Stanniocalcin-1 is a naturally occurring L-channel inhibitor in cardiomyocytes: relevance to human heart failure

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Submitted 10 December 2002; accepted in final form 19 March 2003


Stanniocalcin-1 is a naturally occurring L-channel inhibitor in cardiomyocytes: relevance to human heart failure. Am J Physiol Heart Circ Physiol 285: H442–H448, 2003. First published March 27, 2003; 10.1152/ajpheart.01071.2002.—Cardiomyocytes of the failing heart undergo profound phenotypic and structural changes that are accompanied by variations in the genetic program and profile of calcium homeostatic proteins. The underlying mechanisms for these changes remain unclear. Because the mammalian counterpart of the fish calcium-regulating hormone stanniocalcin-1 (STC1) is expressed in the heart, we reasoned that STC1 might play a role in the adaptive-maladaptive processes that lead to the heart failure phenotype. We examined the expression and localization of STC1 in cardiac tissue of patients with advanced heart failure before and after mechanical unloading using a left ventricular assist device (LVAD), and we compared the results with those of normal heart tissue. STC1 protein is markedly upregulated in cardiomyocytes and arterial walls of failing hearts pre-LVAD and is strikingly reduced after LVAD treatment. STC1 is diffusely expressed in cardiomyocytes, although nuclear predominance is apparent. Addition of recombinant STC1 to the medium of cultured rat cardiomyocytes slows their endogenous beating rate and diminishes the rise in intracellular calcium with each contraction. Furthermore, using whole cell patch-clamp studies in cultured rat cardiomyocytes, we find that addition of STC1 to the bath causes reversible inhibition of transmembrane calcium currents through L-channels. Our data suggest differential regulation of myocardial STC1 protein expression in heart failure. In addition, STC1 may regulate calcium currents in cardiomyocytes and may contribute to the alterations in calcium homeostasis of the failing heart.

METHODS

Patients. As a bridge to heart transplantation, some patients with idiopathic dilated cardiomyopathy and advanced heart failure were supported by mechanical unloading to reduce left ventricular workload and alleviate the symptoms of congestive heart failure (12). At the time of left ventricular assist device (LVAD) placement, a core of the left ventricular tissue (1–1.5 cm in width) was removed. At the time of transplantation, following an average LVAD support period (8), where elevation of serum calcium triggers the release of STC1 from the corpuscles of Stannius (23), organs associated with the kidneys (26). On circulation in the gill and intestine, STC1 inhibits calcium flux from the aquatic environment through these organs, thus maintaining normal calcium concentrations in the blood (15, 24). In mammals, STC1 is expressed in multiple organs, including the heart, skeletal muscle, brain, thyroid, spleen, thymus, parathyroid, lung, kidney, pancreas, small intestine, colon, placenta, ovary, testes, and prostate (4, 5, 22). The wide expression of STC1 suggested that it might function in an autocrine and/or paracrine manner, whereas its localization to the heart and skeletal muscle suggested a role in myocyte function.

Through the evolutionary process from fish to mammals, STC1 appears to have maintained its functional role in calcium regulation, because mammalian STC1 appears to be involved in calcium homeostasis in the normal physiology of the gut (16) and in the adaptive response of brain cells to ischemic injury (28). Because cardiomyocyte calcium homeostasis demonstrates a wide range of abnormalities in patients with heart failure (2, 9, 11, 13, 17, 21), we hypothesized that myocardial expression of STC1 may be relevant to calcium homeostasis in the failing heart. Our current data suggest differential expression of STC1 protein in cardiomyocytes and blood vessel walls of failing hearts and are consistent with a potential role for STC1 in cardiomyocyte calcium homeostasis.

