Coronary artery myogenic response in a genetic model of hypertrophic cardiomyopathy

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Coronary artery myogenic response in a genetic model of hypertrophic cardiomyopathy. Am J Physiol Heart Circ Physiol 283: H2244–H2249, 2002. First published August 29, 2002; 10.1152/ajpheart.00606.2002.—Hypertrophic cardiac myopathy (HCM), first described in 1958, is the most frequent cause of sudden cardiac death in young athletes, with an estimated incidence of 1:200,000 athletes (1, 11, 12). Estimates of HCM can be as high as 5% of young athletes based on data from tertiary referral centers (15). It is usual for sudden cardiac death to be the first sign of undiagnosed HCM. Significant myocyte hypertrophy, myofibrillar disarray, increased interstitial collagen, intramyocardial vessel thickening, and arrhythmias characterize HCM (3). Abnormalities in small intramural coronary arteries (external diameter ≤1,500 μm) are frequently (incidence of 80%) found at autopsy in subjects with HCM. The walls of these intramural vessels, often found in the ventricular septum, are thickened by smooth muscle cells and matrix, and their lumens frequently appear narrowed (14, 19). Several genetic mutations cause HCM, with the most common gene responsible for human HCM being the β-myosin heavy chain (MHC), accounting for 35–50% of HCM cases (22).

A murine model of human HCM containing an arginine-to-glutamine missense mutation at amino acid position 403 R403Q in α-MHC and a deletion in the actin-binding domain is phenotypically identical to the human disease. In rodents, virtually all of the MHC protein is of the α isoform. These animals develop myocardial and myocellular hypertrophy by 4 mo of age and in males progress to end-stage disease by 10–12 mo of age. In addition, these animals express increased levels of atrial natriuretic factor and α-skeletal actin, several markers of cellular hypertrophy.

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Perhaps most relevant to the current study is the decrease in coronary flow per milligram of cardiac tissue noted on Langendorff perfusion when mutant mice are compared with 10-mo-old wild-type controls (17). In the clinical heart failure population, there is evidence of abnormal vascular function in both endothelium-dependent and acetylcholine-stimulated vasodilatation (5) as well as from endothelin-related vasoconstriction (9). In this animal model, it is unclear whether the coronary flow changes are intrinsic to the vascular system or secondary to the myocellular disease. As noted, the majority of human HCM is due to familial autosomal dominant transmission of mutations in sarcomeric proteins (22, 23). There is a smaller population of de novo mutations that cannot be traced via family history. Over 100 mutations in more than 10 genes encoding sarcomeric proteins have been identified in patients with HCM. Approximately two-thirds of these mutations occur in β-MHC, accounting for the most prominent fraction of the affected population (10). One of these mutations is R403Q. In patients, this mutation is associated with the histopathological changes noted above as well as with premature sudden death.

The vascular contribution to ventricular pathophysiology in HCM is unknown. To this end, we investigated the myogenic properties of the small septal coronary arteries in wild-type and mutant MHC mice to determine whether the genetic defect in the cardiac sarcomeres and associated myocardial thickening could be accompanied by functional consequences in vascular smooth muscle cells and the endothelium.

**METHODS**

**Animals**

The transgenic (TG) mouse model of familial HCM used in this study has been described previously. In short, these TG mice express a mutant α-MHC with the expression driven by a rat α-MHC promoter. The transgene coding region contains two mutations: a point mutation (R403Q) and a deletion of 59 amino acids in the actin-binding site of the α-MHC bridged by an addition of 9 nonmyosin amino acids. The mutant α-MHC protein constitutes up to 10–12% of the total myosin in the TG mice (20, 21). Polymerase chain reaction-amplified tail DNA was used to genotype the heterozygous TG mice and their non-TG wild-type littermates in this study. The animals were maintained under specific pathogen-free conditions with food and water ad libidum. The Institutional Animal Use and Care Committee of the University of Colorado (Boulder, CO) and University of British Columbia (Vancouver, British Columbia, Canada) approved all animal protocols.

