Disruption of cardiac Ena-VASP protein localization in intercalated disks causes dilated cardiomyopathy

Martin Eigenthaler,1 Stefan Engelhardt,2 Birgitta Schinke,1 Anna Kobsar,1 Eva Schmitteckert,2 Stepan Gambaryan,1 Catherine M. Engelhardt,1 Veit Krenn,4 Marina Eliava,3 Thomas Jarchau,1 Martin J. Lohse,2 Ulrich Walter,1 and Lutz Hein2

1Institut für Klinische Biochemie und Pathobiochemie, 2Institut für Pharmakologie und Toxikologie, and 3Institut für Anatomie, Universität Würzburg, 97078 Würzburg; and 4Institut für Pathologie der Charité, 10117 Berlin, Germany

Submitted 17 April 2003; accepted in final form 18 August 2003

Eigenthaler, Martin, Stefan Engelhardt, Birgitta Schinke, Anna Kobsar, Eva Schmitteckert, Stepan Gambaryan, Catherine M. Engelhardt, Veit Krenn, Marina Eliava, Thomas Jarchau, Martin J. Lohse, Ulrich Walter, and Lutz Hein. Disruption of cardiac Ena-VASP protein localization in intercalated disks causes dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 285: H2471–H2481, 2003. First published August 21, 2003; 10.1152/ajpheart.00362.2003.—Vasodilator-stimulated phosphoprotein (VASP) and mammalian enabled (Mena) are actin cytoskeleton signaling modulators. Ena-VASP proteins share an identical domain organization with an NH2-terminal Ena/VASP and mammalian enabled (Mena) are actin cytoskeleton signaling modulators. Ena-VASP proteins share highly homologous NH2- and COOH-terminal domains (Ena-VASP homology domains 1 and 2, respectively, designated EVH1 and EVH2) and a proline-rich central segment (Fig. 1A). In addition to VASP, this family includes the Drosophila protein enabled (Ena), the mammalian Ena homolog (Mena), and the Ena-VASP-like protein (7). Direct evidence for a functional overlap among the members of the Ena-VASP family was found in a Drosophila model system, in which VASP could partially substitute for the complete loss of Ena (1). Functional studies of Ena-VASP proteins demonstrated their important role in actin filament formation including the regulation of essential processes, such as cell adhesion, motility, and growth (4, 19; for recent reviews, see Refs. 25 and 26).

Ena-VASP proteins are associated with focal adhesions, cell-cell contacts, microfilaments, and highly dynamic membrane regions (for review, see Ref. 25). Via the proline-rich region, VASP, Mena, and Ena directly bind to profilin, whereas these proteins (via their EVH1 domain) also bind to FPPPPP-motif containing partners, such as the listerial protein ActA and the focal adhesion and cell-cell contact proteins vinculin and zyxin (25). In the heart, VASP is highly concentrated at intercalated disks (21). Proteins in myocyte-myoocyte contacts contribute significantly to the functional and morphological integrity of cardiomyocytes (13, 30). Major components of intercalated disks are N-cadherin, catenins, and vinculin at adherens junctions and connexins at gap junctions (Fig. 8). Changes of the cytoskeleton have been described to contribute to cardiac remodeling in heart failure. Upregulation of vinculin has been found in the failing human heart (14, 27), whereas deficiency of metavinuculin has been observed in dilated cardiomyopathy (20). In a recent study (24), mutational analysis of metavinuculin demonstrated the critical role of this intercalated disk component for cardiac structure and function and suggested that alterations of intercalated disk proteins are directly involved in the pathogenesis of dilated cardiomyopathy.

