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Troponin T Expression in Normal and Pressure-Loaded Fetal Sheep Heart

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ABSTRACT. The effects of cardiac hypertrophy in adult animals on the expression of a number of genes are well established. There is, however, a paucity of information about the effect of pressure overload on the expression of genes coding for the contractile proteins in the prenatal developing heart. The prenatal cardiac muscle can increase cell number in response to stress, whereas the adult heart increases cell mass. Thus, the response of the fetal heart to pressure overload cannot be assumed to be identical to that of adult myocardium. We studied the effect of banding the great vessels of fetal sheep hearts on the expression of troponin T (TNT). In other vertebrates, TNT mRNA is generated by alternative splicing of a primary transcript. Thus, both the levels and patterns of TNT isoforms generated by alternative splicing in the heart could be influenced by pressure overload. The techniques of cDNA library screening and polymerase chain reaction were used to define the influence of *in utero* banding of the great vessels on TNT expression. The data indicate that there is a single dominant isoform of TNT expressed from mid-gestation to adult life in sheep. The pattern of TNT isoform expression in the sheep heart proved to be unique among all animals studied to date. (*Pediatr Res* 29: 580–585, 1991)

Abbreviations

CHD, congenital heart disease
Mhc, myosin heavy chain
Ao, aorta
TNT, troponin T
RV, right ventricle
LV, left ventricle
PA, pulmonary artery
UTR, untranslated region
PCR, polymerase chain reaction

CHD occurs in about 1 of 100 live births in the United States (1). Despite major advances in the anatomic diagnosis and surgical treatment of CHD, there is little known about the biochemical changes that accompany the morphologic and physiologic perturbations observed in the affected neonatal heart. Changes in the contractile proteins of both the atria and ventricles in samples taken from human hearts at autopsy or surgery (2–4) have been noted. For example, Aukland *et al.* (2) found that the pattern of myosin light chain isotype expression was altered in patients with Tetralogy of Fallot, although the pattern of Mhc isotype expression was not changed. More is known about the response of the adult myocardium to pressure overload. It is now well documented, in various animal models of cardiac hypertrophy, that perturbations occur. Often, in the adult models, the reexpression of fetal or perinatal isoforms is observed (5–7). Additionally, changes in the levels of expression of enzymes associated with Ca^{2+} handling, such as the sarcoplasmic reticulum ATPase, have been noted (8).

These alterations in gene expression appear to be an intrinsic property of the myocardium. The change in the pattern of expression of a given protein varies from animal to animal. For example, in the smaller rodents, in response to hypertrophy caused by pressure overload induced by banding, a shift from the V_1 to V_3 Mhc isotype in the loaded ventricle occurs (9); such a shift does not take place in the ventricles of larger animals, such as man (10). However, the human atria, which normally expresses α-Mhc, will respond to pressure overload by expression of β-Mhc. Thus, it appears that the response of the myocardium to pressure overload is also determined in part by the normal patterns of isoform expression, as well as by the plasticity of the myocardial genetic program.

We wished to explore this plasticity during induction of a simulated congenital cardiac lesion. The fetal heart has the ability to increase cell number to compensate for applied stresses, but the adult heart does not (11). We hypothesized that the genetic program of the fetal myocyte might be more plastic than that of the adult heart and that *in utero* pressure and volume loading of the heart might cause expression of contractile isoforms that are not normally expressed. One candidate for modulation of isoform expression is TNT. Generation of mRNA transcripts by alternative splicing of the primary transcript of the cardiac TNT gene appears to be under developmental regulation. Although studies of the pattern of TNT expression at the protein level did not detect any influence of hypertrophy in adult heart (12), such an effect cannot be ruled out *a priori* in the fetal hearts.

Several authors have described models of congenital cardiac lesions using fetal sheep (13–16). The models involve occluding or restricting flow through one of the great vessels with a band or balloon. Conditions that have been modeled include pulmonic stenosis (13–16), pre- and postductal coarctation of the Ao, (16), aortic stenosis, and hypoplastic LV (16). The morphologic changes noted in the fetal lamb hearts are strikingly similar to those seen in human infants with a similar type of CHD (16).

In our present study, we elected to use the fetal lamb as a model for congenital pulmonary stenosis and preductal coarctation of the Ao by banding the great vessels *in utero*. We define at the nucleotide level the primary TNT expressed in the sheep heart during the fetal and adult stages of development. The effects of pressure and volume overload on the expression of TNT in the fetal heart during the phase of rapid interuterine growth was also determined.