Male mice that were genotyped as either wild-type or TG were used. To examine the evolution of potential vascular dysfunction in hypertrophic cardiomyopathy, coronary septal arteries were examined at two time points, the first when the mice were between 10 and 16 wk old (referred to as 3 mo old), and the second when the mice were between 46 and 50 wk old (referred to as 11 mo old).

**Vessel Isolation and Cannulation**

Each mouse was anesthetized with pentobarbital sodium (Somnotol; 30 mg/kg ip) and heparin sodium (Hepalean; 500 U/kg ip). The animals were euthanized, and the heart was removed. The beating heart was placed in physiological salt solution (PSS) at 4°C. The right ventricular cavity was expanded, and the coronary septal artery was carefully dissected and transferred to an arteriograph filled with oxygenated PSS. The arteriograph contained two cannulas (tip diameter 40 ± 5 μm), both filled with oxygenated PSS. The distal cannula was occluded so that experiments were made under conditions of no flow. The proximal cannula was connected to a pressure servosystem (Living Systems, Burlington, VT). The artery was first mounted on the proximal cannula and tied with a single strand of braided 4-0 silk suture (diameter: 15 μm) and carefully emptied of residual blood. The free end of the vessel was then mounted and tied to a distal closed-off cannula. Vessels were checked for leaks, and only vessels without leaks were used for further experiments. After the septal coronary arteries were dissected, the hearts were weighed, and the apex was cut and frozen in OCT compound, whereas the rest of the heart was preserved in formalin for further histopathology.

The PSS in the vessel chamber was recirculated continuously at a flow rate of 20–30 ml/min. The PSS was stored in an external reservoir where it was bubbled with a gas mixture containing 95% O2–5% CO2. The PSS was circulated via a double-jacketed heating coil placed in line from the external reservoir allowing the temperature in the chamber to be maintained at 36.5 ± 0.5°C. The pH in the arteriograph was 7.4 ± 0.05.

After the artery was mounted, the myograph was placed on the stage of an inverted microscope, and the vessel was observed through a videocamera attached to the microscope. Inner diameter and wall thickness were obtained by using a Video Dimension Analyzer (Living Systems). All data were saved cumulatively on a computer. The inner diameter and the wall thickness were measured at a pressure of 10 mmHg after the vessel had acclimatized at 37°C for 15 min.

**Pressure-Diameter Responses and Dilator Responses**

To obtain a pressure-diameter curve, the intraluminal pressure was increased in 10-mmHg increments, and a 5-min period allowed each vessel to reach a new steady-state diameter. In the 3-mo-old mice, the pressure was increased until a maximum pressure of 120 mmHg; in the 11-mo-old mice, the maximum pressure was only 80 mmHg. After the pressure-diameter curve was obtained, the pressure was maintained at 80 mmHg and each vessel rested for 30 min. During this time, the vessel diameter spontaneously reduced and was maintained at a new value representing pressure-induced vasoconstriction. Cumulative concentration-response curves to 10–8–10–6 M ACh and 10–8–10–6 M sodium nitroprusside (SNP) were made by addition of these agents to the external reservoir and by allowing 5-min periods between successive additions.

After an initial concentration-response curve to ACh, tissues from the 11-mo-old mice were incubated with 10–8 M of the dual-endothelin receptor (ETα/ETb) antagonist bosentan for 60 min. A second pressure-diameter curve was obtained, starting at a pressure of 10 mmHg and increasing by 10-mmHg increments until a maximum pressure of 80 mmHg was reached. Also, here the vessel was given a 5-min resting period between each increment, allowing the vessel to reach a new steady-state diameter. At the end of each experiment, tissues were exposed to calcium-free PSS to obtain the maximal passive diameter of each artery.
The intraluminal diameter in Ca\(^{2+}\) expressed as 100% 

**Expression of Results**

The total cross-sectional area of the heart to obtain the percent area of the total cross-sectional area of the heart was quantified and divided by the total cross-sectional area of the heart to obtain the percent area of fibrous tissue.