Gene-targeted mouse models have significantly advanced our understanding of the physiological rele-

Address for reprint requests and other correspondence: L. Hein, Institut für Pharmakologie, Versbacher Str. 9, 97078 Würzburg, Germany (E-mail: hein@toxi.uni-wuerzburg.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
vance of Ena-VASP proteins (2, 7, 10). However, mice lacking either VASP or Mena revealed no obvious cardiac abnormalities (2, 7, 10). To gain further insight into the in vivo function of Ena-VASP proteins in mammals, a combined knockout of VASP and Mena was attempted but double VASP/Mena-deficient mice died during embryonic development (4). As an alternative to gene targeting, overexpression of functional VASP or Mena protein domains ("dominant negative mutants") has been used to disrupt interaction of endogenous Ena-VASP proteins with their binding partners (31). To search for potential cardiac functions of Ena-VASP proteins, we have generated mice that express the EVH1 domain under control of the cardiac-specific α-myosin heavy chain (α-MHC) gene promoter. The VASP EVH1 domain has previously been shown by determination and functional characterization of its solution structure in complex with its FPPPP motif-containing ligands to constitute a structurally independent and functionally active protein module of VASP (3). Our results demonstrate that dominant negative disruption of intercalated disk localization of cardiac Ena-VASP proteins leads to dilated cardiomyopathy with myocyte hypertropy and bradycardia.

MATERIALS AND METHODS

Generation of transgenic mice. For cardiac-specific transgene expression, the coding region of the EVH1 domain of VASP (amino acids 1–115) was ligated downstream to the promoter of the murine α-MHC gene as depicted in Fig. 1. An 8-kb fragment containing the α-MHC promoter, the coding region of the VASP-EVH1 domain, and SV40 t-intron and poly A signal was separated from the plasmid DNA by restriction with NotI and the linear DNA (1 ng/μl) was microinjected into fertilized oocytes from superovulated FVB/N mice according to standard procedures (12). Injected oocytes
were transferred to the oviducts of pseudopregnant CD-1 mice. All mice were maintained in a specified pathogen-free facility. The generation and characterization of these mice was approved by the local animal experimentation and gene technology authorities (protocol no. 621-2531.01-15/97, 10/98, 28/01).

Transgenic founder mice were identified by Southern blotting or by PCR, as detailed in Fig. 1C. For PCR detection of transgenic mice, a sense primer (5'-AGTCAGGACTTCCACATA-GAAGCCTTAG-3') located in the o-MHC promoter and an antisense primer (5'-GGTCCCTTCAAGAGATGCTCGT-3') located in the VASP coding sequence were used. Transgenic mice from the F2 generation were mated with wild-type FVB/N mice, and transgenic and nontransgenic littermates were used for all backcrossing onto a C57BL/6 background for this study.

Transgene expression. Protein analysis was performed as described previously with minor modifications (10). Briefly, tissue samples were homogenized in buffer composed of 10 mM K2HPO4 (pH 7.4), 1 mM EDTA, 1 mM EGTA, and 1% Triton X-100 containing protease inhibitors (400 mM Pefabloc, 25 mM benzamidine, 2 mM pepstatin A, 5 μg/ml leupeptin, and 200 U/ml aprotinin). Protein content was measured by a Bradford assay. The homogenate was mixed with one-half of the volume of a 6% SDS-containing stop solution and analyzed on 8% or 13% SDS-polyacrylamide gels for VASP or the VASP-EVH1 domain, respectively. Proteins were transferred to nitrocellulose membranes, which were then analyzed with the use of a polyclonal anti-VASP or a polyclonal anti-VASP-EVH1 antiserum (10).

Histological analysis. After cervical dislocation, hearts were excised, rinsed briefly in PBS, fixed by immersion in 4% paraformaldehyde in PBS, and embedded in paraffin, and 5-μm-thick sections were stained with hematoxylin-eosin. The samples were examined and photographed at a magnification of ×160 with a Zeiss IM 35 microscope. Sections were analyzed by computer-assisted morphometry using an imaging system with a high-resolution CCD camera (Visitron; Puchheim, Germany). Midequatorial sections from wild-type and transgenic hearts were used for determination of myocyte cross-sectional area (15–20 nucleated myocytes per section; n = 5–10 mice per genotype; for details, see Table 1).

