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EXPERIMENTAL STUDIES

Important Role of Endogenous Norepinephrine and Epinephrine in the Development of In Vivo Pressure-Overload Cardiac Hypertrophy

Antonio Rapacciuolo, MD,* Giovanni Esposito, MD,* Kathleen Caron, PHD,† Lan Mao, MD,* Steven A. Thomas, MD, PHD,‡ Howard A. Rockman, MD*

Durham and Chapel Hill, North Carolina and Philadelphia, Pennsylvania

OBJECTIVES We sought to define the role of norepinephrine and epinephrine in the development of cardiac hypertrophy and to determine whether the absence of circulating catecholamines alters the activation of downstream myocardial signaling pathways.

BACKGROUND Cardiac hypertrophy is associated with elevated plasma catecholamine levels and an increase in cardiac morbidity and mortality. Although considerable evidence suggests that G-protein–coupled receptors are involved in the hypertrophic response, it remains controversial whether catecholamines are required for the development of in vivo cardiac hypertrophy.

METHODS We performed transverse aortic constriction (TAC) in dopamine beta-hydroxylase knockout mice ($Dbh^{-/-}$, genetically altered mice that are completely devoid of endogenous norepinephrine and epinephrine) and littermate control mice. After induction of cardiac hypertrophy, the mitogen-activated protein kinase (MAPK) signaling pathways were measured in pressure-overloaded/wild-type and $Dbh^{-/-}$ hearts.

RESULTS Compared with the control animals, cardiac hypertrophy was significantly blunted in $Dbh^{-/-}$ mice, which was not associated with altered cardiac function, as assessed by transthoracic echocardiography in conscious mice. The extracellularly regulated kinase (ERK 1/2), c-*jun*–NH_2-terminal kinase (JNK) and p38 MAPK pathways were all activated by two- to threefold after TAC in the control animals. In contrast, induction of the three pathways (ERK 1/2, JNK and p38) was completely abolished in $Dbh^{-/-}$ mice.

CONCLUSIONS These data demonstrate a nearly complete requirement of endogenous norepinephrine and epinephrine for the induction of in vivo pressure-overload cardiac hypertrophy and for the activation of hypertrophic signaling pathways. (J Am Coll Cardiol 2001;38:876–82)
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Cardiac hypertrophy occurs in response to increased stress on the heart. Although the development of myocardial hypertrophy is believed to be a compensatory mechanism to normalize wall stress and maintain normal cardiac function, epidemiologic studies have shown an association between ventricular hypertrophy and increased cardiac morbidity and mortality (1). Therefore, investigations that lead to a better understanding of the molecular signals that stimulate myocyte growth would have clear therapeutic implications for diseases characterized by cardiac hypertrophy. In this regard, considerable evidence suggests that G-protein–coupled receptors are involved in the hypertrophic response (2); however, the precise ligands and receptors that are most involved are largely unknown. Catecholamines are one possibility, because they work through G-protein–coupled receptors and are clearly relevant to maintain cardiac function in both health and disease. Nonetheless, whether catecholamines are required for the development of in vivo cardiac hypertrophy remains controversial (3).

Studies in both cultured neonatal rat cardiomyocytes (4) and experimental animals (5) have suggested that stimulation by catecholamines can produce a hypertrophic phenotype. In contrast, in vitro (6) and in vivo studies (7) have suggested that catecholamines are not important mediators of the hypertrophic response. To date, studies of gene-targeted mice have not resolved the issue of whether intracellular signals resulting from catecholamine stimulation are important for cardiac growth. For example, transgenic mice with cardiac overexpression of either the wild-type $beta_{1b}$-adrenergic receptor (AR) or $beta_2$-AR did not result in cardiac hypertrophy (8,9). Likewise, cardiac weight was unaffected in gene-targeted adult mice with disruption of genes encoding the $alpha_{1b}$-AR, $alpha_{1c}$-AR or $beta_1$-AR (10–12).

