Epinephrine Is Required for Normal Cardiovascular Responses to Stress in the Phenylethanolamine N-Methyltransferase Knockout Mouse

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Background—Epinephrine (EPI) is an important neurotransmitter and hormone. Its role in regulating cardiovascular function at rest and with stress is unclear, however.

Methods and Results—An epinephrine-deficient mouse model was generated in which the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase was knocked out (KO). Blood pressure and heart rate were monitored by telemetry at rest and during graded treadmill exercise. Cardiac structure and function were evaluated by echocardiography in mice under 1 of 2 conditions: unstressed and lightly anesthetized or restrained and awake. In KO mice, resting cardiovascular function, including blood pressure, heart rate, and cardiac output, was the same as that in wild-type mice, and the basal norepinephrine plasma level was normal. However, inhibition of sympathetic innervation with the ganglion blocker hexamethonium caused a 54% smaller decrease in blood pressure in KO mice, and treadmill exercise caused an 11% higher increase in blood pressure, both suggesting impaired vasodilation in KO mice. Interestingly, phenylethanolamine N-methyltransferase KO did not change the heart rate response to ganglionic blockade and exercise. By echocardiography, KO mice had an increased ratio of left ventricular posterior wall thickness to internal dimensions but did not have cardiac hypertrophy, suggesting concentric remodeling in the KO heart. Finally, in restrained, awake KO mice, heart rate and ejection fraction remained normal, but cardiac output was significantly reduced because of diminished end-diastolic volume.

Conclusion—Our data suggest that epinephrine is required for normal blood pressure and cardiac filling responses to stress but is not required for tachycardia during stress or normal cardiovascular function at rest. (Circulation. 2007;116:1024-1031.)

Key Words: blood pressure • cardiac volume • catecholamines • exercise • stress

Epinephrine (EPI; also called adrenaline) is preserved throughout vertebrate evolution. In vivo, EPI is synthesized from norepinephrine (NE) in the reaction catalyzed by the final enzyme in the catecholamine pathway, phenylethanolamine N-methyltransferase (PNMT). EPI synthesis has been detected in the embryonic heart before either sympathetic innervation of the heart or production of EPI in adrenal chromaffin cells,1,2 the primary site for EPI synthesis in adult mammals. However, the role of EPI in normal cardiovascular function remains unclear. In humans, resting plasma EPI levels are usually <80 pg/mL, apparently below the threshold at which they influence heart rate (HR) and blood pressure (BP).3 The abolition of EPI release by adrenal medullotomy has no detectable effect in resting humans;4 but the cardiovascular effects of adrenal medullotomy are controversial in various animal models.5-9 On the other hand, the release of EPI from the adrenal medulla is among the first responses to many stressors, including dynamic exercise.10-12 As a major stress hormone, this blood-borne EPI is involved in regulating cardiovascular function during exercise. However, because of the simultaneous release of other hormones and neurotransmitters, especially NE from sympathetic nerves, it remains unclear whether EPI plays a primary role in the cardiovascular response to exercise or even if its role is stimulatory or inhibitory in nature, despite many exercise studies in medullectomized and/or denervated animals.3,13,14

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It has proved difficult to decipher the role of EPI for several reasons. First, the commonly used adrenal medullectomy can damage the adrenal cortex, altering release of corticosteroids. Second, the adrenal medulla releases not
only EPI but also NE, chromogranin A, cestatin, pancreastatin, neuropeptide Y, and other hormones. Third, EPI synthesis is concentrated in the adrenal medulla but also is present in many adult tissues outside the adrenal such as heart and blood vessels. These peripheral tissues might make enough EPI to tonically stimulate adrenergic receptors locally because EPI is a potent agonist of adrenergic receptors, especially the β1-adrenergic receptor, for which it has a much higher affinity than any other known endogenous compound. Fourth, although several PNMT inhibitors currently are available to block EPI synthesis in vivo, most PNMT inhibitors also inhibit other catecholamine processes such as monoamine oxidase and α1 receptors.

