Journal of Cellular Biochemistry 72:8–15 (1999)

ARTICLES

Plakoglobin Is Essential for Myocardial Compliance but Dispensable for Myofibril Insertion Into Adherens Junctions

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Abstract Plakoglobin (γ-catenin), a member of the armadillo family of proteins, is a constituent of the cytoplasmic plaque of cardiac junctions and is involved in anchorage of cytoskeletal filaments to specific cadherins. Its genetic inactivation leads to an embryonic lethal phenotype due to heart dysfunction related to an impairment in the architecture of intercalated discs and in the stability of the heart tissue. To elucidate the functional consequences of the loss of plakoglobin for myofibrillar function, we monitored passive stress–strain relationship and contractility parameters of demembranated embryonic fibers. Heart fibers obtained from plakoglobin-deficient embryonic mice were significantly less compliant than were fibers from wild-type embryos. This difference was especially pronounced at lower fiber extension levels: at 120% of slack length, compliance was 2.5-fold lower in plakoglobin-deficient mice than in the corresponding wild-type group. Contractile paramenters (force per cross-section; Ca^{2+} sensitivity of isometric force and shortening velocity at near-zero load) were comparable in all experimental groups. Therefore, we suggest that plakoglobin is important for cardiac compliance but not necessary for the attachment of the myofibrillar apparatus to adherens junctions. Thus, we conclude that the loss of function of desmosomes and the profound disarrangement of junctional components in plakoglobin *null* embryos is associated with a decreased passive compliance, which may explain the ventricular rupture and consequent pericardial tamponade in embryos lacking plakoglobin. J. Cell. Biochem. 72:8–15, 1999.

Key words: plakoglobin; myofibers; mouse; embryo; heart; compliance; contractility

Plakoglobin (γ-catenin), first identified as a component of the cellular junctions desmosomes, is a member of the armadillo family of proteins [Cowin et al., 1986; Franke et al., 1989]. Armadillo and its vertebrate homologues, plakoglobin and β-catenin, are involved in the Wnt/wingless receptor-mediated signaling pathway, which modulates cell fate in embryos and is activated by a family of secreted glycoproteins encoded by the Wnt genes [Moon et al., 1997]. Plakoglobin is unique among junctional components because it anchors cytoskeletal filaments to both desmosomal and classic cadherins [Mathus et al., 1994; Sacco et al., 1995; Chitaev et al., 1996; Witcher et al., 1996]. The intercalated discs of the heart are extended regions of contact between cardiomyocytes in which different types of junctions occur side by side. Besides gap junctions, these are typical desmosomes that anchor desmin-containing intermediate filaments (IF) and adherens junctions at which the bundles of sarcomeric myofilaments attach [Forbes and Sperelakis, 1985]. Distinct desmosomes have been observed in very early stages of cardiac development of diverse vertebrate species and in cardiomyocyte cultures [Kartenbeck et al., 1983; Atherton et al., 1986; Kuruc and Franke, 1988; Shiozaki and Shimada, 1992; Viragh et al., 1993; Hertig et al., 1996]. Myocardiac desmosomes have been shown to contain cell-type-specific isoforms of the desmosomal cadherins, desmoplakin I, and certain cell-type-specific accessory proteins such as desmoplakin II and plakophilins 1 and 2 [Franke et al., 1982; Schwarz et al., 1990; Gar-

Contract grant sponsor: Deutsche Forschungsgemeinschaft; Contract grant number: DFG Mo 362/17–1.

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Received 23 April 1998; Accepted 2 June 1998

rod, 1993; Hatzfeld et al., 1994; Troyanovsky et al., 1994; Mertens et al., 1996]. By contrast, adherens junctions contain N-cadherin in association with plakoglobin, β- and α-catenin, vinculin, and other actin-binding proteins [Troyanovsky et al., 1994; Nieset et al., 1997].

