Inhibition of Norepinephrine-induced Cardiac Hypertrophy in S100β Transgenic Mice

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Abstract

We have recently reported that the Ca^{2+}-binding protein S100β was induced in rat heart after infarction and forced expression of S100β in neonatal rat cardiac myocyte cultures inhibited α_1-adrenergic induction of β myosin heavy chain (MHC) and skeletal α-actin (skACT). We now extend this work by showing that S100β is induced in hearts of human subjects after myocardial infarction. Furthermore, to determine whether overexpression of S100β was sufficient to inhibit in vivo hypertrophy, transgenic mice containing multiple copies of the human gene under the control of its own promoter, and CD1 control mice were treated with norepinephrine (NE) (1.5 mg/kg) or vehicle, intraperitoneally twice daily for 15 d. In CD1, NE produced an increase in left ventricular/body weight ratio, ventricular wall thickness, induction of skACT, atrial natriuretic factor, βMHC, and downregulation of αMHC. In transgenic mice, NE induced S100β transgene mRNA and protein, but provoked neither hypertrophy nor regulated cardiac-specific gene expression. NE induced hypertrophy in cultured CD1 but not S100β transgenic myocytes, confirming that the effects of S100β on cardiac mass reflected myocyte-specific responses. These transgenic studies complement in vitro data and support the hypothesis that S100β acts as an intrinsic negative regulator of the myocardial hypertrophic response. (*J. Clin. Invest.* 1998. 102:1609–1616.) Key words: Ca^{2+}-binding proteins • gene expression • α_1-adrenergic agents • skeletal α-actin • atrial natriuretic factor

Introduction

The adult cardiac myocyte is terminally differentiated and has lost the ability to proliferate. Therefore, the myocardium adapts to increasing workloads through hypertrophy of individual cells in response to hormonal, paracrine, and mechanical signals (1–2). Whereas this process is initially compensatory, it can progress to irreversible enlargement and dilatation of the ventricle culminating in heart failure (3).

Myocyte hypertrophy is accompanied by a program of fetal gene re-expression including the embryonic β-myosin heavy chain (MHC),[1] the skeletal isoform of α-actin (skACT), and atrial natriuretic factor (ANF) (2, 4, 5). This response can be reproduced in vitro in cultured neonatal cardiac myocytes by treatment with a number of trophic effectors including peptide growth factors and α_1-adrenergic agonists (6–14).

Negative modulators of the hypertrophic response are essential to maintain a balance between compensatory hypertrophy and unchecked progression. We have recently identified the first likely candidate for a negative intrinsic modulator of the myocardial hypertrophic response. This modulator, a brain Ca^{2+}-binding protein, S100β, was found to be induced in rat heart after experimental myocardial infarction and was shown in transfection studies to inhibit the α_1-adrenergic and anoxic-mediated induction of βMHC and skACT in cultured cardiac myocytes (15). Consistent with previous data (16–19), this inhibition involved the interruption by S100β of the protein kinase C (PKC)-signaling pathway in a Ca^{2+}-dependent manner.

In the present communication, we have extended this work by showing that S100β is induced in the heart of human subjects after myocardial infarction. In addition, the availability of a transgenic mouse model (20), in which human S100β is overexpressed, has allowed us to examine the consequences of this overexpression on the response of the myocardium to hypertrophic α_1-adrenergic stimulation in vivo. In agreement with our previous in vitro results (15), we report that the hypertrophic response is blunted by induction of S100β in transgenic mice, as expected for a negative modulating influence of elevated levels of this regulatory molecule.

Methods

Transgenic mice. S100β transgenic mice containing multiple copies (approximately eight) of the human S100β gene under the control of its own promoter were derived on a CD1 background as previously described (20). Transgenic and control CD1-mice (Charles River, St. Constant, Quebec, Canada) were housed in microisolators. Testing was performed at 8 wk of age.

Induction of experimental hypertrophy. Norepinephrine (NE) (1.5 mg/kg) was injected intraperitoneally in ascorbic acid saline twice daily for 15 d. This treatment schedule produces hypertrophy without necrosis (21). Control injections consisted of vehicle alone. The four treatment groups (17 mice per group) included transgenic or control

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1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; GAPDH, glyceraldehyde-3 phosphate dehydrogenase; MHC, myosin heavy chain; NE, norepinephrine; PE, phenylephrine; PKC, protein kinase C; skACT, skeletal α-actin; TG, transgenic.

CD1 mice injected with either NE or vehicle above. Fluid intake and body weight were monitored daily during treatment.

Monitoring of systolic blood pressure and heart rate. On day 16, 1 h after treatment with either NE or vehicle, systolic blood pressure and heart rate were measured using an indirect mouse tail blood pressure system (Harvard Apparatus, South Natick, Massachusetts) according to the instructions supplied by the manufacturer. In brief, mice were placed in restrainers with tails exposed 15–30 min before taking readings. A tail cuff and pulse sensor were applied to the tail and after a 15-min warming period to stimulate vasodilation, heart rate and systolic blood pressure readings were taken.

Echocardiographic assessment of cardiac hypertrophy and function. Four animals in each group underwent transthoracic echocardiography, before time of death, using a 12-mHz probe (Hewlett Packard, Mississauga, Ontario, Canada). End-diatolic and end-systolic diameters, end-diastolic interventricular and posterior wall thickness, and left ventricular percent fractional shortening were measured at the level of the papillary muscles and recorded as the consensus of two blinded observers (J.N. Tsoporis and T.G. Parker) with interobserver variability of $< 10\%$.