To further define the role of EPI in cardiovascular function, we made an EPI-deficient mouse model by selectively inactivating the PNMT gene. A prior report of a PNMT knockout (KO) did not study physiological effects. We used telemetry and echocardiography to study the cardiovascular responses to 2 acute stressors, treadmill exercise and restraint. Dynamic exercise activates both cardiac sympathetic outflow and adrenal medullary stimulation, causing increased BP and HR. Restraint is a potent emotional stressor that preferentially triggers adrenomedullary release of EPI. We also studied the response to ganglionic blockade with hexamethonium (HEX), which normally causes vasodilation. Our findings indicate that EPI is required for normal vasodilation and cardiac filling with stress but has little role during rest.

Methods

Gene Targeting
A 7.6-kb genomic PNMT fragment was cloned from a 129/SvJ mouse genomic DNA library (Incyte Genomics, St Louis, Mo). The targeting vector contained a 5’ arm of ~3.3 kb and a 3’ arm of ~2.9 kb (see Figure 1A). Polymerase chain reaction–based mutagenesis was used to convert the 1.1-kb PNMT fragment between the translational start codon ATG and the exon 3 ClaI site into an XhoI site, and Cre-recombinase cDNA and a PGK-Neo cassette were inserted into the XhoI site. This deleted a 1.1-kb fragment containing exon 1, intron 1, exon 2, intron 2, and part of exon 3 of the PNMT gene. The linearized construct was electroporated into 129/SvJ ES cells, and G418-resistant ES clones were screened for homologous gene targeting. Two of 288 clones had correct 5’ orientation and Cre-recombinase cDNA and the exon 3 ClaI site. Cre-recombinase was inserted into the PNMT gene targeting. A, Cre-Neo cassette replaced the PNMT gene from the translational start codon ATG to the exon 3 ClaI site. Cre-recombinase was inserted into the PNMT 5’ untranslated region sequence so that its transcription was prevented during embryogenesis.

Catecholamine Assay
Catecholamines were measured in plasma and urine from isoflurane-anesthetized mice or from whole-tissue homogenates. Blood was removed by cardiac puncture and preserved with EDTA and reduced glutathione, and organs were harvested immediately thereafter. Tissue catecholamines were determined using solvent extraction of catecholamines from tissue homogenate supernatants and then incubation in the presence of excess H-S-adenosylmethionine and rat liver catechol O-methyltransferase. The resulting H-O-methylated catecholamines were separated by thin-layer chromatography and quantified by liquid scintillation spectrometry. To validate the measurements, urine catecholamines also were determined by high-performance liquid chromatography.

Echocardiography
Transthoracic echocardiography was done either in lightly anesthetized mice with a Sonos 4500 Ultrasound Imaging System (model M2424A, Hewlett-Packard, Palo Alto, Calif) or in conscious, restrained mice with an Acuson Sequoia C256 ultrasound machine (Acuson, Mountain View, Calif). In the present study, light anesthesia was done with isoflurane to minimize any reduction in HR. Conscious echocardiography was done in unrestrained mice that were restrained in a soft plastic cone. Interventricular septal thickness (IVS), left ventricular internal dimension (LVID), and left ventricular posterior wall thickness (LVPW) were determined from 2-dimensional guided M-mode images at the end of diastole (d) and systole (s), with end diastole defined as the maximal LV diastolic dimension and end systole as the most anterior systolic excursion of the LV posterior wall. LV volume was calculated by the cubed method:

\[ \text{volume} = 1.047 \times (\text{LVID})^3. \]
Stroke volume (SV) and cardiac output (CO) were defined as follows:

\[ SV = EDV - ESV, \]

where EDV is end-diastolic volume and ESV is end-systolic volume, and

\[ CO = HR \times SV. \]

Ejection fraction (EF) was defined as follows:

\[ EF = \frac{EDV - ESV}{EDV} \times 100. \]

Fractional shortening (FS) was derived from this equation:

\[ FS = \frac{LVIDd - LVIDs}{LVIDd} \times 100. \]