For electron microscopy, mice were perfused from the abdominal aorta with 2% paraformaldehyde and 0.2% glutaraldehyde in PBS. Heart slices were immersed in this fixative, washed with PBS, postfixed with 1% OsO4 for 1 h, and then embedded in Epon for electron microscopic analysis.

Immunocytochemistry. Tissues from PBS-paraformaldehyde (4%)-perfused mice were prepared and analyzed as described by blocking the samples with 5% goat serum and using affinity-purified polyonal VASP antiserum (1:500), a polyclonal VASP-EVH1 domain antiserum (1:500) and polyclonal Mena antiserum (1:1,500) (kindly provided by Dr. F. B. Gertler, Massachusetts Institute of Technology, and an monoclonal connexin-43 antibody (1:1,000) (10). Monoclonal antibodies against vinculin (clone J-VIN), sarcomeric α-actinin (clone EA-53), and rabbit anti-cadherin antiserum were obtained from Sigma and used at a dilution of 1:80,000, 1:10,000 and 1:1,000, respectively. Monoclonal antibodies against γ-catenin (clone 15, BD Transduction Laboratories) and desmoplakin 1 and 2 (clone DP1 and 2–2.15, Acris) were used at a dilution of 1:1,000 and 1:50, respectively.

ECG and in vivo cardiac catheterization. After anesthesia by intraperitoneal injection of triethoanethanol (13 μl of a 2.5% solution/g body wt), the mice were placed on a temperature-controlled pad (37°C) and ECG electrodes were attached. ECGs were recorded for 5 min and mice were then allowed to recover from anesthesia (12). To determine heart rates in conscious, unrestrained mice, the animals were placed in a Plexiglas box with electrodes attached to the floor of the box. The mice were allowed to accommodate to the new environment for at least 15 min and ECGs were recorded during a 5-min period. For left ventricular catheterization, mice were anesthetized as above and the right carotid artery was cannulated with a 1.4-F high-fidelity micromanometer (Millar Instruments; Houston, TX) (11). To ensure that the catheter was correctly positioned, the catheter in the left ventricle was assessed by continuously monitoring the pressure tracing. The output signal from the micromanometer was digitized at 4,000 Hz with a MacLab system (ADInstruments; Castle Hill, Australia) (6). For isotropic stimulation, dobutamine (20 μg/ml in 0.9% NaCl) was infused intravenously with a microinfusion pump (Braun; Mel- sungen, Germany).

Organ bath studies. Hearts were rapidly excised and placed in carbogenated modified Tyrode solution composed of (in mM) 119 NaCl, 5.4 KCl, 1.2 CaCl2, 1 MgCl2, 22.6 NaHCO3, 0.42 Na2HPO4, 0.025 EDTA, 10 glucose, and 0.2 ascorbic acid, pH 7.4 (6). The right atria were dissected, tied with two 7-0 silk sutures, and placed in a carbogenated 37°C tissue bath with modified Tyrode solution. Atria were allowed to contract spontaneously. Signals from isometric force