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Table 1. *Physiologic parameters of banded and nonbanded hearts**

	Nonbanded control ($n = 3$)	PA banded 85–90 d ($n = 3$)	PA banded 100–105 d ($n = 2$)	Ao/PA banded 100–105 d ($n = 2$)
RV systolic	42 (37–45)	60 (45–75)	62, 75	50, 65
LV systolic	67 (65–67)	60 (50–65)	62, 80	110, 125
RV/LV pressure	0.63 (0.56–0.69)	1.0 (0.9–1.15)†	1.0, 0.9†	0.45, 0.52
Heart wt/body wt (g/kg)	7.6 (7.3–7.8)	9.46 (8.7–10.8)‡	8.5, 9.4‡	13, 9.5‡

* The mean and range ( ) are presented for the nonbanded controls and PA-banded hearts at 85–90 d. For $n = 2$, the two data values are given.
† RV/LV pressure ratio significantly greater than the controls for all PA-banded hearts if grouped together ($p < 0.05$).
‡ Heart wt/body wt ratios for grouped banded hearts are significantly greater than for the nonbanded controls ($p < 0.05$).

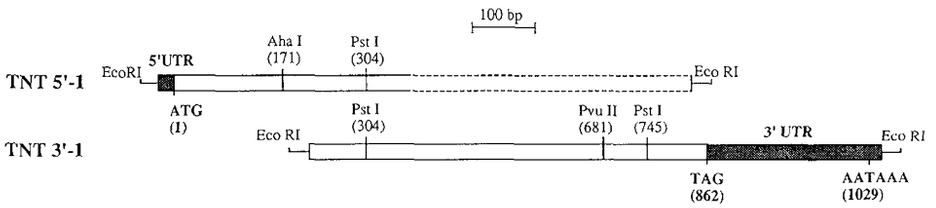

Fig. 1. Restriction map of two overlapping TNT cDNA clones. Clone TNT5′-1 contains the 5′UTR (*shaded*), the translation start site, "ATG," and the 5′ portion of the translated sequence. This clone overlaps clone TNT3′-1 in the region indicated by the *solid lines*. The *dotted lines* indicate a region not sequenced for this clone. Clone TNT3′-1 contains the remainder of the translated region, the stop codon "TAG," the 3′UTR (*shaded*), and the polyadenylation signal "AATAAA." The EcoRI ends are cloning sites artificially introduced. Restriction site locations, in bases from the ATG initiator methionine codon (1), are shown *in parentheses*.