LV mass (LVM in diastole, in mg) was computed by the M-mode (cubed) method as follows:

\[ LVM = 1.05 \times [(IVSd + LVIDd + LVPWd)^3 - (LVIDd)^3]. \]

**BP and HR**

Twenty-four-hour BP and HR were monitored in conscious, unrestrained mice in their home cages using the Data Sciences International (DSI; Transoma Medical, St Paul, Minn) Physio Tel telemetry system. Briefly, the arterial pressure catheter was inserted into the left carotid artery under isoflurane anesthesia, and the catheter was coupled to a TA11PA-C20 transmitter (DSI). The transmitter was then placed in the abdominal cavity and secured to the abdominal musculature. Telemetry signals were received by an antenna below the cage that relayed the data to a signal processor (DataQuest A.R.T. Gold version 2.3; DSI) connected to a Compaq desktop personal computer (Hewlett-Packard). Ten days after the implantation surgery, the basal BP and HR were recorded every 30 minutes for 24 hours with lights off at 7 PM and with lights on at 7 AM. We calculated mean arterial BP (MAP) as follows: MAP = DBP + (SBP - DBP)/3, where DBP and SBP are diastolic and systolic BPs, and we calculated HR over 24 hours and during daytime and nighttime. The mice were then given an intraperitoneal bolus of 30 mg/kg HEX, and their BPs and HRs were recorded by telemetry every 10 seconds over 30 minutes. The maximal changes in values during the 30-minute period are reported. We calculated the average 1-minute values (6 measurements) at 10, 20, and 30 minutes after injection. After at least 1 day of recovery, BP and HR during graded treadmill exercise were then measured in these conscious mice fitted with DSI transmitters. After waiting approximately 30 minutes for the mouse to stay still on the treadmill out of the cage, we measured the baselines of BP and HR at rest for 10 minutes, and then the mouse started to exercise on the treadmill at 0.06 m/s. The BP and HR during exercise were recorded every 10 seconds. The exercise speed was increased by 0.03 m/s every 2 minutes until the mouse did not maintain a given speed because of exhaustion. The average MAP and HR for each speed were calculated.

**Statistics**

Data are presented as mean ± SE and were compared by an unpaired t test or by 2-way ANOVA with repeated-measures test for >2 groups. Values of P<0.05 on a 2-sided test were considered significant.

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

**Results**

The PNMT gene was replaced with Cre by homologous recombination-guided gene targeting to generate a PNMT KO mouse, as illustrated in Figure 1A. After germ-line transmission of the KO allele, heterozygous KO pairs were intercrossed to generate WT, PNMT heterozygous KO (HET), and PNMT KO mice. Figure 1B shows a Southern blot using tail DNA from the offspring of a PNMT HET intercross. From 161 intercross progeny screened at weaning, 39 WT mice, 78 PNMT HET mice, and 44 PNMT KO mice were identified. These results are consistent with the expected mendelian frequency (χ²=0.78; P>0.6). Thus, there was no embryonic or postnatal lethality associated with disruption of the PNMT gene in mice. After maturing into adults, KO mice appeared grossly normal and did not exhibit overtly abnormal behavior. Both KO males and females were fertile, indicating that EPI was not required for prenatal and postnatal development or for reproduction, in agreement with a prior report.

To verify that the genetic modification prevented expression of the PNMT gene, PNMT enzymatic activity was measured in 3 major PNMT-expressing tissues: adrenal gland, heart, and brain. PNMT activity was eliminated in the KO (Figure 2). In the HET, PNMT activity was 50% to 70% of WT (Figure 2).

To test whether the absence of PNMT expression altered catecholamine biosynthesis, catecholamines were determined in adrenal gland, plasma, and urine (Figure 3). The KO eliminated EPI in adrenal gland, plasma, and urine. The KO increased adrenal NE content significantly (Figure 3A), but the KO did not change adrenal dopamine or plasma or urine NE or dopamine (Figure 3). No significant differences in catecholamines were found between the HET and WT mice (data not shown). In summary, the PNMT KO eliminated EPI but did not change circulating NE.

