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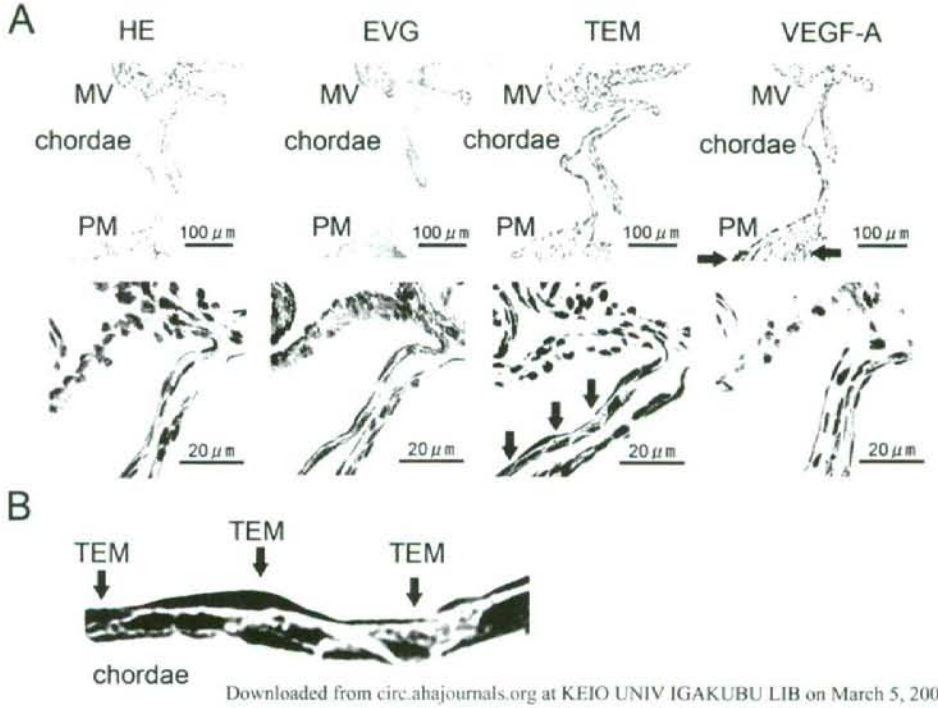
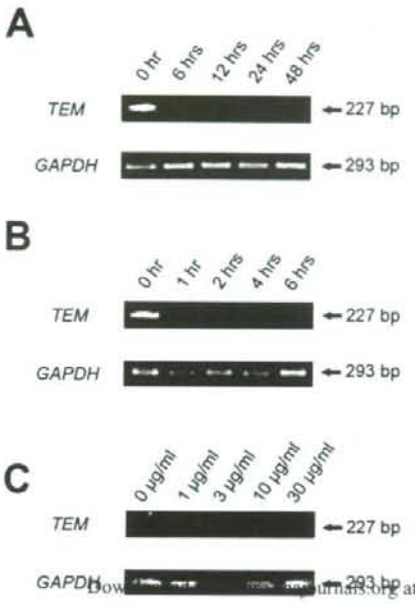
CLINICAL PERSPECTIVE

Valvular heart disease is a life-threatening disease. Although the overall incidence of rheumatic valvular heart disease has been decreasing continuously in developed countries, it has increased with respect to patient age. Rupture of the chordae tendineae cordis (CTC) is a well-known cause of mitral regurgitation, although its etiology remains unknown and surgical procedures have focused exclusively on treatment. Cardiac valves and the CTC are avascular tissues, and we have recently reported that cardiac valves express chondromodulin-I, which is an angioinhibitory factor purified from cartilage that plays a pivotal role in the maintenance of normal valvular function by preventing angiogenesis. In the present study, we show that tenomodulin, which is a chondromodulin-I–related antiangiogenic factor isolated from tendons, is concentrically expressed in normal CTC. Conditioned medium from cultured CTC interstitial cells showed a strong angioinhibitory effect, and the immunohistochemical analysis of human surgical samples showed that tenomodulin was locally absent in the ruptured areas of the CTC, in which abnormal vessel formation, strong expression of vascular endothelial growth factor-A and matrix metalloproteinases, and infiltration of inflammatory cells were observed, whereas these features were not observed in the normal or nonruptured areas. The tenomodulin layers of the tricuspid CTC of dogs were surgically filed, the animals were euthanized after several months, and immunohistological analyses were performed. Angiogenesis and the expression of vascular endothelial growth factor-A and matrix metalloproteinases in the core layer were observed in a time-dependent manner. The present findings support tenomodulin and unknown proteins of similar function as therapeutic agents for the prevention of CTC rupture. Understanding these mechanisms should form the basis for new therapeutic regimens for the treatment of valvular heart disease.

Supplementary Fig. 1



Supplementary Fig. 2



特集

心筋症の最前線

識る *J-d*

特定心筋症update

たこつぼ型心筋障害

Takotsubo (ampulla) cardiomyopathy

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キーワード

ampulla cardiomyopathy，apical ballooning，neuromediated myocardial stunning

本症例がわが国で先駆けて発見されたのは，日本の今までの医療事情（国民皆保険制度下における低検査料・低手術料，冠動脈介入治療の進歩と普及，検査・施術年齢の引き上げの結果，急性冠症候群の多くが早期に冠動脈造影・左室造影検査を受けている事実）がもたらした幸運と想定される。

本病態の左室壁運動異常は短期間で正常化するため，超急性期の観察がなければ，左室機能が短期間に回復した正常冠動脈症例または気絶心筋の典型的臨床例として今まで誤認され，報告されてきていた。本病態が日本人以外にも起こりうると欧米で理解されたのは，2003年のDesmetらの報告[1]以降である。病態は洋の東西を問わずほぼ同一である。

発症機序は，当初日本では冠動脈多枝攣縮によるのがその原因と主張され，その後も冠循環から本疾患をとらえる傾向が主流をなしたが，Tsuchihashiらの論文[2]で本症に接した欧米の研究者は，気絶心筋説を乗り越えた次元から本病態を観察しており，根源的な機序に迫る研究が多い。

名称は，原著者が心尖部バルーン状左室造影右前斜位収縮期像を「ツボ型」と形容したことによるが，Parodi[3]は左心室形態を示す心尖部バルーニングより，より成因論的なneuromediated myocardial stunningが適切であると主張した。

しかし，myocardial stunningを広義に解釈しており[4]，先行する精神肉体的ストレスが確認されない例は半数以上存在し，すべてのたこつぼ症例が神経関連性であるとの証拠はない。

頻度

Desmet[5]およびDec[6]は1%の数字をあげているが，根拠が明らかでない。

1342-6591/08/¥400/論文/JCLS

Bybeeら[7]はST上昇型急性冠症候群の2.3%以下としている。特発性心筋症研究班の多施設全国アンケート調査(2000年8月)で，実数提示施設に限定した有病率は，急性心筋梗塞疑い・緊急冠動脈造影症例の平均2.3%[0.3～6.25%：粗平均1.4%(96/6,774例)]であった。通常，緊急冠動脈造影施行患者は男性が圧倒的に多いので，緊急冠動脈造影施行女性患者に限定すれば，本病態の頻度は2.3%の数倍に当たると推定され，実数を提示した施設の報告は8%を超える[8]。

病態

当初，急性冠症候群に類似する症状(胸痛)で発症すると理解されていたが，急性発症の呼吸困難や無症状発症例も少なくない[9]。逆たこつぼ現象を含めて，心尖部以外にさまざまな部位に収縮異常をみる。