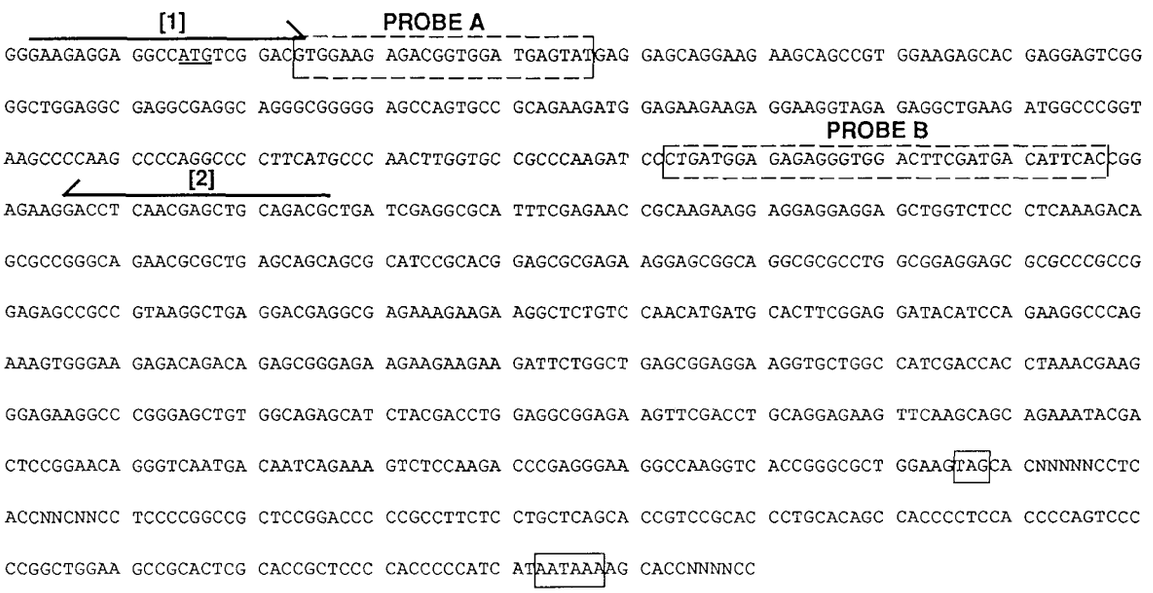

Fig. 2. The nucleotide sequence of the dominant isoform of sheep cardiac TNT. The sequence beneath *arrow [1]* delineates the sequence of PCR primer [1] and that beneath *arrow [2]* the sequence complementary to PCR primer [2]. The locations of the internal oligonucleotide probes used to confirm the identity of the PCR products are *boxed with dotted lines*. The stop codon, TAG, and polyadenylation signal (AATAAA) are *boxed*. *N* denotes a nucleotide not resolved.

MATERIALS AND METHODS

Surgical preparation. Institutional Animal Care and Use Committee approval was obtained for all experiments involving live animals. Ewes of mixed Western breeds, with known breeding dates, were used for all experiments. At either an 85- to 90-d or a 100- to 105-d gestation, the ewes were anesthetized with halothane and a hysterotomy was performed via a midline approach. The fetal lamb skin was marsupialized to the maternal membranes and the fetal chest was entered via a left thoracotomy incision. The pericardium was opened sufficiently to allow access to either the PA or to both the PA and the Ao. PA banding was performed essentially as described by Fishman *et al.* (16).

At term, approximately 141–144 d of gestation, the lambs were delivered by cesarean section, immediately intubated, ventilated, and anesthetized with halothane (0.5–0.7%) in oxygen. Catheters were placed in the femoral artery and vein, followed by a midline sternotomy for placement of catheters in both the RV and LV. Pressures were recorded from the descending Ao, the RV and LV and the PA proximal to the band using a Grass Physiograph (Grass Instrument Co., Quincy, MA) and Edward DTS260 transducers (American Edwards, Irvine, CA). After pressure recording was complete, the hearts were rapidly excised, rinsed in cold saline (150 mM NaCl), and frozen in liquid nitrogen.

Oligonucleotides. Oligonucleotide primers and probes were synthesized on a DNA synthesizer (Applied Biodystems, Inc., Foster City, CA) and purified on a 11% acrylamide-8 M urea

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Sheep:   MSDVEETVDEYEE*QEE**********A**AVEEHEESVEEEAGGEARQ 36
Rat:     ---A--E-V----E---EDWSEEEEDEQEE----EDGEA-PDPE---EA 49
Bovine:  *-----A-E----*---AAEEE*****HEE----EAG*******---EA 35
Rabbit:  *--L--V-E----E---**********QEAGE--EAG***GGAEA--ET 35

Sheep:   GGGASAAEDGEEEEGREAEDGPVEESKPQAPGPFMPNLVPPKIPDGERV 85
Rat:     EEDK*-E-V-PD--A-D-------D---KPSRL---------------- 97
Bovine:  -EPC*T-------------------F--KP*R----------------- 82
Rabbit:  EETQ*-E---Q---DK-D----------KP*R----------------- 82

Sheep:   DFDDIHRKRMEKDLNELQTLIEAHFENRKKEEEELVSLKDRIEKRRAER 134
Rat:     ------------------------------------I------------ 146
Bovine:  ---------------------------------------------------- 131
Rabbit:  ----------------------------------------E--------- 131

Sheep:   AEQQRIRTEREKERQARLAEERARREEEESRRKAEDEARKKKALSNMMH 183
Rat:     -------N-------N-------------N-------------------- 195
Bovine:  -------A-------T---------------------------------- 180
Rabbit:  ---L---A-------N---------------------------------- 180

Sheep:   FGGYIQKAQTERKSGKRQTEREKKKKILAERRKVLAIDHLNEDQLREKA 232
Rat:     ---------------------------------------------------- 244
Bovine:  ---------------------------------------------------- 229
Rabbit:  -------QA********---------------------------------- 221

Sheep:   RELWQSIYDLEAEKFDLQEKFKQQKYEINVLRNRVNDNQKVSKTRGKAK 281
Rat:     K------HN-------------------------I-------------- 293
Bovine:  K----N------------------------------------------- 278
Rabbit:  K-------N------------------------------------------ 270