Table 1. Comparison of heart weight, body weight, and cardiac myocyte size in wild-type and VASP-EVH1 transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type E1</th>
<th>VASP-TG E1</th>
<th>Wild-Type E2</th>
<th>VASP-TG E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 1–2 mo</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>24.4 ± 0.9</td>
<td>11.5 ± 2.9*</td>
<td>23.8 ± 1.0</td>
<td>24.5 ± 1.5</td>
</tr>
<tr>
<td>Heart weight, mg/m2</td>
<td>111 ± 6</td>
<td>141 ± 30</td>
<td>104 ± 5</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>Heart/body weight, mg/g</td>
<td>4.5 ± 0.1</td>
<td>12.8 ± 2.9*</td>
<td>4.4 ± 0.1</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Heart weight/tibia length, mg/mm</td>
<td>6.8 ± 0.5</td>
<td>10.2 ± 2.0*</td>
<td>6.6 ± 0.1</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Myocyte cross section, μm²</td>
<td>395 ± 14</td>
<td>560 ± 22*</td>
<td>440 ± 37</td>
<td>421 ± 42</td>
</tr>
<tr>
<td>Age 3–4 mo</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>ND</td>
<td>ND</td>
<td>27.0 ± 2.4</td>
<td>26.2 ± 2.1</td>
</tr>
<tr>
<td>Heart weight, mg/m2</td>
<td>ND</td>
<td>ND</td>
<td>103 ± 6</td>
<td>117 ± 13*</td>
</tr>
<tr>
<td>Heart/body weight, mg/g</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>4.5 ± 0.2*</td>
<td>4.5 ± 0.2*</td>
</tr>
<tr>
<td>Heart weight/tibia length, mg/mm</td>
<td>ND</td>
<td>ND</td>
<td>5.7 ± 0.2</td>
<td>6.8 ± 0.6*</td>
</tr>
<tr>
<td>Myocyte cross section, μm²</td>
<td>ND</td>
<td>ND</td>
<td>610 ± 345</td>
<td>717 ± 31*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice per group. VASP, vasodilator-stimulated phosphoprotein; EVH1, Drosophila protein enabled (Ena) VASP homology-1; TG, transgenic; ND, not determined due to lethality of the E1 TG mice. *P < 0.05, TG vs. wild type.
transducers were fed via a bridge amplifier to a PowerLab system (ADInstruments).

RESULTS

Generation of VASP-EVH1 transgenic mice. To investigate the in vivo function of Ena-VASP proteins in the heart, we generated transgenic mice with cardiac-specific overexpression of the VASP-EVH1 domain (Fig. 1A). This approach has been shown in other cell systems to have a dominant negative effect on all members of the Ena-VASP protein family (31). For the transgene, we used exactly the part of the VASP protein that contains the EVH1 domain (VASP amino acids 1–115), the structure of which has been deter-

![Image of VASP and Connexin43 distribution in the heart of WT (A and B), VASP-deficient (C and D), and VASP-EVH1 TG mice (E–H). Immunostaining for VASP is shown in A, C, and E, and staining for connexin43 to identify intercalated disks is shown in B, D, and F. In VASP-EVH1-TG and VASP knockout (KO) mouse hearts, VASP was absent in intercalated disks (C and E). In VASP-EVH1-TG hearts, vinculin (G) and N-cadherin (H) showed normal localization in intercalated disks and costameres. Selected intercalated disks are indicated by arrows. Bars, 20 μm.](http://ajpheart.physiology.org/)

Fig. 2. Distribution of VASP in the heart of WT (A and B), VASP-deficient (C and D), and VASP-EVH1 TG mice (E–H). Immunostaining for VASP is shown in A, C, and E, and staining for connexin43 to identify intercalated disks is shown in B, D, and F. In VASP-EVH1-TG and VASP knockout (KO) mouse hearts, VASP was absent in intercalated disks (C and E). In VASP-EVH1-TG hearts, vinculin (G) and N-cadherin (H) showed normal localization in intercalated disks and costameres. Selected intercalated disks are indicated by arrows. Bars, 20 μm.
mined previously in complex with its FP4-containing ligand (3). For cardiac-specific expression, the EVH1 domain was placed under control of the α-MHC gene promoter (Fig. 1B) (22). From pronuclear injections of the linearized transgenic construct, three founder mice were obtained, which could be identified by Southern blotting with an EVH1-specific probe as a 1.7-kb fragment (Fig. 1C). One of the three founder mice died prematurely with a massively dilated heart. From the other founder mice, two independent transgenic mouse lines were established, designated E1 and E2. Approximately one-half of the offspring from these two founder mice were transgenic as detected by Southern blotting or by PCR screening. For this study, only mice from the F1 generation of transgenic lines E1 and E2 were investigated.