Adherens junctions, into which myofibrils selectively project, act as a Z line, providing continuity of sarcomeres between cells [Forbes and Sperelakis, 1985]. The involvement of N-cadherin in myofibrillogenesis has been postulated [Goncharova et al., 1992; Soler and Knudsen, 1994]: antibodies to N-cadherin, which are capable of blocking adherens junction formation in culture, have an inhibitory effect on the development and alignment of myofibrils and on the contraction of both interacting and single myocytes. A recent study, however, has attenuated these speculations: N-cadherin-deficient mice develop several morphological disturbances and die at day 10 of embryonic development, but cardiomyocytes aggregate and beat synchronously [Radice et al., 1997]. The most dramatic defects of these mutant mice affect the primitive heart: in an expanded pericardial cavity a malformed heart tube is composed of myocardial and endocardial layers separated by cardiac jelly. In addition, cardiac myocytes surrounding the endocardium are unable to develop a normal myocardium and tend to dissociate. A more profound analysis of the consequences of N-cadherin loss with regard to myofibrillogenesis and myofibril function is still to be done.

A plakoglobin *null* mutation generated by gene targeting in embryonic stem cells demonstrates its involvement in the arrangement of intercalated discs and in the integrity of cardiac tissue [Ruiz et al., 1996]. Plakoglobin-deficient animals die during embryogenesis due to the disruption of their ventricles, a pathological feature related to the disappearance of cardiac desmosomes and the alteration in the sorting mechanisms of junctional components. The amplitude of heart contraction measured by using a high-resolution ultrasonic echo-tracking device has been found to be reduced in plakoglobin-deficient animals, whereas the heart rate is increased [Ruiz et al., 1996]. The effects of N-cadherin defiency on heart tube formation and myocardium development are more dramatic than those in the plakoglobin-deficient mice, thus emphasizing the role of plakoglobin in the stabilization of cardiac junctions and its maintenance more than in heart development per se.

Specific myofilament protein isoforms modulate myofibrillar activity, i.e., the rate and intensity of contraction. Contractile parameters can be determined by using an experimental system in which muscle fibers are demembranated. By chemical treatment, the external cell membrane is removed, leaving an intact contractile system. Such demembranated fibers represent an ideal model for a detailed analysis of myofibrillar function because isolated myofibrils can be exposed to a well-defined ionic concentration. For instance, the concentration of ATP and of the free calcium ion concentration can be accurately controlled [Fabiato and Fabiato, 1979]. This model offers the opportunity of analyzing the role of plakoglobin in the anchorage of myofibrils into adherens junctions. In addition, it is the first time, to our knowledge, that contractile parameters have been evaluated in embryonic mouse heart fibers.

Passive stress–strain curves, Ca^{2+}-activated force per cross-section, Ca^{2+} sensitivity of isometric force, and shortening velocity at near-zero load were determined by using demembranated multicellular cardiac preparations from 12–13-day-old homozygous and heterozygous mutant and wild-type plakoglobin embryos. No significant changes were found in Ca^{2+} sensitivity, force generation, and shortening velocity in plakoglobin-deficient embryos versus wild-type controls. However, passive stress–strain ratios were impaired in homozygous *null* embryos.

MATERIALS AND METHODS

The plakoglobin-deficient mice were generated by using embryonic stem cell technology; their phenotypic characterization has been described in detail elsewhere [Ruiz et al., 1996]. Cardiac ventricles from adult mice and from 12–13-day-old mutant and wild-type embryos were dissected for biochemical and mechanical analyses as described previously [Morano et al., 1996] and stored at −20°C.

Biochemical Analysis

Triton-demembranated fibers were washed twice in phosphate buffered saline, pH 7.2, and subsequently homogenized in sodium dodecylsulfate (SDS) sample buffer consisting of 5% SDS, 50 mM Tris HCl, pH 7.5, 250 mM sucrose, 75 mM urea, and 60 mM β-mercaptoethanol using a motor-driven glass-teflon homogenizer

three times for 10 sec each. The homogenate was denatured for 2 min at 95°C and cleared by centrifugation at 10.000*g* (Sorvall MC 12V; DuPont, Wilmington, DE) for 5 min. The protein concentration in the supernatant was determined by a modified Lowry method [Wang and Smith, 1975].