To determine whether catecholamines are required for the development of cardiac hypertrophy, we tested whether genetically altered mice that lack endogenous norepinephrine and epinephrine are able to develop cardiac hypertrophy in response to in vivo pressure overload. These mice have been created by targeted deletion of the dopamine beta-

From the *Department of Medicine, Duke University Medical Center, Durham, North Carolina; †University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and ‡Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania. This work was supported in part by Grant HL-61558 (to Dr. Rockman) from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland. Dr. Rockman is a recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

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Abbreviations and Acronyms

AR	= adrenergic receptor
BW	= body weight
$Dbh^{-/-}$	= dopamine beta-hydroxylase knockout mice
ERK	= extracellularly regulated kinase
%FS	= percent fractional shortening
JNK	= c-*jun*–NH_2-terminal kinase
L-DOPS	= L-threo-3,4-dihydroxyphenylserine
LV	= left ventricular
LVW	= left ventricular weight
MAPK	= mitogen-activated protein kinase
TAC	= transverse aortic constriction
TL	= tibial length

hydroxylase gene ($Dbh^{-/-}$), the essential enzyme in the biosynthetic pathway converting dopamine to norepinephrine (13,14). This model has the advantage of totally eliminating norepinephrine and epinephrine, while preserving the release of co-transmitters and other factors derived from the sympathetic nervous system or adrenal gland, or both. By measuring the response to transverse aortic constriction (TAC) in $Dbh^{-/-}$ and littermate control mice, we are able to definitively assess the role of norepinephrine and epinephrine in the development of cardiac hypertrophy and induction of downstream signaling pathways.

METHODS

Experimental animals. Adult (4 to 5 months old) dopamine beta-hydroxylase knockout mice ($Dbh^{-/-}$) were used in this study. Age-matched littermate heterozygotes ($Dbh^{+/-}$) were used as control animals. These $Dbh^{+/-}$ mice have been extensively characterized and shown to have normal levels of norepinephrine and epinephrine (13,14). The animals in this study were handled according to approved protocols and animal welfare regulations by the Institutional Review Board of Duke University Medical Center.

Echocardiography in conscious animals. Transthoracic two-dimensional guided M-mode echocardiography was performed, using an HDI 5000 echocardiograph (ATL, Bothell, Washington), in conscious mice, as previously described (15). In separate conscious $Dbh^{-/-}$ mice, echocardiography was performed before and 5 h after the subcutaneous administration of L-threo-3,4-dihydroxyphenylserine (L-DOPS) (0.4 mg/g) to transiently restore norepinephrine levels. DOPS is a synthetic amino acid that is converted to norepinephrine by aromatic L-amino-acid decarboxylase, which completely restores tissue and circulating norepinephrine levels after dorsal subcutaneous injection (16).

Transverse aortic constriction. After conscious echocardiography, mice were anesthetized and TAC or a sham operation was performed, as previously described (17,18). Seven days after the operation, conscious echocardiography was again performed, and the trans-stenotic gradient systolic pressure was assessed (17,18). The hearts were then excised; the chambers were dissected free and weighed and then frozen in liquid nitrogen. There was no difference in post-TAC mortality between the control and $Dbh^{-/-}$ mice.

In separate anesthetized control and $Dbh^{-/-}$ mice, intravenous infusion of either saline, angiotensin II (0.2 μmol/min) or norepinephrine (0.02 μmol/min) was administered through the jugular vein for 7 to 10 min, while monitoring arterial pressure. The dose of angiotensin II and norepinephrine was chosen to achieve a 50% increase in blood pressure. After infusion, the hearts were harvested and immediately frozen in liquid nitrogen for determination of mitogen-activated protein kinase (MAPK) activity.

Mitogen-activated protein kinase assays. The MAPK assays were performed on myocardial extracts from the left ventricles of the mice, as previously described (19). After immunoprecipitation with antibodies specific for the various MAPKs, reactions were performed in 20 μmol/liter of adenosine triphosphate (ATP), (γ-^{32}P)ATP (20 μCi/ml) and myelin basic protein (0.25 mg/ml) for extracellularly regulated kinase (ERK)/p38 or GST–c-*jun* (10 μg) for c-jun–NH_2-terminal kinase (JNK) and incubated at 30°C for 20 min, then terminated by adding 40 μl of 2× Laemmli buffer. Phosphorylated myelin basic protein and GST–c-*jun* were resolved by polyacrylamide gel electrophoresis and quantified with a phosphorimager.

