Pheochromocytoma-Induced Cardiomyopathy is Modulated by the Synergistic Effects of Cell-Secreted Factors

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Background—Pheochromocytomas are rare tumors derived from the chromaffin cells of the adrenal medulla. Although these tumors have long been postulated to induce hypertension and cardiomyopathy through the hypersecretion of catecholamines, catecholamines alone may not fully explain the profound myocardial remodeling induced by these tumors. We sought to determine whether changes in myocardial function in pheochromocytoma-induced cardiomyopathy result solely from catecholamines secretion or from multiple pheochromocytoma-derived factors.

Methods and Results—Isolated cardiomyocytes incubated with pheochromocytoma-conditioned growth media contracted at a higher frequency than cardiomyocytes incubated with norepinephrine (NE) only. Sprague-Dawley rats and black-6 mice were implanted with aga-rose-encapsulated pheochromocytoma (PC12) cells, dihydroxyphenylalanine decarboxylase knock-out PC12 cells deficient in NE (PC12-KO), or NE-secreting pumps. PC12 cell implantation increased left ventricular dilation by 35±6% and 9.6±1.4% and reduced left ventricular fractional shortening by 20±3% and 28±4% in rats and mice compared with animals dosed only with NE, respectively. Elimination of NE secretion in PC12-KO cells induced neither cardiac dilation (3.9%±1.8% increase versus control) nor changes in (1.9%±0.4% reduction) fractional shortening compared to controls.

Conclusions—Pheochromocytomas induce a greater degree of cardiomyopathy than equivalent doses of NE, suggesting pheochromocytoma-induced cardiomyopathy is not solely mediated by NE, rather pheochromocytoma secretory factors in combination with catecholamines act synergistically to induce greater cardiac damage than catecholamines alone.

Key Words: norepinephrine ■ cardiomyopathy ■ catecholamines ■ heart failure

Pheochromocytomas are rare but devastating tumors arising from chromaffin cells of the adrenal medulla or extra-adrenal paraganglia. These tumors often induce alterations in myocardial structure and function, leading to eventual development of severe cardiomyopathy.1–5 Pheochromocytomas are characterized by hypersecretion of catecholamines, namely norepinephrine (NE) and epinephrine, which are most often hypothesized to be the primary cause of tumor-induced alterations in cardiac function. Excessive adrenergic stimulation can induce and exacerbate cardiovascular disease.6–8 Exogenous epinephrine9,10 and NE11,12 are cardiotoxic in a dose-dependent fashion.13,14 Cardiomyocyte viability is decreased as a function of NE concentration15 mediated by β adrenergic receptor stimulation, increased cAMP, and calcium influx.15 Selective stimulation of β adrenergic receptors mimics NE cardiotoxicity, and β adrenergic receptor blockade significantly attenuates these toxic effects.16 Infusion of NE increases systolic blood pressure, downregulates β adrenergic receptor, and alters left ventricular (LV) contractility. LV hypertrophy is characterized by multifocal mixed inflammatory infiltrates, acute myocardial degeneration,13,17 and increased interstitial fibrosis.18–20

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However, it is still unclear whether catecholamine excess alone can explain the severity of cardiomyopathy with pheochromocytoma and heart failure in the absence of blood pressure effects. Only one third of patients with these tumors are persistently hypertensive and onset of cardiomyopathy does not correlate with blood pressure or circulating catecholamine.21 Previous experiments with pheochromocytoma implants did not control cell growth and consequently NE secretions were excessively high, nor did they directly compare pheochromocytoma effects to equivalent effects of NE alone.13,22,23 The lack of dose control makes direct comparison of cell and drug models problematic. It may be that these tumors secrete other factors that exacerbate catecholamine-
induced damage or are cardiotoxic themselves. To determine the factors secreted by pheochromocytoma cells responsible for cardiomyopathy induction at low levels of NE, the secretion of NE by pheochromocytoma cells must be replicated in vivo at a concentration and rate equal to that of pheochromocytoma-bearing animals. To investigate the development of dilated cardiomyopathy in the presence of a pheochromocytoma, we engineered a novel polymeric encapsulation system enabling the implantation of a pheochromocytoma cell line into a murine model, allowing for the control of tumor cell growth and subsequent factor secretion. The effects of pheochromocytoma cells on cardiac and cellular function and remodeling were compared with the effects of NE alone. Our successful development of a new animal model of pheochromocytoma-induced cardiomyopathy allowed us to demonstrate differential effects of, and responses to, complete pheochromocytoma secretions versus catecholamines, yielding new insight into the etiology, pathogenesis, and approaches for treating pheochromocytoma-induced cardiomyopathy.