Expression of the transgenic VASP-EVH1 protein in the heart was verified by Western blotting (Fig. 1, D–F). The E1 line showed 2.5-fold higher expression of this domain in the heart than the E2 line, whereas no change in the amount of VASP or Mena protein in these lines compared with wild-type mice was observed (Fig. 1D, data for Mena not shown). In both lines, the EVH1 domain was detected in atria as well as in ventricles of transgenic hearts (Fig. 1E). Expression of the transgenic protein could not be detected in the lung, liver, kidney (Fig. 1F), brain, or platelets (data not shown).

Displacement of both VASP and Mena from cardiac intercalated disks in VASP-EVH1 transgenic mice. To determine the subcellular localization of VASP and Mena proteins in the heart of transgenic mice, cryostat sections were incubated with primary antibodies against full-length VASP and Mena proteins, which cannot detect the VASP-EVH1 domain (Figs. 2 and 3). In wild-type control hearts, both endogenous VASP and Mena were localized in intercalated disks of cardiac myocytes (Figs. 2 and 3) as well as in small blood vessels. Transgenic overexpression of the VASP-EVH1 domain led to a complete displacement of endogenous VASP from the intercalated disks (Fig. 2E). Importantly, immunostaining for Mena at this site was also greatly reduced (Fig. 3E), suggesting that the VASP-EVH1 domain has a dominant negative effect on both endogenous EVH1 containing proteins with respect to their localization in cardiac myocytes. In contrast to the VASP-EVH1 transgenic mice, Mena protein was

---

**Fig. 3.** Distribution of Mena and connexin43 in the heart of WT (A and B), VASP-deficient (C and D), and VASP-EVH1 TG mice (E and F). Immunostaining for mammalian Ena (Mena) is shown at left, staining for connexin43 to identify intercalated disks is shown at right. In VASP-deficient mice, a slight up-regulation of Mena in intercalated disks could be observed (C). In contrast, Mena was absent or greatly reduced in most intercalated disks of VASP-EVH1 TG mice (E). Selected intercalated disks are indicated by arrows. Bars, 20 μm.
abundantly expressed at intercalated disks of VASP-deficient mice (VASP-KO, Fig. 3C). Spred-2, a protein that contains an EVH-1 domain with only 30% homology to the VASP-EVH-1 domain (32), is also located at intercalated disks. No changes in the concentration and localization of Spred-2 at intercalated disks of the VASP-EVH1 transgenic mice were observed (data not shown), demonstrating the specificity of the effect of the VASP-EVH1 domain transgene. Furthermore, concentration and localization of vinculin, which is localized at intercalated disks and binds VASP via interaction with the VASP-EVH1 domain (Figs. 1A and 8), was unchanged in the in VASP-EVH1 transgene (Fig. 2G). In addition, localization of connexin43 and N-cadherin, which represent essential intercalated disk proteins, was also unaffected at the light-microscopical level by overexpression of the EVH1-domain (Figs. 2 and 3). Furthermore, localization of the desmosomal proteins desmoplakin and γ-catenin, and the sarcomeric protein α-actinin were not changed in the hearts of transgenic animals (Fig. 4). Taken together, these results support the hypothesis that overexpression of the VASP-EVH1 domain, which is capable to bind in vitro its cognate ligands (as previously shown in Ref. 3) exerts a specific dominant negative effect with respect to proper intercalated disk localization of the cardiac EVH1 domain-containing proteins VASP and Mena.