Sheep:   VTGRWK 287
Rat:     ------ 299
Bovine:  ------ 284
Rabbit:  ------ 276
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Fig. 3. Derived amino acid sequence of the dominant isoform of sheep cardiac TNT compared with other mammalian cardiac TNT amino acid sequences. The embryonic rat sequence (35), the bovine sequence encoding the larger isoform (39), and the adult rabbit sequence (38) are shown. The amino acids encoded by alternatively spliced exons are *underlined*. An *asterisk* indicates that no amino acid is present at that position; the homology algorithm shifts the sequence to maximize perceived identities. A *dash* denotes a residue identical to that of the sheep sequence.

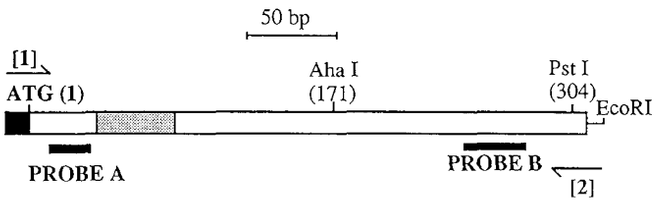

Fig. 4. Strategy of the PCR experiment. The locations and directions of extension of the PCR primers (*[1]* and *[2]*) are shown, as well as the internal oligonucleotide probes (*A* and *B*) used to confirm the identity of the PCR products. The corresponding sequences are denoted in Figure 3. The actual oligonucleotides used, [2] and probe B, are complementary to the sequence shown in Figure 3. Both PCR primers [1] and [2] and probes A and B are located in constitutive exons. The *solid area* indicates 5′UTR sequence. The *stippled area* delineates the region in which alternatively spliced exons are located in other species.

DNA sequencing gel. Oligonucleotides used as probes were end-labeled (17) with [γ-^{32}P]-ATP (6000 Ci/mmol; New England Nuclear, Boston, MA) using T4 polynucleotide kinase.

Unless otherwise noted, hybridizations using oligonucleotide probes were carried out in 6 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate), 1% SDS 1 × BLOTTO (18) (for DNA) or 5 × Denhardt's (for RNA), and 100 μg/mL denatured sonicated salmon sperm DNA at 42°C for 8 h. Probes were present at a concentration of 3 ng/mL.

RNA isolation. Total cellular RNA was isolated from hearts using RNAzol (Cinna Biotecx, Friendswood, TX) essentially as described by Chomczynski and Sacchi (19).

Polyadenylated mRNA was isolated using an oligo dT-cellulose column (20). The integrity of the RNA preparation, the degree of ribosomal RNA contamination, and size range of the mRNA was determined by electrophoresis on a methyl mercury hydroxide agarose gel (21).

cDNA cloning. A cDNA library was prepared using 5 μg of polyadenylated mRNA from a 97-d gestation fetal lamb heart using a Pharmacia cDNA synthesis kit (Pharmacia-LKB, Piscataway, NJ) following the manufacturer's protocol. The 0.5–3.7 kbp DNA fraction was isolated on agarose gels and ligated (17) to λ-gt11 arms with T4 ligase at 14°C overnight and packaged in Gigapack Gold extracts (Stratagene, LaJolla, CA). The library was screened using the oligonucleotide 5′CTGITCAGCCCICTCTGCCCGICGCTTTTCIATCCTG-TCITTGAGAGAAATGAGCTCCTCTTCCTCCTTCTTCCT 3′, which corresponds to a region that has been conserved between different TNT sequences (see Results). Single-stranded sequencing was performed by the dideoxy chain termination method (22) using Sequenase (United States Biochemicals, Cleveland, OH).