**Morphometric Analysis of Hypertrophic Hearts**

Hearts from TG and wild-type mice were harvested, formalin fixed, and paraffin embedded. With the use of the software Image Pro Plus, ventricular transverse sections of TG and wild-type hearts stained with Masson’s trichrome were used to visualize fibrosis. A color segmentation file was created that recognized light blue staining, and this was used to determine the fibrous area (mm\(^2\)) in the myocardium. The total area of light blue staining was quantified and divided by the total cross-sectional area of the heart to obtain the percent area of fibrous tissue.

**Expression of Results**

Vessel constriction in the pressure-diameter curves was expressed as 100% × [(\(D_{\text{Ca free}} - D_{\text{PSS}}\)/\(D_{\text{Ca free}}\)], where \(D\) is the intraluminal diameter in Ca\(^{2+}\)-free PSS or standard PSS. Vessel constriction was expressed as 100% × (\(D_{\text{conc of drug}} - D_{\text{PSS}}\)/\(D_{\text{PSS}}\)). The effect of bosentan on the myogenic tone was expressed as percent constriction at 80 mmHg minus percent constriction at 80 mmHg with bosentan.

**Statistics**

All results are expressed as means ± SE; \(n\), number of mice. 3WT, 3-mo-old male wild-type (WT) mice; 3TG, 3-mo-old transgenic (TG) mice; 11WT, 11-mo-old WT mice; 11TG, 11-mo-old TG mice.

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**Table 1. Morphometric measurements in arteries taken from 3-mo-old WT and TG and 11-mo-old male WT and TG mice**

<table>
<thead>
<tr>
<th></th>
<th>Internal Diameter, (\mu)m</th>
<th>Wall Thickness, (\mu)m</th>
<th>Wall Thickness/ Internal Diameter, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3WT</td>
<td>6–8</td>
<td>93.6 ± 5.6</td>
<td>17.6 ± 1.1</td>
</tr>
<tr>
<td>3TG</td>
<td>5–7</td>
<td>93.3 ± 8.4</td>
<td>18.1 ± 1.9</td>
</tr>
<tr>
<td>11WT</td>
<td>6</td>
<td>106.2 ± 7.4</td>
<td>17.2 ± 1.1</td>
</tr>
<tr>
<td>11TG</td>
<td>6</td>
<td>111.3 ± 7.8</td>
<td>18.0 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), number of mice. 3WT, 3-mo-old male wild-type (WT) mice; 3TG, 3-mo-old transgenic (TG) mice; 11WT, 11-mo-old WT mice; 11TG, 11-mo-old TG mice.
age-gated calcium channels, leading to contraction. To eliminate tone due to myogenic constriction, responses to 8–114 mM KCl were studied in coronary septal arteries at an intraluminal pressure of 30 mmHg. There were no differences in the maximal vasoconstriction induced by K^+/H11001, and no difference was found in sensitivity between wild-type and TG mice (Table 2).

ENDOTHELIN. The vasoconstrictor effects of the peptide endothelin-1 are receptor mediated; responses to cumulative additions of 10^{-11}–10^{-8} M endothelin-1 were studied in coronary septal arteries at 30 mmHg. There were no differences in the endothelin-induced vasoconstriction, and no difference in sensitivity was observed when wild-type and TG mice were compared (Table 2 and Fig. 4).

Dilation. SODIUM NITROPRUSSIDE. Coronary septal arteries with spontaneous myogenic tone (80 mmHg) dilated to 10^{-8}–10^{-5} M of the nitric oxide donor SNP. The response to SNP was similar in arteries from TG and wild-type mice (Table 2).

ACETYLCOLINE. ACh produces an endothelium-dependent, nitric oxide-mediated vasodilatation in blood vessels. Coronary arteries, which spontaneously developed myogenic tone (80 mmHg), were exposed to 10^{-9}–10^{-5} M ACh and vasodilatation recorded. There were no differences between TG and wild-type mice (Table 2).