Harvesting of cardiac tissue. On day 16, after treatment with NE or vehicle as outlined above, eight mice from each group were killed to harvest fresh cardiac tissue, and three mice were killed to obtain fixed cardiac tissue. To harvest fresh cardiac tissue, the mice were killed by opening the chest cavity under chloroform anaesthesia and arresting the heart in diastole by a retrograde injection of 1 mmol/liter KCl through a cannulated aorta. The heart was rapidly excised, the great vessels and atria removed, and the ventricle immersed in ice-cold DEPC-PBS. Of the 14 samples of freshly harvested cardiac tissue, 8 were used for measurement of cardiac hypertrophy and 6 for RNA and protein isolation. To fix cardiac tissue, a similar technique was used as previously described for vessels (22). Briefly, the heart was perfused at a constant pressure (50 mm Hg) with oxygenated Krebs solution (pH 7.4, 37°C) for 10 min at 37°C via an aortic catheter, followed by 20 min of 2.5% glutaraldehyde in 0.03 M phosphate buffer and 15 min of phosphate buffer wash. After initial fixation by perfusion, the heart was excised and further fixed by immersion in 2.5% glutaraldehyde for an additional 48 h at 4°C.

Morphometric measurement of cardiac hypertrophy. For fresh cardiac tissue, the left and right ventricles were weighed and a central full-thickness slice of the wall of the left ventricle was obtained by two transverse cuts at levels corresponding to the one-third and two-thirds of the length of the ventricle (23). This midventricular slice was used for measurement of left ventricular wall thickness by light microscopy, using a calibrated ocular lens as described previously (23). Fixed cardiac tissue was divided into two equal blocks by a central cross-sectional cut perpendicular to the long axis of the heart. Each block was dehydrated and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin and examined microscopically as previously described (24).

Ribonuclease protection assay. RNA was isolated from mouse and human tissues by a one-step acid guanidinium phenol method (25). RNase protection assays to determine steady-state levels of S100β mRNA, skACT mRNA, ANF mRNA, βMHC/αMHC mRNAs, and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) mRNA were performed by modifying published conditions (26). Antisense riboprobes for mouse skACT (a gift from Dr. L.K. Karns, Laboratory of Molecular Neuro-Oncology, University of Virginia Health Sciences, Charlottesville, VA), mouse/rat ANF (27), mouse/rat βMHC/αMHC (28) (a gift from Dr. C.S. Long, Division of Cardiology and Research, Veterans Affairs Medical Center, San Francisco, CA) and GAPDH (Ambion, Austin, TX) were labeled with [α ^{32}-P] UTP (800 Ci/mmol, Amersham, Oakville, Ontario) by in vitro transcription with T7 (skACT, βMHC/αMHC, GAPDH) or SP6 (ANF, GAPDH) RNA polymerase of appropriate RNA synthesis vectors. The ^{32}P-UTP-labeled S100β antisense riboprobe was derived from pKN3 (20) by in vitro transcription with T3 RNA polymerase. The S100β, skACT, ANF, βMHC/αMHC, and GAPDH probes (2 ng, 10^6 dpm) were hybridized with 25 ng (S100β) or 15 ng (skACT, ANF, βMHC/αMHC, GAPDH) of total RNA from mouse or human tissues, or net tRNA, for 18 h at 45°C and RNase-resistant hybrids were recovered using a commercial kit (Ambion), analyzed on 8 M urea, 6% polyacrylamide sequencing gels, and visualized by autoradiography.

Western blotting. Mouse hearts were obtained as described, heart lysates were prepared from frozen heart powder, and Western blotting for detection of S100β protein was performed as described previously (20). Briefly, aliquots of extracts containing 75 μg of total protein or samples of purified bovine S100 protein (1 or 10 ng) were dissociated and subjected to electrophoresis in 15% sodium dodecyl sulfate-polyacrylamide slab gels under reducing conditions. The proteins were transferred electrophoretically to membranes (PVDF; Millipore Corp., Milford, MA) and the blots were incubated with anti-S100 protein monoclonal antibody G12.B8 (a generous gift from Dr. B. Boss, Salk Institute for Biological Studies, La Jolla, CA) at a 1:2,000 dilution followed by peroxidase-conjugated rabbit anti–mouse Ig antibody (Dako, Carpinteria, CA) at a 1:10,000 solution. The blots were developed using enhanced chemiluminescence (Pierce, Rockford, IL) and exposed to X-ray film (Biomax; Kodak, Rochester, NY).

Cardiac myocyte cultures. Neonatal cardiac myocytes were isolated from the ventricles of 2-d-old control CD1 and S100β transgenic mice and established in primary culture essentially as previously described for neonatal rat cardiac myocytes with $< 5\%$ contaminating fibroblasts (6, 8, 9, 13, 15). The cells were seeded in medium supplemented with 10% FBS. The next day, the cultures were changed to serum-free medium supplement with 0.1 mM bromodeoxyuridine, 1 mg/ml BSA, and 10 μg/ml (each) transferrin and insulin. Cell numbers and myocyte size were determined after a 3-d treatment with either 5% FBS, or 20 μM NE or phenylephrine (PE) in 100 μM ascorbic acid. Control cultures were treated with vehicle alone. Cell numbers were determined by counting cells in randomly selected microscopic fields (6). Cell size was quantified by continuous labeling with ^{14}C-phenylalanine as previously described (6). There were at least three cell culture dishes in each group. There was no change in the number of cells after treatment of cultures with any agent.