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STANNIOCALCIN-1 (STC1) is a homodimeric glycoprotein hormone involved in calcium regulation in bony fish (8), where elevation of serum calcium triggers the release of STC1 from the corpuscles of Stannius (23), organs associated with the kidneys (26). On circulation in the gill and intestine, STC1 inhibits calcium flux from the aquatic environment through these organs, thus maintaining normal calcium concentrations in the blood (15, 24). In mammals, STC1 is expressed in multiple organs, including the heart, skeletal muscle, brain, thyroid, spleen, thymus, parathyroid, lung, kidney, pancreas, small intestine, colon, placenta, ovary, testes, and prostate (4, 5, 22). The wide expression of STC1 suggested that it might function in an autocrine and/or paracrine manner, whereas its localization to the heart and skeletal muscle suggested a role in myocyte function.

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of 2.3 mo, a second core was obtained from the diseased heart at a region close to the site of the original core (LVAD implant site). All patients had a left ventricular ejection fraction of <20%. Control left apical cardiac samples were obtained from accident victims. An institutional review committee approved studies with human subjects, and where applicable, informed consent was obtained.

**Materials.** All chemicals were purchased from Sigma Chemical (St. Louis, MO) except where stated. Fluorescent probes were purchased from Molecular Probes (Eugene, OR). STC1-specific rabbit polyclonal antibodies (that do not cross react with STC2) and recombinant hSTC1 protein were kindly provided by Dr. Henrik Olsen, Human Genome Sciences (Rockville, MD). Recombinant human STC1 protein was expressed in a baculovirus expression system and is >90% pure (6, 27).

**Immunohistochemistry staining.** Human left ventricular tissue samples were fixed in 2% paraformaldehyde followed by dehydration in graded alcohols and embedded in paraffin using standard techniques. Five-micrometer sections were cut, dried, and rehydrated for labeling with affinity-purified α-Ax (Boehringer Mannheim; Indianapolis, IN) sense and anti-sense canine STC1-specific probes (22) was carried out using MEGAscript kit (Ambion; Austin, TX). These probes are unique and share no homology with other known calcium channels, including transient receptor potential channels. Parafomaldehyde (4%)-fixed and paraffin-embedded normal human heart sections were rehydrated and washed for 10 min in PBS. Sections were then treated with 50 μg/ml proteinase K (Promega; Madison, WI) for 12 min in Tris-EDTA buffer (20 mM Tris·HCl, pH 8, 20 mM EDTA) and washed again in PBS for 10 min. Repeated fixation in 4% paraformaldehyde was carried out, followed by treatment with acetic anhydride (125 μl acetic anhydride added to 50 ml of 0.1 M triethanolamine·HCl (Fisher Scientific; Pittsburgh, PA), pH 8), for 10 min, and a 5-min wash in PBS. Sections were prehybridized for 4 h at 42°C in a prehybridization solution [50% formamide (Fluka; Milwaukee, WI), 5× SSC (0.75 M NaCl and 0.75 M trisodium citrate, pH 7), 1 mg/ml transfer RNA (Boehringer Mannheim; Indianapolis, IN), 100 μg/ml heparin, 1× Denhardt’s solution, 1 Tween-20 (Fisher), 1% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (Fisher), and 5 mM EDTA). Hybridization was carried out overnight at 42°C in prehybridization solution containing 1 μg/ml antisense or sense STC1 probe. Slides were washed sequentially in 0.2× and 0.1× SSC (carried out at 42°C for 15 and 30 min, respectively), followed by a 20-min wash in PBT [PBS containing 0.2% BSA and 0.1% Triton X-100] at room temperature. Slides were then incubated for 1 h in PBT containing 20% heat-inactivated sheep serum, followed by 2-h incubation with preadsorbed anti-digoxigenin antibody. The slides were then washed for 10 min in alkaline phosphatase buffer [50 mM MgCl2, 100 mM NaCl, 0.1% Tween-20, and 100 mM Tris, pH 9.5] followed by a 10-min wash in alkaline phosphatase buffer containing 0.5 mM levamisole. Signal was detected after a 48-h incubation in alkaline phosphatase buffer containing 0.5 mM levamisole, using nitroblue tetrazolium chloride and N’-2-hydroxyethylpiperaazine-N’-2-ethanesulfonic acid as substrates. Staining was stopped by a final wash in PBS.