Vascular Function in 11-Mo-Old Mice

To determine whether age is an important factor in the pathophysiological state and development of hypertrophic cardiomyopathy, a series of experiments was performed on 11-mo-old animals.

**Constriction. MYOGENIC REACTIVITY.** The myogenic response in 11-mo-old TG mice is impaired. The onset of myogenic tone occurs at similar transmural pressures in arteries from both wild-type and TG mice, but the TG mice are not able to develop as powerful a myogenic response as their wild-type littermates. The 11-mo-old TG mice reached a maximum constriction of only 32.3 ± 3.6% compared with 45.8 ± 4.0% in their wild-type littermates at a transmural pressure of 80 mmHg (P < 0.05, Fig. 3B). After incubation of the endothelin dual-receptor antagonist bosentan, the myogenic response in both wild-type mice and TG mice was substantially inhibited. The myogenic response was now 11.9 ± 1.7% in the wild-type mice and 11.3 ± 2.7% in the TG mice at a pressure of 80 mmHg (P > 0.05, Fig. 3B).

POTASSIUM. Exposing the coronary septal arteries to KCl (8–84 mM) resulted in a similar response at maximal concentration of KCl, but a slight difference was seen in pD2 (P < 0.05) when TG mice were compared with their wild-type littermates (Table 2).

ENDOTHELIN. When arteries from the 11-mo-old mice were exposed to 10^{-11}–10^{-8} M endothelin-1, both wild-type and TG mice showed constriction, but the TG mice showed an impaired sensitivity to endothelin-1 with a...
The wild-type littermates (P

Maximum responses and pD2 in arteries from male 3- and 11-mo-old WT and TG mice

Table 2. Maximum responses and pD2 in arteries from male 3- and 11-mo-old WT and TG mice exposed to KCl, ET-1, SNP, and ACh

<table>
<thead>
<tr>
<th></th>
<th>Maximum Response, %</th>
<th>pD2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>WT</td>
</tr>
<tr>
<td>Vasodilators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNP</td>
<td>3</td>
<td>88.1±3.6</td>
</tr>
<tr>
<td>SNP</td>
<td>11</td>
<td>94.8±2.3</td>
</tr>
<tr>
<td>ACh</td>
<td>3</td>
<td>88.9±3.7</td>
</tr>
<tr>
<td>ACh</td>
<td>11</td>
<td>76.9±6.8</td>
</tr>
<tr>
<td>Vasoconstrictors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>3</td>
<td>72.7±2.4</td>
</tr>
<tr>
<td>KCl</td>
<td>11</td>
<td>44.6±3.9</td>
</tr>
<tr>
<td>ET-1</td>
<td>3</td>
<td>46.1±4.6</td>
</tr>
<tr>
<td>ET-1</td>
<td>11</td>
<td>51.8±8.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of mice. ET-1, endothelin-1; SNP, sodium nitroprusside; ACh, acetylcholine. *P < 0.05 compared with WT.

pD2 = 8.8 ± 0.4 compared with a pD2 = 10.3 ± 0.3 in the wild-type littermates (P < 0.05) (Fig. 4).

Dilation. Sodium Nitroprusside. The maximum dilation of the coronary septal arteries in the 11-mo-old mice to 10^-8–10^-5 M of the nitric oxide donor SNP was similar in the TG mice compared with their wild-type littermates, and there were no differences in pD2 either (Table 2).

Acetylcholine. ACh-induced nitric oxide release from the endothelium was similar in both HCM and wild-type mice as gauged by equivalent maximal dilation and pD2 values (Table 2).