Immunoperoxidase staining. Archival blocks of formalin-fixed, paraffin-embedded cardiac tissue from subjects deceased after myocardial infarction (8 patients) and control subjects (14 patients) were obtained through the Department of Pathology, The Toronto Hospital. Rabbit anti-S100 protein antiserum, specific for S100β, was purified by affinity chromatography on a column of bovine brain S100 protein coupled to CNBr-activated Sepharose. The column was extensively washed with PBS and the antibody eluted with 100 mM sodium citrate, pH 3.0, and neutralized with 1.0 M Tris. Immunoperoxidase staining was performed as described (29) using a primary anti-S100β antibody at a 1:4,000 dilution. Stained sections were read by two pathologists (H.J. Kahn and J.W. Butany) blinded to the diagnosis, and staining intensity was graded semi-quantitatively by consensus as mild (+), moderate (++), or severe (+++).

Statistical analysis. Treated/control ratios were tested for deviation from unity by calculation of confidence limits. Mean values were compared by analysis of variance, followed by Student-Newman-Keuls test with Bonferroni correction for multiple comparisons, with significance defined as $P < 0.05$.

Results

Induction of S100β in the heart of human subjects after myocardial infarction. Immunoperoxidase staining of normal human myocardium from a control subject with anti-S100β antibody showed an absence of staining of cardiac myocytes and only Schwann cells in a nerve bundle staining positively (Fig. 1 *B*). This is consistent with the previously reported (29) selective expression of S100β in astrocytes and Schwann cells in the central and peripheral nervous systems, respectively. In contrast,

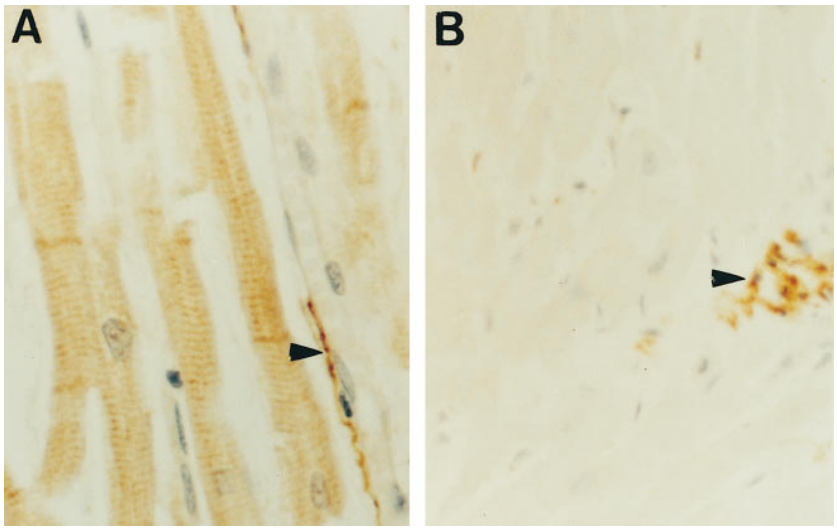

Figure 1. Induction of S100β in myocardial infarction. Sections of formalin-fixed paraffin-embedded heart tissue from a human subject deceased after myocardial infarction (*A*) or a non-cardiac cause (*B*) were stained with a polyclonal antibody specific for S100β using the immunoperoxidase reaction. *A* shows diffuse positive immunostaining of cardiac myocytes for S100β with accentuation of Schwann cells in a nerve twig running parallel to a blood vessel (*arrowhead*) ×500. *B* shows absence of staining of myocytes; *arrowhead* indicates staining of Schwann cells in a nerve bundle.

cardiac myocytes in the myocardium of a subject deceased after myocardial infarction were stained positively with the anti-S100β antibody (Fig. 1 *A*). In 8 subjects (7 males, 1 female) with myocardial infarction (mean age 66.5 yr, range 59–85 yr), intense S100β staining (+++) was apparent in the majority of myocytes in the peri-infarct region of healed infarcts (> 3-mo-old) in 7/8. One subject with acute infarction 1 wk before death had only mild (+) staining in peri-infarction myocytes. Staining was less intense to absent in all patients in myocardium remote from the infarcted territory such as the right ventricular free wall. Myocyte staining was absent in 14 control subjects (9 males, 5 females, mean age 62.9 yr, range 16–87 yr) including 7 with malignant disease, 2 with intracranial pathology, 2 with chronic pulmonary disease, and 1 each with sepsis, ruptured aortic aneurysm, and post abdominal surgery. Two of the control subjects with no myocyte staining had coronary artery disease in the absence of myocardial infarction.

