Troponin T– and Troponin I–like Proteins in Bovine Vascular Smooth Muscle

Anna M.C. Zanellato, Anna C. Borrione, Leopoldo Saggin, Luca Giuriato, Stefano Schiaffino, and Saverio Sartore

We have tested the hypothesis whether proteins with biochemical and immunochemical properties similar to those of troponin T (TnT) and troponin I (TnI) are expressed in bovine vascular smooth muscle (SM). Three monoclonal anti-TnT antibodies (TT-1, TT-2, and RV-C2) specific for the two isoforms of TnT present in the bovine cardiac muscle and two monoclonal antibodies (TI-1 and TI-5) reacting with cardiac TnI were used in this study. Anti-TnT antibodies were found to be unreactive with 1) skeletal and nonmuscle isoforms of glyceraldehyde-3-phosphate dehydrogenase, a glycolytic enzyme that shares some structural homologies with skeletal TnT, and 2) calponin, a TnI-like calmodulin/troponysin binding protein with some antigenic properties in common with TnT. When tested on SM extracts from aorta or coronary arteries by Western blotting, the anti-TnT antibodies were able to react exclusively with one or two polypeptides whose electrophoretic mobility corresponds to the cardiac TnT subunits. Similarly, anti-TnI antibodies specifically recognized a component in the aortic or coronary SM extracts with electrophoretic properties identical to the cardiac TnI. Immunofluorescence analysis performed on the vascular SM cells of bovine aorta, coronary arteries, and intramural branches of coronary vessels confirmed the existence of cardiac troponin immunoreactivity in these tissues. In addition, differences in the distribution of cardiac TnT- and TnI-like proteins were evidenced in nonvascular and vascular SM cells. This study shows for the first time that polypeptides with some structural properties in common with cardiac TnT and TnI can be found in the vascular SM system. (Circulation Research 1991;68:1349–1361)

In the sarcomeric muscles, actomyosin ATPase activity is regulated by conformational changes in the troponin-tropomyosin complex that occur when Ca" binds to troponin C.1–3 In the smooth muscle (SM) system, activation of myosin ATPase activity by actin is achieved by a phosphorylation of the 20-kDa myosin light chain through a Ca"-calmodulin–dependent myosin light chain kinase.1,4–6 Enzymatic activation induces an increase of crossbridge cycling rate, leading to tension development.1–4 Thus, myosin light chain phosphorylation and tension development are strongly related in several preparations of SM tissues.7–9 The crucial role played by the phosphorylation process of myosin in the regulation of SM contractility is confirmed by the different motility displayed by phosphorylated and unphosphorylated myosin on oriented actin filaments derived from the algae Nitella.10 While a general consensus exists about the biochemical mechanism that underlies the initiation step of SM contraction,4 the subsequent phase concerning stress maintenance appears less clear. Active stress in a variety of intact SM preparations can be maintained independent of phosphorylation.8,11–15 Thus, phosphorylation of SM myosin correlates better with the shortening velocity of SM tissue.16 It has been suggested that a second Ca"-dependent regulatory mechanism could be involved in the stress maintenance, that is, to keep crossbridges attached when myosin is dephosphorylated.17 Several regulatory, thin filament–linked mechanisms have been proposed as alternative or complementary to the phosphorylation of thick filaments.18 In particular, “leiotonin A,” an 80-kDa actin-binding protein, in combination with Ca"-dependent regulator “leiotonin C” would activate a normally inactive SM actin.19 Caldesmon, an actin-tropomyosin–binding and calmodulin-binding protein acts by inhibiting the phosphorylated actin-activated myosin ATPase activity.3,18 In vertebrate striated muscle, sensitivity to Ca" and regulation of the contractile performance is achieved through the troponin-tropomyosin complex. In the search for an “SM troponin,” Lim et al20
have recently found a troponin T (TnT) immunoreactivity in the chicken gizzard. In addition, other authors\textsuperscript{21-23} have demonstrated the presence of a calmodulin- and tropomyosin-binding protein, named calponin, in vascular, nonvascular, and non-muscle tissues. Phosphorylation of this protein by either protein kinase C or Ca\textsuperscript{2+}-calmodulin-dependent protein kinase II is able to regulate actin-myosin interaction.\textsuperscript{24} The vascular isoform of calponin purified from bovine aortic SM shares some structural properties in common with rabbit skeletal and bovine cardiac TnT and is able to bind troponin C (TnC) but not troponin I (TnI).\textsuperscript{25} The existence of amino acid sequence homology between TnT and the ubiquitous glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (G3PD) has prompted Sanders et al\textsuperscript{26} to consider immunological data about TnT-like protein as due to cross-reactivity with this enzyme.