多くの症例で，情動ストレスが誘因であることは，米国でも同様[10]である。Giannniら[11]は精神的ストレスが26.8%，肉体的なストレスが37.8%との数字をあげている。わが国の症例では女性では精神的ストレス，男性では肉体的なストレス優位である[12]。しかし，自覚的にはなんのストレスをも訴えない症例もある。

可逆的流出路狭窄例は左室のみならず右室にもみられ，同一施設連続72例中12例を数える。頻度は明らかではないが，sigmoid septumをもち，左室容量が小さい(主に女性)人が強力な交感神経刺激や脱水に曝された場合に左室流出路障害が生じる可能性が想定されている。

病理

心筋組織障害が生じていることは心筋逸脱酵素値の上昇より推定されるが，心筋生検を施行した報告は多くなく，剖検例を含めた複数の組織病理所見を詳細に観察した研究は少ない[13]。生検例では早期は細胞浸潤，次いで心筋脱落，巣状の線維症との経時的変化がみられ，心室瘤を後遺する例もある。剖検例では，好酸性染色性の亢進(図1)，筋収縮帯形成，融解などの単一心筋細胞障害像，それらの集簇像，および傷害された心筋細胞に対する細胞反応もしばしば認められる。

壁構成心筋細胞数に対する障害心筋細胞数は，心基部に比して心尖部の心筋細胞障害が高率で，壁運動異常と関連する。本症の心筋病変の特徴は，心筋障害が個々の心筋細胞を標的として

図1 たこつぼ心筋障害組織像
好酸性染色性の亢進を示す単一心筋細胞障害像(→)。PAS染色，400倍。

生じており，病変は単一心筋細胞の障害およびその集合像よりなることである。

Nefら[14]は急性期と回復期生検により，急性期では心筋細胞肥大，さまざまなサイズのグリコーゲン顆粒を認め，収縮蛋白，細胞骨格の障害，細胞外マトリックスの増加を認めたが，oncoticな壊死，アポトーシスは認めなかった。障害像は回復期生検で完全に回復していたと述べ，たこつぼ心筋障害にみられる形態変化はカテコラミン過剰による微小循環障害とカテコラミンの直接心筋障害であると結論している。

成因

Wittsteinら[4]は13名の急性情動ストレス後の左室機能異常例（平均63歳，12名女性，平均駆出率0.20）を7名のKillip Ⅲ群の心筋梗塞例と比較した。血漿カテコラミン値高値，小円形細胞浸潤，筋収縮帯壊死をみており，交感神経刺激の過剰興奮を介して，引き金因子としての情動ストレスは左室心尖部の可逆的高度機能低下をもたらしうるとしている。

その論文で彼らは推定される機序を
1 多枝攣縮による心筋気絶
2 心筋内微小血管攣縮（心筋気絶）
3 心筋炎
4 カテコラミンによる心筋障害。例：褐色細胞腫
5（交感）神経性心筋障害。例：くも膜下出血
6 心尖部心筋におけるβアドレナリン受容体密度の高値
の6説を枚挙している。

(1) 多枝攣縮による気絶心筋

多枝攣縮による気絶心筋は，虚血が証明されてなく，心筋気絶の定義に合致しない。また，慢性期の低冠攣縮誘発率（1/3例以下），心筋障害・壊死像および一過性の左右室流出路閉塞の説明が困難であることより，否定的である。心筋内の冠血流測定で所見が示されている心筋微小循環障害は一時的に広範な心尖部収縮不全を起こし，心基部を過収縮にする機序の説明は困難であり，二次的な変化である可能性は否定できない。

(2) 心筋炎

心筋炎は，先行感染欠如，心筋細胞障害の先行，心電図経過，回復過程などの諸点から否定的と考える。

(3) カテコラミンによる心筋障害

基礎病態にカテコラミン・β刺激薬使用，Guillain-Barre症候群の存在は，発症における心臓自律神経の異常を推定させる。Ueyamaら[15]はラット無動拘束実験で4割に心尖部バルーニング，4割にびまん性収縮低下をつくり，アドレナリン受容体遮断薬前投与で病変を予防しうることを示した。左室心尖部における交感神経刺激に対する不均一反応が明らかにされ，左室心尖部における疎な交感神経線維密度を代償する，より高いβ受容体密度と交感神経刺激に対する高い心筋反応性を有し，これが各種ストレス下での心筋障害・機能低下の成因と考えられている。

また，Ueyamaら[16]は卵巣切除ラットと卵巣切除エストロゲン補充療法ラットに無動拘束負荷を与え，エストロゲン補充は情動誘発性心血管反応を，神経系に対する間接的なそして，心臓に対する直接的な作用により，病変発症を防止するとした。

(4) (交感) 神経性心筋障害

Ortakら[17]は，本症9例における心臓交感神経活動を心拍変動により解析し，たこつぼ心筋障害急性期では虚血心に比較して，緊張性ならびに反射性自律神経活動が保持されることを示した。この観察は，たこつぼ心筋障害亜急性期における心臓自律神経障害の急速な回復，障害部心筋の電気的安定性ならびに酸素需要の減少を説明する。過剰な交感神経活動性，心尖部における高いβ受容体密度と交感神経刺激に対する高い心筋反応性が本病態の形成に重要な役割をなすと考えられている。Akashiら[18]は急性期と慢性期症例の心拍変動解析より，たこつぼ現象は急性自律神経障害下の神経性心筋気絶でおきるとの説を支持した。

(5) βアドレナリン受容体密度の高値

動物では心筋アドレナリン受容体分布は不均一で，基部に低く，心尖部に高い[19]。心臓交感神経は冠動脈に沿い，心基部から心尖部に分枝する[20]。神経伝達物質のノルエピネフリンは神経末端presynapse cleftから放出され，

後シナプスβ受容体に作用する。放出されたノルエピネフリンは再利用される。後シナプスβ受容体活動はguanine nucleotide binding protein（G-proteins）を介して，心筋細胞内のcyclic adenosine monophosphate（cAMP）濃度を上げる。adenyl cyclaseは刺激性（Gs），抑制性（Gi）G-proteinsにより調節されている。Kumeら[21]は，連続5症例で大動脈基部と冠静脈洞からのカテコラミン濃度を測定し，ノルエピネフリン，ドパミン値が上昇することを示した。

左室心尖部における交感神経刺激に対する不均一反応が明らかにされ，左室心尖部における疎な交感神経線維密度を代償する，より高いβアドレナリン受容体密度と交感神経刺激に対する高い心筋反応性を有し，これが各種ストレス下での心筋障害・機能低下の成因と考えられている。

stimulus trafficking

最近，Lyonら[22]は高濃度エピネフリンが心筋細胞内でのβ1-Gsシグナル伝達系からβ2-Giシグナル伝達系への転換により陰性変力作用を起こすことを，たこつぼ現象の原因とする説（図2）を唱えている。それは以下のような説明になる。

生理的および上昇した濃度では交感神経末端から放出されたエピネフリンは心室筋のβ1受容体を介して，陽性変力作用と変弛緩反応（lusitropic response）を起こす。この効果はβ1受容体がGs protein family（adenylyl cyclase-intracellular cyclic AMP-protein kinase A）と結合することで生じる。エピネフリンはβ1受容体と結合しこの効果を起こす。しかし，エピネフリンはβ2受容体により強い親和性をもつ。ヒトは他種動物に比べより高密度のβ2受容体をもつ。正常のヒト心室筋のβ1：β2受容体比はおおよそ4：1で，ヒトβ2受容体を心室に発現させトランスジェニックマウスの研究では，正常濃度エピネフリンでは陽性変力作用を示すが，超正常濃度エピネフリンは陰性変力作用を起こす。これはβ1-Gsシグナル伝達系からβ2-Giシグナル伝達系への転換による。これはstimulus traffickingとよばれる。