Induction of S100β in transgenic mice. Multiple copies of the human S100β gene under the control of its own promoter confers copy number-dependent high basal expression exclusively in glial cells of the central and peripheral nervous system in transgenic mice (20). Basal expression is not seen in cardiac myocytes (Fig. 2). After treatment with NE, the induction of the human S100β transgene in ventricular myocardium is shown at the mRNA and protein levels based on RNase protection (Fig. 2 *A*) and Western blotting analyses (Fig. 2 *B*), respectively. There was no induction of the endogenous mouse S100β protein in control CD1 mice treated with NE (Fig. 2 *B*).

NE increases systolic blood pressure and heart rate in CD1 and transgenic (TG) mice. In CD-1 and TG mice, similar systolic blood pressures (106±3 mmHg and 102±3 mmHg, respectively) and heart rates (286±9 bpm and 298±7 bpm, respectively) were observed at baseline. NE increased systolic blood pressure ~ 25% in both CD-1 and TG animals (127±2 mmHg and 124±2 mmHg, respectively, $P < 0.05$ relative to control, $n = 4$) and heart rate ~ 12% (341±4 bpm and 335±9 bpm, respectively, $P < 0.05$ relative to control, $n = 4$).

Absence of cardiac hypertrophy in response to NE in S100β transgenic mice. S100β TG animals had lower body weights than CD1 control animals (28.68±0.90 g versus 33.30±0.44 g, $P < 0.05$, $n = 8$). Fluid intake was unchanged in any group over two weeks of treatment with NE or vehicle, and NE produced no significant increase in body weight. Similarly, postmortem morphometry (Fig 3.) demonstrated that vehicle-treated TG mice had a slightly lower mean wall thickness compared to CD1 controls (0.95±0.021 mm versus 1.068±0.032 mm, $P < 0.05$) but equivalent left ventricular weight to body weight ratio (3.249±0.106 g/kg versus 3.115±0.063 g/kg). NE significantly increased left ventricular weight to body weight ratio by an average of 21% (Fig. 3 *A*), and wall thickness (Fig. 3 *B*) by 31% in CD1 compared with vehicle alone (both $P < 0.05$). Treatment of S100β transgenic mice with NE did not result in any statistically significant changes in left ventricular weight to body weight ratio or wall thickness in comparison with vehicle control (Figs. 3 and 4 *A*). Treatment with NE also increased right ventricular weight only in CD1 mice (data not shown).

Echocardiography demonstrated the development of concentric left ventricular hypertrophy in CD1 with significant increases in septal and posterior wall thickness in the absence of alterations in left ventricular diastolic diameter (Table I; Fig. 4 *B*). TG animals demonstrated no significant changes in septal or posterior wall thickness. The relatively smaller wall thicknesses demonstrated by echocardiography in vivo highlight

Table I. Echocardiographic Measures in Control and Treated Mice

	CD1		TG	
	Control	NE	Control	NE
IVSWT*	0.81±0.02	1.20±0.10*	0.75±0.03	0.77±0.02
PWT	0.75±0.03	1.10±0.02*	0.71±0.02	0.76±0.03
EDD	3.85±0.05	3.62±0.10	3.55±0.07	3.48±0.04
ESD	2.19±0.06	2.00±0.07	2.03±0.06	2.03±0.06
FS (%)	43.1±2.3	44.4±2.6	42.6±2.8	43.3±1.7

*IVSWT indicates interventricular septal wall thickness; PWT, posterior wall thickness; EDD and ESD, end-diastolic and end-systolic diameter, respectively, all in millimeters (means±SEM); FS, left ventricular percent fractional shortening; TG, S100β transgenic animals; NE, norepinephrine treatment. *$P < 0.05$ compared to vehicle control, $n = 4$ in all groups.