To characterize the hemodynamic consequences of EPI deficiency, BP and HR were monitored by telemetry in conscious and unrestrained PNMT KO mice. As shown in Table 1, the basal 24-hour MAP and HR did not differ significantly between KO and WT mice. Furthermore, the nighttime MAP and HR were significantly higher than the daytime MAP and HR for both KO and WT mice (P<0.05 for all), and they were comparable between the 2 genotypes, suggesting that the diurnal variations of BP and HR were preserved in EPI-deficient mice. In summary, PNMT KO did not change resting BP or HR.

To begin to test the role of EPI in the regulation of BP and HR with stress, we studied the BP and HR responses to HEX, a ganglionic blocking agent. HEX blocks autonomic nervous system input at nicotinic ganglionic receptors, which are common to both the sympathetic and parasympathetic nervous systems. In mice, administration of HEX at the doses used in the present study causes a decrease in BP and HR. In contrast to humans, resting vagal tone is known to be minimal in mice, and the HR decrease after HEX is most likely due to blockade of β-adrenergic stimulation by HEX. After administration of HEX, BP and HR were measured every 10 seconds by telemetry over a period of 30 minutes (Figure 4 and Table 1). In WT mice, HEX produced a bradycardia and hypotension, as expected. In PNMT KO
mice, the bradycardic response to HEX was preserved (Figure 4 and Table 1), suggesting that when autonomic outflow is blocked, the intrinsic HR is comparable between KO and WT mice. However, the hypotensive response over time was significantly diminished ($P<0.05$; Figure 4), and the maximum BP drop after drug injection also was dramatically reduced by 54% of that in WT mice ($P<0.05$; Table 1). The reduced hypotensive response to HEX in the KO mouse suggested that vasodilation by EPI is required for the decrease in BP when sympathetic vasoconstriction is inhibited.

To investigate whether EPI deficiency in the PNMT KO mouse changed the cardiovascular response to the stress of exercise, BP and HR were measured by telemetry during graded treadmill exercise (Figure 5). PNMT KO and WT mice had similar HR increases during the exercise protocol. A significant difference, however, was observed in the BP response to exercise. During exercise, PNMT KO mice became hypertensive compared with WT mice. At the initial exercise level of 0.06 m/s, PNMT KO mice had an MAP of 136±4 mm Hg; this high level of BP remained throughout the exercise session, whereas the average MAP in WT mice (123±1 mm Hg) was 11% lower than that in KO mice. In summary, PNMT KO caused a hypertensive response to exercise but did not change exercise-induced tachycardia.

We used echocardiography to determine the effects of EPI deficiency on cardiac structure and function. Echocardiography was done in mice that were unstressed, anesthetized, or restrained and awake. HR in lightly anesthetized mice was nearly identical to that by telemetry in awake, unrestrained mice (Table 1 and data not shown), suggesting that cardiac function assessed by echocardiography under light anesthesia was comparable to basal cardiac function at rest. Under light anesthesia, HR and all echocardiographic variables, including CO, were identical in PNMT KO and WT mice, except that the KO heart had a small but significant increase in the ratio of LVPW to LVID in diastole (6% to 11%; $P<0.05$ versus KO vs WT for $P<0.05$).
WT: n=4 to 5; data not shown). In awake, restrained mice, HR by echocardiography was 21% to 51% faster than that in awake, unrestrained mice (Tables 1 and 2), suggesting elevated sympathoadrenal activation during echocardiography while restrained. During restraint, HR was the same in KO and WT mice (Table 2). LVPW and IVS, LVID, and LV weight (LVM and LVM index) did not differ significantly between KO and WT mice (Table 2), indicating no overall hypertrophic changes. However, as under light anesthesia but more prominently, the relative diastolic wall thickness, the ratio of LVPW to LVID, was significantly increased in KO mice (20% to 25%; Table 2). Furthermore, under the restrained condition, the EDV was significantly lower in KO mice than in WT mice (22% to 25%; Table 2). The smaller EDV caused a significantly smaller SV in KO mice (23% to 24%; Table 2), and as a result, CO was significantly decreased (26% to 29%), even though there was no change in HR or in cardiac contractility, as demonstrated by normal FS and EF. In summary, PNMT KO caused concentric LV remodeling and decreased EDV during stress.

