Activation of the Cardiac Proteasome During Pressure Overload Promotes Ventricular Hypertrophy

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Background—The adaptation of cardiac mass to hemodynamic overload requires an adaptation of protein turnover, ie, the balance between protein synthesis and degradation. We tested 2 hypotheses: (1) chronic left ventricular hypertrophy (LVH) activates the proteasome system of protein degradation, especially in the myocardium submitted to the highest wall stress, ie, the subendocardium, and (2) the proteasome system is required for the development of LVH.

Methods and Results—Gene and protein expression of proteasome subunits and proteasome activity were measured separately from left ventricular subendocardium and subepicardium, right ventricle, and peripheral tissues in a canine model of severe, chronic (2 years) LVH induced by aortic banding and then were compared with controls. Both gene and protein expressions of proteasome subunits were increased in LVH versus control (P<0.05), which was accompanied by a significant (P<0.05) increase in proteasome activity. Posttranslational modification of the proteasome was also detected by 2-dimensional gel electrophoresis. These changes were found specifically in left ventricular subendocardium but not in left ventricular subepicardium, right ventricle, or noncardiac tissues from the same animals. In a mouse model of chronic pressure overload, a 50% increase in heart mass and a 2-fold increase in proteasome activity (both P<0.05 versus sham) were induced. In that model, the proteasome inhibitor epoxomicin completely prevented LVH while blocking proteasome activation.

Conclusions—The increase in proteasome expression and activity found during chronic pressure overload in myocardium submitted to higher stress is also required for the establishment of LVH. (Circulation. 2006;114:1821-1828.)

Key Words: heart diseases ■ hypertrophy ■ physiology ■ pressure ■ stress ■ proteins

Left ventricular hypertrophy (LVH) is a key compensatory mechanism in response to pressure or volume overload that involves alterations in the regulation of signal transduction pathways, transcription factors, excitation-contraction coupling, contractile proteins, and energy metabolism. One key element of cardiac hypertrophy is an adaptation in protein turnover. Protein turnover refers to protein synthesis and degradation, and both mechanisms are activated by increased cardiac workload.1,2 Although multiple studies have addressed the activation of protein synthesis during the acute phase of LVH that follows aortic banding, the mechanisms controlling protein degradation in the hypertrophied myocardium, especially over the long term, remain largely unknown.

A key mechanism involved in protein degradation is the ubiquitin/proteasome system (UPS),3 which is known to be an important mechanism mediating muscle atrophy.4,5 Proteolytic substrates are ligated to multiple ubiquitin (Ub) moieties that are assembled into a chain that binds the proteasome with high affinity. The 26S proteasome contains multiple subunits in the regulatory (19S) particle that can bind multiubiquitinated (multi-Ub) proteins.6,7 The composition of the proteasome is highly dynamic8 and can be rapidly tailored to respond to environmental signals.

Although the role of the proteasome in the heart remains largely linked to decreased mass, its activation in the process of hypertrophy has also been the subject of speculation,7,9 because hypertrophy involves activation of protein synthesis and degradation.1,2,7,9 For example, the proteasome promotes the degradation of inhibitors of hypertrophy, such as the inducible cyclic adenosine monophosphate early repressor.10 Another potential role for the proteasome in cell growth would be to recycle the denatured proteins that result from an increased turnover of peptide synthesis.11 Accordingly, in the

Received November 1, 2005; de novo received May 2, 2006; revision received July 24, 2006; accepted July 27, 2006.

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Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.106.637827

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present study, we tested the hypothesis that the increased protein turnover during LVH involves an activation of the proteasome. In addition, we addressed whether this potential activation would begin in the myocardium submitted to the highest wall stress, ie, the subendocardium.12 The abnormal response to exercise of the hypertrophied subendocardium in LVH, ie, impaired coronary blood flow and contraction that occurs selectively in the subendocardium,13 suggests that this area is particularly vulnerable to stress signals and would be more susceptible to protein degradation. The results of the present study point to an increase in the activity of the proteasome during LVH, as well as elevated expression and novel modification of proteasome subunits. Significantly, in the large mammalian model studied, in which it was possible to discriminate transmural changes, these increases were confined to subendocardial myocardium and were not observed in unrelated tissues examined from the same animals. We then tested the hypothesis that activation of the proteasome was necessary for the development of LVH in response to pressure overload in a mouse model of thoracic aortic constriction in which the increase in proteasome activity was prevented by chronic application of a proteasome inhibitor. Our results demonstrate that increased protein degradation must coordinate with the increase in protein synthesis during cardiac overload to achieve an increase in heart mass.