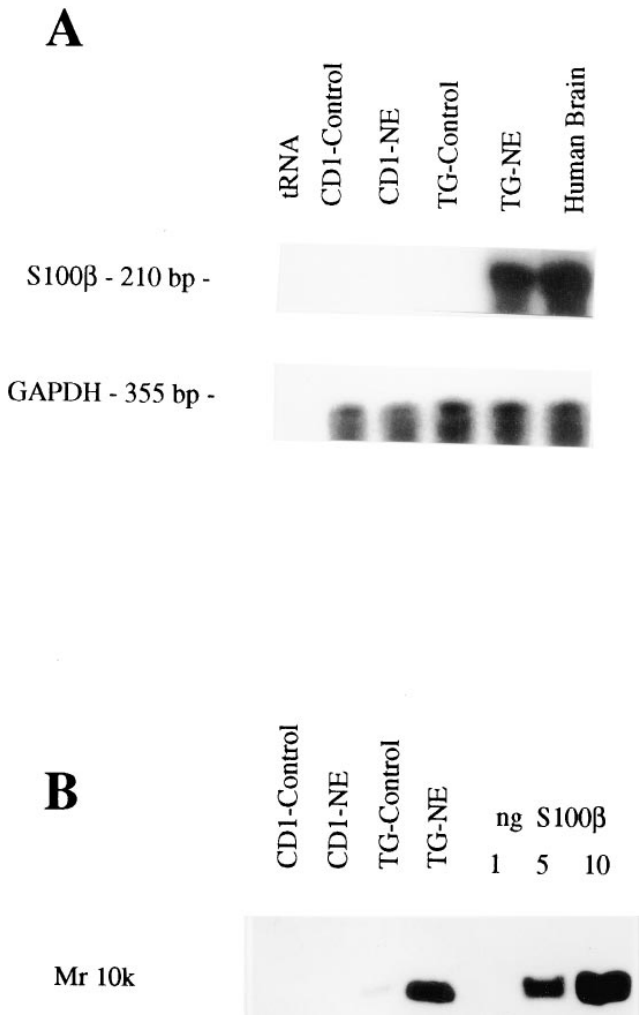

Figure 2. Analysis of the human S100β transgene expression in CD1 and transgenic (*TG*) mice. CD1 and TG mice were killed after 15 d of norepineprine (*NE*) (1.5 mg/kg, intraperitoneally, twice daily) or diluent (ascorbic acid saline) treatment. (*A*) Steady state levels of human S100β, and mouse GAPDH mRNAs were determined by RNase protection. Protected fragments specific for S100β (*210 bp*), and GAPDH (*355 bp*) mRNAs are indicated in composite figures depicting results of a representative experiment using RNA from NE or vehicle-treated mice hearts, human brain, and tRNA. (*B*) Western blotting was performed on heart extracts. The primary antibody used was mouse monoclonal anti-S100β protein antibody, followed by rabbit anti–mouse Ig antibody and ^{125}I-labeled protein A. The position of migration of S100β protein (M_r *10k*) is indicated in a representative blot. Purified bovine S100β protein is used as a quantitative control.

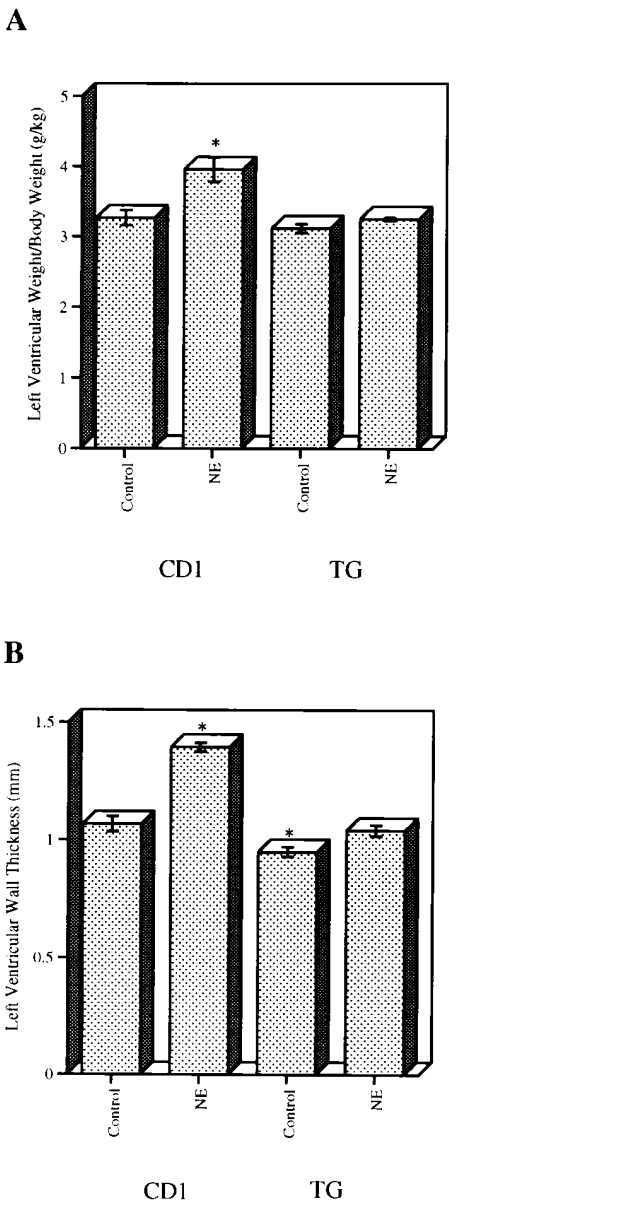

Figure 3. Norepinephrine (*NE*) induction of left ventricular hypertrophy is inhibited in S100β transgenic (*TG*) mice. 8-wk-old S100β transgenic (*TG*) and age-matched CD1 mice were treated with NE (1.5 mg/kg intraperitoneally) or diluent (ascorbic acid saline) twice daily for 15 d. Bars are the mean±SEM of left ventricular weight to body weight ratios (*g/kg*) (*A*) and left ventricular wall thickness (*mm*) (*B*). *$P < 0.05$ versus CD1 control, $n = 8$/group.

limitations in the assessment of wall thickness in even freshly isolated postmortem hearts. Echocardiography is also less subject to variations in sectioning and alterations in perfusion-fixed sections (Fig. 4). In addition this non-invasive in vivo assessment demonstrated comparable normal baseline systolic function in CD1 and TG animals as measured by left ventricular percent fractional shortening with preservation of function in both groups after NE treatment.