たこつぼ心筋障害例でしばしばみられるエピネフリン上昇後，Gi結合βアドレナリン受容体はGs couplingに代わり，変力作用を示す。

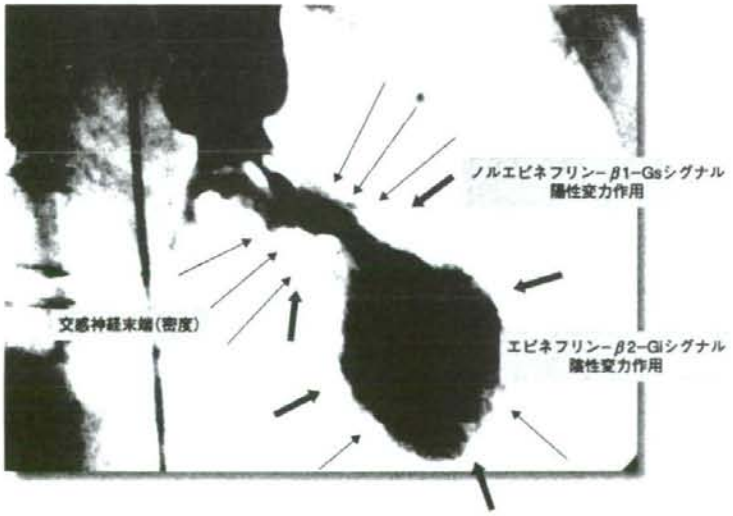

図2 stimulus trafficking
（左室造影像は板橋中央病院循環器内科田村勤先生，東京大学循環器内科渡辺昌文先生のご厚意による）
→：交感神経密度を表す。
➡：ノルエピネフリン-β1-Gsシグナル陽性変力作用。
➡：エピネフリン-β2-Giシグナル陰性変力作用。

β2-Giによる陰性変力作用の存在もしくは機序については議論が分かれる。エピネフリン-β2-Giによる陰性変力作用は，心基部を除く，心尖部抑制のpropensityを説明する。心室筋におけるアドレナリン受容体に対する交感神経刺激は2経路を経る。1つは直接に心筋を神経支配する交感神経末端および局所におけるエピネフリン放出である。ほかの1つは冠循環を介し心筋に拡散する循環カテコラミンである。ヒト剖検例の検討では交感神経末端密度は心基部で4割多く，イヌも同様であるとされる。イヌでは心尖部にβ受容体密度が高く，循環エピネフリンの心尖部に対するより強い作用を示し，高濃度カテコラミン値における心室局所反応の違い(β2-Gi couplingによる機能異常)を説明する。通常の生理的状態ではノルエピネフリンの大部分は神経末端より放出され，副腎髄質から放出される循環ノルエピネフリン濃度はわずかである。神経パターンはstress cardiomyopathyの機能異常とはあわない。

機能異常を示すβ2-Gi couplingは，カテコラミン高上昇に防御的に働くと考えられる。卵巣切除はβ1アドレナリン受容体発現を増加させる。卵巣切除動物におけるこの反応はエストロゲン補充で逆転する。したがって，エストロゲンはカテコラミン高上昇に防御的β2-Gi couplingに働く，女性におけるβ1β2シグナル比に影響を及ぼしうる。このことは最もβ1β2受容体密度が高い心尖部において，陰性変力作用という機械的代償として現れる。このβ1-Gi signalingのprotective dampening効果を欠くオスではカテコラミン高上昇における強力なβ1-Gs signalingによる急性心筋障害を生じ，致死的となり生存しないのではないかと推察している。

高濃度循環エピネフリンがGsからGi signalingへの細胞内signaling traffickingへの変換トリガーとなり，β2アドレナリン受容体を介し，陰性変力作用を示し，この作用はβアドレナリン受容体密度の高い心尖部で最も強いと仮説を出している。

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遺伝

Nefら[23]は，3例の急性期，回復期患者の心筋生検資料を用いたmicroarray解析で酸化ストレスに関与する遺伝子などの過剰発現を見出し，過剰なカテコラミンが酸化障害過程の引き金を引く可能性を述べている。

予後

たこつぼ心筋障害例は数週から1月以内にほぼ正常化し，予後良好な病態として，医師やマスコミにも理解されている。しかし，過去10年間のたこつぼ心筋障害死亡例・剖検例の報告抄録，論文に記録された死亡例数は37例ある。年齢は神経性食思不振症の34歳を除き，61歳から90歳まで(平均76歳)で，1～33病日(平均10日，1週間以内15例)で死亡していた。死因は肺炎，敗血症，多臓器不全などの基礎疾によるものと，心破裂(6例)，心タンポナーデ，心室不整脈によるものもみられた。全国アンケート調査(2002年施行79施設)で618例中死亡は25例(4.45%)，重度後遺症(心室瘤，完全房室ブロック，心不全再発など)は8例(1.3%)にみられた。死亡・重度後遺症は5%ほどにみられ，死因として早期の心破裂が重要である。

おわりに

本病態は従来注目されていなかった，未知の非虚血性心筋障害であり，呼吸困難，肺水腫，ショックで発症する重症例，死亡例も存在する。しかし，潜在性(無症候性)症例の有無，心基部過収縮と心筋障害との関連，巨大陰性T波の出る機序と左室内層外層別の病変の差異，活動電位持続時間，QT延長など不明な問題点が残されている。

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JACC CARDIOVASCULAR IMAGING
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PUBLISHED BY ELSEVIER INC.

VOL. 1, NO. 6, 2008
ISSN 1936-878X/08/$34.00
DOI:10.1016/j.jcmg.2008.04.012

Dobutamine Stress Testing as a Diagnostic Tool for Evaluation of Myocardial Contractile Reserve in Asymptomatic or Mildly Symptomatic Patients With Dilated Cardiomyopathy

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OBJECTIVES We performed dobutamine stress testing for evaluation of myocardial contractile reserve in asymptomatic or mildly symptomatic patients with dilated cardiomyopathy (DCM).

BACKGROUND Catecholamine sensitivity is reduced in failing hearts as a result of myocardial abnormalities in the beta-adrenergic receptor signaling pathway. However, little is known about adrenergic myocardial contractile reserve in asymptomatic or mildly symptomatic patients with DCM.

METHODS The maximal first derivative of left ventricular pressure (LV dP/dt_{max}) was determined during infusion of dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$) in 46 asymptomatic or mildly symptomatic (New York Heart Association functional class I or II) patients with DCM. The expression of messenger ribonucleic acid (mRNA) for contractile regulatory proteins in endomyocardial biopsy specimens was quantified by reverse transcription and real-time polymerase chain reaction analysis. Plasma norepinephrine levels were measured in all patients and [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy performed.

