and 20% acetic acid for at least 2 h followed by overnight staining with colloidal Coomassie blue stain. After being destained, visualized gel bands were individually cut from the gel and subjected to in-gel trypic digestion according to Shevchenko et al. (16) with modifications. Briefly, gel bands were dehydrated using 100% acetonitrile and incubated with 10 mM DTT in 100 mM ammonium bicarbonate (pH ~8) at 56°C for 45 min, dehydrated again, and incubated in the dark with 50 mM iodoacetamide in 100 mM ammonium bicarbonate for 20 min. Gel bands were then washed with ammonium bicarbonate and dehydrated again. Sequencing grade modified trypsin was prepared to 0.01 μg/μl in 50 mM ammonium bicarbonate, and ~50 μl of this were added to each gel band so that the gel was completely submerged. Bands were then incubated at ~37°C overnight. Peptides were extracted from the gel by water bath sonication in a solution of 60% acetonitrile-1% trichloroacetic acid and vacuum dried to ~2 μl.

Mass spectrometric analysis. Peptides were resuspended in 2% acetonitrile and 0.1% trifluoroacetic acid to 20 μl, and from this, 10 μl were automatically injected by a nanoAcquity Sample Manager (Waters, Milford, MA) and loaded for 5 min onto a Waters Symmetry C18 peptide trap (5 μm, 180 μm × 20 mm) at 4 μl/min in 2% acetonitrile and 0.1% formic acid. Bound peptides were then eluted using a Waters nanoAcquity UPLC (buffer A: 99.9% water and 0.1% formic acid and buffer B: 99.9% acetonitrile and 0.1% formic acid) onto a Waters BHM130 C18 column (1.7 μm, 100 μm × 100 mm) and eluted over 60 min with a gradient of 5% buffer B to 30% buffer B over 47 min, ramped to 90% buffer B at the 49th minute, held at 90% buffer B for 1 min, and then dropped back to 5% buffer B at the 50.1 minute, all at a constant flow rate of 0.8 μl/min. Eluted peptides were sprayed into either a LTQ-FT Ultra mass spectrometer (ThermoFisher, Waltham, MA) or a ThermoFisher LTQ Linear Ion-Trap mass spectrometer, both using a Microm ABANCE nanospray source. For LTQ-Fourier transformation (FT) analysis, survey scans were taken in the FT (25,000 resolution determined at a mass-to-charge ratio of 400), and the top five ions in each survey scan were then subjected in a data-dependent fashion to automatic low energy collision.
DECREASED TG1 AND TG2 IN AORTAS FROM HYPERTENSIVE RATS

Table 1. Morphological measurements of the aorta from hypertensive and control rats

<table>
<thead>
<tr>
<th></th>
<th>Wall Thickness, μm</th>
<th>Lumen Diameter, μm</th>
<th>Wall-to-Lumen Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-salt</td>
<td>180.9 ± 13.57*</td>
<td>1862 ± 46.26</td>
<td>0.0971 ± 0.0065*</td>
</tr>
<tr>
<td>Sham</td>
<td>127.5 ± 7.764</td>
<td>1814 ± 22.77</td>
<td>0.0702 ± 0.0039</td>
</tr>
<tr>
<td>SHRSP</td>
<td>158.2 ± 22.16*</td>
<td>1863 ± 70.65*</td>
<td>0.0841 ± 0.0085*</td>
</tr>
<tr>
<td>WKY</td>
<td>91.47 ± 5.120</td>
<td>1569 ± 42.29</td>
<td>0.0582 ± 0.0027</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 for all groups. SHRSP rats, spontaneously hypertensive stroke-prone rats; WKY, Wistar-Kyoto rats. *Significantly different (P < 0.05) from sham/WKY rats.

RESULTS

Decrease in TG1 and TG2 IHC staining in the aorta from DOCA-salt rats. An increase in the wall-to-lumen ratio of the aorta, measured by bright-field microscopy, was used as an indicator of smooth muscle hypertrophy/hyperplasia and confirmed that arterial remodeling had occurred (Table 1). IHC detection of TG1 (Fig. 1A) and TG2 (Fig. 1B) protein in the aorta and vena cava from DOCA-salt and sham rats was performed, with human skin (TG1) and human breast tumor (TG2) as positive controls. Staining was absent in sections lacking the primary antibody (insets in Fig. 1). For both TG1 and TG2, staining was significant in smooth muscle layers of both vessels, although it was not exclusive to those areas. This...
was particularly apparent in the vena cava, which contain only one layer of luminal smooth muscle. Qualitative decreases in both TG1 and TG2 staining were observed in aortas from DOCA-salt rats compared with aortas from sham rats. This decrease was not observed in the vena cava.