**Real time PCR.** Left ventricular tissue (apex) was obtained from normal hearts (n = 6) and from patients with dilated cardiomyopathy (n = 6). STC1 mRNA level in the myocardium was measured using real-time quantitative RT-PCR as previously described (7). Results were normalized to the expression of the housekeeping gene, β-actin, in the same sample. RT-PCR primers used were forward: forward-primer 5’-CCCTGCACCACCCAGCAGC-3’ and reverse-primer 5’-GGCGATCCACAGGGATGAC-3’, corresponding to bases 956–971 and 1008–1128 of human actin mRNA, respectively (GenBank Accession no. BC014861). STC1 primers used were the following: forward-primer 5’-CAGCT-GCCCAATCACTTCTC-3’ and reverse-primer 5’TCTCCAT-CAGGCTGTCTCTGTA-3’, corresponding to bases 736–755 and 821–841 of human STC1 mRNA, respectively (GenBank Accession no. XM-011704). Stanniocalcin primers were unique to mammalian STC1 and shared no homology with STC2.

**Preparation of cultured rat cardiomyocytes.** Adult rat cardiomyocytes were harvested as previously described (14, 19). Briefly, after anesthesia, rats (average weight of 200 g) were given 3,000 IU of heparin by intravenous injection. The heart was exposed by a longitudinal thoracotomy incision, and the thymus and fascia were cleared from the aorta with a sterile swab. The aorta was cross-clamped and cut distally, and the heart was removed and placed in 50 ml of Joklik’s media (1 package of Joklik’s media powder (GIBCO-BRL), suspended in 50 ml water, and supplemented with 3.91 g taurine, 2.0 g NaHCO3, 0.391 g L-glutamine, and 0.282 g adenosine). The heart was rinsed and transferred to fresh ice-cold Joklik’s media. The aorta was cannulated and flushed with cold Joklik’s media by using a syringe, followed by perfusion with a perfusion pump at a rate of 12–15 ml/min, for 5 min. The heart was transferred to a Langendorff apparatus, flushed with warm Joklik’s media, and further digested by perfusion with Joklik’s media containing 0.1% collagenase and 0.1% trypsin for 45 min.

The ventricles were minced and placed in digestion buffer containing 0.1% collagenase (in Joklik’s media). Mincing heart tissue was incubated in a shaking water bath at 37°C for 30 min. The supernatant was transferred to a conical tube and centrifuged (for 3 min at 50 g). The resultant pellet was washed twice in 4% BSA solution and once in 2% BSA solution. The pellet was then suspended in 20 ml Joklik’s media (pH 7.2), containing 2% BSA, followed by slow addition of CaO2 to yield a final concentration of 1.25 mM. The cells were then pelleted (as above) and suspended by gentle pipetting in 4 ml of warm, serum-free DMEM medium.

The cell suspension (1–2 drops) was then layered onto sterile laminin-coated coverslips. After 30 min of incubation at 37°C in 5% CO2-95% O2 (to allow cell attachment), plating media (DMEM containing 10% fetal bovine serum,
3 μg/ml Ara-C (to inhibit fibroblast growth), 10 μg/ml insulin, and 5 U/ml each of penicillin and streptomycin were gently added. Cells were fed with fresh plating media every other day (19). The cells began beating after 5–7 days of incubation, and the experiments were carried out between days 7 and 14. Animal experiments and care were in compliance with National Institutes of Health and institutional guidelines.

Measurement of intracellular Ca\(^{2+}\) by fluorescence spectro-photometry. For calcium measurements, we have defined a calcium transient as a spike, or spikes, that increase in intensity to such an extent that the majority of the volume of a cardiomyocyte fluoresces at maximum intensity and is followed by cell contraction.