Absence of induction of fetal genes by NE in the heart of S100β transgenic mice. In agreement with previously published data (20, 31, 32), adult mouse myocardium demonstrated absent ANF expression, low basal skACT and βMHC expression, and high basal level αMHC expression. Treatment of control CD1 mice with NE resulted in an induction of ANF, an increase in skACT and βMHC, and a decrease in αMHC expression in myocardium (Fig. 5). These results are in agreement with the previously described program of fetal gene induction associated with experimental myocyte hypertrophy in response to α_1-adrenergic stimulation in both in vitro (6, 34, 35, 36) and in vivo (37) models. In contrast, there was no induction of skACT, βMHC, or ANF, and no downregulation of αMHC in the myocardium of S100β transgenic mice

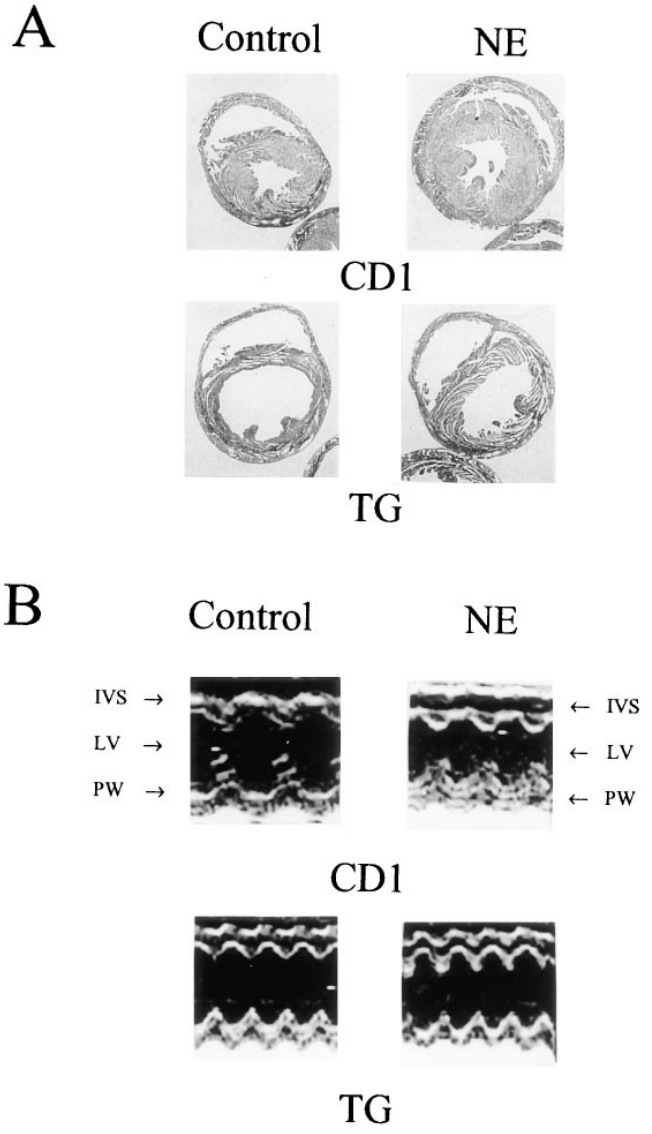

Figure 4. Lack of hypertrophy in S100β transgenic (*TG*) mice treated with norepinephrine (*NE*). (*A*) Photomicrograph depicting perfusion-fixed mid-level left and right ventricular transverse slices in CD1 and TG mice treated with vehicle (*Control*) or NE for 15 d; (*B*) M-mode echocardiography depicting representative midventricular images over a minimum of two cardiac cycles imaging the interventricular septum (*IVS*), left ventricular cavity (*LV*), and left ventricular posterior wall (*PW*). CD1 tracings were recorded at a speed of 100 mm/s and TG tracings at 50 mm/s at the same depth setting and scale.