**Decreased TG activity in the aorta from both DOCA-salt and SHRSP rats.** In addition to the aorta and vena cava from DOCA-salt and sham rats, we investigated the aorta and vena cava from SHRSP rats and their WKY controls using the in situ TG activity assay and in situ BAP assay. In situ incorporation of a fluorescently labeled isozyme-specific glutamine donor peptide (K5 for TG1 and T26 for TG2) was used to measure in situ TG1 activity (Figs. 2A and 3A) and TG2 activity (Figs. 2B and 3B). Negative control peptides (K5QN or T26QN, insets in Figs. 2 and 3), which have reactive glutamine replaced by nonreactive asparagine, were used in parallel with active peptides. The wavy lines seen between smooth muscle layers in the aorta are caused by elastin autofluorescence. The graphs in Figs. 2 and 3, right, quantify fluorescence for each set of tissues. The results of the in situ glutamine donor peptide incorporation support a significant decrease in TG1 and TG2 activity in aortas from DOCA-salt rats compared with aortas from sham 

\( P < 0.05; \) Fig. 2). No significant differences in TG1 or TG2 activity were observed in the vena cava. Similarly, in situ glutamine donor peptide incorporation showed a significant decrease in TG1 and TG2 activity in the aorta but not in the vena cava from SHRSP rats compared with the aorta from WKY rats 

\( P < 0.05; \) Fig. 3).

In situ incorporation and fluorescent detection of an amine donor substrate (BAP assay) was used to measure general in situ TG activity (Fig. 4). As a negative control, parallel sections were included without the addition of BAP in the reaction solution 

\( \text{insets in Fig. 4). The signal was not present in negative control sections. The graphs in Fig. 4, right, quantify the fluorescence of images. Aortas from DOCA-salt rats had decreased incorporation of BAP compared with aortas from sham rats 

\( P < 0.05; \) Fig. 4A). Similarly, aortas from SHRSP rats had decreased incorporation of BAP compared with aortas from WKY rats 

\( P < 0.05; \) Fig. 4B). Incorporation of BAP was not reduced in the vena cava of either DOCA-salt or SHRSP rats compared with control rats. We performed an endothelial marker (RECA-1)/BAP colocalization to verify that TG activ-

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**Fig. 2.** In situ activity assay for TG1 (A) and TG2 (B) activity in sham and DOCA-salt rat aortas and vena cavas. Left: sham rat images are representative of \( n = 4 \) and DOCA-salt rat images are representative of \( n = 5 \). Negative controls are included in the inset images. Arrows indicate areas of interest. Right: quantification results. *Significantly different 

\( P < 0.05 \) from sham rats; **significantly different 

\( P < 0.05 \) from active peptide.
ity in the vena cava was present in the luminal smooth muscle layer as well as the endothelial layer (Fig. 5). We found that TG activity was present in both the endothelial layer and the luminal smooth muscle layer. This was consistent with IHC staining for TG protein.

**Decreased TG1 and TG2 protein expression in the aorta from DOCA-salt rats.** Western blot analysis was used to measure TG1 and TG2 protein expression in the aorta and vena cava from DOCA-salt and sham rats, with protein levels normalized to β-actin (TG1, Fig. 6A; TG2, Fig. 6B). When we measured TG1 protein expression, bands of generally lower intensity were seen in the aorta from DOCA-salt rats compared with the aorta from sham rats, but this difference was not statistically significant. TG2 protein expression was decreased in the aorta from DOCA-salt rats compared with the aorta from sham rats ($P < 0.05$). No significant difference in either TG1 or TG2 protein expression was seen in the vena cava from DOCA-salt rats compared with control rats.