RESULTS Patients were classified into 3 groups based on the percentage increase in LV dP/dt_{max} induced by dobutamine (ΔLV dP/dt_{max}) and on LV ejection fraction (LVEF) at baseline: group I ($n = 18$): ΔLV $dP/dt_{max} > 100\%$ and LVEF $> 25\%$; group IIa ($n = 17$): ΔLV $dP/dt_{max} \leq 100\%$ and LVEF $> 25\%$; and group IIb ($n = 11$): ΔLV $dP/dt_{max} \leq 100\%$ and LVEF $\leq 25\%$. The amounts of $beta_1$-adrenergic receptor, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase, and phospholamban mRNA were significantly smaller in groups IIa and IIb than in group I. The plasma norepinephrine level was increased and the delayed heart/mediastinum count ratio in MIBG scintigraphy was decreased in both groups IIa and IIb.

CONCLUSIONS Dobutamine stress testing is a useful diagnostic tool for identifying reduced adrenergic myocardial contractile reserve related to altered myocardial expression of $beta_1$-adrenergic receptor, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase, and phospholamban genes even in asymptomatic or mildly symptomatic patients with DCM. (J Am Coll Cardiol Img 2008;1:718–26) © 2008 by the American College of Cardiology Foundation

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Manuscript received February 29, 2008; revised manuscript March 27, 2008; accepted April 17, 2008.

Dilated cardiomyopathy (DCM) is characterized by progressive left ventricular (LV) dilation and greatly impaired LV systolic function. In spite of progress in pharmacotherapy for end-stage heart failure, the overall prognosis of individuals with DCM is still poor. It is therefore important that DCM patients who are refractory to standard medical treatment be placed under strict management as early as possible.

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Catecholamine sensitivity is reduced in failing hearts as a result of myocardial abnormalities in the beta-adrenergic receptor (AR) signaling pathway, most prominently down-regulation of the $beta_1$-AR (1,2). Dobutamine is a relatively selective $beta_1$-AR agonist with weak $beta_2$- and alpha-AR agonistic activity. Dobutamine stress testing (DST) is performed widely, mostly in patients with coronary artery disease, to assess myocardial viability, and its safety has been well established (3,4). Previous studies have determined myocardial contractile reserve in DCM patients by DST and found that the response to dobutamine is associated with clinical prognosis (5–7). Although the mechanisms responsible for this association have remained unclear, it is possible that adrenergic myocardial contractile reserve revealed by DST reflects molecular biological changes in the myocardium.

We have now evaluated DST as a diagnostic tool for identifying individuals with a reduced adrenergic myocardial contractile reserve among patients with DCM and a New York Heart Association (NYHA) functional class of I or II. We have also examined the relations of such adrenergic myocardial contractile reserve to activity of the sympathetic nervous system and to myocardial expression of genes for contractile regulatory proteins related to beta-AR signaling or intracellular Ca^{2+} handling.

METHODS

Patients. The study protocol was approved by the Ethics Review Board of Nagoya University School of Medicine (approval #359), and written informed consent was obtained from all study participants. We studied 46 DCM patients with a NYHA functional class of I or II. Dilated cardiomyopathy was defined by a left ventricular ejection fraction (LVEF) of <50% (as determined by contrast ventriculography) in the absence of coronary artery stenosis of >50% (as determined by coronary angiography), valvular heart disease, arterial hypertension, and cardiac muscle disease secondary to any known systemic condition (8). Endomyocardial biopsy at the LV posterior wall was performed to exclude myocarditis or specific heart muscle disease. All patients were in normal sinus rhythm.

The patients were hospitalized for examinations and underwent laboratory measurements including neurohumoral factors, echocardiography, resting myocardial [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy, and cardiac catheterization. Fourteen patients had been treated with beta-blockers, 11 with digitalis, 34 with diuretics, and 33 with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (or both). All medications were discontinued at least 4 days before the study. Sixteen patients had been hospitalized because of heart failure with dyspnea on exertion or peripheral edema, but they had been in a stable condition for at least 3 months and were classified as NYHA functional class II at the time of hospitalization for the study.

Myocardial [^{123}I]MIBG scintigraphy. Myocardial [^{123}I]MIBG scintigraphic imaging was performed as previously described (9). A dose of 148 MBq of [^{123}I]MIBG was injected intravenously with the patient in a supine position. Myocardial [^{123}I]MIBG uptake was quantified in anterior planar views at 15 min (early image) and 4 h (delayed image) after tracer injection. The heart/mediastinum count ratio was determined from the delayed anterior planar [^{123}I]MIBG image. The washout rate was calculated with the following formula: $100\% \times [(H - M)_{early} - (H - M)_{delayed}]/(H - M)_{early}$, where H is the mean counts per pixel in the left ventricle and M is the mean counts per pixel in the upper mediastinum. In our laboratory, the normal range for the delayed heart/mediastinum count ratio is 1.8 to 2.7 and that for the washout rate is 16% to 27%.

Cardiac catheterization. All patients initially underwent routine diagnostic left and right heart catheterization by the femoral approach. A 6-F fluid-filled pigtail catheter with a high-fidelity micromanometer (CA-61000-PLB Pressure-tip Catheter, CD Leycom, Zoetermeer, the Netherlands) was placed in the LV cavity for measurement of LV pressure. We evaluate the maximal first derivative of LV pressure (LV dP/dt_{max}) as an index

ABBREVIATIONS AND ACRONYMS

AR = adrenergic receptor
DCM = dilated cardiomyopathy
DST = dobutamine stress testing
GRK2 = G-protein–coupled receptor kinase 2
LV = left ventricular
LV dP/dt_{max} = maximal first derivative of left ventricular pressure
LVEF = left ventricular ejection fraction
MIBG = metaiodobenzylguanidine
mRNA = messenger ribonucleic acid
NYHA = New York Heart Association
SERCA2a = sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a

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of contractility and the pressure half-time ($T_{1/2}$) as an index of isovolumic relaxation as previously described (10). After collection of baseline hemodynamic data, dobutamine was infused intravenously at incremental doses of 5 and 10 $\mu g\ kg^{-1}\ min^{-1}$, and hemodynamic measurements were made at the end of each 10-min infusion period. After hemodynamic values had returned to baseline, endomyocardial biopsy was performed. Several (at least 3) endomyocardial biopsy specimens for messenger ribonucleic acid (mRNA) analysis were frozen immediately in liquid nitrogen and stored at −80°C until the analysis.

Quantitative reverse transcription–polymerase chain reaction analysis. Total RNA was isolated from 1 to 2.5 mg of frozen LV biopsy specimens and subjected to quantitative reverse transcription and polymerase chain reaction analysis, as previously described (10,11), with primers and TaqMan probes (Nippon EGT Co. Ltd., Toyama, Japan) specific for human complementary DNA encoding $beta_1$-AR, $beta_2$-AR, sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a (SERCA2a), phospholamban, ryanodine receptor-2, calsequestrin, and the Na^+/Ca^{2+} exchanger. The primer sequences (forward and reverse, respectively) for G-protein–coupled receptor kinase 2 (GRK2) and for the alpha subunits of G_s and G_{i2} (G_s alpha, G_{i2} alpha) were as follows: GRK2, 5′-TGAGAGCGATAAGTTCACACGG-3′ and 5′-CGCTTTTTGTCCAGGCACT-3′; G_s alpha, 5′-GTACTCCCTGGACAAGATCGACG-3′ and 5′-GCAGTCACATCGTTGAAGCACT-3′; and G_{i2}alpha, 5′-GGAATACCAGCTCAACGACTCA-3′ and 5′-CTGACCACCCACATCAAACATCT-3′. The amount of each mRNA was thus determined with a fluorogenic 5′-nuclease polymerase chain reaction assay and an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, California). All determinations were performed in triplicate. The abundance of each mRNA was normalized by the corresponding amount of glyceraldehyde-3-phosphate dehydrogenase mRNA.