In this work, we have re-examined the problem of the existence of troponin or troponinlike proteins in vascular SM. Three monoclonal antibodies (TT-1, TT-2, and RV-C2\textsuperscript{26}) specific for the two TnT isoforms present in the adult bovine ventricle and unreactive with G3PD and calponin were used in this study. Moreover, two other monoclonal antibodies (TI-1\textsuperscript{27} and TI-5) reacting with bovine cardiac TnT were also used to evaluate the putative presence of a TnT-like protein in vascular SM. We present experimental evidence that indicates that TnT- and TnI-like proteins are expressed in bovine aorta and coronary arteries.

Materials and Methods

Antigen Preparation

Bovine cardiac troponin complex (TnT+TnI+TnC) was prepared as described in Reference 26. G3PD from human erythrocytes and porcine skeletal muscle was purchased from Sigma Chemical Co., St. Louis. Crude calponin was obtained from bovine aortic SM following the procedure of Takahashi et al.\textsuperscript{23} Purified calponin from pig stomach was a generous gift of Dr. J.V. Small, Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria. Crude sodium dodecyl sulfate (SDS) extracts from bovine left ventricular tissue, aortic SM (about 5 cm below the aortic arch), and left descending coronary artery SM (about 10 cm from the bifurcation) were prepared according to Sartore et al\textsuperscript{28} and used within 1 week.

Monoclonal Antibodies

TT-1 and TT-2 monoclonal anti-TnT antibodies were obtained by fusing splenocytes from mice immunized with bovine atrial troponin complex and plasmacytoma cells (NS-O cell line) according to a procedure used in our laboratory.\textsuperscript{26,27} BF-49 monoclonal anti-myosin antibody was obtained using an actomyosin preparation from bovine fetal skeletal muscle as the immunogen. Screening and cloning of hybridomas were performed as previously described.\textsuperscript{26,27} The other monoclonal anti-TnT antibody used in this study (RV-C2) has been described elsewhere.\textsuperscript{26}

TI-5 anti-TnI antibody was obtained using the immunogen and the procedure described above for anti-TnT antibodies. TT-1, TT-2, and TI-5 antibodies were found to be of the immunoglobulin G\textsubscript{1} (IgG\textsubscript{1}) type and contained \(\kappa\) light chains, as determined by the Amersham antibody typing kit (Amersham, Little Chalfont, England). BF-49 anti-myosin antibody was of the IgG\textsubscript{2} class with the \(\lambda\) light chain complement. Purification of IgG\textsubscript{1}-type antibodies was carried out according to Russo et al.\textsuperscript{29} Details about the other anti-TnI antibody used in this study (TI-1) have been published elsewhere.\textsuperscript{27}

Monoclonal anti-desmin and anti- vimentin antibodies were obtained from Boehringer Mannheim, Mannheim, FRG; the commercially available monoclonal anti-TnT antibody\textsuperscript{30,31} was purchased from Amersham. SM-E7 anti-SM myosin antibody has been characterized elsewhere.\textsuperscript{32}

Electrophoresis and Western Blotting

SDS–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out following the procedure of Sartore et al.\textsuperscript{28} Antigens were electrophoresed in 10\% SDS slab gels at 200 V/slab. Gels were then stained with Coomassie brilliant R, and the background was removed after several changes of destaining solution. Apparent molecular mass was determined by the high and low molecular mass standard kit of Bio-Rad, Richmond, Calif. These kits included rabbit skeletal myosin (200 kDa), \(\beta\)-galactosidase (116 kDa), phosphorylase \(b\) (92.5 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.5 kDa).