Methods

Cell Culture and Encapsulation

Rat pheochromocytoma cells (PC12, ATCC, Manassas, Va) were maintained in F12K media (ATCC) with 10% horse serum (Hyclone, Logan, Utah), 5% FBS (Hyclone) and 100 U/mL penicillin/streptomycin (Invitrogen, Carlsbad, Calif).25,26 Cells were grown at 37°C and 5% CO2, scraped and resuspended in a preheated solution of 2.5% (w/w) agarose (type VII, Sigma-Aldrich, St Louis, Mo) in 0.9% NaCl. The mixture was drawn into an Eppendorf Repeater Pipette with a 0.5 mL Combitip (VWR, Earth City, Mo) and 10 µL aliquots sheared into mineral oil (~600 mL) forming cell-encapsulating agarose beads as described.27 The beads were separated using a 1000 µm pore size mesh (Small Parts, Miami Lakes, Fla) and washed with PBS. Cell number increased from 104 to 1.8×106 cells per bead more than 21 days and following growth kinetics of PC12 cells on tissue culture polystyrene plates. Each bead secreted 460 pg/day of NE by ELISA. Dopamine levels in PC12 cells were undetectable,28 as were epinephrine levels given the low or nonexistent expression of phenylethanolamine-N-methyltransferase, the enzyme responsible for converting NE to epinephrine.29 The lack of epinephrine or dopamine secretion makes these cells then an ideal side-by-side comparison to the NE-secreting pumps.

Cardiomyocyte Contractility

Cardiomyocytes were obtained from 2-day-old neonatal Sprague-Dawley rats (Taconic, Hudson, NY). Cardiac ventricles were minced, incubated in trypsin (0.6 mg/mL in HBSS) for 16 hours at 4°C and digested with collagenase type II (Sigma-Aldrich), 1 mg/mL. Cells were resuspended in DMEM supplemented with 10% FBS, 25 mmol/L HEPES and penicillin (100 U/mL). Each ventricle yielded ~6×106 cells with viability between 88% and 94%. Myocytes were plated on 60-mm culture dishes and incubated with varying concentrations of control media, NE media, or PC12-conditioned media for 20 minutes. PC12 media was collected from separate dishes containing varying numbers of beads, the media was assayed for NE and matched with newly prepared NE media. Contractility was recorded in a temperature-controlled chamber mounted with a digital video camera (Olympus, DP70, New York, NY). Cardiomyocyte contractility was quantified with MatLab (Mathworks, Natick, Mass). All in vitro contractile studies were performed at constant temperature and CO2 to reduce environmental impact on contractile function.

Transfection of Dihydroxyphenylalanine Decarboxylase Short Hairpin RNA

Phoenix cells (Origen, San Diego, Calif) were transfected with a single dihydroxyphenylalanine decarboxylase short hairpin RNA construct (Origen, Rockville, Md) and were selected for 3 to 4 weeks in 2 µg/mL of puromycin to generate stably transfected, retrovirus-producing cells. Constructs used for short hairpin RNA were GGTGTATGGCTGACATTGTAGCTGCTGATA. PC12 cells were exposed to retrovirus expressing dihydroxyphenylalanine decarboxylase short hairpin RNA or empty vector in the presence of 0.5 µg/mL polybrene (Sigma-Aldrich) for 4 to 6 hours. The media was replaced with media containing retrovirus and the transfectants were incubated overnight. Transfected cells were then selected with 1 µg/mL puromycin for 4 to 5 weeks and used in experiments.