Statistical analysis. Data are presented as means ± standard deviation. For comparisons among groups, 1-way analysis of variance was used for continuous variables when appropriate and contingency table analysis was used for categorical variables. Given that the plasma concentration of brain natriuretic peptide was not normally distributed, we assessed differences in such values with the nonparametric Kruskal-Wallis test. When a significant difference was detected, intergroup comparisons were performed with Bonferroni's multiple comparison test. A p value of <0.05 was considered statistically significant.

RESULTS

Patient classification. Patients were divided into groups on the basis of the adrenergic myocardial contractile response revealed by DST, specifically the percentage change in LV dP/dt_{max} during dobutamine infusion at a dose of 10 $\mu g\ kg^{-1}\ min^{-1}$ relative to the baseline value. Those who showed an increase of >100% were assigned to group I (n = 18), and those who showed an increase of ≤100% were assigned to group II (n = 28). In addition, the patients in group II were further assigned to 2 subgroups according to the LVEF at baseline: those with an LVEF of >25% (group IIa, n = 17) and those with an LVEF of ≤25% (group IIb, n = 11). All of the patients in group I had an LVEF of >25% at baseline (Fig. 1).

Baseline data. Baseline clinical characteristics of the patients are shown in Table 1. The LVEF was significantly decreased in group IIb compared with that in group I or in group IIa, which is consistent with the criterion for subgroup classification. The plasma concentration of brain natriuretic peptide was significantly higher in group IIb than in either of the other 2 groups. Although there were no significant differences in the plasma concentration of this peptide or in resting cardiac function as evaluated by echocardiography or cardiac catheter-

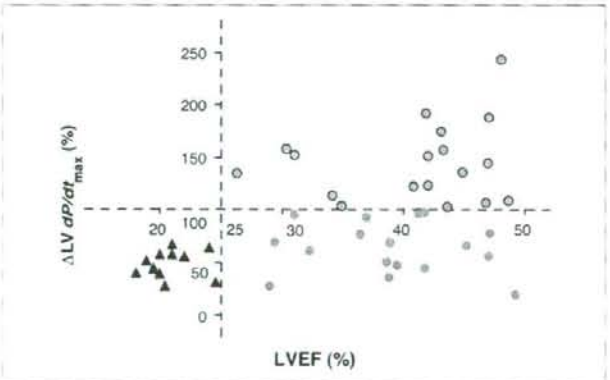

Figure 1. **Relation Between Baseline LVEF and ΔLV dP/dt_{max}**

We calculated the percentage change in maximal first derivative of left ventricular pressure (LV dP/dt_{max} [ΔLV dP/dt_{max}]) induced by intravenous infusion of dobutamine (10 $\mu g\ kg^{-1}\ min^{-1}$). Patients were classified into 3 groups: group I (**orange with black circles**, n = 18), ΔLV dP/dt_{max} >100% (left ventricular ejection fraction [LVEF] >25%); group IIa (**orange circles**, n = 17), ΔLV dP/dt_{max} ≤100% and LVEF >25%; and group IIb (**brown triangles**, n = 11), ΔLV dP/dt_{max} ≤100% and LVEF ≤25%.

ization between groups I and IIa, the plasma norepinephrine level was significantly higher (Fig. 2) and the delayed heart/mediastinum count ratio was significantly lower (Fig. 3) in group IIa than in group I.

Hemodynamic response to intravenous dobutamine. No complications occurred in any of the study subjects during the dobutamine stress protocol. The effects of intravenous dobutamine infusion on hemodynamics are shown in Table 2. The heart rate at baseline was significantly higher in group IIb than in group I, although that at a dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$ was similar among the 3 groups. There were no significant differences in LV dP/dt_{max} or $T_{1/2}$ among the 3 groups at baseline. However, LV dP/dt_{max} was significantly higher in group I than in group IIa or in group IIb at dobutamine infusion rates of 5 or 10 $\mu g\ kg^{-1}\ min^{-1}$, and $T_{1/2}$ was significantly greater in group IIb than in group I at a dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$.

Myocardial [^{123}I]MIBG scintigraphy and the changes in LV dP/dt_{max} during dobutamine infusion of 2 typical cases are presented in Figures 4 and 5.

Expression of contractile regulatory protein genes. The amount of $beta_1$-AR mRNA was significantly reduced in group IIa and in group IIb compared with that in group I (Table 3). There were no significant differences in the expression of $beta_2$-AR, GRK2, G_s alpha, and G_{i2} alpha genes among the 3 groups. With regard to intracellular Ca^{2+}-handling proteins, the abundance of SERCA2a and phospholamban mRNAs was significantly reduced in group IIa and in group IIb compared with that in group I. The abundance of ryanodine receptor-2, calsequestrin, and Na^+/Ca^{2+} exchanger mRNAs did not differ significantly among the 3 groups.

Table 1. Characteristics of the 3 Patient Groups at Baseline

Characteristic	Group I (n = 18)	Group IIa (n = 17)	Group IIb (n = 11)
Age (yrs)	51 ± 9	52 ± 13	50 ± 15
Gender, male/female	12/6	13/4	9/2
NYHA functional class I/II	10/8	8/9	2/9
Medication, n (%)			
Digitalis	3 (17%)	3 (18%)	5 (45%)
Diuretics	10 (56%)	13 (76%)	11 (100%)*
ACE inhibitors or ARBs	13 (72%)	14 (82%)	6 (55%)
Beta-blockers	4 (22%)	5 (29%)	5 (45%)
Spironolactone	6 (33%)	6 (35%)	7 (64%)
LV end-diastolic dimension (mm)	59 ± 4	60 ± 9	72 ± 13*†
LV end-systolic dimension (mm)	47 ± 5	50 ± 10	65 ± 13*†
IVS thickness (mm)	9 ± 1	9 ± 2	8 ± 2
LVPW thickness (mm)	9 ± 1	9 ± 2	9 ± 2
LVEF (%)	41 ± 7	39 ± 6	21 ± 2*†
LV end-diastolic pressure (mm Hg)	17 ± 5	15 ± 7	18 ± 9
PAWP (mm Hg)	11 ± 4	11 ± 4	17 ± 8*†
Cardiac index (l min^{-1} m^{-2})	3.1 ± 0.5	3.0 ± 0.7	2.7 ± 0.5
Plasma BNP (pg/ml)	59 ± 76	140 ± 147	421 ± 442*†
Plasma norepinephrine (pg/ml)	403 ± 143	693 ± 293*	655 ± 267*
Delayed H/M ratio	1.9 ± 0.2	1.6 ± 0.3*	1.5 ± 0.3*
Washout rate (%)	24.7 ± 12.6	35.6 ± 16.6	42.8 ± 15.4*

Data are means ± SD. *p < 0.05 versus group I; †p < 0.05 versus group IIa.
ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker; BNP = brain natriuretic peptide; H/M = heart/mediastinum; IVS = interventricular septum; LV = left ventricular; LVEF = left ventricular ejection fraction; LVPW = left ventricular posterior wall; NYHA = New York Heart Association; PAWP = pulmonary arterial wedge pressure.