**Decreased TG expression in cultured VSMCs.** Western blot analysis of fresh whole tissue and cultured aorta and vena cava VSMCs from normal Sprague-Dawley rats was performed, with protein levels normalized to β-actin. Cultured rat aorta VSMCs showed a complete loss of TG2 protein expression compared with the whole rat aorta, but TG1 was not decreased (Fig. 7A). Additionally, cultured rat vena cava VSMCs showed a decrease in TG1 and a loss of TG2 protein expression compared with the fresh rat vena cava (Fig. 7B). Figure 7C shows the quantification of these data. Seventy-six kilodaltons is the generally accepted molecular weight for TG2. The 52-kDa band may be due to alternate posttranslational modifications and is not indicative of TG2 expression. These data suggest that actively proliferating VSMCs undergo a phenotypic change associated with a decrease in TG1 and, in particular, TG2 expression.

**Decreased TG function in the aorta from DOCA-salt rats.** Cystamine, a nonisozyme selective TG inhibitor, was used in an isometric contraction assay to measure relative TG function. The purpose of this experiment was to assess TG function in the remodeled aorta from hypertensive rats. 5-HT was used as an agonist given its recognized dependence on TG function for contraction (19). Contraction to 5-HT was reported as a percentage of the initial contraction to PE (sham rat aorta: $1,273.4 \pm$
71.8 mg, sham rat aorta + cystamine: 1,018 ± 163 mg; DOCA-salt rat aorta: 1,366 ± 33.96 mg, and DOCA-salt rat aorta + cystamine: 1,300 ± 133 mg). ACh-induced relaxation was 37.8 ± 6.24% (EC50 PE contraction in the sham rat aorta, average of vehicle and cystamine) and 82.47 ± 13% (EC50 PE contraction in the DOCA-salt rat aorta, average of vehicle and cystamine). The ability of cystamine to reduce 5-HT-induced maximal contraction was significantly diminished in the aorta from DOCA-salt rats compared with the aorta from sham rats (Fig. 8). These data suggest that TG function may be decreased in the aorta from DOCA-salt rats.

Aorta- and vena cava-specific TG substrates. Because aortic TG activity was selectively decreased in hypertension compared with vena cava TG activity, we examined the targets of TG activity that were aorta and vena cava specific using BAP to capture proteins. Seven TG substrates were identified that were specific to the aorta and six TG substrates were identified that were specific to the vena cava (Table 2). Proteins that had three hits in the aorta and zero or one hits in the vena cava or three hits in the vena cava and zero or one hits in the aorta as well as the associated full name and function of each protein are shown in Table 2. These proteins play roles in cytoskeletal organization, redox regulation, DNA/RNA/protein synthesis and regulation, coagulation regulation, and cell metabolism.

**DISCUSSION**

Small artery inward remodeling in response to low blood flow is dependent on TG2 (1), and work in our laboratory has shown the presence of TG1 and TG2 in the aorta and vena cava (10). Additionally, TG activity is required for multiple steps in the pathogenesis of atherosclerosis (4) and the small artery remodeling seen in mice in response to L-NAME-induced hypertension (15). Despite this, no research has investigated the role of TGs in the remodeling of large arteries and veins in response to high blood pressure. Large arteries experience the greatest pressures in hypertension, so they may be the most challenged. We investigated the expression, activity, and function of TG1 and TG2 in the aorta and vena cava from hypertensive rats and their normotensive counterparts. We hypothesized that protein expression, activity, and functionality of TG1 and TG2 would be increased in the aorta but not in the vena cava. Contrary to our hypothesis, we found that protein expression, activity, and function of TG1 and TG2 were...
decreased in the aorta from hypertensive rats compared with normotensive rats, whereas no difference was seen in the vena cava between the two groups.

A two-model, two-vessel approach. We used two rat models of hypertension to test our hypothesis. This was done to determine whether the results were specific to a specific model of hypertension.

The DOCA-salt model is an experimental model based on hyperaldosteronism and is characterized by increased vasoconstrictor efficacy (21), reduced vasodilator efficacy, endothelial dysfunction, and centrally mediated increases in vascular smooth muscle tone (17). DOCA-salt hypertensive rats exhibit increased aortic superoxide production and renal inflammation (12) and undergo vascular remodeling in hypertension (20).