SDS-extracted proteins were separated on 8% polyacrylamide gels and transferred to nitrocellulose (Hybond C, Amersham, Arlington Heights, IL). Nitrocellulose filters were stained for protein with Ponceau S (Sigma, St. Louis, MO), scanned densitometrically, and processed for Western blot analysis. Briefly, the blots were blocked with ovalbumin and incubated with 1 µg/ml of an affinity-purified polyclonal rabbit anti-plakoglobin antibody generated against a peptide corresponding to amino acids 731–745 [Aberle et al., 1994]. Following subsequent incubation with peroxidase-conjugated anti-rabbit IgG antibody (BioGenes, Germany) diluted 1:10,000 for 60 min at room temperature, the immunoreactive protein was visualized by enhanced chemiluminiscence reaction kit (ECL) using an X-ray film (X-Omat, Kodak, Rochester, NY). Ponceau-stained myosin heavy chain (MHC) and plakoglobin ECL signals were evaluated by computer-assisted scanner densitometry (Epson GT 8000 Scan Pack, Biometra, Germany). Densitometrical analysis of the 200-kD MHC band and of the plakoglobin immunostaining was within the linear range of detection.

Mechanical Analysis

Demembranated multicellular fibers were prepared from embryonic ventricles as descibed previously [Morano et al., 1996]. Fiber bundles (approximately 1–4 mm long and 150 µm thick) were mounted to a force transducer and immersed in relaxation solution. Relaxation solution contained 40 mM imidazole, 10 mM ATP, 12.5 mM $MgCl_2$, 10 mM creatine phosphate, 5 mM NaN_3, 5 mM EGTA, 1 mM DTE, and 350 U/ml creatine kinase, pH 7.0. Contraction solution was the same as the relaxation solution except that EGTA was substituted with 5 mM Ca EGTA. Ionic strenght was 130 mM. The desired Ca^{2+} concentrations were obtained by mixing relaxation and contraction solutions in the appropiate proportions. Free Ca^{2+} concentration was calculated according to the method of Fabiato and Fabiato [1979].

Passive stress–strain curve. To analyze the relation between passive tension (mN/mm^2) and fiber length (stress–strain curves), fibers were mounted to a force transducer in relaxation solution. Using a microscrew, length was increased in 56-µm steps to an extent where passive force was threshold (slack length = 100%). Passive stress–strain relation was obtained by stepwise increasing fiber length to approximately 200% and measuring the corresponding passive steady-state tension that developed subsequent to an initial peak tension. Three independent samples per experimental group were analyzed, and data were plotted as milli-Newtons per square millimeter versus percentage of slack length.

Compliance (C) of the passive stress–strain ratios of wild-type and homozygous knockout embryos were calculated at two different fiber lengths, 120% and 200% of slack length (six animals per group) by using equation 1,

$$C = \Delta L/\Delta P \quad (1)$$

where L is fiber length and P is the force per diameter.

Ca^{2+}-activated isometric force and shortening velocity. Isometric force and shortening velocity measurements were performed by mounting the fibers between a force transducer and a length-step generator by using microsyringes in relaxation solution. Length was adjusted to slack position. The fibers were transferred from relaxation solution pCa 8 [$pCa = -\log_{10}(Ca^{2+})$] into maximal Ca^{2+} activation solution pCa 4.5, and isometric steady-state force was registered. Shortening velocity was determined by isotonic quick releases under constant load at 21°C by using a commercially available setup (Scientific Instruments, Heidelberg, Germany). Load clamping for isotonic shortening was achieved by changing the mode of operation from length control to force during isometric steady-state tension. The force during isotonic contraction was set to zero load and held constant by the controlled motion of the length-step generator, which followed the contracting fiber with the appropiate velocity. The force control mode was maintained for 250 msec. The velocity of the length step was determined with an optoelectronical position detector. Both force and velocity signals were displayed on a storage digital oscilloscope (HAMEG, HM 408) and analyzed with an IBM-compatible PC using Proscope Hameg Software SP91. Velocity was measured 25–50 msec after the onset of the quick release.

For statistical analysis, means ± SEM of six animals per embryonic group (one fiber per animal) were used. For adult ventricles, six fibers of a 7-week-old animal were analyzed. Ca^{2+} sensitivity was expressed as pCa_{50}. Cooperativity factor was calculated by fitting the Hill coeficient (n_H) and pCa_{50} to equation 2:

$$y = \frac{(Ca^{2+})n_H}{(pCa_{50})n_H \times (Ca^{2+})n_H} \qquad (2)$$

where y is the fractional force, pCa is the negative decadic logarithm of the Ca^{2+} concentration required for half-maximal activation, and n_H the Hill coeficient as an index for cooperativity. Ca^{2+}-sensitivity values are expressed as the means of five to eight fibers. Student's *t*-test was performed for significance analysis.