Western blotting procedures were as in Reference 28, with a few modifications. Polypeptides were transferred electrophoretically from the gel to the nitrocellulose paper (0.45 \(\mu\)m, Bio-Rad) for 3 hours at 400 mA in the presence of 20\% methanol. Under these conditions polypeptides below 100 kDa of apparent molecular weight were completely transferred to the paper. Bound antibody was revealed by rabbit anti-mouse IgG conjugated with alkaline phosphatase (Dakopatts, Glostrup, Denmark). The substrate solution contained 5 mg 5-bromo-4-chloro-3-indolyl phosphate and 10 mg nitroblue tetrazolium blue, in 0.1 M NaCl, and 0.1 M Tris-HCl, pH 9.\textsuperscript{33}

Immunofluorescence

Indirect immunofluorescence assays were performed on cryosections (4 \(\mu\)m thick)\textsuperscript{34} from bovine aorta and coronary arteries isolated from 21-month-old animals and weighing about 500 kg. The fluorescent antibody (anti-mouse IgG conjugated with fluorescein isothiocyanate) was obtained from Dako. Specificity of immunoreaction was tested as follows: 1) the second fluorescent antibody alone and 2) nonimmune mouse IgG in the first step followed by the fluorescent antibody. Sections were then fixed in 1.5\%
p-formaldehyde in phosphate buffered saline, pH 8.0, to prevent fading and examined under epifluorescence illumination on a Zeiss Axioplan microscope (Carl Zeiss, Oberkocken, FRG). Photographs were taken using Kodak Technical Pan Film (Eastman Kodak Co., Rochester, N.Y.) set at 100 ASA and subsequently developed in Kodak HC110 solution.

Results

Immunochromatographic Analysis of Troponin Antigenicity in Vascular SM

Aorta. Specificity of the three anti-TnT antibodies (TT-1, TT-2, and RV-C2) was determined by Western blotting analysis (Figure 1). All three antibodies (lane 1, panels B–D) selectively recognize the two TnT isoforms of bovine ventricle (mass, 41.5 and 41 kDa, respectively). No reactivity was detected with G3PD (mass, 36 kDa) from porcine skeletal muscle (lane 2) or from human erythrocytes (not shown). Even if the amount of G3PD blotted on nitrocellulose is increased up to five times that shown in lane 2, no binding of anti-TnT antibodies can be demonstrated with these enzymes (not shown). Similarly, no immunostaining was detected with calponin (lane 3; mass, about 34 kDa) in the crude extract from bovine aorta or with purified calponin from porcine stomach (not shown).

Immunoreactivity of the three anti-TnT antibodies with crude extracts from bovine aortic SM was analyzed using Western blotting procedures. As shown in Figure 2, the three anti-TnT antibodies are able to recognize a polypeptide that is present in trace amounts in the Coomassie blue–stained gel (less than 1%). The electrophoretic mobility of this aortic polypeptide is similar to that of the TnT isoform with higher apparent molecular weight (TnT-1). The intensity of immunostaining of anti-TnT antibodies with this 41-kDa polypeptide is, however, much lower than the corresponding band in the ventricular extract (panels B–D).

The aortic extract was also examined for TnI immunoreactivity by Western blotting procedures (Figure 3). A band of about 31.5 kDa, corresponding to the TnI subunit of the Tn complex, was identified in the cardiac extract by both TI-1 and TI-5 (lane 1, panels B and C). A polypeptide with similar electrophoretic mobility but showing less reactivity was recognized in the aortic SM extract by both anti-TnI antibodies (lane 2).

Coronary arteries. The possible contamination of the coronary artery preparation with ventricular myocardium was ruled out using an antibody specific for cardiac myosin heavy chain, BF-49. As shown in Figure 4, three different concentrations of SM extract were analyzed for the presence of contaminating cardiac tissue (panel B). In the limits of the sensitivity of our detection system, there is no evidence for cardiac tissue contamination in the coronary artery preparation. The same coronary artery extract was examined for the presence of TnT-like proteins using TT-1 (panel C). Two bands of different intensity but comigrating with the two cardiac TnT subunits were recognized by this antibody (panel C). Similar results...
were obtained with TT-2 and RV-C2 anti-TnT antibodies (not shown). The nitrocellulose paper reacted with TT-1 was incubated subsequently with TI-5 anti-TnI antibody (panel D). This antibody was able to stain a band whose electrophoretic mobility corresponded to the cardiac TnI component (panel D). The other anti-TnI antibody (TI-1) used in this study gave the same immunostaining pattern (not shown).