**Methods**

**Canine Model of LVH**

Chronic pressure-overload LVH induced by thoracic aortic banding was performed as described previously.13,14 Eight mongrel puppies (Marshall Farms, North Rose, NY) of either sex at 8 to 10 weeks of age were anesthetized with 12.5 mg/kg sodium thiamylal and maintained with isoflurane (0.5 to 2.0 vol%). With a sterile surgical technique, a 1-cm-wide polytetrafluoroethylene cuff was placed around the aorta and tightened until a thrill was palpated over the aortic arch, and then the chest was closed. The polytetrafluoroethylene band created a fixed supravalvular aortic lesion that became more stenotic as the puppies grew. Eight normal dogs served as controls. At ≈2 years after banding, the animals were surgically instrumented to study left ventricular (LV) function in the conscious state with LV pressure transducers and aortic, LV, and left atrial catheters.13,14 Global LV function (LV pressure, dP/dt, and heart rate) was measured by implanting a solid-state miniature pressure gauge in the apex of the ventricle. Fractional shortening was determined by echocardiography. During euthanasia, myocardial samples were taken separately from the LV subendocardium, the LV subepicardium, and the right ventricle (RV) of the beating hearts and were immediately frozen in liquid nitrogen. Samples were also taken from the quadriceps and the kidneys.

**Murine Model of LVH**

Three-month-old male S6BJL mice (The Jackson Laboratory, Bar Harbor, Me) under anesthesia (65 mg/kg ketamine/2 mg/kg acepromazine/13 mg/kg xylazine) were submitted to lateral thoracotomy followed by transverse aortic constriction with a 28-gauge needle as described previously.15 Shams were treated similarly but without a ligature. Five days after surgery, the animals were anesthetized (2.5% tribromoethanol) for hemodynamic measurements and 2-dimensional echocardiography as described previously,16 followed by tissue harvest. Heart weight, LV weight, body weight, and tibial length were measured in all cases. Epoxomicin (Peptide International, Louisville, Ky) was diluted in saline/10% dimethyl sulfoxide and injected at a daily dose of 0.5 mg/kg intraperitoneally. Shams were injected with the vehicle only.

**Subtractive Hybridization**

The subtractive hybridization was performed as described previously17 with messenger RNA extracted from the myocardial samples from dogs in both control and LVH conditions. After hybridization, the subtracted products were further amplified by polymerase chain reaction (PCR), subcloned into the pGEM-Teasy vector (Promega, Madison, Wis) and transformed into SURE2 cells (Stratagene, La Jolla, Calif). The clones were sequenced by standard procedure (CEQ 8000, Beckman Coulter, Fullerton, Calif). Sequences were queried in a public database to determine the identity of the genes.

**Quantitative Reverse Transcription–PCR**

Quantitative reverse transcription–PCR (7700 Prism, PerkinElmer/ Applied Biosystems Division, Foster City, Calif) was performed with specific primers and fluorogenic probes (derivated with 5′-FAM and 3′-TAMRA) designed from the sequences obtained in the subtractive hybridization. For each measurement, the mRNA of interest was reverse-transcribed and subsequently used for quantitative 2-step PCR. Internal standards were prepared for each transcript from its PCR-amplified cDNA after ligation of the T7 promoter (Ambion, Austin, Tex). Because of variation in sample-to-sample loading, PCR data are normalized per number of 36B4 transcripts measured as housekeeping gene in each sample. Expression of 36B4 transcript was 1.4±0.4×10^4 and 1.8±0.2×10^4 copies per nanogram of total RNA in control and LVH samples, respectively (P=NS).

**Protein Extracts and Immunoblotting**

Tissue samples were homogenized in 50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 5 mmol/L EDTA, and protease inhibitors. Cell debris was removed by centrifugation for 2 minutes at 12 000g, and protein concentration was determined with the Bradford reagent (Bio-Rad Laboratories, Hercules, Calif). Extracts were normalized to equal protein amounts and separated by SDS-PAGE. The resolved proteins were immunoblotted with appropriate antibodies. Proteasome-specific antibodies were from BIOMOL International, LP (Plymouth Meeting, Pa; Rpt1, Rpn2) and Boston Biochem Inc (Cambridge, Mass; α-4, α-6, and α-7). All antibodies were used as recommended by the manufacturer. After incubation with the secondary antibody and washing, the signal was detected with enhanced chemiluminescence (PerkinElmer Life Sciences, Boston, Mass) and quantified by densitometry.