Quantitative measurement of kidney renin ribonucleic acid (RNA). Total kidney RNA was prepared using TRIzol reagent (GibcoBRL, Grand Island, New York), according to manufacturer's instructions. Amounts of renin RNA per 0.5 mg of total RNA were determined using the ABI 7700 real-time reverse-transcription polymerase chain reaction system (Perkin Elmer Applied Biosystems, Foster, California). Primers and Taqman probes were determined by using Primer Express software (Perkin Elmer Applied Biosystems). The primers and sequences for renin amplification were RenF-5′-ACAGTATCCCAACAGGAGAGACAAG-3′; and RenR-5′-CACCCAGGACCCAGACA-3′. The renin Taqman probe sequence was 5′-FAM-TGGCTCTCCATGCCATGGACATCC-Tamra-3′. Beta-actin was used as the internal standard control agent in each sample. The primers and sequences for beta-actin amplification were beta-actinF-5′-CTGCCTGACGGCCAAGTC-3′; and beta-actinR-5′-CAAGAAGGAAGGCTGGAAAAGA-3′. The beta-actin Taqman probe sequence was 5′-TET-CACTATTGGCAACGAGCGGTTCCG-Tamra-3′. Each sample was measured twice.

Statistical analysis. Data are expressed as the mean value ± SEM. For comparison of the echocardiographic variables before and after TAC or treatment with L-DOPS, twice repeated measures analysis of variance (ANOVA) was used. When appropriate, post hoc analysis was performed using the Scheffé test. Analysis of the slopes of the regression lines for the relationship between cardiac mass and pressure gradient was performed using a test of parallelism

Table 1. Physiologic Variables in Control Mice

	Sham Operation (n = 12)		7 Days TAC (n = 13)	
	Before	After	Before	After
Physiologic variables				
BW (g)	31.92 ± 1.87	30.77 ± 1.96*	32.13 ± 1.85	29.76 ± 1.77*
LVW/BW ratio (mg/g)		3.04 ± 0.05		4.86 ± 0.28†
LVW/TL ratio (mg/mm)		5.1 ± 0.33		8.27 ± 0.68†
TSPG (mm Hg)				70.7 ± 5.6
LV/BW vs. sham operation (% change)				60
LV/TL vs. sham operation (% change)				62
Echocardiographic variables				
LVEDD (mm)			3.36 ± 0.12	3.25 ± 0.07
LVESD (mm)			1.80 ± 0.11	1.65 ± 0.09
FS (%)			46.8 ± 2.23	49.8 ± 2.22
SWT (mm)			0.79 ± 0.05	1.0 ± 0.06‡
PWT (mm)			0.76 ± 0.04	0.79 ± 0.05
HR (beats/min)			562 ± 38	592 ± 19
Mean Vcfc (circ/s)			2.64 ± 0.15	2.83 ± 0.17

*$p < 0.05$ for after vs. before either sham operation or TAC; †$p < 0.001$ for after TAC vs. after sham operation; ‡$p < 0.005$ for after TAC vs. before TAC. Data are presented as the mean value ± SEM.

BW = body weight; %FS = percent fractional shortening, calculated as (LVEDD − LVESD)*100/LVEDD; HR = heart rate; LVEDD = left ventricular end-diastolic dimension; LVESD = left ventricular end-systolic dimension; LVW = left ventricular weight; PWT = posterior wall thickness; SWT = septal wall thickness; TAC = transverse aortic constriction; TL = tibial length; TSPG = trans-stenotic systolic pressure gradient, calculated as the difference between right and left carotid artery systolic pressure; Vcfc = heart rate–corrected mean velocity of circumferential fiber shortening, calculated as fractional shortening divided by ejection time multiplied by the square root of the R-R interval.

by multivariate ANOVA. For heart weight and kinase activity data, unpaired *t* tests were performed. For all analyses, $p < 0.05$ was considered significant.

RESULTS

Evaluation of the hypertrophic response seven days after aortic constriction. To evaluate the hypertrophic response after TAC, we measured the increase in the left ventricular weight (LVW) to body weight (BW) ratio and the LVW to tibial length (TL) ratio after seven days of in vivo pressure overload. Seven days after TAC, control mice with normal norepinephrine and epinephrine levels developed significant left ventricular (LV) hypertrophy, with a 60% increase in the LVW/BW ratio and a 62% increase in the LVW/TL ratio, compared with sham-operated control mice. This degree of cardiac hypertrophy was induced with a mean trans-stenotic systolic pressure gradient of 70.7 ± 5.6 mm Hg (Table 1, Fig. 1). In marked contrast, the hypertrophic response after TAC was significantly blunted in the littermate *Dbh*$^{-/-}$ mice compared with those undergoing a sham operation, as measured by LVW/BW (19.7%) or LVW/TL (14%), even though the trans-stenotic pressure gradient remained elevated at 67.9 ± 5.5 mm Hg (Table 2, Fig. 1). In addition, peak LV systolic pressure, as measured by the carotid artery pressure proximal to the stenosis, was not different in the *Dbh*$^{-/-}$ mice compared with the control mice (174 ± 8.2 vs. 172 ± 5.6 mm Hg), indicating that the two groups of banded mice had matched hemodynamic loads. The LVW/BW and LVW/TL ratios in the sham-operated animals were not different between the two groups (Tables 1 and 2, Fig. 1).