**Immunofluorescence Analysis of Troponin Antigenicity in Striated and SM Tissues**

Before undertaking the in vivo study about TnT- and TnI-like protein expression in aorta and coronary arteries, we have tested whether 1) the immunostaining of the cardiac muscle tissue with the fluorescent anti-troponin antibodies is influenced by a variety of detergents (SDS, Tween 20, Nonidet P-40, and Triton X-100) and fixatives (1.5% p-formaldehyde, acetone, and methanol), and 2) the five monoclonal antibodies displayed a different distribution pattern of immunofluorescence when applied to cryosections from bovine striated muscles as well as to nonvascular and vascular SM tissues. It is important, in fact, to ascertain whether differences in immunoreactivity can be attributable to a selective masking of the epitopes recognized by the anti-troponin antibodies. Persistence of immunoreactivity after denaturing treatments would indicate that the epitopes are not masked. In this circumstance, we can assume that eventually distinct immunostaining patterns on different muscle tissues with the anti-troponin antibodies are likely to be due to particular localizations of the antigenic epitopes.

The reactivity of the five monoclonal anti-troponin antibodies with skeletal, cardiac, and SM tissues are summarized in Table 1. The antibodies display different patterns of immunofluorescence with the antigens tested, and the epitopes are relatively stable even after 15 minutes of treatment at 37°C with detergents or fixatives (not shown).

To determine the extent of similarity between the cardiac TnT and the putative TnT-like protein of vascular SM type, we have applied the JLT-12 anti-TnT antibody to cryosections from aorta and coronary artery. This antibody, which is known to be directed against an epitope localized in a highly conserved region of striated muscle TnT, is unreactive with both the vascular SM tissues used in this study.

**Immunofluorescence Analysis of TnT- and TnI-like Protein Distribution in Aortic and Coronary Artery SM**

**Aorta.** The expression of TnT- and TnI-like proteins at the cellular level was studied by the indirect immunofluorescence technique and by using antitroponin antibodies. The results of this study are presented in Table 2. TnT-like immunoreactivity was present in all aortic SM cells examined and localized at the cytoplasm level (Figure 5B). Serial sections showed that TnT-immunostaining codistributed with that of SM-E7 anti-SM myosin antibody (Figure 5A).
TABLE 1. Immunoreactivity of Skeletal, Cardiac, and Smooth Muscle Tissues With the Five Monoclonal Anti-Troponin Antibodies as Determined by Western Blotting and Immunofluorescence Staining

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td></td>
<td>TT-1  TT-2 RV-C2 TI-1 TI-5</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>Fetal skeletal muscle</td>
<td>++    -    -    -</td>
</tr>
<tr>
<td>Fetal ventricular myocardium</td>
<td>++    +    -    -</td>
</tr>
<tr>
<td>Adult skeletal type 1</td>
<td>++    -    -    -</td>
</tr>
<tr>
<td>Adult skeletal type 2</td>
<td>+    +    -    -</td>
</tr>
<tr>
<td>Adult ventricular muscle</td>
<td>+    +    +    +</td>
</tr>
<tr>
<td>Chicken</td>
<td>++    -    -    -</td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
</tr>
<tr>
<td>Pectoralis muscle</td>
<td>+    -    *    +*</td>
</tr>
<tr>
<td>Bronchial smooth muscle</td>
<td>-    -    +</td>
</tr>
<tr>
<td>Pulmonary artery (small branches)</td>
<td>-    -    +    -</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
</tr>
<tr>
<td>Inner muscular layer</td>
<td>+    -    -    -</td>
</tr>
<tr>
<td>Outer muscular layer</td>
<td>+    -    -    -</td>
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+ and -, positive or negative reaction, independent from the level or intensity of antibody immunostaining.
*Immunofluorescence staining is restricted to type 1 fibers.