VASP-EVH1 transgenic mice develop cardiac dilatation and hypertrophy. Cardiac overexpression of the VASP-EVH1 domain led to perinatal lethality of transgenic offspring from the E1 founder within 2–6 wk after birth. However, transgenic mice from the E2 line showed normal survival until adulthood. Pathological investigation of transgenic hearts from the E1 line revealed massive dilatation of atria and ventricles compared with wild-type mice (Fig. 5). Many transgenic atria were greatly distended and filled with thrombi, which were mostly organized with fibroblasts. At the light microscopic level, myocytes were hypertrophied (Fig. 5). Morphometric analysis of cardiac myocytes revealed increased myocyte cross sectional areas in hearts from 1- to 2-mo-old E1 transgenic mice, but myocyte hypertrophy in the E2 line was not apparent before 3–4 mo of age (Table 1). Consistent with these histological results, heart weight normalized to body weight (line E2) or tibia length (lines E1 and E2) indicated the presence of cardiac hypertrophy in E1 and E2 transgenic mice (Table 1). Electron microscopical observation of transgenic hearts revealed normal myofilament architecture (not shown), but >40% of the intercalated disks were disorganized and fasciae adherentes were frequently interrupted in transgenic mice compared with wild-type cardiomyocytes (Fig. 5, E–G).

![Distribution of desmoplakin, γ-catenin, and α-actinin in the hearts of WT (A–C), VASP-deficient (D–F), and VASP-EVH1 TG mice (G–I). Immunostaining for desmoplakin (desmosomal protein) is shown in A, D, and G, staining for γ-catenin (plakoglobin, desmosomal protein) is shown in B, E, and H, and α-actinin (sarcomeric protein) is shown in C, F, and I. No changes in the localization of these proteins could be observed in VASP-deficient or in EVH1-TG mice. Desmoplakin and γ-catenin are localized in intercalated disks (arrows), whereas α-actinin shows the typical sarcomeric pattern of distribution. Bars, 20 μm.](http://ajpheart.physiology.org/)
Bradycardia in VASP-EVH1 transgenic mice. Several lines of evidence suggested that E1 transgenic offspring died from early heart failure and arrhythmia. E1 transgenic mice showed clinical signs of heart failure with dyspnea, ascites, congested lungs, and reduced weight gain after birth. In addition, we recorded ECGs from wild-type and transgenic mice under anesthesia. All transgenic mice had significantly lower heart rates than nontransgenic littermates at baseline (Fig. 6, A and B). In transgenic mice from the E1 line, basal heart rate was decreased to 50% of the respective control value. Furthermore, analysis of the ECGs of E1 transgenic animals revealed first-degree atrioventricular conduction block, as evidenced by a significant prolongation of the PR interval (Fig. 6A). Bradycardia was also apparent in E2 transgenic mice, with a 15% decrease of heart rate in these mice compared with control littermates (Fig. 6B). In contrast, VASP-deficient mice had normal heart frequency and atrioventricular conduction (Fig. 6B). Bradycardia was also observed in conscious, unrestrained mice of the E2 transgenic line (Fig. 6C). Mice of the E1 line were too young to obtain reliable ECG recordings without anesthesia.

These findings were further supported by in vitro studies on spontaneously beating isolated right atria which were mounted in an organ bath. Similar to the in vivo situation, isolated right atria from VASP-EVH1 transgenic mice were beating at a lower rate than the respective control atria (Fig. 6D). Again, bradycardia was also apparent in E2 transgenic mice, with a 15% decrease of heart rate in these mice compared with control littermates (Fig. 6B). In contrast, VASP-deficient mice had normal heart frequency and atrioventricular conduction (Fig. 6B). Bradycardia was also observed in conscious, unrestrained mice of the E2 transgenic line (Fig. 6C). Mice of the E1 line were too young to obtain reliable ECG recordings without anesthesia.