The SHRSP model is a genetic model characterized by increased sympathetic activity (18), elevated renin levels, increased VSMC growth and proliferation (17), and increased vascular release of superoxide (12). Importantly, the SHRSP model exhibits pathophysiological changes similar to those seen in human essential hypertension (18) and has been used to investigate the role of TG2 in small artery remodeling (6).

The thoracic aorta and inferior vena cava each have fundamentally different roles in cardiovascular physiology. The aorta is an elastic conduit vessel that, due to the cushioning function of large arteries, experiences high blood pressures, contributes to systolic blood pressure (17), and undergoes significant remodeling in response to hypertension (20). In contrast, the vena cava carries a high blood volume, is highly compliant, contributes greatly to vascular capacitance, is a low-pressure vessel (17), and undergoes little remodeling in response to hypertension (20).

A five-method approach. The multiple approaches taken (IHC, in situ activity assay using a glutamine donor, in situ BAP assay using an amine donor, Western blot analysis, and

![Fig. 5. BAP assay/RECA-1 colocalization in the SHRSP rat vena cava. Red, BAP; green, RECA-1; yellow, colocalization. Negative controls are included in the inset images. All images are representative of n = 4. Arrows indicate areas of interest.](image)

![Fig. 6. Western blots (left) and densitometric analysis (right) of TG1 (A) and TG2 (B) protein expression in sham and DOCA-salt rat aortas (n = 4) and vena cavas (n = 4). Densitometric analysis of TG1 and TG2 protein expression was performed in both sham and DOCA-salt aortas and vena cavas corrected to α-actin. *Significantly different (P < 0.05) from sham rats.](image)
contractility are internally consistent. However, the outcome refutes our hypothesis. In direct contrast to research in small arteries, the protein expression, isozyme specific activity, general activity, and functionality of TGs were decreased in the aorta from hypertensive rats, but not in the vena cava of these rats, compared with normotensive rats. These changes in TG activity are meaningful to aortic function because contraction to 5-HT, a TG-dependent process (19), was inhibited to a lesser degree in the aorta from DOCA-salt rats compared with sham rats. This suggests that TG function is decreased in these vessels.

Arteries versus veins. TG activity in the vena cava of hypertensive rats, unlike in the aorta, was not reduced compared with normotensive control rats. The aorta experiences higher pressures and typically remodels to a greater extent than the vena cava (20). If remodeling were associated with decreased TG activity, this would explain the decrease in TG activity seen in the aorta but not in the vena cava. The aorta has seven layers of smooth muscle, whereas the vena cava only has one layer of smooth muscle. If remodeling were related to phenotypic changes in VSMCs, characterized by a decrease in TG activity, the difference in amount of vascular smooth muscle would explain the significant decrease in TG activity seen in the aorta. In support of this idea, cultured VSMCs from both the aorta and vena cava lost TG expression. Vena cava smooth muscle cells have the potential to lose TG expression and enter a proliferative state similar to aortic smooth muscle cells, and do so in culture, but do not do so in vivo in response to hypertension.

A third factor that could account for the difference in TG1 and TG2 activity between the aorta and vena cava of hypertensive rats is the difference in TG substrates in each vessel. Three functional groups of aorta-specific TG protein substrates were identified: cytoskeletal proteins (destrin, profilin 1, and myosin heavy chain 10), a redox regulation protein (glutathione-S-transferase-μS), and DNA/RNA/protein synthesis and regulation proteins (cysteine and glycine-rich protein 1, GTP-binding nuclear protein RAN, and protein niban). The vena cava does not lose TG activity and does not undergo remodeling during hypertension, so vena cava-specific TG substrates may account for the stabilization and lack of remodeling in this vessel. Three functional groups of vena cava-specific TG protein substrates were identified: cytoskeletal proteins (vimentin, dihydropyrimidinase-related protein 2, and tubulin-β2A-chain), coagulation regulation proteins (fibrinogen γ-chain and α1-macroglobulin), and a cell metabolism protein (guanine deaminase). Literature searches indicated that no research has been performed investigating how TG-mediated modifications affect the activity or function of these proteins, so the discussion of these proteins’ roles in arterial remodeling is speculative. Our findings raise the question of how TG-driven modification of these proteins through TG-mediated reactions changes their activity such that the loss of TG activity in the aorta is associated with remodeling.