**Discussion**

Using gene targeting, we generated mice with a disrupted PNMT gene. Based on functional analyses, this mutation abolished expression of the PNMT gene and thus prevented EPI synthesis in vivo, with no effect on circulating NE. By evaluating PNMT KO mice under unstressed, resting, and stressed conditions, we found that total elimination of EPI by PNMT gene KO had little impact on basal cardiovascular function at rest. However, denervation by ganglionic blockade, treadmill exercise, and restraint stress revealed functional deficits in vasodilation, BP response, and cardiac filling and CO. These results support the hypothesis that EPI is a significant modulator of cardiovascular function during stress but is not required for maintaining function at rest.

Central adrenergic neurons generally are thought to decrease sympathetic outflow by releasing EPI on preganglionic sympathetic neurons. However, loss of EPI in our PNMT KO mice did not significantly change sympathetic activity because the plasma and urine NE levels were the same in KO and WT mice, and the KO mice had normal HR and BP at rest. Therefore, our data suggest that central EPI is not important in maintaining overall sympathetic tone and cardiovascular function at rest. On the other hand, EPI is also

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**TABLE 1. Cardiovascular Indexes at Rest and Their Changes After Administration of HEX**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
<th>KO/WT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>11</td>
<td>12</td>
<td>...</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>98±3</td>
<td>100±2</td>
<td>102</td>
</tr>
<tr>
<td>Daytime</td>
<td>95±2</td>
<td>95±2</td>
<td>100</td>
</tr>
<tr>
<td>Nighttime</td>
<td>103±2</td>
<td>106±3</td>
<td>103</td>
</tr>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>497±15</td>
<td>522±10</td>
<td>105</td>
</tr>
<tr>
<td>Daytime</td>
<td>487±13</td>
<td>499±8</td>
<td>102</td>
</tr>
<tr>
<td>Nighttime</td>
<td>544±16</td>
<td>561±13</td>
<td>103</td>
</tr>
<tr>
<td><strong>Change after HEX</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔMAP, mm Hg</td>
<td>-35±9</td>
<td>-16±3</td>
<td>46*</td>
</tr>
<tr>
<td>ΔHR, bpm</td>
<td>-116±27</td>
<td>143±27</td>
<td>123</td>
</tr>
</tbody>
</table>

MAP and HR were measured with telemetry in adult male mice (age, 16 to 52 weeks; average, 27 weeks). ΔMAP and ΔHR represent maximum changes between 20 and 30 minutes after drug administration. Values are mean±SEM. *P<0.05 vs WT.

**Figure 4.** In vivo cardiovascular responses to HEX in PNMT KO and WT mice. After at least a 10-day recovery from implantation surgery, mice were given an intraperitoneal bolus of 30 mg/kg of HEX. HR (A) and MAP (B) were then monitored by telemetry over 30 minutes. Values shown represent the mean±SEM for WT (n=6) and PNMT KO (n=7) mice. P<0.05 for difference between MAP by repeated-measures ANOVA (time-by-genotype interaction).