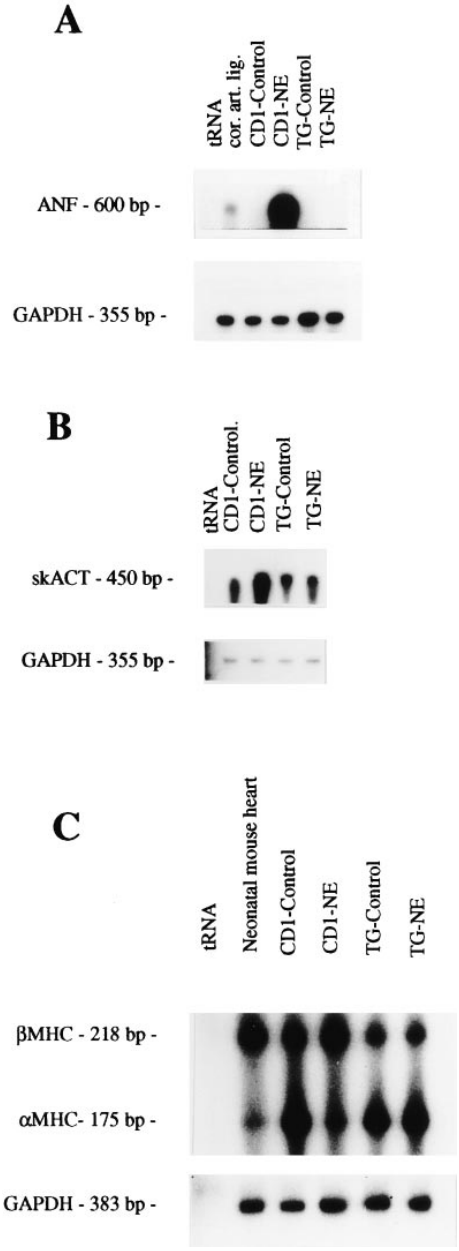

Figure 5. Norepinephrine (*NE*) induction of (*A*) atrial natriuretic factor (*ANF*), (*B*) skeletal α-actin (*skACT*), and (*C*) induction of βMHC and downregulation of αMHC mRNAs is inhibited in transgenic (TG) mice. CD1 and TG mice were killed after 15 d of NE (1.5 mg/kg, intraperitoneally, twice daily) or diluent treatment (*Control*). Steady state levels of mouse skACT, ANF, βMHC, αMHC, and GAPDH mRNAs were determined by RNase protection. Protected fragments specific for skACT (*450 bp*), ANF (*600 bp*), βMHC (*218 bp*), αMHC (*175 bp*) and GAPDH (*355* or *383 bp*) mRNAs are indicated in composite figures depicting results of a representative experiment using RNA from NE or vehicle-treated mice hearts, neonatal mouse hearts, and RNA prepared from a rat heart 35 d after coronary artery ligation (*cor. art. lig.*) or tRNA.

after treatment with NE (Fig. 5). The lack of repression of αMHC in TG animals treated with NE demonstrates that these animals do not exhibit a non-specific block to cardiac-specific transcription but rather an inhibition of a more complex hypertrophic phenotype.

Absence of the α_1-adrenergic hypertrophic response in myocyte primary cultures from S100β transgenic mice. To examine the direct action of α_1-adrenergic agonists on cardiac myocytes, primary myocyte cultures were established from hearts of neonatal control CD1 and S100β transgenic mice. In agreement with previously published data in rodent myocyte cultures (6, 36), treatment of control CD1 murine myocyte cultures with NE or PE induced hypertrophy manifested by a 1.3-fold increase in myocyte protein (Fig. 6). This is the first demonstration of α_1-induced murine myocyte hypertrophy. In contrast, these α_1-adrenergic agonists did not induce hypertro-

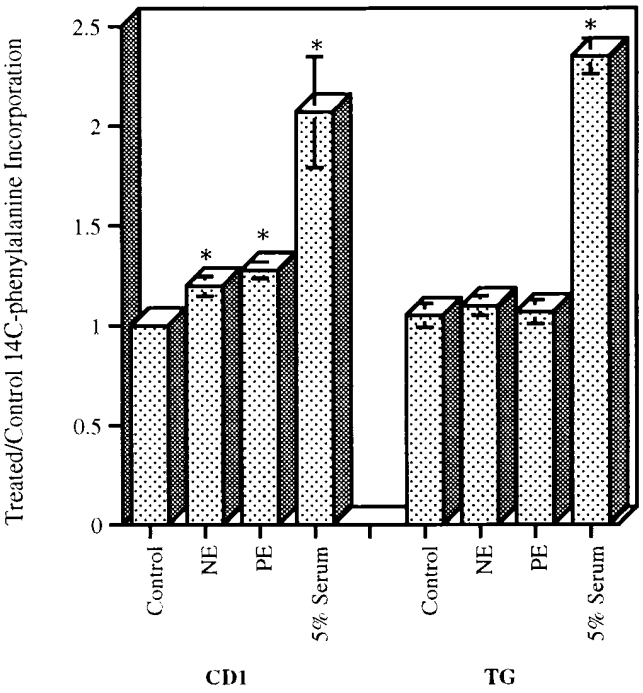

Figure 6. Inhibition of α_1-adrenergic myocyte growth in S100β transgenic–cultured neonatal mouse myocytes (*TG*). Cardiac myocytes from TG and CD1 animals were treated with either norepinephrine (*NE*) (20 μM), phenylephrine (*PE*) (20 μM), or 5% serum. Incorporation of [^{14}C]phenylalanine into newly synthesized protein after 72 h was determined. Values are the mean±SEM from five separate experiments normalized to protein incorporation in vehicle control. *$P < 0.05$ versus control.

phy in primary myocyte cultures derived from S100β transgenic mice (Fig. 6). This was not due to the incapacity of these cells to respond to alternate hypertrophic stimuli as primary myocyte cultures from both control CD1 and S100β transgenic mice responded to 5% FBS treatment with a 2.5-fold increase in protein content (Fig. 6). Analogous to the in vivo response (Fig. 2), cultured TG myocytes exhibit no basal transgene expression with induction of the human transgene within 24 h by α_1-adrenergic agonists, whereas wild-type myocytes exhibit no endogenous S100β expression at baseline or during the time frame of these experiments (data not shown).

Discussion

We have previously suggested that intrinsic negative modulators of the cardiac hypertrophic response tempered the action of trophic effectors that sustain this response in animal models of acute pressure overload and experimental myocardial infarction (15). Intriguingly, the first likely candidate for a negative modulator was identified as S100β, a Ca^{2+}-binding 10-kD protein, which is normally expressed as a dimer in brain astrocytes and Schwann cells of the peripheral nervous system (38). The corresponding cardiac-specific isoform S100α is expressed as a dimer in cardiac myocytes which normally do not express S100β (38). We reported (15) a delayed induction of S100β in surviving rat myocardium after experimental myocardial infarction resulting from coronary artery ligation. This induction of S100β coincided with the downregulation of skACT, one of the fetal genes re-expressed in the context of the hypertrophic response triggered by the myocardial infarction (15). An indication of a possible underlying biochemical pathway modulated by S100β was obtained experimentally in cultured neonatal rat cardiac myocytes. In this in vitro model system, the hypertrophic response can be reproduced by treatment with a number of effectors including α_1-adrenergic agonists. Using a co-transfection strategy, we demonstrated that human S100β inhibited the α_1-adrenergic induction of the skACT and βMHC promoters mediated by β-protein kinase C (15). This prototypical pathway is an integral part of the program of fetal gene re-expression associated with the hypertrophic response.