**Proteasome Assay**

Myocardium and other canine tissues were homogenized as described above. The chymotryptsin-like and trypsin-like activities of proteasomes were determined with 15 μg of total protein extracts and the fluorogenic substrates Suc-LLVY-AMC and BOC-LLR-AMC (Boston Biochem), with a Turner TD-700 fluorometer (Turner Designs, Inc, Sunnyvale, Calif). All measurements were performed in duplicate and were further replicated in independent experiments.

**Two-Dimensional Gel Electrophoresis**

LV tissues from control and LVH dogs were homogenized in 50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 5 mmol/L EDTA, and protease inhibitor cocktail (Sigma, St. Louis, Mo). Cell debris was removed by centrifugation at 12 000g for 30 minutes at 4°C. Equal amounts of protein (50 μg) were separated in the first dimension by isoelectric point (isoelectric focusing) and in the second dimension by molecular weight (sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS-PAGE]). Isoelectric focusing was performed with immobilized pH gradient strips (pH range of 5 to 8) on a Bio-Rad isoelectric focusing cell with a programmed voltage gradient. SDS-PAGE was performed in 12.5% polyacrylamide gels, and the separated proteins were transferred to nitrocellulose and incubated overnight at 4°C with mouse monoclonal antibodies against 203 proteasome subunits α-6 and α-7 (Biomol Research Laboratories, Inc, Plymouth Meeting, Pa). Immunoreactive spots were detected with enhanced chemiluminescence (PerkinElmer Life and Analytical Sciences, Wellesley, Mass).
Myocyte Cross-Sectional Area

Myocyte cross-sectional area was determined on digitized images of rhodamine–labeled wheat germ agglutinin–stained sections of paraffin-embedded samples. The myocyte outlines were traced and the cell areas measured with Image-Pro Plus (Media Cybernetics, Inc, Silver Spring, Md). In cross sections of LV, the measurable cross sections of the myocytes are found in the endocardial one third and in epicardial one third of myocardium. At least 50 myocytes were routinely measured in each region.

Statistical Analysis

Results are mean±SEM for the number of samples indicated in the figure legends and were compared with the Student t test. ANOVA with post hoc Bonferroni correction was used when necessary. A probability value <0.05 was considered significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Characteristics of the Canine Model

Physiological parameters were measured 1 to 2 years after application of aortic banding that resulted in LVH and compared with controls. The results were similar to those reported previously in the same model. The LV weight/body weight ratio was significantly increased in LVH (10.6±0.2) compared with control (5.8±0.4, P<0.05). LV end-diastolic pressure was 5.0±0.2 versus 5.1±0.3 mm Hg and LV dP/dt was 2900±125 versus 3860±350 mm Hg/s in control and LVH hearts, respectively. Therefore, the LVH hearts were studied during a period of functional compensation.

Genomic Analysis of UPS in LVH

The genomic profile of the hypertrophied canine heart was analyzed by subtractive hybridization. A number of genes involved in the UPS were found in the library of genes potentially upregulated in the LVH group compared with shams, and the true regulation of these genes was confirmed by quantitative reverse transcription–PCR (Figure 1). Genes encoding polyubiquitin and the 26S proteasome subunit p40 were detected at significantly higher levels during stable LVH than in sham hearts (P<0.05), whereas the Ub mRNA showed a nonsignificant trend toward an increase in regulation. Subtractive hybridization also revealed increased expression of genes encoding Ub-processing enzymes (Usp4, Usp14, Usp24, and Usp28), which are required to detach Ub from proteolytic substrates that are targeted to the proteasome, and therefore for restoration of the Ub pool.

Increased Proteasome Activity in Hypertrophied Myocardium

The increased expression of mRNA encoding proteasome components led us to examine proteasome function. Chymotryptic and tryptic activities of the proteasome were measured by monitoring fluorescence after hydrolysis of a test substrate in extracts prepared from a variety of tissues derived from normal and LVH animals. Significantly elevated proteasome activity was detected in subendocardium but not subepicardium derived from LVH hearts compared with control when both the chymotryptic (Figure 2A) and tryptic activities (Figure 2B) were measured. Furthermore, the addition of epoxomicin, an inhibitor of chymotrypsin activity, dramatically inhibited hydrolysis of the test substrate in the chymotryptic assay (Figure 2A), which confirmed the specificity of the measurement. In contrast, proteasome activity was unaffected in RV and in other tissues from the same animals, such as the quadriceps muscle or the kidney (Figure 2C). These results show that a cardiac-specific activation of the proteasome during LVH is limited to the subendocardium, which represents the part of the hypertrophied muscle where wall stress is the highest.