The impaired hypertrophic response in the *Dbh*$^{-/-}$ mice occurred over a wide range of pressures, as shown in Figure 2. In the control group, a strong correlation between the index LV mass (LVW/BW) and trans-stenotic systolic pressure gradient was observed (Fig. 2). In contrast, *Dbh*$^{-/-}$ mice showed no relationship between the pressure load and degree of hypertrophy (Fig. 2).

Evaluation of cardiac function before and after aortic constriction. To eliminate the effect of anesthesia on LV performance, we evaluated LV function by echocardiography in conscious mice. As shown in Table 1, seven days of pressure overload in control mice did not affect the chamber diameters or percent fractional shortening (%FS). Septal wall thickness was increased, consistent with the induction of concentric LV hypertrophy after pressure overload.

Although the hypertrophic response was blunted in the *Dbh*$^{-/-}$ mice, cardiac function after TAC remained normal. The *Dbh*$^{-/-}$ mice showed no change in LV end-diastolic dimension after TAC. Furthermore, a small but significant increase in %FS after TAC was found in the *Dbh*$^{-/-}$ mice, as measured by noninvasive echocardiography (Table 2). Lower basal %FS, heart rate and heart rate–corrected mean velocity of circumferential fiber shortening (Vcfc) in the pre-TAC *Dbh*$^{-/-}$ mice are also shown in Table 2, compared with the pre-TAC littermate control animals (Table 1).

To determine whether the reduced basal cardiac function in the *Dbh*$^{-/-}$ mice reflects a developmental defect caused by norepinephrine deficiency, or one that results from a physiologic deficit of norepinephrine, we quickly restored norepinephrine levels by administering L-DOPS to sepa-

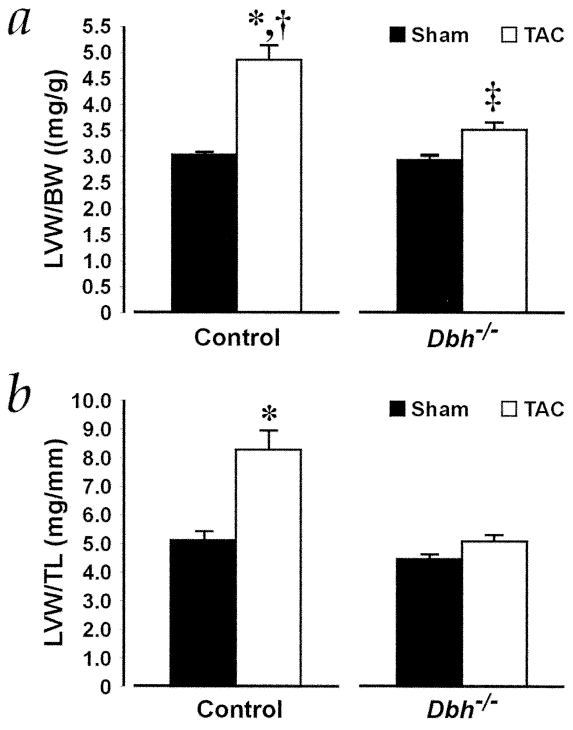

Figure 1. Effect of pressure overload on development of cardiac hypertrophy in control and $Dbh^{-/-}$ mice. **(a)** The LVW/BW ratio was measured in control and $Dbh^{-/-}$ mice seven days after either a sham operation or TAC. *p < 0.00001 for TAC control group vs. sham operation control group. †p < 0.005 for TAC control group vs. TAC $Dbh^{-/-}$ group. ‡p < 0.01 for TAC $Dbh^{-/-}$ group vs. sham operation $Dbh^{-/-}$ group. **(b)** The LVW/TL ratio was used as an index of the hypertrophic response after TAC. *p < 0.001 for TAC control group vs. either sham operation control group or TAC $Dbh^{-/-}$ group. Sham operation control group (n = 12); TAC control group (n = 13); sham operation $Dbh^{-/-}$ group (n = 13); and TAC $Dbh^{-/-}$ group (n = 11). Data are from the control and $Dbh^{-/-}$ mice with pressure gradients >40 mm Hg.