Animal Experiments

Female Sprague-Dawley rats (8 weeks old, 250 g) and female black-6 mice (7 to 8 weeks old, 15 to 20 g) were obtained from Taconic. All animal studies were performed in accordance with protocols approved by the MIT Institutional Animal Care and Use Committee and Harvard Medical School’s IACUC and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Osmotic pumps (model 2004 [rats] and Model 1004 [mice], Alzet, Cupertino, CA) or agarose-encapsulated PC12 cells were implanted in the retroperitoneal cavity, mimicking the spatial release of pheochromocytoma cells. A total of 20 agarose-encapsulated PC12 or PC12-KO beads were implanted. The 9.2 ng/day NE secretion rate of the 20 agarose-PC12 beads was matched with osmotic pumps (Alzet) delivering 0.25 µL/hour (rats) and 0.11 µL/hour (mice) loaded with 9.06 and 21 µmol/L solution of NE in acidic saline (0.1 mg/mL ascorbic acid in saline) for rats and mice, respectively. The ascorbic acid solution retards catecholamine oxidation. Catecholamine levels were tracked through weekly blood draws via the retro-orbital plexus and quantified by ELISA (Rocky Mountain Diagnostics, Colorado Springs, Colo). Control animals received osmotic pumps loaded with acidic saline carrier solution alone. Animals implanted with empty agarose beads and nonsurgical, nonimplanted animals had statistically identical cardiac dimensions and mRNA levels.

Echocardiographic and Hemodynamic Measurements

Echocardiography of anesthetized rats (pentobarbital 30 mg/kg IP) was performed at the 56-day end point with a linear array probe (RMV710B, Visual Sonics, Toronto, Canada) and a Visual Sonics Vevo 770. Cardiac dimensions were obtained from M-mode tracings using measurements averaged from 3 separate cardiac cycles by an echocardiographer blinded to the rat’s genotype. Arterial pressure was recorded by inserting a pressure-conductance catheter (SPR-878, Millar Instruments, Houston, Tex) into the LV via the right internal carotid artery. The catheter was connected to a pressure-conductance unit (MPV5–400, Millar Instruments) and waveforms were recorded using Chart software (AD Instruments, Colorado Springs, Colo). Data were analyzed with Millar PVAN software (Millar Instruments). Four randomly selected rats from each group were chosen for analysis.

Transthoracic echocardiography was performed in anesthetized mice (2% isoflurane) using a 12-MHz probe and a Sonos 5500 ultrasonograph (Hewlett-Packard, Marlborough, Mass). LV parameters and heart rates were obtained from M-mode interrogation in a short-axis view, averaged from 3 separate cardiac cycles at heart rates greater than 400 bpm. The echocardiographer was blinded to mice genotypes. Cardiac contractile function was represented by the parameter LV fractional shortening (FS) (percentage), calculated as [LV diastolic diameter − LV systolic diameter]/LV diastolic diameter × 100.
Cardiomyocyte Contractility

Freshly isolated neonatal cardiomyocytes were used to determine whether PC12-conditioned media induced a greater effect at the single cell level than cardiomyocytes dosed with identical concentrations of only NE. PC12-conditioned media caused cardiomyocytes to contract at a higher frequency than those incubated with identical NE doses and with nearly 2-fold greater contractility at 0.07 nM (P<0.01) (Figure 1).

Histological Analyses

Animals were killed, hearts excised, rinsed in PBS, weighed, and then pressure perfused (100 mm Hg) with PBS for 5 minutes followed by 10% neutral buffered formalin (NBF) until visibly firm and pale. The hearts were placed in 10% NBF overnight, processed, paraffin fixed (Polysciences Inc, Warrington, Pa), and serial coronal sections cut and stained with hematoxylin-eosin (Sigma-Aldrich) and Gomori trichrome (American HistoLabs Inc, Gaithersburg, Md). A pathologist blinded to the treatment groups graded the tissues. TUNEL assay was performed with an apoptosis kit (Millipore, Danvers, Mass) according to manufacturer’s instructions. Six images per heart were acquired on Leica microscope. Results were expressed as the number of apoptotic nuclei per total nuclei per image field.

RNA Preparation and Real-Time Polymerase Chain Reaction

Excised hearts were perfused with PBS, and a biopsy (8×8 mm) was taken. Total RNA was isolated with the use of Qiashredder and RNeasy spin columns (Qiagen, Valencia, Calif), cDNA was synthesized using a Taqman real-time polymerase chain reaction (RT-PCR) kit (Applied Biosystems, Foster City, Calif). Specific primers were designed using Primer3.30 RT-PCR was performed with an Opticon RT-PCR machine (Biorad, Hercules, Calif) using an SYBR Green PCR Master Mix Reagent Kit (Applied Biosystems). All samples (n=5 per group) were measured in triplicate and the expression level was normalized to GAPDH expression.