Increased Abundance of Proteasome Subunits in Hypertrophied Myocardium

To further elucidate the mechanisms of activation of proteasome function, we measured the levels of several proteasome subunits. Rpn2 and Rpt1 reside in the 19S regulatory particle, which is required for recognition of multi-Ub substrates. Rpt1 is an adenosine triphosphatase that promotes substrate unfolding, whereas Rpn2 promotes transfer of the target protein into the catalytic 20S particle. To monitor levels of the 20S catalytic particle, we also examined the levels of its α6 subunit. Equal amounts of protein extract prepared from control and LVH animals were resolved by SDS-PAGE, transferred to nitrocellulose, and incubated with specific antibodies. In agreement with the elevated proteasome activity in endocardial tissue in LVH, we observed an increased
expression of all 3 proteasome subunits in LVH samples that was significantly different from controls (Figure 3A).

To test whether activation of the proteasome might reflect a general stress-response mechanism, we measured the expression of proteasome subunits in unrelated tissues. Total protein extracts were prepared from quadriceps muscle and resolved by SDS-PAGE. Immunoblotting showed that the levels of Rpn2, Rpt1, and \( \alpha_6 \) were unaffected in this tissue (Figure 3B). Immunoblotting of a protein extract of kidney also did not show any significant change (not shown). These findings suggest that activation of the UPS during hypertrophy is largely confined to the myocardium and could represent a localized response to accumulation of damaged proteins.

**Two-Dimensional Electrophoresis of Proteasome Subunits**

Phosphorylation of certain \( \alpha \)-subunits can increase proteasome function.\(^{22,23} \) To investigate the underlying mechanism of proteasome activation in LVH, we examined protein extracts that were prepared from control and LVH subendocardium and separated by high-resolution 2-dimensional gel electrophoresis. In preliminary studies, proteins were characterized with antibodies that simultaneously recognized 6 of the 7 \( \alpha \)-subunits in the proteasome (\( \alpha_1, \alpha_2, \alpha_3, \alpha_5, \alpha_6, \) and \( \alpha_7 \)). These exploratory studies showed that the subunit \( \alpha_7 \) displayed altered mobility (data not shown). On the basis of these studies, we characterized subendocardial tissues from a set of 5 control and 5 LVH animals. Nitrocellulose filters were reacted with antibodies against the subunits \( \alpha_4, \alpha_6, \) or \( \alpha_7 \) (Figure 4). The 2-dimensional profile of \( \alpha_4- \) and \( \alpha_6- \) specific spots was similar in control and LVH specimens (Figure 4A and 4B). However, a marked alteration in migration of the \( \alpha_7 \) species was evident in all 5 LVH samples (Figure 4C), indicative of hypophosphorylation. These findings are in agreement with earlier findings, which showed that the \( \alpha_7 \) subunit can be phosphorylated.\(^{24} \) As expected, treatment of protein extracts with lambda phosphatase, before 2-dimensional electrophoresis, increased the isoelectric point of the protein spots (data not shown), which demonstrates that a decreased phosphorylation of \( \alpha_7 \) is associated with chronic LVH.

**Proteasome Inhibition Prevents Pressure Overload–Induced LVH in Mice**

We tested next whether proteasome activation is required for LVH during pressure overload. Mice were submitted to transverse aortic banding for 5 days. Starting 1 day before
surgery, the mice were treated with the proteasome inhibitor epoxomicin (0.5 mg/kg per day) or with vehicle and compared with sham-operated animals. The LV/aortic pressure gradient was similar in aortic-banded mice treated with epoxomicin (63±6 mm Hg) or with vehicle (70±3 mm Hg). Compared with shams, vehicle-treated banded mice showed a 50% increase in LV/body weight and LV/tibial length ratios (P<0.05 versus shams), which indicates significant LVH after banding (Figure 5A). In banded mice treated with epoxomicin, the LV/tibial length ratio was not significantly different from sham animals but was significantly lower (P<0.05) than the ratio in vehicle-treated banded mice (Figure 5A). The increase in heart mass in vehicle-treated banded mice was accompanied by a 2-fold increase in proteasome activity (Figure 5B), which was abolished by epoxomicin treatment (Figure 5B). The increase in proteasome activity found in the vehicle-treated group 5 days after pressure overload was maintained to a comparable level in mice killed 2 or 3 weeks after banding (data not shown).