DISCUSSION

The main findings of the present study include the following: 1) All patients with severe LV systolic dysfunction (LVEF ≤25%) showed a reduced adrenergic myocardial contractile reserve. 2) In some patients with mild to moderate LV systolic dysfunction (25 < LVEF <50%), adrenergic myocardial contractile reserve was preserved, whereas in others it was reduced. 3) The abundance of mRNA not only for the $beta_1$-AR but also for SERCA2a and phospholamban was reduced in patients with reduced adrenergic myocardial contractile reserve, even in those in whom LV systolic dysfunction was only mild to moderate, like that in the comparison group.

Evaluation of myocardial contractile reserve. Determinants of myocardial contractile reserve include

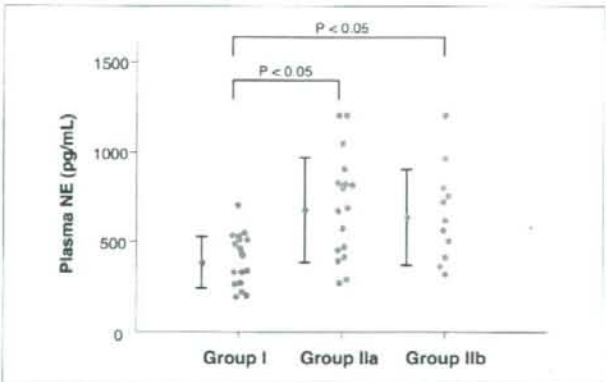

Figure 2. Comparison of Plasma NE Levels Among the 3 Patient Groups

Plasma norepinephrine (NE) levels measured at baseline were significantly higher in group IIa and in group IIb compared with that in group I. Data for individual subjects and the corresponding mean ± SD values are shown.

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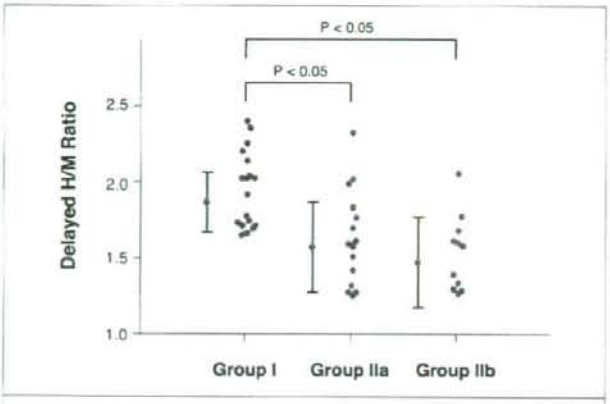

Figure 3. Comparison of the Delayed H/M Ratio in [^{123}I]MIBG Scintigraphy Among the 3 Patient Groups

We determined the heart/mediastinum (H/M) count ratio from the delayed planer [^{123}I]metaiodobenzylguanidine (MIBG) image. The delayed H/M ratio was significantly lower in group IIa and in group IIb compared with that in group I. Data for individual subjects and the corresponding mean ± SD values are shown.

the Frank-Starling mechanism, the force-frequency effect, and adrenergic stimulation (12,13). The myocardial contractile response to adrenergic stimulation is impaired in DCM. Several studies based on dobutamine stress echocardiography have demonstrated a relation between prognosis and adrenergic myocardial contractile reserve, as determined by measurement of indices of LV systolic function

Table 2. Hemodynamic Response of the 3 Patient Groups to Intravenous Dobutamine Infusion

Parameter	Group I	Group IIa	Group IIb
Heart rate (beats/min)			
Baseline	69 ± 7	76 ± 12	86 ± 13*
Dobutamine (5 μg kg^{-1} min^{-1})	75 ± 10	82 ± 16	90 ± 13*
Dobutamine (10 μg kg^{-1} min^{-1})	98 ± 14	98 ± 20	99 ± 16
LVSP (mm Hg)			
Baseline	122 ± 15	113 ± 16	112 ± 26
Dobutamine (5 μg kg^{-1} min^{-1})	137 ± 21	123 ± 17*	115 ± 23*
Dobutamine (10 μg kg^{-1} min^{-1})	133 ± 16	123 ± 16	124 ± 27
LV dP/dt$_{max}$ (mm Hg/s)			
Baseline	1131 ± 178	1053 ± 190	1048 ± 224
Dobutamine (5 μg kg^{-1} min^{-1})	1746 ± 298	1340 ± 246*	1193 ± 247*
Dobutamine (10 μg kg^{-1} min^{-1})	2741 ± 417	1692 ± 300*	1544 ± 342*
$T_{1/2}$ (ms)			
Baseline	41 ± 6	43 ± 8	44 ± 5
Dobutamine (5 μg kg^{-1} min^{-1})	35 ± 8	37 ± 10	41 ± 5
Dobutamine (10 μg kg^{-1} min^{-1})	25 ± 5	29 ± 11	34 ± 7*

Data are means ± SD. *p < 0.05 versus group I.
LV dP/dt$_{max}$ = maximal first derivative of left ventricular pressure; LVSP = left ventricular systolic pressure; $T_{1/2}$ = pressure half-time.

such as LVEF or cardiac output (5,6). However, these indices are relatively load-dependent. Few studies have evaluated the adrenergic contractile response to dobutamine infusion by measurement of the increase in LV dP/dt$_{max}$ in patients with nonischemic LV systolic dysfunction (7,14). These studies applied dobutamine stress with atrial pacing or intracoronary dobutamine infusion to avoid the effects of a change in heart rate. In the present study, we evaluated adrenergic myocardial contractile reserve on the basis of the percentage change in LV dP/dt$_{max}$ at an intravenous dobutamine infusion rate of 10 μg kg^{-1} min^{-1}, with a cutoff of 100% based on the median value for patients with mild to moderate LV systolic dysfunction (25 < LVEF <50%). Differences in the increase in heart rate among the 3 groups may have influenced the change in LV dP/dt$_{max}$; patient classification in our study was therefore based on the frequency-dependent inotropic response to dobutamine infusion, which is clinically more useful as a measure of adrenergic myocardial functional reserve.

End-systolic wall stress as assessed by echocardiography has been shown to be a quantitative index of true myocardial afterload and an important determinant of systolic function independent of loading conditions (15). Twenty-three of the 46 patients in the present study (6, 10, and 7 patients in groups I, IIa, and IIb, respectively) underwent echocardiography during DST. The percentage increase in end-systolic wall stress determined by echocardiography during dobutamine infusion tended to be higher in group I than in group IIa or in group IIb (data not shown).

Relation of altered expression of contractile regulatory protein genes to adrenergic myocardial contractile reserve. The failing human heart is characterized by a reduced sensitivity to beta-AR agonists, which may contribute to the loss of cardiac contractility (1,2,14). The decrease in cardiac responsiveness to beta$_1$-AR stimulation appears to be due in part to a reduction in the number of beta$_1$-ARs (14), which in turn is thought to result from a reduction in the amount of the corresponding mRNA (16,17). Our results now suggest that evaluation of adrenergic myocardial contractile reserve by DST might be a means of identifying down-regulation of the beta$_1$-AR in patients with DCM. On the other hand, we did not detect changes in the abundance of GRK2 or G_{i2}alpha mRNAs in patients with a reduced adrenergic myocardial contractile reserve, although the expression of GRK2 and G_{i2}alpha

genes was previously found to be increased in the heart of patients with end-stage heart failure (17,18). This apparent discrepancy might be explained by differences in the patient populations of the studies, with our study including a less severely afflicted ambulatory population of patients with a NYHA functional class of I or II.