In the present communication, we have extended these observations on a possible participation of human S100β in a negative feedback regulatory pathway modulating the hypertrophic response. First, we showed that S100β expression was detected in human cardiac muscle fibers after myocardial infarction (Fig. 1). Expression was highest in surviving peri-infarction myocytes in the setting of fully healed infarction with less expression in acute infarction or in myocytes remote to the infarct. This is in agreement with our previous observation in a rat coronary artery ligation model in which S100β expression was not seen acutely but appeared at day 7 to 14 and peaked at 7 wk after infarction. Second, we used a transgenic mouse model in which human S100β is overexpressed in proportion to transgene dosage (20) to examine the involvement of S100β in cardiac hypertrophy induced by the α_1-adrenergic agonist NE.

As described previously in several mammalian species (21, 39, 40, 41), treatment with NE induced concentric hypertrophy in control CD1 mice, manifested by a 21% increase of left ventricular to body weight ratio (Fig. 3 *A*) and 31% increase in wall thickness (Fig. 3 *B* and Fig. 4, *A* and *B*). These increases are comparable with those seen in other experimental murine models of cardiac hypertrophy, including pressure overload in response to microsurgical banding of the aortic or pulmonary arteries (33, 34). The cardiac hypertrophy induced by NE in normal CD1 mice was accompanied by an induction of ANF, skACT, βMHC, and repression of αMHC (Fig. 5). These observations are in agreement with the previously reported program of fetal gene induction associated with the hypertrophic response in cardiac myocytes as a consequence of treatment with α_1-adrenergic agonists in other model systems (6, 34, 35, 36).

The induction of human S100β in the myocardium of S100β transgenic mice in response to NE treatment (Fig. 2, *A* and *B*) and the concomitant absence of the cardiac hypertrophic response in these mice (Figs. 3–5) satisfy the following two criteria required to implicate this protein as an intrinsic negative feedback modulator of this response. First, the action of an intrinsic negative feedback modulator must be somehow triggered by the same positive effectors that sustain the response. Second, increased levels of the negative modulator (as in the case of human S100β in the transgenic mice) should blunt the response in comparison with controls.

Induction of human S100β in transgenic mice by NE is also consistent with our previous suggestion that the observed induction of S100β in the myocardium of human subjects with chronic lung disease is mediated by elevated circulating levels of catecholamines (30). Our inability to detect the induction of the endogenous mouse S100β gene by NE may be due to a limit of detection which is overcome by multiple copies of the

transgene in the case of human S100β. Thus, the lack of induction of cardiac hypertrophy and the associated pattern of cardiac-specific gene expression by NE in S100β transgenic animals should be viewed in the context of concomitant inducible forced expression of S100β in myocardium. Transgenic animals, lacking basal S100β expression, exhibit a normal pattern of skACT, MHC, and ANF expression. Unlike the recently described Gαq-overexpressing mice (42), echocardiography demonstrates normal systolic ventricular function in these animals at baseline with no decompensation of function after trophic stimulation (Table I). Thus, blunting of increases in wall thickness in TG animals does not reflect the presence of myocardial failure or development of ventricular dilation.

The above results in transgenic mice suggest that the global blunting of cardiac hypertrophy in response to NE by elevated levels of human S100β is a reflection of the absence of the hypertrophic response in individual myocytes. To confirm this possibility, we established primary myocyte cultures from neonatal hearts of control CD1 and S100β transgenic mice. Primary cultures from control CD1 mice responded to α_1-adrenergic treatment with NE or PE with a 1.3-fold increase in myocyte protein (Fig. 6). However, a similar hypertrophic response was not seen in primary myocyte cultures derived from S100β transgenic mice (Fig. 6). The direct demonstration that these cells did not respond normally to a hypertrophic stimulus also extends our previous observations in cultured rat myocytes. Specifically, the use of the endogenous hypertrophic response as the endpoint in the present cultured model system complements the previously demonstrated inhibition by S100β of the induction of the skACT and βMHC promoters in cotransfection experiments, which was used as surrogate marker of the hypertrophic response (15).

Whereas we have previously provided experimental evidence that S100β inhibits the β-PKC signaling pathway in cardiac myocytes (15), the exact molecular mechanisms through which S100β modulates the hypertrophic response remain to be defined. In this respect both S100α and S100β have been independently implicated in the regulation of intracellular pathways in cardiac myocytes (15, 43). We suggest that the newly induced S100β is part of an intrinsic countervailing pathway that attenuates the hypertrophic response, which, in its chronic state, can lead to ventricular dysfunction and heart failure. This regulatory function of S100β could be exerted as a homodimer or a heterodimer with the endogenous S100α subunit. In fact, a significant reduction in S100α in left ventricular specimens of patients with heart failure has recently been reported (44). This raises the possibility that the inverse regulation of expression of these two proteins in cardiac disease could be part of a reciprocal compensatory mechanism.

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