PCR. The PCR (23) was used to determine if more than one isoform of TNT was present in the fetal lamb heart. A 1-μg aliquot of total RNA was annealed with 50 pmol of an antisense oligonucleotide primer in 50 μL of 50 mM KCl, 10 mM Tris (pH 8.3), and 1.5 mM $MgCl_2$ at 65°C for 10 min, followed by slow cooling to 40°C. First, strand synthesis was initiated using deoxynucleotide triphosphates and avian myeloblastosis virus reverse transcriptase. The reaction was terminated by boiling for 3 min and cooling on ice for 4 min. Fifty pmol of the second oligonucleotide primer was added, and 2.5 units of Taq polymerase (U.S. Biochemical Corp., Cleveland, OH) were used in the amplification. The program steps were: one cycle at 94°C for 3 min; 23 cycles of 94°C for 60 s, 58°C for 30 s, and 72°C for 30 s; and a final cycle of 94°C for 60 s, 58°C for 30 s, and extension at 72°C for 10 min. The reaction products were separated on a 2.5% agarose gel, transferred bidirectionally to nylon membranes for 45 min (24), and hybridized with the end-labeled oligonucleotide probes as described above. After hybridization, the filters were washed in 1 × SSC with 1% SDS at 50°C for a total of 30 min and exposed at −80°C for variable periods of time without intensifying screens. Only those bands that hybridized to two oligonucleotides, both corresponding to internal sequence in the expected product, were considered to be authentic TNT transcripts.

RESULTS

Physiologic studies of banded and nonbanded hearts. The hearts of all animals used in the study were examined *in situ* at the time of ventricular catheterization for direct chamber pressure measurements. All of the banded hearts used exhibited evidence of stenosis of one of the great vessels. This included poststenotic dilation, enlargement of the vessel diameter distal to the band, and increased heart weight to body weight ratio. The heart weight to body weight ratios of the hearts included in our study are shown in Table 1. The heart to body weight ratios shown for the nonbanded control hearts (7.6 g heart wt/kg body wt) are in good agreement with previously published data (7.4 g heart wt/kg body wt) (13). It should be noted that to obtain meaningful statistics it was necessary to group the banded hearts. Both the early (85–90 d gestation) and late (105 d gestation) banded hearts exhibited a trend to increased heart/body weight ratios as compared to the controls. Additionally, the mean RV systolic pressure was greater in the banded hearts than for the nonbanded controls (Table 1).

The RV/LV pressure ratio is 1.0 for the PA-banded hearts, which is greater than for the nonbanded controls. The Ao/PA-