Statistical Analysis

Comparisons of data from 2 groups used Student t test and for multiple groups, 1-way ANOVA or 2-way ANOVA for repeated measurements (Minitab, State College, Pa). Contractility and LV mRNA data were analyzed for statistical significance using 2-way ANOVA followed by a Tukey post hoc test to determine significance. A value of P<0.05 was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Cardiomyocyte Contractility

The increased beating frequency induced by PC12-conditioned media compared with identical doses of NE occurred in a dose-dependent fashion.

Rat and Mouse Models, Cardiac Morphology, and Function

Experiments were performed in 2 species to verify the nature of the effects. NE secretion in pheochromocytoma-implanted (Pheo) animals was matched with the implantation of NE secreting pumps (Figure 2). Implanted pumps and PC12 cells in rats raised NE plasma values by 0.4 and 0.3 ng/mL, respectively. Implanted pumps, PC12 cells, and PC12-KO cells (Pheo-KO) in mice raised NE plasma values by 4, 3, and 0.7 ng/mL, respectively. To ensure the cardiac pathology observed was not a result of the host-PC12 cell interaction instead of PC12 secreted factors, empty agarose beads were implanted and their effects on cardiac pathology were compared with control rats. The heart weight normalized to body weight of rats implanted with empty agarose beads (6.0±0.5 μg/kg) was statistically identical to control rats (6.3±0.4 μg/kg). There was no detectable histological difference in the cellular and tissue response, indicating that host-cell interactions would not be a factor cardiomyopathy development.

Cardiac dilation was more pronounced in Pheo rats and mice (P<0.01 versus control, P<0.01 versus NE) compared with NE rats and mice (Figure 3A). The hearts of Pheo rats were 47% larger than controls (P<0.01) and NE rats (P<0.01), the latter statistically indistinguishable from controls (Table 1). Similarly, Pheo mice developed a 22% greater degree of dilation over NE mice (P<0.01), 17% dilation over Pheo-KO mice and 61% greater than controls (P<0.01).
Furthermore Pheo mice experienced a greater loss of cardiac function, evident in the 10.8±1.9% (P<0.01 versus control, P<0.05 versus NE) decrease in FS compared with NE mice that experienced a 6.3±2.9% (P<0.01) decrease in FS. These changes occurred despite the absence of significant increases in the systolic blood pressure or heart rates, and both were statistically indistinguishable from Pheo, Pheo-KO, and NE mice (Figure 3B and 3C). Of note, Pheo-KO mice did exhibit slight increase in cardiac dilation but no discernible loss of FS compared with controls (Table 2). LV end-systolic volume scaled linearly with NE levels (R²=0.916, P<0.0001) in control, pheo and pheo-KO mice but not in NE pump animals whose catecholamine levels were all at the upper limit and without correlating effect. Histological analyses of cardiac tissue showed little microscopic difference between Pheo, NE, Pheo-KO and control animals (Figure 4). TUNEL-positive apoptotic cells were identified in greatest density in Pheo animals (1.53±0.90 apoptotic nuclei/total nuclei, P=0.25 versus NE, P=0.10 versus control), intermediate density in NE animals (0.97±0.60 apoptotic nuclei/total nuclei, P=0.79 versus control), and lowest density in control animals (0.86±0.60 apoptotic nuclei/total nuclei).

Cardiac function was further characterized by LV catheterization 56 days after cell implantation or NE infusion. At the doses tested, NE increased myocardial function above control, with a steeper slope of the end-systolic pressure–volume relationship (P<0.05), decreased end diastolic pressure (P<0.05), and a higher maximum ventricular elastance (P<0.01) (Figure 5D through 5F). In contrast, at the same doses of NE released, pheo cells reduced all indices of cardiac function. There was a 73% reduction in the slope of the end-systolic pressure–volume relationship (P<0.01 versus NE rats, P<0.01 versus control), a 24% decrease in end diastolic pressure (P>0.05 versus NE rats, P<0.01 versus controls), and a 70% decrease in ventricular elastance (P=0.017 versus NE rats, P<0.01 versus controls) (Table 3).