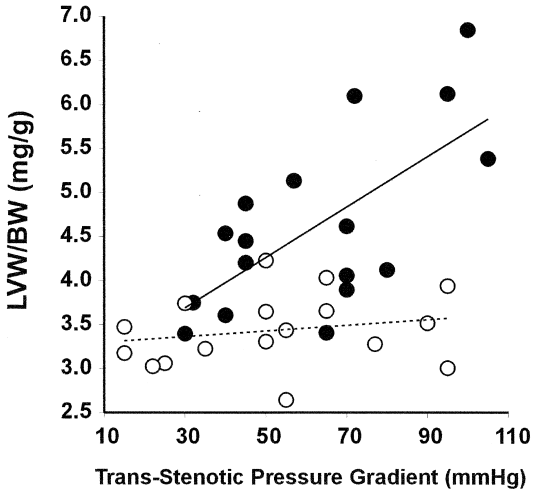

Figure 2. Relationship between pressure load and induction of cardiac hypertrophy for all banded mice. The index of LV mass (LVW/BW) is plotted against the trans-stenotic systolic pressure gradient produced by TAC for all of the banded control (n = 17, **solid circles**) and $Dbh^{-/-}$ (n = 17, **open circles**) animals. Linear regression analysis was significant for the control mice (y = 0.0286x + 2.8325, r = 0.66, p < 0.01 by ANOVA), but not for the $Dbh^{-/-}$ mice (0.0032x + 3.2665, r = 0.20, p = NS). The slopes of the two regression lines were significantly different (p < 0.005) by multivariate ANOVA.

rate out $Dbh^{-/-}$ mice, and then we measured cardiac function. DOPS is a synthetic amino acid that is converted to norepinephrine by aromatic L-amino-acid decarboxylase and restores tissue and circulating norepinephrine levels in the mouse 5 h after subcutaneous injection (16). As shown in Table 3, LV end-systolic dimension, heart rate, mean Vcfc and %FS significantly increased in the $Dbh^{-/-}$ mice 5 h after the early administration of DOPS. These results indicate that differences in basal function between control

Table 2. Physiologic Variables in $Dbh^{-/-}$ Mice

	Sham Operation (n = 13)		7 Days TAC (n = 11)	
	Before	After	Before	After
Physiologic variables				
BW (g)	28.03 ± 1.74	27.66 ± 1.79*	26.64 ± 1.00	25.57 ± 0.77*
LVW/BW ratio (mg/g)		2.94 ± 0.14		3.52 ± 0.14†
LVW/TL ratio (mg/mm)		4.45 ± 0.18		5.07 ± 0.24
TSPG (mm Hg)				67.9 ± 5.52
LV/BW vs. sham operation (% change)				19.7
LV/TL vs. sham operation (% change)				13.9
Echocardiographic variables				
LVEDD (mm)			3.36 ± 0.10	3.32 ± 0.08
LVESD (mm)			2.18 ± 0.10‡	1.95 ± 0.10
FS (%)			35.3 ± 1.53‡	41.6 ± 1.98§
SWT (mm)			0.70 ± 0.04	0.82 ± 0.03
PWT (mm)			0.68 ± 0.02	0.75 ± 0.01
HR (beats/min)			426 ± 13‡	433 ± 11
Mean Vcfc (circ/s)			2.11 ± 0.09‡	2.42 ± 0.14

*p < 0.05 for after vs. before either sham operation or TAC. †p < 0.05 for after TAC vs. after sham operation. ‡p < 0.005 for after TAC vs. before $Dbh^{-/-}$ mice vs. before TAC in control mice (see Table 1). §p < 0.05 for after TAC vs. before TAC. Data are presented as the mean value ± SEM. As a group, LVESD, %FS, SWT and HR were significantly different in the *Dbh* mice compared with the control mice at baseline (p < 0.01 by ANOVA).

Abbreviations as in Table 1.