**Figure 5.** Cardiovascular response to exercise in PNMT KO and WT mice. During a graded treadmill exercise program, HR (A) and MAP (B) were monitored by telemetry. Values shown represent the mean±SEM for WT (n=7) and PNMT KO (n=8) mice. P<0.01 for difference between MAP by repeated-measures ANOVA (treadmill speed–by-genotype interaction).
well known to regulate vascular tone by stimulating the peripheral β2-adrenergic receptors.19 The peripheral β2-adrenergic receptors mediate vascular smooth muscle relaxation, which leads to a decrease in total peripheral resistance and a lower BP. Indeed, physiological concentrations of plasma EPI decrease vascular resistance in humans.30 In addition to circulating EPI, EPI also is synthesized in endothelial cells of blood vessels.18 Thus, EPI may regulate vascular tone and BP locally. When sympathetic innervation was inhibited by ganglionic blockade with HEX in the present study, BP decreased in both KO and WT mice, but the BP decrease was significantly less in KO mice. Although HEX reduced BP mainly by blocking sympathetic innervation of arterioles,31 the reduced BP fall with ganglionic blockade in KO mice was likely due to the loss of EPI-mediated vasodilation rather than less sympathetic vasoconstriction because the sympathetic activity was normal in KO mice. Furthermore, and consistent with the smaller BP decrease with ganglionic blockade, PNMT KO mice had significantly higher BP during treadmill exercise. Vasodilation by EPI and metabolic byproducts are both thought to play a role in limiting the BP increase during exercise.32 The metabolic vasodilation did not appear to be altered in the KO mice because the levels of exercise achieved in WT and KO mice were the same. Therefore, the likely explanation of the higher BP in exercising KO mice is less vasodilation because of EPI deficiency. In agreement with this explanation, β2-adrenergic receptor KO mice also have a normal basal resting BP and become hypertensive during exercise.33 Taken together, these observations suggest that EPI-induced vasodilation is required to prevent BP overshoot during exercise but plays only a minimal role in maintaining BP at rest. Finally, the exaggerated BP response to exercise in EPI-deficient mice was associated with increased relative LV wall thickness but not overall cardiac hypertrophy, suggesting a concentric remodeling of the left ventricle of EPI-deficient mice. Although an exaggerated BP response to exercise can be used to predict new-onset hypertension in humans,34 EPI-deficient mice did not develop hypertension even at 1 year of age in the present study.

In addition to the β2-adrenergic receptor, EPI is also known as a potent agonist of cardiac α and β receptors, which can explain in part why intravenous infusion of EPI increases HR and CO in humans.30,35 In addition, EPI synthesis in the heart has been associated with the development of pacemaking and cardiac conduction in recent studies.1,2 However, total loss of EPI in our PNMT KO mice had little influence on resting cardiac function, including HR, EF, and CO. The intrinsic HR in EPI-deficient mice remained normal when autonomic innervation of the heart was inhibited by ganglionic blockade, and the positive cardiac chronotropic responses to both exercise and restraint in EPI-deficient mice were similar to those seen in WT mice. The identical HR responses to 2 acute stressors in KO and WT mice, as demonstrated here, argue against the conventional view of EPI as an important neurohormonal modulator of HR during stress and suggest that sympathetic neural control of HR (eg, through NE on β1-adrenergic receptors) is dominant in both KO and WT mice or at least compensates, as a redundant pathway, for the loss of cardiac stimulation by EPI in KO mice.

Despite the normal chronotropic response to restraint, CO in EPI-deficient mice was dramatically reduced during restraint in the present study. The decreased CO could be explained by decreased cardiac filling with a smaller EDV. Although the concentric remodeling of the left ventricle in our PNMT KO mice might somehow restrict venous return