TI-5 anti-TnI antibody (Figure 5D) stained some peculiar aortic regions (small areas of SM cells surrounded by a network of fibroelastic fibers) in a manner similar to that of SM-E7 antibody (Figure 5C). Conversely, aortic areas particularly enriched with SM cells (Figure 6B) are heterogeneously labeled by TI-5. Serial sections stained with TT-1 (Figure 6A) showed that all aortic SM cells displayed a homogeneous staining with this antibody. Higher magnification analysis of these peculiar regions in serial sections with TT-1 and TI-5 antibodies confirmed the existence of heterogeneous patterns of TnI-like immunoreactivity both among and within aortic SM cells (Figures 6C and 6D). In particular, TI-5 immunostaining appeared to be distributed intracellularly in a punctate manner (Figure 6D). TT-2 gave an immunostaining pattern with the aortic SM tissue similar to that of TT-1 (see Table 2). On the contrary, RV-C2 and TI-1 were almost negative with the vascular SM cells of aorta by the indirect immunofluorescence technique (see Table 2) but positive when revealed by the indirect immunophosphatase reaction (not shown).

Coronary arteries. Large, medium, and small branches of coronary arteries as well as intramural coronary vessels of left ventricular myocardium were examined (see Table 2). In particular, we studied the following segments of the left coronary artery: 1) 2 cm before the bifurcation; 2) the circumflex branch, about 10 cm from the bifurcation; and 3) the descending branch, 10 cm from the bifurcation; the right coronary artery, about 10 cm from the origin, was also studied. Small branches of coronary arteries were about 1 mm in diameter.

The results of immunofluorescence experiments are shown in Table 2. All the segments of left and right coronary arteries were found to be homogeneously positive with TT-1 (Figure 7). Conversely, the other anti-TnT antibodies (TT-2 and RV-C2) were weakly reactive with the large segments of coronary arteries (Table 2). The anti-TnI antibody TI-5 gave a heterogeneous pattern of immunostaining in all the coronary artery segments examined (Figure 8). A number of vascular SM cells appeared to be negative (Figure 8D), and in cells positive with TI-5 (Figures 8A–8C) only part of the cytoplasm was labeled by the antibody in serial sections experiments (not shown).

Surprisingly, when the small coronary arteries were examined, all vascular cells were found to be homogeneously and brightly reactive with all the five anti-TnT and anti-TnI antibodies (Figure 10 and Table 2). No difference in the distribution of immunoreaction was evident in the subendocardium or in subepicardium regions (not shown).

TABLE 2. Distribution of Troponin T– and Troponin I–like Immunostaining in Vascular Smooth Muscle Cells From Bovine Aorta, Coronary Arteries, and Intramural Coronary Vessels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT-1  TT-2 RV-C2 TI-1 TI-5</td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
</tr>
<tr>
<td>Coronary arteries (large vessels)</td>
<td></td>
</tr>
<tr>
<td>Left coronary artery</td>
<td></td>
</tr>
<tr>
<td>Before the bifurcation</td>
<td>++    +    +    +†</td>
</tr>
<tr>
<td>Descending branch</td>
<td>++    +    +    +†</td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>++    +    +    +†</td>
</tr>
<tr>
<td>Coronary arteries (small branches)</td>
<td></td>
</tr>
<tr>
<td>Intramural vessels</td>
<td>+++    +++    +++    +++</td>
</tr>
</tbody>
</table>

Reactivity of vascular smooth muscle cells. +++, strong; +, intermediate; +, weak.
*Positive staining can be demonstrated using the indirect immunophosphatase technique (see "Results").
†Heterogeneous immunostaining (see "Results").