Table 3. Echocardiographic Variables in $Dbh^{-/-}$ Mice Before and After Repletion of Norepinephrine with L-Threo-3,4-Dihydroxyphenylserine

	Before	After
LVEDD (mm)	3.30 ± 0.09	3.14 ± 0.05
LVESD (mm)	2.11 ± 0.12	1.43 ± 0.10*
FS (%)	36.11 ± 2.83	54.67 ± 2.36*
SWT (mm)	0.61 ± 0.04	0.69 ± 0.06
PWT (mm)	0.51 ± 0.03	0.57 ± 0.04
HR (beats/min)	405 ± 23	578 ± 23*
Mean Vcfc (circ/s)	2.12 ± 0.12	4.01 ± 0.22*

Echocardiography was performed in conscious mice (n = 7) before and 5 h after the dorsal subcutaneous injection of L-threo-3,4-dihydroxyphenylserine (L-DOPS, 0.4 mg/g). *p < 0.005 for after vs. before L-DOPS. Data are presented as the mean value ± SEM.

Abbreviations as in Table 1.

and $Dbh^{-/-}$ mice are the result of the physiologic loss of norepinephrine, rather than being secondary to a fixed developmental abnormality.

Role of norepinephrine and epinephrine in pressure-overload–induced MAPK activation. In hearts from six sham and six TAC control animals, the activity of ERK 1/2, JNK, p38 and p38-beta was evaluated seven days after the operation. As shown in Figure 3, a significant increase in activity of the three known MAPK pathways was found after TAC in hypertrophied control mice. In contrast, despite a comparable and persistent pressure load in the $Dbh^{-/-}$ mice, the induction of ERK 1/2, p38, p38-beta and JNK activation was completely abolished (Fig. 3). In a separate set of animals, the basal MAPK activity was not found to be different between the control group and the $Dbh^{-/-}$ group (n = 3 each).

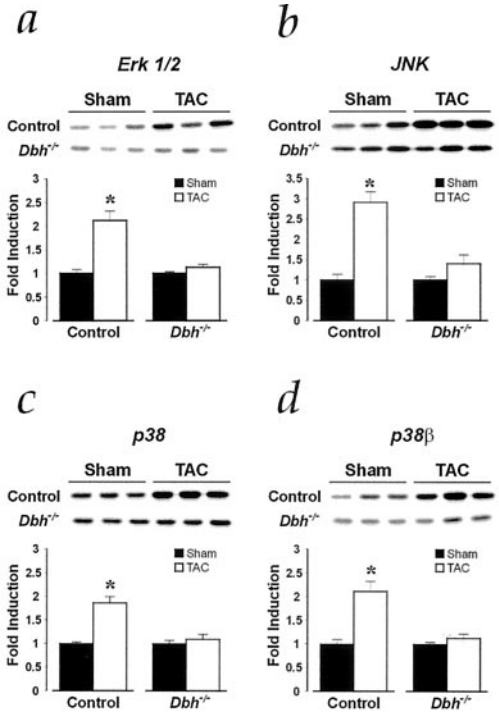

Figure 3. Effect of pressure overload on activation of MAPK pathways. Representative autoradiograms and summary data of MAPK activity for each of the three major pathways are shown. **(a)** ERK 1/2: *p < 0.0005 for sham operation control group vs. TAC control group. **(b)** JNK 1/2: *p < 0.0001 for sham operation control group vs. TAC control group. **(c)** p38: *p < 0.0005 for sham operation control group vs. TAC control group. **(d)** p38*β*: *p < 0.001 for sham operation control group vs. TAC control group. p = NS for sham operation $Dbh^{-/-}$ group vs. TAC $Dbh^{-/-}$ group for the aforementioned MAPKs.

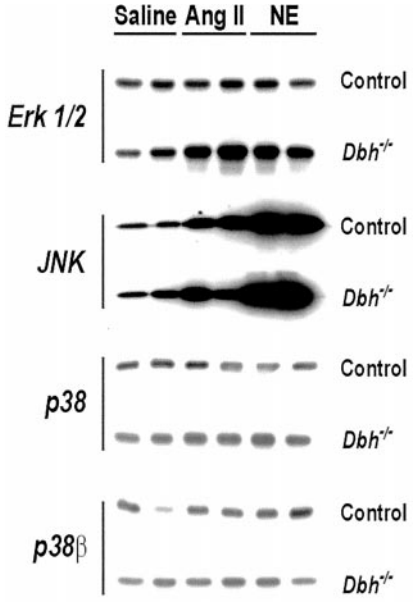

Figure 4. Effect of exogenous administration of norepinephrine (NE) and angiotensin II (Ang II) on activation of the MAPK pathways. Representative autoradiograms for each of the three major MAPK pathways are shown. The increase in ERK and JNK activity in the hearts of $Dbh^{-/-}$ mice after infusion of norepinephrine and angiotensin II was equal to or greater than that of the control hearts.