Measurements of fluorescence intensity of calcium fluoroprobe (Fluo3 at 3 μmol/l concentration) and sequential image recording of contractile events were made on a Wallac/Perkin-Elmer (Gaithersburg, MD) Concord system incorporating a SpectraMaster multiwavelength controller and temperature-controlled stage (Melville, NY). To detail at least one calcium transient, sequential images captured over a 2- to 5-s span were selected, and video recordings of these events were made for a number of minutes (average of 25,000 image acquisitions) using an Olympix AstroCam CCD4100 Fast Scan (12 bit; 768 × 576; 1,000 frames/s; 9-μm resolution; (3)). Cell contraction studies were performed by using a Wallac/Perkin-Elmer Concord system, employing Fluo3 (3 μmol/l final concentration) as a fluorescent molecular probe. Contractile-fluorescence data were captured with a Merlin High Performance Ratio Fluorescence Workstation, utilizing a SpectraMaster monochromator and Rainbow Multi-wavelength Filter Wheel (Olympus America; Melville, NY).

Whole cell patch-clamp studies. Calcium currents (\(I_{\text{Ca}}\)) were recorded from single cultured rat cardiomyocytes at room temperature (20–23°C), using an EPC-9 amplifier (HEKA, Lambrecht, Germany (10)). The recording chamber was perfused with Tyrode solution [in mM: 140 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 10 glucose, and 10 HEPES, pH 7.4] at a rate of 1.5–2.0 ml/min. The pipettes had resistances of 3–5 MΩ when filled with pipette solution [in mM: 130 cesium gluconate, 10 tetraethylammonium chloride, 10 HEPES, 10 EGTA, 3 MgATP, and 0.3 Na\(_3\)GTP]. The membrane capacitance and series resistance were electronically compensated to minimize the capacitive transient and to improve the dynamic response. Membrane currents were filtered at 3 kHz, recorded, and analyzed with PULSE & PULSEFIT software (HEKA).

Statistical analysis. The means ± SE were calculated using paired and unpaired t-test as appropriate. Results were considered statistically significant if \(P < 0.05\).

Fig. 1. Increased stanniocalcin-1 (STC1) protein labeling in cardiomyocytes of failed human heart. Top: paired left ventricular tissue samples [before and 2 mo after left ventricular assist device (LVAD)] from a representative patient with dilated cardiomyopathy (B and C) and from normal left ventricular tissue (A) were subjected to immunohistochemistry using anti-hSTC1 antibody. Arrowheads point to nuclei in cardiomyocytes. Arrows point to arterial walls. Negative control for staining utilizing normal rabbit IgG showed no labeling (not shown). All sections were processed identically. X400. Bottom: representative staining for STC1 in rat myocardium is shown. Negative control for staining performed with omission of the primary antibody (D). Staining with anti-hSTC1 antibody (E and F). Arrowheads in D and E point to nuclei in cardiomyocytes. Arrowhead in F points to an endothelial cell. Arrows in E point to interstitial cells (fibroblasts and possibly inflammatory cells). Arrow in F points to a smooth muscle cell in the arterial wall. All sections were processed identically. ×1,000 (D and E). ×1,200 (F). *Interstitial cells.
RESULTS