Bradycardia in VASP-EVH1 transgenic mice. Several lines of evidence suggested that E1 transgenic offspring died from early heart failure and arrhythmia. E1 transgenic mice showed clinical signs of heart failure with dyspnea, ascites, congested lungs, and reduced weight gain after birth. In addition, we recorded ECGs from wild-type and transgenic mice under anesthesia. All transgenic mice had significantly lower heart rates than nontransgenic littermates at baseline (Fig. 6, A and B). In transgenic mice from the E1 line, basal heart rate was decreased to 50% of the respective control value. Furthermore, analysis of the ECGs of E1 transgenic animals revealed first-degree atrioventricular conduction block, as evidenced by a significant prolongation of the PR interval (Fig. 6A). Bradycardia was also apparent in E2 transgenic mice, with a 15% decrease of heart rate in these mice compared with control littermates (Fig. 6B). In contrast, VASP-deficient mice had normal heart frequency and atrioventricular conduction (Fig. 6B). Bradycardia was also observed in conscious, unrestrained mice of the E2 transgenic line (Fig. 6C). Mice of the E1 line were too young to obtain reliable ECG recordings without anesthesia.
was more pronounced in atria from the E1 mouse line than in the E2 transgenic mice.

Left ventricular function of VASP-EVH1 transgenic mice. To directly assess left ventricular function, the mice were anesthetized, and a high-fidelity microtip catheter was advanced via the carotid artery into the left ventricle. At baseline, left ventricular contractility and heart rate were significantly lower in 3- to 4-month-old E2 transgenic mice than in matching littermates (Fig. 7, A and B). Aortic diastolic and systolic pressures did not differ between genotypes (data not shown). Maximal contractility stimulated by intravenous infusion of dobutamine reached significantly higher values in wild-type mice than in transgenic mice, despite similar maximal heart rates (Fig. 7, A and B). We were unable to obtain similar data from E1 transgenic mice, as they were too small for cardiac catheterization with a 1.4-F catheter before they developed severe heart failure (average body weight: 11.0 ± 2.9 g). Thus, in addition to basal bradycardia, VASP-EVH1 transgenic mice had decreased cardiac contractility.

DISCUSSION

The present study demonstrates for the first time in an in vivo model that proteins of the Ena-VASP family play an important role in maintaining the structural and functional integrity of cardiac myocytes. Mice with cardiac-specific overexpression of the VASP-EVH1 domain showed displacement of both Mena and VASP proteins from intercalated disks as observed by immunohistochemistry. Transgenic mice developed a severe dilated cardiomyopathy early after birth with dilatation of all cardiac chambers. These results demonstrate that the interaction of cardiac Ena-VASP proteins with components of the intercalated disk, e.g., vinculin (see Fig. 8), may play an important role in the control of electrical conduction and force transmission between cardiac myocytes.

In this study, we demonstrate that VASP and Mena are localized together with vinculin, cadherin, and con-
nexitin43 in intercalated disks, which suggests that Ena-VASP proteins may play a role in intercellular communication mediated by these subcellular structures. Intercalated disks are composed of three distinct structures including adherens junctions, desmosomes, and gap junctions (Fig. 8). Whereas gap junctions mediate direct electrical contact between adjacent myocytes, desmosomes and adherens junctions anchor thin filaments and intermediate filaments and transmit contractile force generated by actin-myosin filaments in the heart. Vinculin and its splicing variant metavinculin, which are the major cardiac binding partners of Ena-VASP proteins, are located at intercalated disks (5). VASP binds to vinculin via its EVH1-domain and the direct interaction between these two proteins has been demonstrated in the heart by coimmunoprecipitation (15). Targeted disruption of vinculin in mice causes loss of cardiac contractility during embryonic development (33). Most recently, metavinculin mutations have been shown to cause dilated cardiomyopathy in humans (24). Dilated cardiomyopathies are mostly associated with dysfunction of or mutations in cytoskeletal proteins (13, 28, 30). In our transgenic model system, desmosomal proteins that are associated with dilated left ventricular cardiomyopathy (23) were unchanged. Furthermore, no changes in the subcellular localization of the sarcomeric protein α-actinin could be detected.