To verify that the differences in heart mass between vehicle-treated and epoxomicin-treated animals reflect an adaptation in cardiac cells, myocyte cross-sectional area was measured in animals from each group (Figure 5C). In sham animals, the cross-sectional area was comparable between the vehicle group (283±5 μm²) and the epoxomicin-treated mice (279±5 μm²). After banding, the cross-sectional area significantly increased (P<0.05) in vehicle-treated mice (372±5 μm²), whereas it remained unaffected with epoxomicin treatment (244±4 μm²). Altogether, these results indicate that proteasome activity increases acutely during pressure overload and that blocking this activation prevents LVH.

Cardiac function was measured in the different groups by echocardiography and hemodynamics (Table). The difference in hypertrophic response on banding between vehicle-treated

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Figure 3. Increased expression of proteasome subunits in heart during LVH. A, Protein extracts were separated in an SDS-polyacrylamide gel, transferred to nitrocellulose, and incubated with antibodies against the 19S proteasome subunits Rpn2 and Rpt1, as well as the 20S subunit α6. Band intensities were quantified by densitometry, and results are shown for each protein in subendocardium (Sub-Endo) from both control (CTRL) and LVH hearts. n=8 per group. B, Tissue extracts prepared from quadriceps were examined by immunoblotting with the same antibodies. Glucose-6-phosphate dehydrogenase (G6PDH) was used as a loading control. n=5/group. *P<0.05 vs control.
and epoxomicin-treated mice was confirmed by the difference in wall thickness (Table). Despite the fact that banded mice treated with the proteasome inhibitor did not develop hypertrophy, cardiac function was remarkably similar compared with the vehicle-treated banded mice (Table), which indicates that the major compensatory mechanism for chronic pressure overload, LVH, is not required to preserve cardiac function, at least during the first week of pressure overload.

**Discussion**

Although the role of the UPS in the heart remains largely studied in conditions of atrophy, an emerging literature in other systems demonstrates an activation of the UPS in actively growing cells compared with quiescent cells. Higher levels of proteasome activity promote the degradation of tumor suppressors and eliminate the denatured proteins that result from an increased turnover during protein synthesis. Remarkably, as much as 50% of nascent proteins are cotranslationally degraded, and the levels of translationally damaged proteins increases on stimulation of translation.

In the present study, we demonstrate through several independent methods that the UPS is activated in the hypertrophied heart. First, subtractive hybridization revealed increased expression of multiple components of the proteasome pathway in LVH. Second, we subjected normal and hypertrophied myocardium to biochemical analysis and detected increased proteasome-specific activity. Third, we compared subendocardial, subepicardial, and noncardiac (quadriceps and kidney) tissue and observed increased abundance of multiple subunits of the proteasome only in the subendocardium derived from LVH, not in noncardiac tissue. These findings suggest that increased proteasome abundance in compensated LVH might be necessary to successfully promote rapid elimination of damaged proteins. The increased expression of proteasome subunits in LVH could reflect a compensatory mechanism in response to reduced phosphorylation, to maintain adequate levels of activity. Previous studies also showed that hyperphosphorylation increases proteasome stability and increases activity.

The role of the UPS in the control of muscle mass has been studied most extensively in skeletal muscle, where it has been shown to play an important role in mediating proteolysis
during muscle atrophy as a response to denervation, fasting, metabolic disease, or during cachexia induced by cancer or heart failure. Regulation of proteasome activity in the heart has been demonstrated in a context of idiopathic cardiomyopathy, in myocardial hibernation, with aging, and in the regression of cardiac mass after unloading. Recently, another study demonstrated an increased transcript expression of Ub and Ub-conjugating enzymes in a rat model of hypertrophy, in agreement with the present results. Although cardiac hypertrophy has been known for some time to activate both protein synthesis and protein degradation, no previous studies demonstrated either an increase in proteasome activity or, more importantly, a mechanism for this increased proteasome activity. In the present investigation, aortic banding for 5 days in mice increased LVH and proteasome activity by 50%, whereas this LVH was not observed in mice with a similar level of pressure overload, as assessed by direct measurement of LV/aortic pressure gradients, when treated with the proteasome inhibitor epoxomicin. The unexpected results from these experiments indicate that activation of the UPS may be required for the development of LVH in response to pressure overload. Interestingly, decreased activity of the proteasome was observed during the transition into heart failure after pressure overload in the mouse.