To test whether the MAPK pathways in the hearts of $Dbh^{-/-}$ mice could respond to the exogenous administration of neurohormones, MAPK activity was assessed after a 7- to 10-min infusion of either norepinephrine or angiotensin II in separate $Dbh^{-/-}$ and control mice. As shown in Figure 4, a robust increase in ERK and JNK MAPK activity was seen in the $Dbh^{-/-}$ mice, which was equal to or even greater than that observed in the control mice. Neither p38 nor p38-beta was activated by short infusion of norepinephrine or angiotensin II in either control or $Dbh^{-/-}$ mice. These data demonstrate that the hearts of $Dbh^{-/-}$ mice are able to respond to extracellular signals that activate MAPK pathways and support our hypothesis that endogenous norepinephrine and epinephrine are required for the development of cardiac hypertrophy in response to in vivo pressure overload.

Activation of the renin-angiotensin system after aortic constriction. The sympathetic nervous system is a very potent stimulatory factor for the control of renin secretion, which is mediated primarily by stimulation of $beta_1$-ARs on juxtaglomerular cells (20). To determine whether the sympathetic nervous system plays a prominent role in activation of the renin-angiotensin system during development of in vivo pressure-overload cardiac hypertrophy, renal renin messenger RNA (mRNA) levels were measured by real-time reverse-transcription polymerase chain reaction. It has

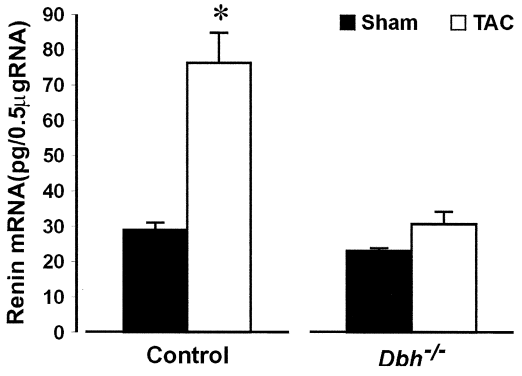

Figure 5. Role of norepinephrine and epinephrine in regulating activation of the renin-angiotensin system after aortic constriction. The renal renin messenger ribonucleic acid (mRNA) level was significantly increased in the banded control mice compared with the sham-operated mice, but not in the banded $Dbh^{-/-}$ mice. *$p < 0.001$ for sham operation control group vs. TAC control group; p = NS for sham operation $Dbh^{-/-}$ group vs. TAC $Dbh^{-/-}$ group. Sham operation: n = 6 in control group and n = 4 in $Dbh^{-/-}$ group; TAC: n = 4 in control group and n = 6 in $Dbh^{-/-}$ group.

been shown that renal renin mRNA levels accurately reflect homeostatic changes in the renin-angiotensin system (21). As shown in Figure 4, the renin level was significantly increased (2.5-fold) in the banded control mice compared with the sham-operated animals, indicating a robust activation of the renin-angiotensin system after TAC. In contrast, no increase in kidney renin levels was found after aortic banding in the $Dbh^{-/-}$ animals (Fig. 5).

DISCUSSION

The main purpose of this study was to define the role of norepinephrine and epinephrine in the development of cardiac hypertrophy and to determine whether the absence of circulating norepinephrine and epinephrine alters the activation of downstream signaling pathways. The first finding is that endogenous norepinephrine and epinephrine are required, to a large extent, for the development of cardiac hypertrophy in response to the physiologic stress of pressure overload. Most likely, this is the result of both a direct effect of norepinephrine and epinephrine on cardiac adrenergic receptors and an indirect effect through stimulation of renal renin release. Although autonomic regulation of cardiac contractile function is important, sympathetic nervous system hyperactivity can also initiate or accelerate cardiovascular pathology. The detrimental effects of catecholamines are likely due, in part, to the induction of myocyte apoptosis by chronic beta-AR stimulation (22).