### TABLE 2. Echocardiography in Conscious, Restrained PNMT KO Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male</th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>KO</td>
<td>KO/WT, %</td>
</tr>
<tr>
<td></td>
<td>WT</td>
<td>KO</td>
<td>KO/WT, %</td>
</tr>
<tr>
<td>No.</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>23±2</td>
<td>22±1</td>
<td>97</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>705±13</td>
<td>681±10</td>
<td>97</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.76±0.02</td>
<td>0.83±0.01</td>
<td>109</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.77±0.01</td>
<td>0.85±0.02</td>
<td>111</td>
</tr>
<tr>
<td>LVIdd, mm</td>
<td>3.55±0.10</td>
<td>3.27±0.04</td>
<td>92</td>
</tr>
<tr>
<td>LVIds, mm</td>
<td>1.99±0.13</td>
<td>1.91±0.03</td>
<td>96</td>
</tr>
<tr>
<td>LVPWd/LVIdd</td>
<td>0.26±0.01</td>
<td>0.22±0.01</td>
<td>120†</td>
</tr>
<tr>
<td>FS, %</td>
<td>44±2</td>
<td>41±1</td>
<td>94</td>
</tr>
<tr>
<td>EDV, μL</td>
<td>47±4</td>
<td>37±1</td>
<td>78†</td>
</tr>
<tr>
<td>ESV, μL</td>
<td>8.6±1.7</td>
<td>7.4±0.3</td>
<td>85</td>
</tr>
<tr>
<td>SV, μL</td>
<td>38±2</td>
<td>29±1</td>
<td>76†</td>
</tr>
<tr>
<td>EF, μL</td>
<td>82±2</td>
<td>80±1</td>
<td>97</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>27±2</td>
<td>20±1</td>
<td>74†</td>
</tr>
<tr>
<td>LVM, mg</td>
<td>90±4</td>
<td>90±1</td>
<td>100</td>
</tr>
<tr>
<td>LVM index, mg/g×10⁻³</td>
<td>4.0±0.2</td>
<td>4.1±0.2</td>
<td>104</td>
</tr>
</tbody>
</table>

BW indicates body weight. Two-dimensional guided M-mode echocardiography was performed on awake, restrained PNMT KO mice (age range, 7 to 16 weeks).

*P<0.05; †P<0.01, KO vs WT.
during diastole, EDV is also determined by venous tone. The reduced EDV during the stress of restraint in our EPI-deficient mice is consistent with previous studies\textsuperscript{36–40} that suggest that EPI has potent effects to constrict veins in vivo, mobilizing blood from the venous compartment and augmenting central venous pressure by decreasing venous compliance.

In summary, EPI deficiency has no impact on basal cardiovascular functions such as HR, BP, EF, and CO. However, the exaggerated BP response to exercise and reduced cardiac filling with restraint found in our PNMT KO mice indicate that EPI is required for maintaining normal cardiovascular function during stress. The adrenaland EPI response to various stressors is reduced with aging,\textsuperscript{41} and the salutary effects of EPI on cardiovascular adaptation to acute stress, as revealed here, might partly explain decreased cardiovascular homeostasis with stress in advancing age.\textsuperscript{42} The PNMT KO mouse will allow more thorough studies involving different types of stressors (such as induced heart failure) and longitudinal studies (eg, chronic stress) to further clarify the role of EPI in cardiovascular adaptation to stress.

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Disclosures
None.

References
Epinephrine, also called adrenaline, is the prototypical stress hormone. Blood epinephrine levels are quite low at rest but increase rapidly during restraint, exercise, and emotion. Epinephrine can cause muscle tremor and lower plasma potassium levels and can precipitate cardiac arrhythmias, leading one to question its benefits. A knockout mouse that lacked the enzyme needed to produce epinephrine was vital and grossly normal. The epinephrine-deficient mice had normal resting blood pressure and heart rate by 24-hour monitoring, and their chronotropic responses to stress such as exercise and restraint also were normal. However, during exercise, the blood pressure of epinephrine-deficient mice was 11% higher than in normal mice. Epinephrine improves vasodilation by stimulating $\beta_2$-adrenergic receptors. Epinephrine-induced vasodilation may not only help direct blood flow to exercising muscles but also temper the blood pressure increase associated with exercise. Furthermore, mice lacking epinephrine had poor cardiac filling, and their cardiac output was diminished by 25% during restraint. Therefore, the present study suggests that EPI is required for maintaining normal blood pressure and cardiac filling responses to stress. Human adrenal release of epinephrine decreases with age. The salutary effects of epinephrine on cardiovascular adaptation to acute stress, as revealed here, might partly explain decreased cardiovascular homeostasis with stress in advancing age.
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