Discussion

In this work we present experimental evidence for the presence of TnI– and TnI–like proteins in vascular SM cells from bovine aorta and coronary arteries. Three monoclonal anti-TnI and two anti-TnI antibodies have revealed that polypeptides with electrophoretic and immunochemical properties in common with bovine ventricular TnT and TnI subunits are expressed in vascular SM tissues. Because the five monoclonal anti-Tn antibodies show distinct immunoreactivities on the different types of SM tissue (Table 1) and the immunostaining pattern displayed on cardiac muscle tissue is not affected by denaturing treatments, it seems reasonable to assume that these antibodies are di-
FIGURE 5. Serial cryosections through bovine aortic smooth muscle (SM) tissue treated with SM-E7 anti-SM myosin antibody (panel A) and TT-1 anti-troponin T antibody (panel B); in the lower panels is shown the immunostaining pattern of aortic SM tissue with SM-E7 anti-SM myosin (panel C) and TI-5 anti-TnI (panel D) antibodies. Arrows in panels A and B indicate the cytoplasm of the same cell labeled with both antibodies. Bar, 40 μm.
Figure 6. Serial cryosections from bovine aortic smooth muscle treated with TT-1 anti-troponin T (panels A and C) and TI-5 anti-troponin I (panels B and D) antibodies. Only part of vascular smooth muscle cells stained by TT-1 (panel A) are also labeled by TI-5; arrows in panels A and B indicate that at the single cell level, the cytoplasm is stained differently by the two antibodies. This peculiar immunofluorescence pattern is confirmed by the analysis at higher magnification (panels C and D). Troponin I-like immunoreactivity is of punctate type (asterisks) and localized in some regions of the smooth muscle cell cytoplasm. Bars: 40 μm (panels A and B) and 18 μm (panels C and D).
Figure 7. Indirect immunofluorescence staining of vascular smooth muscle from coronary arteries stained with TT-I anti-troponin T antibody. Left coronary artery before the bifurcation (panel A), the circumflex branch (panel B), the descending branch (panel C), and the right coronary artery (panel D). A bright immunostaining of vascular smooth muscle cells is visible in all the coronary artery segments examined. Bar, 45 μm.

Rected against distinct antigenic epitopes. Given the size of the antigenic epitope is about five to six amino acid residues, the structural similarity between cardiac TnT and TnI proteins, on the one hand, and SM TnT- and TnI-like proteins might be of at least 10 amino acid residues.

Sequencing of striated muscle TnT and cDNA analysis have demonstrated marked conservation of large portions among skeletal and cardiac muscles from different species. The fact that JLT-12 anti-TnT antibody is unreactive with vascular SM tissue indicates that the amino acid sequence shared by cardiac TnT and SM TnT-like protein must be localized outside residues 71–151. It is interesting in this respect that the CNBr fragment CB2 (residues 71–151) shows a marked degree of sequence homology with G3PD, for which the antibodies used in this study are unreactive.

In addition, TT-1, TT-2, and RV-C2 do not cross-react with the Ca2+ and calmodulin-binding protein calponin. This protein present in SM tissues shares some structural characteristics with skeletal TnT in the COOH-terminal region (between residues 242 and 259). Thus, it is likely that the anti-TnT antibodies used in this study recognize antigenic epitopes that are localized in the TnT regions other than of residues 71–151 and residues 242–259. Obviously, we cannot rule out the possibility that TnT or TnI antigenicities in aortic and coronary artery SM can be due to cross-reactivity with unknown nontroponin proteins. However, the fact that the bands recognized by the anti-troponin...
antibodies in the blotted extracts comigrate with the respective cardiac troponin subunits argues against this hypothesis.

In the aortic SM, the anti-TnT antibodies can recognize a TnT-like polypeptide that displays an electrophoretic mobility identical to the slower migrating component (TnT-i) of the two cardiac TnT isoforms. Conversely, in the vascular SM from coronary arteries both TnT-1 and TnT-2 (the faster migrating component of the TnT doublet) are present, although they are present or react in a different manner. The TnT-1 isoform shows unique structural and functional characteristics compared with the faster migrating TnT variant (TnT-2). For example, the TnT-1 isoform contains five residues (positions 15–19) that are absent in the TnT-2 variant and needs slightly more Ca$^{2+}$ than does the TnT-2 isoform to activate the Mg$^{2+}$-ATPase activity of the cardiac myosin. In addition, we have also identified an SM TnI-like protein that shares some immunological characteristics with the cardiac counterpart. A TnI-like protein has also been described for porcine platelets.