Role of norepinephrine and epinephrine in development of cardiac hypertrophy. Early studies by Simpson (4) showed that cultured neonatal rat cardiomyocytes respond to norepinephrine stimulation, with an increase in myocyte size. Furthermore, long-term infusion of catecholamines in experimental animals can induce cardiac hypertrophy (5). In contrast, in experiments using loaded and unloaded papillary muscles in a cat model of ventricular overload, Cooper et al. (7) showed that catecholamines may not be important mediators of the hypertrophic response. Other studies (6) have also supported the conclusion that mechanical factors predominate in the development of cardiac hypertrophy, and, while catecholamines may play a role, their importance is not certain (3). The data from the present study clearly establish the important role of norepinephrine and epinephrine in the induction of cardiac hypertrophy and suggests that blocking the action of norepinephrine and epinephrine would not only be favorable with regard to cell survival, but it would also inhibit cardiac hypertrophy.

Role of norepinephrine and epinephrine on induction of MAPK activity. The second finding is that induction of a hypertrophic response is associated with activation of the three major MAPK pathways, and these MAPK signaling pathways may be activated both directly and indirectly by norepinephrine and epinephrine. G-protein–coupled receptors and G proteins are involved in regulation of the hypertrophic response through activation of MAPK pathways (19,23). Among the G proteins, Gq has been shown to be associated with the development of in vivo cardiac hypertrophy (17,24) and induction of MAPK activity, particularly ERK and JNK (19). A variety of ligands can activate Gq-coupled receptors, such as the $alpha_1$-AR (norepinephrine and phenylephrine), the angiotensin II type-1a receptor (AT_{1a}) and endothelin-1 receptor, which have all been shown to trigger cellular hypertrophy in cultured neonatal rat ventricular myocytes (4,25,26). The role of beta-ARs in the development of cardiac hypertrophy is more controversial. Early in vitro studies did not show that beta-AR stimulation contributes to myocyte hypertrophy (4), whereas more recent studies suggest that beta-AR stimulation can lead to both cellular hypertrophy and MAPK activation (27,28). In this regard, recent evidence suggests that the phosphorylated beta-ARs can activate the ERK pathway by switching coupling to the G protein called Gi, a process that involves the beta-gamma subunits (29).

Activation of the renin-angiotensin system by norepinephrine and epinephrine. The small residual hypertrophy observed in the pressure overloaded $Dbh^{-/-}$ mice could be due to autocrine or paracrine signaling mediated by fibroblast growth factor-2 (30) and/or other peptide growth factors, such as angiotensin II and endothelin I. Although it is interesting to note that the AT_{1a} knockout mice still develop robust hypertrophy in response to pressure overload (31,32), supporting our data of an important role for catecholamines, it is possible that signaling through either AT_{1b} (33) or AT_2 (34) receptors is sufficient for the development of hypertrophy. However, it is unlikely that the circulating renin-angiotensin system is responsible for the low residual hypertrophy in the $Dbh^{-/-}$ mice, on the basis of our third finding in this study. That is, endogenous norepinephrine and epinephrine are necessary for the activation of the renin-angiotensin system after imposition of mechanical pressure overload. Previous work has shown that release of renin from juxtaglomerular cells is under the control of $beta_1$-adrenergic stimulation (20). In this study,

we show that renin release, in response to hemodynamic stress, is dependent on activation of the sympathetic nervous system. It is interesting that the basal renin levels were normal in the $Dbh^{-/-}$ mice, suggesting that under normal homeostatic conditions, regulation of the renin-angiotensin system does not require endogenous norepinephrine or epinephrine.

It has been previously demonstrated that LV mass in normotensive and hypertensive patients is closely coupled to an increased cardiac sympathetic activity (35). In the same study, arterial blood pressure did not correlate with LV mass (35). Data from our study suggest that normalization of cardiac sympathetic tone is extremely important to reduce or prevent LV hypertrophy. Finally, this model will be valuable to determine whether preventing cardiac hypertrophy has adverse long-term consequences on cardiac function.

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Reprint requests and correspondence: Dr. Howard A. Rockman, Department of Medicine, Duke University Medical Center, DUMC 3104, Durham, North Carolina 27710. E-mail: h.rockman@duke.edu.

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