### Table 1. Cardiac Morphology and Function of Control, NE, and Pheo Rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>NE</th>
<th>Pheo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. rats</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Age, wk</td>
<td>10±2</td>
<td>10±2</td>
<td>10±2</td>
</tr>
<tr>
<td>HW:TL, g/cm</td>
<td>0.17±0.04</td>
<td>0.19±0.02</td>
<td>0.25±0.02§</td>
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<tr>
<td>HR, bpm</td>
<td>317±15</td>
<td>360±45</td>
<td>362±19</td>
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<td>SBP, mm Hg</td>
<td>104±17</td>
<td>84±18</td>
<td>94±30</td>
</tr>
<tr>
<td>LVWT, mm</td>
<td>1.16±0.12</td>
<td>1.18±0.16</td>
<td>1.00±0.19</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>5.5±0.1</td>
<td>6.0±0.6§</td>
<td>8.1±0.3 §</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.4±0.6</td>
<td>3.4±0.6§</td>
<td>5.3±0.31 §</td>
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<tr>
<td>FS, %</td>
<td>49±4</td>
<td>43±2</td>
<td>34±3§</td>
</tr>
<tr>
<td>EF, %</td>
<td>39±6</td>
<td>36±4</td>
<td>27±4§</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD.

SBP indicates systolic blood pressure; LVWT, LV wall thickness at end diastole; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; EF, ejection fraction.

*P<0.05, †P<0.01 versus NE rats; ‡P<0.05, §P<0.01 versus controls.

### Table 2. Cardiac Morphology and Function of Control, NE, Pheo and Pheo-KO Mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>NE</th>
<th>Pheo</th>
<th>Pheo-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Mice</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Age, wk</td>
<td>7±2</td>
<td>7±2</td>
<td>7±2</td>
<td>7±2</td>
</tr>
<tr>
<td>DLWT, mm</td>
<td>-0.03±0.01</td>
<td>-0.13±0.03†</td>
<td>-0.09±0.03</td>
<td>-0.04±0.02</td>
</tr>
<tr>
<td>∆LVEDD, mm</td>
<td>-0.03±0.01</td>
<td>0.24±0.09†</td>
<td>0.34±0.04§</td>
<td>0.26±0.08†</td>
</tr>
<tr>
<td>∆LVESD, mm</td>
<td>-0.01±0.01</td>
<td>0.43±0.11§</td>
<td>0.63±0.07§</td>
<td>0.2±0.07†</td>
</tr>
<tr>
<td>∆FS, %</td>
<td>-1.2±0.3</td>
<td>-6.3±2.9</td>
<td>-10.8±1.9§</td>
<td>-1.0±1.5</td>
</tr>
<tr>
<td>∆EF, %</td>
<td>3.4±0.6</td>
<td>8.8±3.8</td>
<td>21.5±4.2</td>
<td>-1.6±1.9</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD.

∆LWT, change in LV wall thickness at end diastole; ∆LVEDD, change in LV end-diastolic diameter; ∆LVESD, change in LV end-systolic diameter; ∆FS, change in fractional shortening; ∆EF, change in ejection fraction.

*P<0.05, †P<0.01, §P<0.01 versus controls.
The decreased end-systolic pressure–volume relationship slope reflects a decrease in the heart’s inotropic capabilities. To maintain stroke volume under these circumstances, the ventricle will often operate at higher volumes in a process of compensatory dilation. This mechanism is further validated by the 96% and 36% increase in stroke volume for Pheo rats over controls and NE rats, respectively.