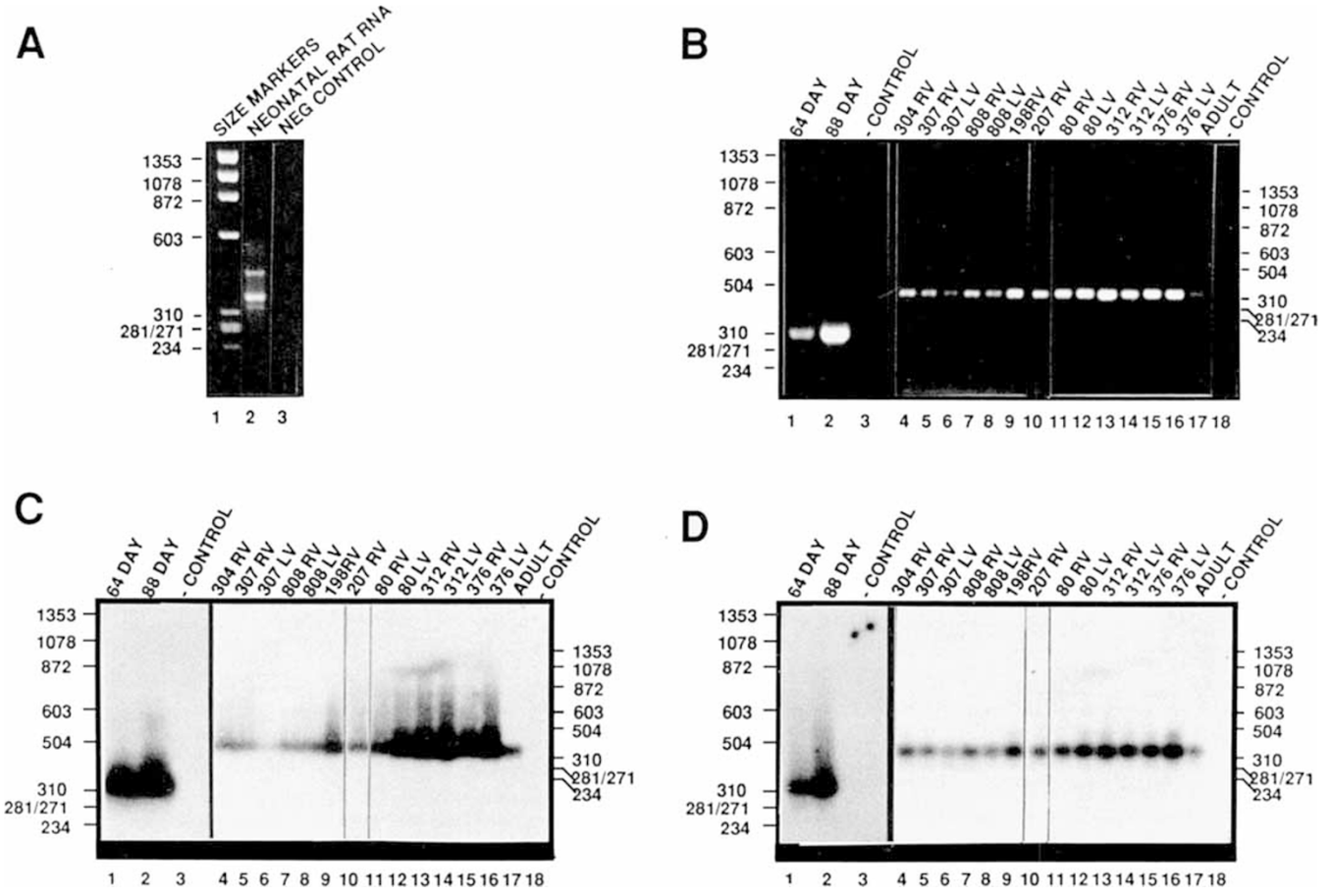

Fig. 5. PCR analysis of TNT isoforms. *Panel A*, the multiple isoforms of rat cardiac TNT generated by the amplification of total cellular RNA from neonatal rat heart. The TNT transcripts were amplified using the primers described in the text. The products were electrophoresed in agarose, transferred to nylon membranes, and hybridized to internal TNT oligonucleotide probes. *Lane 1* contains molecular weight standards; *lane 2* contains the PCR products. *Lane 3* contains the material from a PCR reaction without RNA template (negative control). *Panel B*, TNT transcripts present in fetal sheep heart. Total cellular RNA from fetal sheep hearts was used. The designation above the data (*e.g. 304RV*) refers to the individual sheep heart and the particular chamber used for the RNA isolation. The samples are as follows: *Lane 1*, 64 d gestation; *lane 2*, 88 d gestation; *lanes 4–8*, 140–144 d (term) gestation nonbanded controls; *lanes 9–10*, term, banded at 85–90 d gestation; *lanes 11–12*, term, PA/Ao-banded at 100–105 d gestation; *lanes 13–16*, term, PA-banded at 100–105 d gestation; and *lane 17*, total cellular RNA from adult sheep heart. The RNA samples were amplified as described in Materials and Methods and separated electrophoretically in agarose gels. The ethidium bromide-stained products are shown in *panel B*. *Lanes 3* and *10* are negative controls (no RNA template). *Panel C*, the PCR products in *panel B* were transferred to nylon membranes and hybridized to probe A. *Panel D*, the PCR products in *panel B* were transferred to nylon membranes and hybridized to probe B. The lane assignments in *panels C* and *D* are the same as in *panel B*. In *panels B*, *C*, and *D*, the samples present in *lanes 1–3* were electrophoresed on a different agarose gel than those present in *lanes 4–18*; hence, the lower relative position of the bands. The size markers in bp for *lanes 1–3* are shown to the left, for *lanes 4–18*, to the right.

banded hearts exhibit a dramatically elevated LV pressure and a large LV-descending Ao pressure gradient (70 mm Hg). A summary of the pressure ratios is given in Table 1. The animals that underwent PA banding at a 100–105 d gestation also had an RV/LV pressure ratio of 1.0. The two animals that underwent both PA and Ao banding and survived to term had an average LV systolic pressure of 120 mm Hg with an LV-Ao gradient of 70 mm Hg. The normal systolic pressure for a fetal lamb under anesthesia is 70 mm Hg (25). The measured pressures in the LV of the PA/Ao-banded hearts represent a significant increase in load on the myocardium.