DISCUSSION

The main finding of the study was that in 11-mo-old mice the pressure-induced response of isolated coronary small arteries was reduced in the TG mice compared with their wild-type littermates, and that this difference in responsiveness disappeared after treatment with the dual-endothelin receptor antagonist bosentan. These observations suggest that an abnormality in the vascular endothelin system is present in the TG mice. To address the background for this, we investigated the responsiveness to exogenously applied endothelin and found a reduced responsiveness to endothelin. These results provide strong evidence for the possibility that the reduced pressure-induced response is reflecting a dependence on this response on vascular endothelin production and that a reduction of this responsiveness to endothelin is involved in the reduced pressure-induced response in the TG mice. The dependence of the pressure-induced response on vascular endothelin system (presumably release of endothelin from the artery wall) confirms our previous observations in CD-1 mice (8) and extends thereon to the C57BL6 mice used in this study. Others (2, 4, 16) have also reported a role for endothelin in the myogenic response in a number of laboratory animals as well as in humans (7). In hypertensive animals, an abnormality of the vascular endothelin physiology has also been suggested to play a role (18). In hypertension, though, the system may be responsible for an enhancement of the pressure-induced response (2, 4). This is in contrast to the reduced responsiveness we report in coronary arteries from HCM (also known as TG) mice.

It is also of substantial importance to appreciate that apart from the change in myogenic tone, which could be ascribed to a change in endothelin responsiveness, there were no other differences between the coronary arteries from 11-mo (and 3 mo)-old mice. The responses to potassium were identical indicating that depolarization-induced contractility is unaffected, supporting the interpretation that the reduced response to endothelin is not the result of a general reduction of the contractility of smooth muscle. Furthermore, the responses to ACh and to SNP were identical in the two groups indicating that the endothelium, nitric oxide, cGMP axes were not affected.

To assess the time course for the development of the abnormality in the vascular endothelin system, 3-mo-old mice were also investigated. The observation that the pressure-induced response was not impaired in 3-mo-old HCM mice indicates that the vascular expression of the disease may not be completely manifest until later in adulthood when growth and development have occurred. In this regard, it is of interest that, whereas coronary blood flow (in ml·min⁻¹·g heart wt⁻¹) was similar in 3-mo-old wild-type and HCM

Fig. 4. Concentration-constriction curves to ET in 3- (n = 6–8) and 11-mo-old (n = 6) WT and TG mice. Results are normalized to resting tone at 30 mmHg.
mice, there was a nearly 50% reduction of resting coronary blood flow in HCM mice at 10 mo of age (17), and these data strongly implicate a late development of vascular changes in this model of HCM.

The pathophysiological consequence of a reduced pressure-induced response may be important if coronary autoregulation is impaired in HCM. In particular, if a fall in coronary perfusion pressure does not lead to an appropriate reduction of vascular tone, an ischemic response may more easily be elicited. Of interest in this context are the findings of Krams et al. (6), who found that coronary resistance values in HCM patients were lower under resting conditions but similar to control patients during reactive hyperemia. Although this finding may have many explanations, it is also consistent with reduced pressure-induced tone of the coronary arteries in these patients. The reduced pressure-induced response would also tend to increase capillary pressure for a given change in blood pressure, potentially leading to edema formation in the heart. An aberrant vasoregulation may thus lead to ischemia and in part be responsible for the observation that many patients with HCM have small vessel coronary disease (13, 14, 19). It should be appreciated, though, that the in vitro experiments reported here are made in the absence of flow and without the influence of neural and hormonal input. Whereas this has some advantages, it also calls for caution in interpretation in terms of pathophysiological importance in vivo.

The main finding in this study, that there is an abnormality in the endothelin physiology of the coronary arteries, which has consequences for the pressure-induced response in this model of HCM, leads to a number of new questions. Two very important questions that need to be addressed are 1) which part of the excitation-contraction coupling for endothelin is abnormal and 2) which mechanisms are of importance for the change in the endothelin responsiveness between the age of 3 and 11 mo? Our observations of a reduced pressure-induced response, which is bosentan sensitive, and a reduced responsiveness to exogenous endothelin thus provide evidence for an important aspect of the pathophysiology of HCM and provides the background for further intensive investigation.

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