Differences between large and small artery TGs in hypertension. Our results are in direct contrast with previous work that has shown that the inward remodeling of small arteries is TG2 dependent (1). Bakker et al. (1) found that both the inward remodeling of rat mesenteric arteries induced by low blood flow and the inward remodeling of rat skeletal muscle arteries induced by endothelin-1 were decreased by the addition of the TG inhibitor cystamine. Additionally, inward remodeling of rat mesenteric arteries in response to both L-NAME-induced hypertension and low blood flow is delayed in TG2 knockout mice (3, 15). Our results may be different because the vessels our laboratory and other laboratories used have fundamentally different physiological roles. Small arteri-
ies contribute to total peripheral resistance. Large arteries, on the other hand, are highly elastic conduit vessels. It is not unreasonable to suggest that remodeling of these vessels plays different roles in the regulation of blood pressure and that TGs have different roles in this remodeling.

Limitations. The results and conclusions of this research are limited to vessels of similar size and caliber as those used: the aorta and vena cava. Indeed, our research is in direct contrast to similar research using smaller vessels. The use of sections and standardized conditions to test for TG activity may remove or modify regulators of TG activity, such as oxidation-sensitive factors. We standardized conditions to test for TG activity may, therefore, be different in large and small arteries. Moreover, the role of TGs in arterial remodeling may differ in large and small arteries.

Table 2. Identification of rat aorta-specific and vena cava-specific TG substrates using BAP as a nonisozyme-specific substrate linker to endogenous proteins

<table>
<thead>
<tr>
<th>Hits</th>
<th>Abbreviation</th>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hits RA</td>
<td>NIBAN</td>
<td>Protein niban</td>
<td>DNA/RNA/protein synthesis and regulation</td>
</tr>
<tr>
<td>1 hit RVC</td>
<td>PROFI</td>
<td>Profilin-1</td>
<td>Cytoskeletal polymerization</td>
</tr>
<tr>
<td>0 hit RVC</td>
<td>CSRPI</td>
<td>Cysteine and glycine-rich protein 1</td>
<td>Development</td>
</tr>
<tr>
<td>0 hit RVC</td>
<td>GSTM5</td>
<td>Glutathione-S-transferase-μs</td>
<td>Redox regulation</td>
</tr>
<tr>
<td>0 hit RVC</td>
<td>DEST</td>
<td>Destrin</td>
<td>Actin depolymerization</td>
</tr>
<tr>
<td>0 hit RA</td>
<td>MYH10</td>
<td>Myosin-10</td>
<td>Cytokinesis and cell shape</td>
</tr>
<tr>
<td>0 hit RVC</td>
<td>RAN</td>
<td>GTP-binding nuclear protein RAN</td>
<td>G protein signaling</td>
</tr>
<tr>
<td>3 hits RVC</td>
<td>VIME</td>
<td>Vimentin</td>
<td>Intermediate filament component</td>
</tr>
<tr>
<td>1 hit RA</td>
<td>DPYL2</td>
<td>Dihydropyrimidinase-related protein 2</td>
<td>Development</td>
</tr>
<tr>
<td>0 hit RA</td>
<td>TBB2A</td>
<td>Tubulin-β 2A-chain</td>
<td>Microtubule component</td>
</tr>
<tr>
<td>0 hit RA</td>
<td>FIBG</td>
<td>Fibrinogen γ-chain</td>
<td>Blood coagulation</td>
</tr>
<tr>
<td>0 hit RA</td>
<td>A1M</td>
<td>α1-Macroglobulin</td>
<td>Plasma protease inhibitor</td>
</tr>
<tr>
<td>0 hit RA</td>
<td>GUAD</td>
<td>Guanine deaminase</td>
<td>Cell metabolism</td>
</tr>
</tbody>
</table>

Proteins that were present in the treatment group at a level greater than two times the control group were considered a “hit.” Proteins were considered “aorta specific” if they registered three hits in the rat aorta (3 hits RA) and zero or one hits in the rat vena cava (0 and 1 hit RVC, respectively). Proteins were considered “vena cava specific” if they registered three hits in the rat vena cava (3 hits RVC) and zero or one hits in the rat aorta (0 or 1 hit RA, respectively).

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