**Myocardial Gene Expression**

Cardiac extracellular matrix biomolecules play a central role in maladaptive myocardial remodeling and cardiac decompensation. Clinical studies during the past decade have shown increased levels of chemokine (CC) ligand 2 (CCL2, MCP-1), matrix metalloproteinase 3 (MMP3), collagen-1, and decreased levels of tissue inhibitor of matrix metalloproteinases 3 (TIMP3) to be associated with cardiovascular disease and cardiac dysfunction. Pheo-implants raised MMP3 (P<0.01), collagen-1 (P<0.05), CCL2 (P<0.01), and reduced TIMP3 (P<0.01) mRNA levels compared with NE rats (Figure 6). BNP mRNA levels were elevated 2.8±0.2-fold above control animals 28 days after implantation and remained elevated for the duration of the experiment. It took twice as long for NE rats to exhibit the same level of elevation. Levels of mRNA were identical to controls (100±20% over controls) at 28 days and became elevated (290±50% over controls) only at 56 days. These statistically significant changes highlight the ability of pheochromocytomas to induce cardiac pathology with accelerated kinetics versus NE alone. The changes in gene expression values bear a strong correlation to the heart’s morphological and functional changes as diagnosed echocardiographically. Specifically, increasing CCL2 levels correlate strongly with increasing LV end diastolic diameter (R²=0.92, P<0.0001) and decreasing FS (R²=-0.84, P=0.000). Similarly, increase in MMP3 and collagen correlate with increasing LV end diastolic diameter (R²=0.86, P<0.0001 for MMP3 and R²=0.73, P=0.003 for collagen), with increases in collagen highly correlative with decreases in FS (R²=-0.94, P<0.0001). TIMP3 mRNA levels further correlate with LV end diastolic diameter (R²=-0.81, P<0.0001) and FS (R²=0.66, P=0.010).

**Discussion**

Although catecholamines may dominate the cardiotoxic effects of late-stage pheochromocytomas, the secretion of low levels of catecholamines during early tumor development is insufficient to induce hemodynamic effects. Indeed, only 29% of all pheochromocytoma patients are persistently hypertensive, and only another 30% demonstrate episodic hypertension. Previous research has attributed the cardiotoxic-
Table 3. Hemodynamic Measurements of Control, NE and Pheo Rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>NE</th>
<th>Pheo</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESPVR slope, mm Hg/L</td>
<td>1.49±0.16</td>
<td>1.04±0.33</td>
<td>2.58±0.20†</td>
</tr>
<tr>
<td>Stroke volume, μL</td>
<td>53.3±1.4</td>
<td>76.4±6.0§</td>
<td>104.3±7.6†</td>
</tr>
<tr>
<td>End diastolic pressure, mmHg</td>
<td>4.51±0.14</td>
<td>3.47±0.61‡</td>
<td>3.41±0.25†</td>
</tr>
<tr>
<td>End diastolic volume, μL</td>
<td>275.5±3.5</td>
<td>284.3±3.4†</td>
<td>392.2±28.3‡†</td>
</tr>
<tr>
<td>Elastance, mm Hg/μL</td>
<td>2.19±0.32</td>
<td>0.954±0.18†</td>
<td>0.64±0.10‡†</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD (n=5 for all groups).
*P<0.01 versus NE rats; †P<0.05, ‡P<0.01 versus controls.

icity of pheochromocytomas to catecholamines but has not addressed whether other tumor secreted factors act to induce myocardial remodeling. Here we use agarose-encapsulation of PC12 cells that enables quantifiable and reproducible control of cell growth and NE release allowing for the matched secretion of NE by the PC12-agarose beads with implanted NE releasing osmotic pumps. We focused on the impact of low-grade, low-NE secreting tumors to separate the effects of noncatecholamine secretory products of early-stage pheochromocytomas from those of late-stage tumors with NE hypersecretion.

In vitro, pheochromocytoma cell-conditioned media induced more frequent cardiomyocyte contractions than NE alone suggesting that catecholamines are not solely responsible for the effects of pheochromocytomas on cardiomyocyte physiology. Animals implanted with PC12 cells exhibited far greater structural and functional impairment than animals with NE implants, all without changes in blood pressure or heart rate. In these similar hemodynamic domains, only pheochromocytoma cells induced significant cardiomyopathy in mice and rats. These changes closely mimic the human consequences of these tumors, as LV chamber size was enlarged, FS was reduced, and hemodynamic function altered, all to a far greater degree than with equivalent NE doses. These effects were absent with the elimination of NE secretion from PC12-KO cells, whose implantation failed to induce a cardiomyopathic state. The induction of cardiomyopathy therefore likely results from the confluence of pheochromocytoma-secreted factors, rather than from a single factor alone. Though NE is necessary to induce cardiomyopathy, it is not sufficient to explain the full force of the effects of pheochromocytoma on the heart.