Several findings suggest that cardiac dilatation and hypertrophy in EVH1 transgenic mice is caused by dominant negative disruption of Ena-VASP protein function in cardiac myocytes. Functional studies of Ena-VASP proteins demonstrated their important role in essential processes like cell adhesion, cell motility, cell growth, and regulation of signal transduction in a variety of cells (for recent reviews, see Refs. 25 and 26). The VASP-EVH1 domain, which was overexpressed in the transgenic mice, is a well-characterized protein domain with a structure that has been determined recently in a complex with one of its FPPP-containing ligands (3). It has also been shown to bind in vitro to FPPP-containing peptides of zyxin with an affinity nearly identical to that of the Mena EVH1 domain as judged by quantitative fluorescence spectroscopy (3). Furthermore, detailed structural comparison of the VASP and Mena EVH1 domains showed that the ligand binding sites are almost identical in both domains (3). Despite the fact that the transgenic EVH1 domain displaced VASP and Mena from their binding partners in intercalated disks, the major binding protein vinculin was not altered in its subcellular localization. Several lines of evidence suggest that the interaction between VASP and vinculin result in conformational changes in these proteins. Studies (17) in bacteria indicate that a structural change in the VASP protein is necessary for binding to vinculin, zyxin, and related proteins. At focal contacts, phosphatidylinositol 4,5-bisphosphate may lead to unmasking of VASP-vinculin interaction sites (15).

In our transgenic mice, cardiac-specific overexpression of the VASP-EVH1 domain was found to displace endogenous VASP and Mena proteins from intercalated disks in cardiac myocytes in vivo. Previous reports using peptides derived from a critical binding epitope of the EVH1 domain as well as overexpression of an EVH1 domain (or EVH2 domain) in vitro and in an in vivo mouse model resulted in the disturbance of cell interactions in epithelial sheet formation probably by a dominant negative effect on Ena-VASP proteins (31). However, cardiac structure and function as well as subcellular localization of Mena were essentially normal in mice carrying a deletion in the VASP gene (2, 10, 15, and this study). On the basis of results obtained from gene-targeted mice lacking VASP or Mena, cardiac functions of VASP and Mena may be at least partially overlapping. Genetic deletion of both VASP and Mena genes was lethal during late embryonic development (4). In contrast, our mice with α-MHC promoter-driven overexpression of a dominant negative EVH1-domain were expected to survive embryonic development because the α-MHC gene promoter is only expressed in the inflow tract of the developing heart which gives rise to the cardiac atria (22).

The bradycardia observed in E1 transgenic mice was quite severe as average heart rate was ~50% of the rate observed in wild-type littermates. Bradycardia was already detectable in 1-wk-old E1 transgenic mice before these mice showed clinical signs of heart failure including reduced weight gain. Postnatal heart rate is an essential determinant of cardiac output and severe bradycardia may precipitate acute
clinical heart failure (12). In the E2 transgenic mice, basal heart rate was only 20% lower than in wild-type littermates. This finding is in agreement with the observation that E2 transgenic mice survived to adulthood.

In summary, signaling from the cardiac plasma membrane to the actin cytoskeleton at intercalated disks may be, at least in part, modulated by Mena and VASP, which appear to have an essential role for cardiac structure and function in vivo. Although it is tempting to speculate that vinculin is the major EVH1 binding partner at intercalated disks, future studies will have to elucidate the precise identity of cardiac Mena-VASP binding proteins and their potential role in cardiac disease.

The authors thank Kerstin Hadamek for assistance with the electron microscopic analysis.

DISCLOSURES

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 355, TP C3, C9, and C10) and the EURCAR program of the European Union. A. Koobar was supported by the Interdisziplinäres Zentrum für Klinische Forschung Würzburg Teilprojekt E 15.

REFERENCES


29. Smolenski A, Burkhardt AM, Eigenthaler M, But E, Gambaryan S, Lohmann SM, and Walter U. Functional analysis of cGMP-dependent protein kinases I and II as mediators of...