Isolation of sheep cardiac TNT cDNA. To define the cardiac TNT isoforms present in the normal and banded sheep hearts, a molecular genetic approach was undertaken. The gene(s) and cDNA encoding sheep cardiac TNT have not been isolated and, to be able to probe for the (putative) different TNT in a definitive manner, it was necessary to isolate and characterize the TNT cDNA. To initiate these studies, we prepared a cDNA library from a 96-d gestation fetal sheep heart, assuming, on the basis of other systems (26), that if multiple TNT isoforms were generated in sheep cardiac muscle, most or all of the TNT isoforms would be present at this developmental stage. The library was initially screened (5×10^5 recombinants) with a 75-base oligonucleotide probe complementary to a region that exhibits maximum homology between the rat cardiac (27) and chick cardiac (28) cDNA sequences (see Materials and Methods). Five of the bases were inosine residues; these were used to reduce destabilization of the hybrids due to mismatches (17).

The screening yielded 53 clones. One of these was found to encode cardiac TNT, as based on sequence homologies, and was used to exhaustively rescreen (1×10^6 recombinants) the sheep heart cDNA library. The rescreening yielded 252 clones. Seventy-five of these were analyzed further. Restriction fragment length analysis and sequencing indicated that all clones appeared to encode an identical transcript, whose organization is shown in Figure 1. Two overlapping clones, TNT5′-1 and TNT3′-1, encompass the entire transcript. TNT mRNA consists of a 24-base 5′ UTR, an 867-base translated sequence, and a 211 base 3′ UTR. The sequence is shown in Figure 2. A single polyadenylation signal, AATAAA, is present, but a polyadenylated tail is

not because the cloning site abuts the sequence. It is likely that some of the sequence present between the polyadenylation signal and the polyadenylated tail may be deleted from the cDNA. Thus, the 211-base 3′ UTR encoded by TNT 3′-1 may not represent the entire 3′ UTR.

A comparison of the derived amino acid sequence of sheep cardiac TNT with other published cardiac TNT sequences is shown in Figure 3. The sheep sequence shares 92.5% homology with rat cardiac TNT in the region containing exons 8–18 (encoding amino acids 48–288); this sequence is thought to encode the tropomyosin binding domain(s) of TNT. Similar sequence conservation with the other mammalian cardiac TNT is observed in this region. The sheep cardiac TNT contains a proline rich region (eight residues) between amino acids 57 and 81. These residues are a common feature to all TNT sequences and are thought to be a critical part of one of the tropomyosin binding domains of the TNT molecule (27). As noted in other TNT, there is a region of amino acid divergence near the amino terminus. The sheep sequence appears to lack residues encoded by exon 4 and/or 5 when compared to the embryonic rat sequence. Depending upon the particular species, the products of alternatively spliced exons are found in the region corresponding to amino acids 16–32 (28–30).

PCR. The above data are consistent with the presence of a single dominant isoform of TNT in the fetal sheep heart. However, a second isoform, if present in low abundance, might not be detected. PCR experiments were performed to detect any rare messages coding for other isoforms of TNT in the normal or banded fetal sheep hearts. The PCR strategy is schematically depicted in Figure 4. The sense and antisense primers are shown in Figures 2 and 4 and are located in constitutive exons (28, 31). The region amplified by these oligonucleotides includes the part of the TNT transcript that is subject to alternative splicing (*stippled area*, Fig. 4). These primers should yield a product of 324 bp; larger or smaller products would represent (potential) alternatively spliced TNT transcript.

Hybridizations were carried out with internal oligonucleotide probes (probes A and B in Figs. 2 and 4) to confirm the PCR products' authenticity. In preliminary experiments, the expected band was generated and sequencing confirmed that the fragment corresponded to the 5′ end of the coding region of cardiac TNT.

We tested the ability of the general primer and internal oligonucleotide probe strategy to detect known multiple isoforms of TNT present in total cellular RNA isolated from newborn rat heart. Previous studies have shown that two TNT are present in this tissue (27). The primer used to initiate reverse transcription of the rat RNA was identical to [2] in Figures 2 and 4. The sequence of the second primer, 5′GACACCTCAAGTCCTGAGTGC3′, corresponds to bases 4–24 of the rat cardiac TNT cDNA sequence (27). The internal oligonucleotide probes used were probe B and a 24-base oligonucleotide complementary to bases 54 to 77 in the rat cardiac TNT cDNA sequence, 5′CCACCACCTCTTCCTCGGCGTCAG3′, which is analogous to probe A (Fig. 2).