The altered balance of the cytoskeletal proteins, MMP3, TIMP3, and collagen, creates maladaptive myocardial remodeling that mediates the transition from compensated to decompensated heart failure.31–33,35 MMP3 elevation and reduction in its inhibitor, TIMP3, exacerbates ventricular dilation by disintegrating the collagen network.32 High dose NE infusion increased myocardial collagen, myocyte diameter, and fibrosis with elevated TIMP3 and MMP3 mRNA levels.17 In line with these observations, collagen and MMP3 mRNA levels were significantly increased and TIMP3 levels decreased in pheochromocytoma implanted animals. The hearts of animals exposed to low doses of NE alone expressed minimal alterations in collagen, cardiac function, MMP3 levels, and TIMP3 levels. Other inflammatory mediators involved in the pathogenesis and progression of heart failure followed suit to provide further mechanistic insight. CCL2 mediates myocardial remodeling by promoting the attraction and invasion of activated leukocytes into damaged or inflamed tissue. Circulating CCL2 levels rise significantly in advanced dilated cardiomyopathy, congestive heart failure, and ischemic reperfusion injury.36,37 Similarly, CCL2 levels increased significantly in Pheo implanted rats at day 28 and continued to rise at day 56. In contrast, NE rats expressed normal levels of CCL2 at day 28 compared with controls, with levels only rising at 56 days. Our data demonstrating that CCL2 mRNA levels were upregulated to a greater extent in Pheo versus NE rats coincides with the changes seen in a variety of clinical structural pathologies.38

Figure 6. Rats implanted with pheochromocytoma cells increase mRNA levels of cardiac dysfunction associated proteins. Quantification of LV mRNA levels for Pheo and NE rats at 28 and 56 days. The mRNA levels were normalized to percent controls. CCL2, MMP3, and collagen mRNA levels were upregulated to a greater degree in rats implanted with pheochromocytomas than those implanted with NE pumps. TIMP3 levels were downregulated to a greater degree in Pheo rats than NE rats (n=5 rats per group). *P<0.05, **P<0.01 versus NE rats; †P<0.05, ††P<0.01 versus controls.
Taken together, our findings suggest that pheochromocytoma tumor cells in combination with their catecholamines act synergistically to induce greater cardiac damage than catecholamines alone. Numerous factors secreted by pheochromocytoma cells can directly or indirectly alter heart function.\textsuperscript{9,40} Our work suggests that it is the confluence of these factors that enable the synergistic induction of myocardial injury. The implications of our work lie not only in a better understanding of pheochromocytomas and their secretory products but also in the differences between cell-secreted substances and their exogenous analogues in isolation. Given that secondary factors are sufficient to induce pheochromocytoma-induced cardiomypathy in early-stage patients, these data further highlight the need for understanding the fundamental development of early pathology for the purpose of basic insight into cardiomyopathies, developing novel therapeutic approaches, and identifying pheochromocytoma screening tools other than catecholamine metabolites.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

The autonomic nervous system is an important regulatory system in heart failure. Circulating catecholamines are markers of and causal contributors to progression of myocardial disease. In this regard, the pheochromocytoma state presents a fascinating form of catecholamine excess. Pheochromocytomas induce a devastating cardiomyopathy, but a paradox remains in understanding the means by which these tumors induce cardiotoxicity. Previous work attributed the induction of cardiomyopathy by pheochromocytomas to the hemodynamic and direct toxic effects that follow the hypersecretion of catecholamines. Yet, only a fraction of patients with these tumors are hypertensive, and cardiac toxicity does not correlate with catecholamine levels. We show that catecholamine excess can induce heart failure but only at doses of catecholamines sufficiently high to induce hypertension and tachycardia. Pheochromocytoma cell implants in contrast induce cardiomyopathy, even when they secrete low levels of catecholamines that alone have no effect. These cell implants produce early and profound cardiac dilation and loss of contractility without hemodynamic changes. In this article, we demonstrate for the first time that it is the synergistic effect of the total secretory cocktail that induces cardiomyopathy, not catecholamines alone. Infusion of catecholamines alone cannot recapitulate the pheochromocytoma’s profound toxic effects. This work represents the first controlled and reproducible model of pheochromocytoma-induced cardiomyopathy and demonstrates that the effects of pheochromocytomas are induced by the synergistic effect of cell-secreted factors, not catecholamine excess alone. These results further our understanding of the general notion of myocardial disease generation, the course of cardiomyopathies, and the particular toxicity of pheochromocytomas and secretory tumors.
Pheochromocytoma-Induced Cardiomyopathy is Modulated by the Synergistic Effects of Cell-Secreted Factors


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