In the SM system, intracellular [Ca$^{2+}$] and myosin phosphorylation, via calmodulin-dependent myosin light chain kinase, are not directly proportional to force maintenance. Other Ca$^{2+}$-dependent regulatory mechanisms have been proposed (see references in Reference 45) to explain the dynamic properties of SM contraction. It might be possible that thin filament–linked regulatory proteins are involved in this phenomenon. TnT- and TnI-like proteins...
proteins might be, in principle, among the candidates in this specific role. Several variants of TnT and TnI proteins, which are the product of an alternative pre-mRNA splicing process, have been described for the striated muscle system.46 The recent report47 of the existence of a slow skeletal TnC-like mRNA in human fibroblasts is in keeping with the possibility that the proteins of the troponin complex may be present in nonsarcomeric muscle and in the nonmuscle system. In SM the presence of proteins that show immunological and biochemical characteristics in common with cardiac TnT and TnI does not prove that they have a direct role in the regulation of the contractile performance. The existence of two structurally related TnT regulatory proteins such as caldesmon and calponin, which are functionally related to the TnT-TnI complex of striated muscle,18,24 indicate that the troponinlike activity in SM is not incompatible with the physiology of SM contraction. According to the current model of SM contraction, the trace amounts of TnT- and TnI-like proteins found in the present study would suggest, however, that these proteins are unlikely to be functionally significant.
Conversely, the presence of troponinlike proteins in vascular SM cells could be related to a unique structural role. In some regions of aorta, the distribution of TnI immunoreactivity is distinct from that of TnT. In the regions where the muscular component is more abundant, the anti-TnI antibody stains vascular SM cells quite heterogeneously, whereas in other regions almost devoid of muscle (“fibroelastic regions”) SM cells are stained homogeneously. We have recently demonstrated that in developing and adult bovine aorta these two regions display a different combination of SM and nonmuscle myosin content. Similarly, TnI-like protein is another marker that is able to identify different SM cell populations. Convincing evidence has been accumulated that indicates that vascular SM is heterogeneous as concerns cytocontractile and cytoskeletal proteins such as actin, myosin, desmin, and vimentin. It turns out that by using anti-TnI antibodies on aorta and large and medium coronary arteries, the immunofluorescence staining is not uniform among and within vascular SM cells. In fact, only part of the cytoplasm labeled by SM-E7 anti-SM myosin antibody or anti-TNT antibody is also stained with anti-TnI antibody (see Figures 6 and 8). Furthermore, preliminary data obtained with primary cultures of aortic SM cells indicate a different intracellular distribution of TnT- and TnI-like proteins. This finding would fit better with the hypothesis of a structural role for troponinlike proteins in vascular SM cells. In vitro experiments are under way to clarify whether these proteins are associated with cytoskeletal or cytocontractile structures.

The other important point raised by the data presented in this study is the difference in troponinlike composition of large and medium coronary arteries on the one hand and the small coronary branches and intramural vessels on the other. TnI-like heterogeneity in the vascular SM cells of large vessels completely disappears in small coronary branches or in intramural vessels, with all the vascular SM cells uniformly reactive. SM cells present in the left or right coronary artery show a low degree of staining with TT-2, RV-C2, TI-1, or TI-5; on the contrary, SM cells from small coronary artery branches or intramural vessels are strongly reactive with these antibodies. The existence of unique antigenic properties in SM cells of coronary artery compared with other vascular and nonvascular SM tissues has been preliminarily reported by other authors. Vascular SM cells are not homogeneous with regard to cytoskeletal protein composition at different levels of the same vessel wall. Force development and maximum values of shortening appear to be quantitatively different in coronary arteries compared with other conduit arteries and veins. The size-specific change in troponinlike immunoreactivity of the vessel wall of coronary arteries might be related to the peculiar aspects of coronary circulation associated with a unique autoregulatory capacity. It would be of interest to the understanding of the structural/functional role of the troponinlike proteins in the vascular SM cells to ascertain whether the expression of these proteins is coordinated with specific patterns of cytoskeletal or cytocontractile proteins.

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KEY WORDS • troponin T • troponin I • vascular smooth muscle • aorta • coronary arteries
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