Total cellular RNA from the heart of a 1-d-old rat was amplified and analyzed by gel electrophoresis and flat hybridization (Fig. 5*A*). The products (*lane 2*) consist of three bands, of 410, 356, and 326 bp. The 356- and 326-bp products hybridize to the two internal oligonucleotide probes, but the 410-bp product does not (data not shown). The 356- and 326-bp products correspond to those expected on the basis of the rat cardiac TNT cDNA sequence (27). The relatively greater abundance of the larger transcript is consistent with studies of TNT expression in neonatal rat heart at the protein level (12). This result indicated that the PCR strategy should detect any transcripts generated by alternative splicing of the 5′ end of the primary TNT transcript.

The TNT transcripts present in the RNA isolated from both the normal and banded sheep hearts were amplified (Fig. 5*B*). All of the lanes contain the expected 324-bp fragment. The DNA was blotted onto nylon membranes and hybridized to both of the internal oligonucleotide probes (Fig. 5*C* and *D*). The 324-bp fragment annealed to both probes. A minor product, 30–45 bp longer, was sometimes observed in the banded hearts taken at terms, but could not be consistently well resolved from the major isoform (Fig. 5*C*, *lanes 12–16*). Although this longer product was not observed in the term control hearts nor in the adult heart, we were unable to confirm its identity as a separate isoform by sequencing techniques. In any case, these data show that a single isoform is the dominant TNT to ovine heart from midgestation to adult.

DISCUSSION

This report details the effects of banding the great vessels of the heart *in utero* on the expression of a contractile protein. Previous studies in which the great vessels of the fetal lamb were banded or otherwise flow-restricted described changes in blood flow, cardiac output, and morphologic characteristics of the heart and great vessels (14–17). We did not include morphometric calculations in our present study because the liquid nitrogen preservation of the tissue precluded such measurements. However, prior studies by Shapiro *et al.* and others (14–17) provide a basis for interpreting the present report. These authors found that PA-banded fetal sheep hearts had a diminished overall cardiac output with a dramatic reduction in RV output as a fraction of total cardiac output. The shunting from right to left atrium tends to reduce the pressure loading of the RV and increase the volume loading of the LV. The hearts of the two fetal lambs that underwent banding of both vessels could not offload in a similar manner. The result was a significant increase in LV work for pressure work and probably volume work as well. Thus, in the case of the PA-banded hearts, the stimulus to the RV to change protein expression patterns is a mild increase in pressure. For the LV, the stimulus is an increase in chamber volume. For the Ao/PA-banded hearts, the stimulus for the RV is a mild pressure increase and, for the LV, it is a larger pressure increase.

The pressure measurements made in our study did not reveal any large gradients across the pulmonary outflow tract despite the PA banding. Prior studies have found gradients varying from 15–45 mm Hg (13–14). In the study of Shapiro *et al.* (15), the RV pressure was found to be 9–93% greater than LV pressure in five of six animals studied, but 18% less than LV pressure in one animal. The RV/LV pressure ratio in our present study is 1.0 for the banded hearts and less than 0.6 for the nonbanded controls. The use of halothane at 0.5–0.7% may, in part, explain some of the differences we observed in pressure measurement, inasmuch as halothane at the concentration used can depress cardiac output in newborn lambs nearly 50% by direct myocardial depression (25, 32). Halothane anesthesia was not used in the studies of banded fetal sheep hearts cited above. It is noteworthy that, despite the lack of a significant RV to PA gradient, the PA-banded hearts exhibited increased heart to body weight ratios as compared to the controls and to published data for nonbanded fetal sheep heart at term. The finding of increased body weight ratio is consistent with the development of hyperplasia or hypertrophy of the fetal heart in response to the PA band. The Ao/PA-banded hearts were clearly subjected to a major pressure overload.

The data indicate that the sheep has a "mature" TNT isoform pattern at birth, unlike the rabbit (33) or rat (27), animals that express fetal isoforms for some time after birth. In one other ruminant species, the cow, two isoforms of cardiac TNT are expressed throughout postnatal life (30). Thus, the sheep appears unique in that only one isoform of cardiac TNT is normally observed from late in gestation to adulthood, even using the very sensitive technique of PCR as a means of identifying multiple isoforms.

The study was undertaken to determine if the neonatal heart would respond to cardiac loading in a manner different from

that reported for the adult. The definition of the sheep cardiac TNT at the nucleotide level allowed us to design a PCR strategy for detecting both abundant and rare TNT transcripts. The data from the cDNA and PCR analyses are consistent with the expression of a single, dominant TNT message during gestation of both the normal and banded hearts.

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