Stanniocalcin protein expression in human heart correlates with ventricular load. We examined myocardial tissue expression of stanniocalcin protein in patients with advanced heart failure before and after LVAD support. Paired left ventricular samples (pre- and post-LVAD) were compared with each other and to normal heart tissue, obtained from accident victims. Normal hearts showed little STC1 expression (Figs. 1 and 2), whereas myocardium from heart failure patients showed marked increase in the expression of STC1 in cardiomyocytes and an intense staining in the walls of arteries and veins (Fig. 1). Of interest, the expression of STC1 in cardiomyocytes and blood vessel walls was markedly attenuated following LVAD support (Figs. 1 and 2). In cardiomyocytes, staining was predominant in the nucleus and was paralleled by diffuse, albeit weaker, cytoplasmic labeling. In addition, cardiac STC1 expression was present in interstitial cells, likely representing fibroblasts and/or inflammatory cells. In control experiments, staining was carried out in the presence of excess exogenous STC1 protein and showed no labeling (data not shown). Examination of STC1 expression in the normal rat myocardium reveals a similar staining pattern; in addition to its presence in interstitial cells and cardiomyocytes (where it assumes a nuclear and cytoplasmic distribution), STC1 is strongly expressed in the endothelium and smooth muscle cells of the arterial wall. Our data suggest that myocardial STC1 is upregulated in heart failure and is normalized (downregulated) following mechanical unloading. In this respect, STC1 can now be added to a list of proteins that respond to mechanical unloading in patients with heart failure, such as tumor necrosis factor-α (25), atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) (1). The localization of STC1 to blood vessel walls and cardiomyocytes suggests a role for STC1 in vascular tone and cardiomyocyte function.

Expression of STC1 mRNA in myocardial tissue, including cardiomyocytes, arterial walls, and fibroblasts. Stanniocalcin expression is ubiquitous in mammals, and its mRNA has been detected in a large number of tissues, including heart, kidney, lung, and liver. In the heart, STC1 mRNA is expressed in cardiomyocytes, smooth muscle cells of arterial walls, and fibroblasts in surrounding connective tissue. Real-time quantitative RT-PCR was applied to measure STC1 mRNA level (normalized to β-actin mRNA in the same sample) in left ventricular tissue (apex) from 6 normal hearts and 6 dilated cardiomyopathy hearts. Differences were not statistically significant (NS).

Fig. 2. Increased staining for STC1 protein in cardiomyocytes and cardiac vessels in patients with dilated cardiomyopathy compared with controls. Stained area (for STC1) and density of staining were measured and analyzed using ImagePro Plus software. Data were generated from 5 separate patient sample pairs (pre- and post-LVAD) and 4 normal hearts. Average LVAD support duration was 2.3 mo. Error bars denote SE. Differences between normal and pre-LVAD as well as between pre-LVAD and post-LVAD were statistically significant (*P < 0.01).

Fig. 3. STC1 mRNA is expressed in cardiomyocytes and smooth muscle cells of arterial walls in heart tissue. Normal human heart tissue was subjected to in situ hybridization using sense (C and D) and antisense (A and B) biotin-labeled canine STC1 cDNA probe. Staining is detected with antisense probe only (A and B). STC1 mRNA is expressed in cardiomyocytes (arrows in A point to nuclei), smooth muscle cells (arrows in B) of the arterial walls (art) and fibroblasts (fib) in surrounding connective tissue (×300). E: real-time quantitative RT-PCR was applied to measure STC1 mRNA level (normalized to β-actin mRNA in the same sample) in left ventricular tissue (apex) from 6 normal hearts and 6 dilated cardiomyopathy hearts. Differences were not statistically significant (NS).
number of organs, including the heart (4, 5, 22). Using in situ hybridization, we determined the cellular elements that express STC1 in normal human heart tissue. STC1 mRNA is expressed in cardiomyocytes, smooth muscle cells of the arterial walls, and connective tissue fibroblasts (Fig. 3). To investigate the mechanism of cardiomyocyte STC1 protein upregulation in heart failure, we performed real-time quantitative RT-PCR on RNA obtained from left ventricular tissue samples of patients suffering from heart failure. Results were compared with those observed in the heart tissue of accident victims and showed no difference in STC1 mRNA levels (Fig. 3). Thus our data suggest that upregulation of STC1 protein in heart failure occurs at a posttranscriptional level.