In summary, we show that the establishment of stable, compensated hypertrophy is accompanied by significantly increased activity of the proteasome, especially in the myocardium submitted to the highest wall stress. We also show in a mouse model that such activation is required for the development of cardiac hypertrophy. These findings are counterintuitive to the belief that proteasome activation is involved solely in mechanisms of muscle loss, such as cardiac atrophy or skeletal muscle wasting. Rather, we show that protein degradation pathways must also be activated to promote an increase in cardiac muscle mass. The present results also illustrate that preventing LVH by proteasome inhibition does not affect cardiac function at the early stage of pressure overload. Therefore, the manipulation of proteasome activity in the heart may represent a novel therapeutic pathway to control cardiac hypertrophy.

### LV Function in Aortic-Banded Mice Treated With Epoxomicin or Vehicle and Compared With Respective Shams

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<tr>
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<td>Vehicle (n=5)</td>
<td>Epoxomicin (n=4)</td>
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<tr>
<td>LV ejection fraction, %</td>
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<td>LV fractional shortening, %</td>
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<td>Heart rate, bpm</td>
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<td>LV septal wall thickness, mm</td>
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<td>LV posterior wall thickness, mm</td>
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<td>LV systolic pressure, mm Hg</td>
<td>145±4*</td>
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*P<0.05 vs corresponding sham.
†P<0.05 vs banded mice with vehicle.

### Acknowledgments

We thank Dr David Lagunoff for morphometric measurements.

### Sources of Funding

This work was supported by National Institutes of Health grants HL59139, HL69020, AG23137, AG14121, HL65183, HL65182, AG028854, HL069752, and HL33107 (Dr Stephen and Dorothy Vatner), HL067724 and AG023039 (Dr Sadoshima), and CA83875 (Dr Madura).

### Disclosures

None.

### References

Left ventricular hypertrophy, a key compensatory mechanism in response to pressure or volume overload, involves protein turnover, ie, not only protein synthesis but also degradation. Although multiple studies addressed the activation of protein synthesis during hypertrophy, the mechanisms controlling protein degradation in the hypertrophied myocardium remain largely unknown. A key mechanism involved in protein degradation is the ubiquitin/proteasome pathway. In this study, we show that the establishment of stable, compensated hypertrophy is accompanied by significantly increased activity of the proteasome, especially in the myocardium submitted to the highest wall stress. We also show in a mouse model that such activation is required for the development of cardiac hypertrophy. These findings are counterintuitive to the belief that proteasome activation is involved solely in mechanisms of muscle loss, such as cardiac atrophy. In contrast, we show that protein degradation pathways must also be activated to promote an increase in cardiac muscle mass. Our results also illustrate that the prevention of hypertrophy by proteasome inhibition does not affect cardiac function at the early stage of hypertrophy. Therefore, the manipulation of proteasome activity in the heart may represent a novel therapeutic pathway to control cardiac hypertrophy in patients with cardiac overload.

**CLINICAL PERSPECTIVE**

Left ventricular hypertrophy, a key compensatory mechanism in response to pressure or volume overload, involves protein turnover, ie, not only protein synthesis but also degradation. Although multiple studies addressed the activation of protein synthesis during hypertrophy, the mechanisms controlling protein degradation in the hypertrophied myocardium remain largely unknown. A key mechanism involved in protein degradation is the ubiquitin/proteasome pathway. In this study, we show that the establishment of stable, compensated hypertrophy is accompanied by significantly increased activity of the proteasome, especially in the myocardium submitted to the highest wall stress. We also show in a mouse model that such activation is required for the development of cardiac hypertrophy. These findings are counterintuitive to the belief that proteasome activation is involved solely in mechanisms of muscle loss, such as cardiac atrophy. In contrast, we show that protein degradation pathways must also be activated to promote an increase in cardiac muscle mass. Our results also illustrate that the prevention of hypertrophy by proteasome inhibition does not affect cardiac function at the early stage of pressure overload. Therefore, the manipulation of proteasome activity in the heart may represent a novel therapeutic pathway to control cardiac hypertrophy in patients with cardiac overload.
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_Circulation_. 2006;114:1821-1828; originally published online October 16, 2006; doi: 10.1161/CIRCULATIONAHA.106.637827

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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