The amounts of SERCA2a and phospholamban mRNAs were found to be decreased in endomyocardial biopsy specimens from patients undergoing heart transplantation (19–21). We previously showed that a reduction in the myocardial abundance of SERCA2a mRNA was apparent in patients with impaired myocardial contractile reserve, as revealed by determination of the force-frequency relation, before the onset of detectable LV systolic dysfunction (10). As far as we are aware, however, the relation between adrenergic myocardial contractile reserve and the abundance of mRNAs for Ca^{2+}-handling proteins has not previously been examined. We have now shown that a reduction in adrenergic myocardial contractile reserve appears to be associated with down-regulation not only of $beta_1$-AR mRNA but also of SERCA2a and phospholamban mRNAs, even in asymptomatic or mildly symptomatic patients with DCM. On the other hand, we did not detect changes in the abundance of ryanodine receptor-2, calsequestrin, or Na^+/Ca^{2+} exchanger mRNA in patients with a reduced adrenergic myocardial contractile reserve, which may be considered consistent with previous findings (21–23).

Plasma norepinephrine level and quantitative [^{123}I]MIBG scintigraphy parameters. In heart failure, increased cardiac spillover of norepinephrine and depletion of myocardial catecholamine result in an increased plasma concentration of norepinephrine (24) and decreased cardiac uptake of [^{123}I]MIBG, an analog of norepinephrine (25). In addition, both the plasma norepinephrine level (26) and [^{123}I]MIBG imaging (27) have been found to provide prognostic information in patients with heart failure. We previously showed that decreased [^{123}I]MIBG uptake in DCM patients was associated with a reduced myocardial contractile reserve as evaluated by atrial pacing (9). In the present study, all patients with a greatly increased plasma level of norepinephrine and a greatly reduced [^{123}I]MIBG uptake had a reduced adrenergic myocardial contractile reserve. However, there was marked overlap in moderately increased levels of plasma norepinephrine or moderately reduced levels of [^{123}I]MIBG uptake among patient groups with reduced or preserved adrenergic myocardial contractile reserve. These findings suggest that these resting parameters alone do not fully reflect adrenergic myocardial contractile reserve.

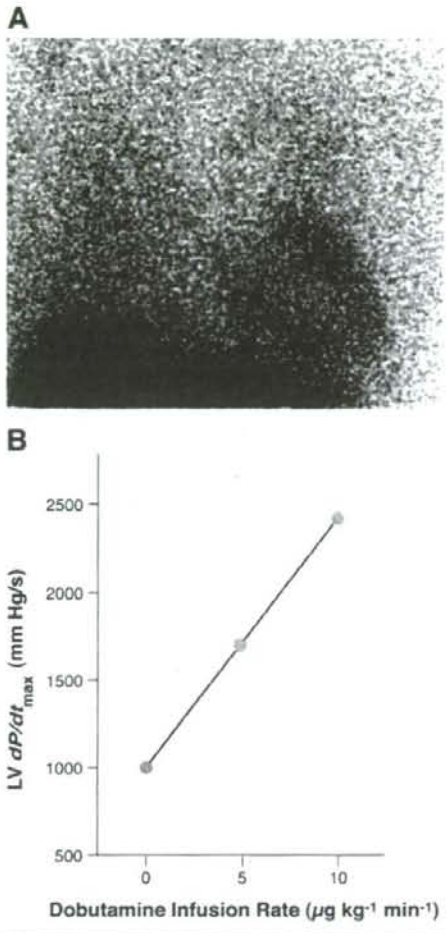

Figure 4. A Representative Case of Group I

This patient was a 43-year-old woman in New York Heart Association functional class I, and she had an LVEF of 43% and a plasma norepinephrine level of 328 pg/ml. (A) Mildly reduced myocardial uptake was observed, and the delayed H/M ratio was 2.2. (B) The relationship between dobutamine infusion rate and LV dP/dt_{max} of this patient is shown. The LV dP/dt_{max} at baseline and at a dobutamine infusion rate of 10 $\mu g\ kg^{-1}\ min^{-1}$ were 986 and 2,415 mm Hg/s, respectively (ΔLV dP/dt_{max} = 145%). Abbreviations as in Figures 1 and 3.

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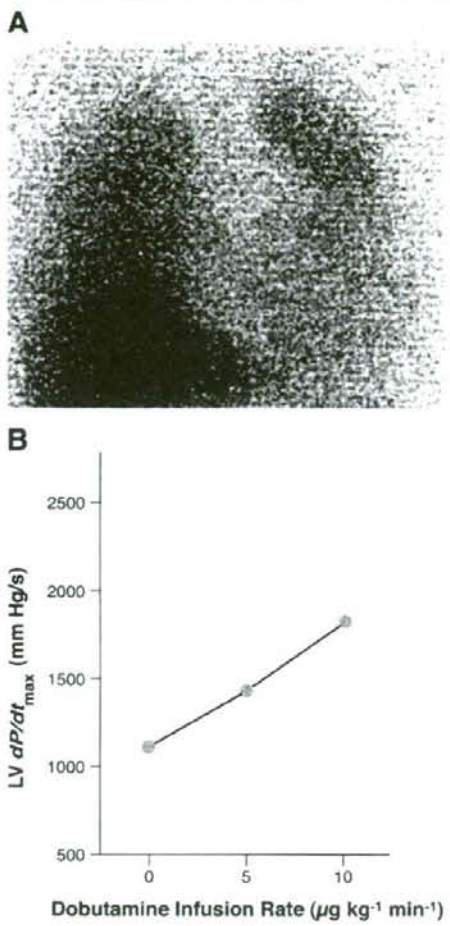

Figure 5. A Representative Case of Group IIa

This patient was a 58-year-old woman in New York Heart Association functional class II, and she had an LVEF of 42% and a plasma norepinephrine level of 1,200 pg/ml. (**A**) Increased lung uptake and severely reduced myocardial uptake are observed, and the delayed H/M ratio was 1.3. (**B**) The relationship between dobutamine infusion rate and LV dP/dt_{max} of this patient is shown. The LV dP/dt_{max} at baseline and at a dobutamine infusion rate of 10 μg kg $^{-1}$ min $^{-1}$ were 1,089 and 1,791 mm Hg/s, respectively (ΔLV dP/dt_{max} = 64%). Abbreviations as in Figures 1 and 3.

Clinical implications. We have evaluated adrenergic myocardial contractile reserve as revealed by DST in DCM patients with a NYHA functional class of I or II. Even among such asymptomatic or mildly symptomatic patients, those individuals with a reduced adrenergic myocardial contractile reserve also manifested activation of the sympathetic nervous system as well as altered myocardial expression of the genes for the $beta_1$-AR, SERCA2a, and phospholamban. Our results suggest that DST is able to identify functional and molecular remodeling associated with overactivation of the sympathetic nervous system in asymptomatic or mildly symptomatic patients with DCM, even when resting parameters still show normal values.

Pharmacological agents that reduce adrenergic hyperactivity, such as beta-blockers or angiotensin-converting enzyme inhibitors, may improve adrenergic myocardial contractile reserve, given that they have been shown to induce up-regulation of myocardial beta-ARs or recovery of the cardiac adrenergic nervous system (28–30). Detrimental changes in cardiac gene expression in patients with DCM have also been shown to be reversed by treatment with beta-blockers (31,32). Although further studies will be necessary to clarify whether abnormalities of intracellular signal transduction related to myocardial contractility influence the prognosis of asymptomatic or mildly symptomatic patients with DCM, DST may provide valuable information for management of these patients.