STC1 slows endogenous beating rate of and diminishes intracellular Ca\textsuperscript{2+} transients in cultured cardiomyocytes in a manner that involves L-channel inhibition. Because of the technical difficulties and ethical issues that are associated with establishing cultured human cardiomyocytes, we performed the following experiments in cultured dedifferentiated adult rat cardiomyocytes, which have been used as a model for the study of heart failure (19). In the following set of experiments, we measured cytoplasmic calcium transients in cultured contractile rat cardiomyocytes, using fluorescence microscopy (Fluo3). Previous studies to determine the baseline spontaneous beating rate in these cells revealed no significant rate variations, with continuous tracings extending for up to 1 h (19). Initial dose-response experiments showed that STC1 at a concentration of 50 ng/ml or greater provides an equivalent decrease in the rate of contraction through the first 10 min. Thus the remaining experiments were carried out using 50 ng/ml STC1. Treatment with STC1 for 10 min produces a statistically significant reduction (75%) in the frequency of cardiomyocytes contraction compared with heat-denatured STC1-treated cells (Fig. 4A), which was associated with 50% attenuation in the peak calcium rise (amplitude of whole cell Fluo3 fluorescence, recorded with each tran-

**Fig. 4.** STC1 decreases the frequency and amplitude of cytoplasmic calcium rise (transients) in cultured rat cardiomyocytes. Cultured "dedifferentiated adult" rat cardiomyocytes were loaded with Fluo3 followed by incubation with STC1 (25, 50, or 125 ng/ml), and calcium transients were recorded. A: time-dependent decrease in the frequency of calcium transients following STC1 treatment. The x-axis shows time in minutes, whereas the y-axis represents number of contractions per minute. Data represent means ± SE of 6 independent determinations (*P < 0.001). B: peak calcium rise 10 min after addition of STC1 (50 ng/ml) or heat-denatured STC1 (50 ng/ml). The y-axis represents intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}), (measured as Fluo3 fluorescence ratio), relative to controls (heat-denatured STC1-treated cells). Data represent means ± SE of 6 independent determinations (+P < 0.01). Peak calcium values approximate 2,000 nM.

**Fig. 5.** Effects of STC1 on calcium current (I\textsubscript{Ca}) through L-type channels. Sodium and T-type I\textsubscript{Ca} were inactivated using 300-ms long preconditioning pulses at −40 mV, from a holding potential of −60 mV. L-type Ca\textsuperscript{2+} currents were then elicited by various depolarizing pulses (voltage increments of 10 mV, beginning at −30 mV and ending at +40 mV, with a pulse duration of 300 ms) at 0.1 Hz to obtain the current-voltage curve under control condition, 5 min after application of STC1 (100–500 ng/ml), and 5-min after STC1 washout. Application of nimodipine (5 \textmu M) completely blocked the I\textsubscript{Ca}. A: representative I\textsubscript{Ca} tracing; vertical and horizontal lines (below nimodipine tracing) represent scales in nanoamps and milliseconds, respectively. B: normalized currents (y-axis) are plotted against membrane potential (x-axis). Values represent means ± SE compared with control (n = 3–7, *P < 0.05, **P < 0.01).
sient; Fig. 4B). Some of the cells, however, showed partial rate recovery after 10 min but virtually all cells ceased to contract after 25 min, and fluorescence intensity (corresponding to intracellular Ca\(^{2+}\) concentration) approached the background.

To further define the mechanism of attenuation of calcium transients by STC1, we measured transmembrane calcium currents using the whole cell patch-clamp method. Addition of nimodipine to the bath solution blocks L-channel-mediated calcium currents in depolarized cardiomyocytes. Similarly, addition of STC1 for 5 min provides \(>50\%\) reduction in transmembrane calcium currents through the L-channels (Fig. 5). This inhibitory effect was significantly diminished after a washout period, suggesting that the inhibitory effect of STC1 on the channel is reversible. In conclusion, STC1 is a potent inhibitor of the L-type calcium channels in cardiomyocytes.