Study limitations. Concomitant medical treatment may have had an impact on the myocardial abundance of mRNA, although the percentage of patients who had been treated with beta-blockers did not differ significantly among the 3 patient groups. However, given that long-term withholding of medical treatment may be harmful to such individuals, drug treatment was discontinued for only 4 days before the study. We were not able to examine healthy subjects for ethical reasons. In addition, it is difficult to analyze protein abundance and function in the small amounts of tissue obtained by percutaneous endomyocardial biopsy. We therefore assessed contractile regulatory protein expression only by reverse transcription–polymerase chain reaction analysis and exclusively in asymptomatic or mildly symptomatic patients with DCM. Further evaluation of patients with a wider range of heart failure severity may provide insight into the chronology of changes in the expression of contractile regulatory proteins. It remains to be determined whether adrenergic myocardial contractile reserve as assessed by noninvasive diagnostic tools such as dobutamine stress echocardiography is also associated with molecular remodeling of contractile regulatory proteins. Finally, prognostic and therapeutic rel-

evance of DST in DCM remains unclear and deserves further investigation.

CONCLUSIONS

The present study suggests that DST is a useful diagnostic tool for identifying reduced adrenergic myocardial contractile reserve related to altered myocardial expression of the $beta_1$-AR, SERCA2a, and phospholamban genes even in asymptomatic or mildly symptomatic patients with DCM.

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Table 3. Relative Abundance of Contractile Regulatory Protein mRNAs in Endomyocardial Biopsy Specimens Relative to the Corresponding Amount of Glyceraldehyde-3-Phosphate Dehydrogenase mRNA

mRNA	Group I	Group IIa	Group IIb
$Beta_1$-AR	1.39 ± 0.68	0.71 ± 0.19*	0.66 ± 0.29*
$Beta_2$-AR	1.29 ± 0.92	0.95 ± 0.18	0.91 ± 0.40
GRK2	1.54 ± 0.63	1.53 ± 0.26	1.59 ± 0.58
G_i alpha	1.18 ± 0.40	0.94 ± 0.17	1.04 ± 0.34
G_{12} alpha	0.78 ± 0.35	0.77 ± 0.15	0.85 ± 0.25
SERCA2a	0.60 ± 0.29	0.36 ± 0.08*	0.37 ± 0.12*
Phospholamban	0.82 ± 0.28	0.56 ± 0.12*	0.36 ± 0.16*
Ryanodine receptor-2	0.74 ± 0.42	0.56 ± 0.17	0.69 ± 0.23
Calsequestrin	1.34 ± 0.58	1.16 ± 0.25	1.30 ± 0.44
Na^+/Ca^{2+} exchanger	1.69 ± 0.76	1.14 ± 0.14	1.46 ± 0.84

Data are means ± SD. *$p < 0.05$ vs. group I.
AR = adrenergic receptor; GRK2 = G protein-coupled receptor kinase 2; mRNA = messenger ribonucleic acid; SERCA2a = sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a.

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Key Words: cardiomyopathy ■ contractility ■ heart failure ■ biopsy ■ dobutamine.

►APPENDIX

For an accompanying slide set, please see the online version of this article.

JACC: CARDIOVASCULAR IMAGING
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VOL. 1, NO. 6, 2008
ISSN 1936-878X/08/$34.00
DOI 10.1016/j.jcmg.2008.09.001

EDITORIAL COMMENT

Contractile Reserve: Are We Beginning to Understand It?*

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The concept of contractile reserve is an old idea garnered from observations made by many investigators over the years. Various techniques have been used to study left ventricular (LV) performance in either the true failing heart or the heart with impaired systolic function, including post-systolic accentuation, epinephrine ventriculography, and various hemodynamic and echocardiographic responses to direct inotropic stimulation with dobutamine (1–3). The idea behind the test is to provide an objective means of determining the point at which the patient's heart has irreversibly failed. The identification of irredeemable heart damage could (for example) allow the physician to refer the patient for heart surgery or heart transplantation.

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In this issue of *iJACC*, Kobayashi et al. (4) have further investigated the basis of diminishment in contractile reserve in 46 asymptomatic or mildly symptomatic patients with dilated cardiomyopathy. Patients were classified depending on their response of dP/dt max to dobutamine, as well as on the basis of their baseline ejection fraction. Plasma norepinephrine and myocardial [^{123}I] metaiodobenzylguanidine scans were obtained, and an LV biopsy was done to characterize a number of molecular markers associated with the inotropic state. Patients less responsive to dobutamine demonstrated more abnormalities of sympathetic activity (higher plasma norepinephrine levels or less myocardial catecholamine tissue density) *despite a similarly reduced ejection fraction*. These patients also had evidence for reduced molecular markers of LV contractile state, indirectly assessed by measuring messenger ribonucleic acid from LV biopsies. *This raises the possibility of using noninvasive imaging to characterize and possibly type subsets of intracellular abnormalities that underlie contractile dysfunction in the future.*

However, the longstanding and vexing question of what is driving the central theme of heart failure remains. Are the abnormal molecular markers a consequence of the impaired myocardial function, or are the molecular changes actually causing the decreased LV function? As the authors point out, markers such as myocardial tissue norepinephrine has long been known to be depleted in human failing hearts (5), and this is usually accompanied by increased plasma levels of norepinephrine (6). Likewise, numerous previous studies have demonstrated reduced beta-adrenergic receptors along with altered sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a (SERCA2a) and phospholamban in failing hearts (7). The fact that these changes were observed in relatively early stages of heart failure, and were more marked in those patients with reduced myocardial contractile reserve, would suggest that changes observed at the molecular level may actually be the cause of at least some of the impairment in LV function. Likewise, blocking the sympathetic nervous system at the beta-receptor level or replacing SERCA2a with gene therapy can reverse the reduced contractile state, thus fulfilling at least one of Koch's postulates.

Assuming that a more noninvasive approach to defining "contractile reserve" or some surrogate thereof in patients with heart failure is preferable, is there a need and a real benefit from such measurements? If so, what is the current status of such noninvasive approaches? Most cardiologists would like to know if there is such a point from which a

*Editorials published in *JACC: Cardiovascular Imaging* reflect the views of the authors and do not necessarily represent the views of *JACC: Cardiovascular Imaging* or the American College of Cardiology.

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return to more normalcy is either likely or not likely. Of course, this will always be a probability estimate, since no such test is likely to be precise in an individual patient. Moreover, for patients being considered for heart surgery, predicting which LV will recover depends to some extent on the quality of the operation in addition to the basal inotropic state of the LV before surgery. Nevertheless, such a need for defining "recoverability" seems self-evident. Two classic examples are patients with severe cardiomyopathy and severe regurgitant valvular disease, or patients with low gradient aortic stenosis and very poor LV function. This area needs much further study, and the study of Kobayashi et al. (4) is only a start in this direction.

In summary, estimating contractile reserve in patients with impaired LV function is worthwhile, particularly when making decisions about valve surgery, coronary revascularization, and candidacy for heart transplantation. The basis for contractile reserve probably resides in several molecular markers of contractility, including beta-receptor density, altered SERCA2a, and phospholamban, as measured by myocardial biopsy by Kobayashi et al. (4). Imaging of molecular markers is on the horizon, but until it is more routine, cardiologists will continue to rely on those techniques that are performed in their own hospital laboratories with the most expertise and experience. For many, this will include the traditional assessment of contractile reserve.

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