**DISCUSSION**

In the failing heart, cardiomyocytes increase in size and display myofibril disorganization and changes in the expression and function of calcium homeostatic proteins (2, 9, 11, 21). These phenotypic and structural changes are accompanied by metabolic alterations, which might further aggravate the structural and functional abnormalities of cardiomyocytes in the failing heart (20). Some of these changes might be adaptive at the protein level, whereas others may result from initiation and/or modification of the genetic program in these cardiomyocytes (20). However, the underlying mechanism for these abnormalities and the contribution of each to the final phenotype remain unclear.

STC1 is a calcium-regulating hormone in bony fish (8). It maintains calcium homeostasis by inhibiting calcium influx from the aquatic environment through the gill and intestine to the blood stream (15, 24). Similarly, the mammalian STC1 is involved in calcium homeostasis in the normal physiology of the gut (16) and in the adaptive response of brain cells to ischemic injury (28). Thus, through the evolutionary process from fish to mammals, STC1 appears to have maintained its functional role in calcium homeostasis.

Because STC1 is expressed in the heart where calcium flux across cellular membranes is essential for cardiac function, we reasoned that STC1 might have a role in calcium homeostasis in this organ, either in normal physiology, or in the adaptive/maladaptive processes that lead to the heart failure phenotype. Our current data reveal major alterations in the level of STC1 protein in the failing heart, where it is markedly elevated in cardiomyocytes and arterial walls. In contrast, STC1 levels are downregulated in these sites following ventricular unloading with LVAD. Because myocardial levels of STC1 mRNA are not altered in patients with cardiomyopathy, we reason that the increase in STC1 protein in the failing heart is regulated at a posttranscriptional level. Furthermore, our data suggest that STC1 may be added to the list of genes that respond to left ventricular workload and may resemble TNF-\(\alpha\), atrial natriuretic peptide, and brain natriuretic peptide in the manner in which it is regulated (1, 25).

The significance of STC1 upregulation in heart failure is not clear at present. However, our data provide some clues to its function. First, treatment of cultured contractile cardiomyocytes with recombinant STC1 reduces the rate of cell contraction and diminishes the rise in cytoplasmic calcium with each contraction. This effect appears to be mediated at least in part through inhibition of L-channels. Using whole cell patch-clamp studies, we demonstrate that addition of STC1 to the bath reduces transmembrane calcium currents in cardiomyocytes in a manner that is similar to treatment with nimodipine (an L-channel blocker). Inhibition of transmembrane calcium currents by STC1, however, was not complete at the concentrations employed and appeared to lag in time (see Fig. 4), suggesting the involvement of an intracellular signaling cascade in its effect. This possibility is supported by recent findings by McCudden et al. (18) that suggest the existence of STC1 receptors in mammalian liver and kidney. However, we cannot presently rule out the likelihood of a direct effect on the channel or of a combination of direct and indirect (signaling-mediated) regulatory inputs. Through its effects on L-channels, STC1 is expected to reduce myocardial rate and contractility. Thus we propose that upregulation of STC1 in the failing heart may be cardioprotective initially, because it would reduce ventricular workload. However, sustained up-regulation of STC1 in the failing heart may become maladaptive, because it could potentially reduce ejection fraction.

Finally, the expression of STC1 in myocardial tissue is not confined to one cell type. In addition to its expression in cardiomyocytes, STC1 is also present in interstitial fibroblasts, smooth muscle cells of the arterial wall, and endothelium; cells that use calcium for regulation of intracellular processes. Together, our findings indicate that STC1 may have a unique role in the adaptive/maladaptive phenotype of heart failure and may contribute to its pathogenesis. Additional studies are needed to further define this role and the suitability of STC1 as a therapeutic target in heart failure.

We are deeply grateful to Dr. Henrik Olsen (Human Genome Sciences, Rockville, MD) for providing rabbit anti-human STC1 antibodies and recombinant human STC1 protein and to Dr. Doug Mann for critical reading of the paper.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-42550 and funds from the Renal Section and DeBakey Heart Center, Baylor College of Medicine and the Methodist Hospital, Houston, Texas.

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