RESULTS

Biochemical Analysis

To assess plakoglobin content in the adult and embryonic demembranated ventricular fibers, we conducted Western blot analysis. Mice were genotyped by following the protocol of Ruiz et al. [1996]. Although demembranated ventricular fibers of wild-type and heterozygous animals contained plakoglobin protein, this protein was not detected in homozygous mutant embryos (Fig. 1).

Table 1 shows a statistical analysis of plakoglobin and MHC expression of adult and embryonic demembranated fibers. MHC content was significantly higher in adult samples than in all embryonic experimental groups. However, there was no significant difference in MHC content within the embryonic fibers. Plakoglobin expression was reduced in heterozygous mutant embryos in comparison with the wild-type embryos.

Mechanical Analysis

To quantify fiber elasticity, we calculated the compliance at two different fiber lengths, namely at 120% and 200% (Table 2). Compliance of homozygous plakoglobin knockout animals was not statistically significant at both fiber lengths measured, suggesting a linear relationship of the stress–strain behavior of the fibers. In contrast, compliance of wild-type fibers at 120% fiber length was significantly higher than in the plakoglobin-deficient group ($P < 0.001$). In the more elongated state (200%), compliance decreased significantly ($P < 0.001$) in wild-type mice to around one-half of the value observed at 120% in the same group (Table 2). Complete passive stress–strain rela-

TABLE I. Relative Content of Plakoglobin and Myosin Heavy Chains (MHC) in Demembranated Mouse Cardiac Fibers[a]

	Plakoglobin		MHC	
Mouse	Arbitrary units	%	Arbitrary units	%
Adult +/+	2,162 ± 229	100	2,276 ± 303	100
e12 +/+	1,659 ± 221	76	1,340 ± 161***	59
e12 +/−	1,245 ± 338*	58	1,589 ± 81**	70
e12 −/−	0	0	1,446 ± 157***	64

[a]Sodium dodecylsulfate (SDS)-extracted proteins from the different experimental groups were processed for SDS–polyacrylamide gel electrophoresis and for Western blotting. Ponceau staining and reactivity with an affinity-purified anti-plakoglobin antibody were used for quantification. Statistical comparisons were performed between samples of adult and 12-day embryonic (e12) mice. +/+, wild-type mutant embryos; +/−, heterozygous mutant embryos; −/−, homozygous mutant embryos.

TABLE II. Compliance (C) of Chemically Demembranated Cardiac Fibers of Wild-type (wt) and Homozygous Plakoglobin Knockout (−/−) Mice at Two Fiber Extensions[a]

Fiber length (%)	C −/−	C wt
120	58.5 ± 5.87**	135.17 ± 12.16
200	50 ± 6*	72.6 ± 5.08

[a]Compliance is expressed in (μm × mm^2)/mN. Values are means ± SEM, with six fibers per group. Statistical comparisons were performed between samples of wild-type and plakoglobin-deficient embryos.
*$P < 0.01$.
**$P < 0.001$.

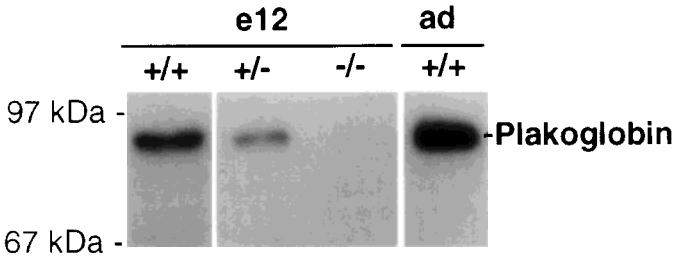

Fig. 1. Western blot analysis of plakoglobin content in demembranated ventricular fibers from 12–13-day-old embryos. Thirty milligrams of total protein were loaded per lane. Plakoglobin was probed with antibody E1021. ad, fibers from 7-week-old adult mice; +/+, +/− and −/− represent embryonic fibers from wild-type, heterozygous, and homozygous mutant plakoglobin animals, respectively. Molecular mass references are indicated in kilodaltons.

tionships of demembranated fiber preparations of plakoglobin-deficient and wild-type embryonic hearts are shown in Figure 2. In agreement with calculated values shown in Table 2, the ratio between passive tension and fiber length of plakoglobin-deficient fibers was close to a Hookean spring. Wild-type embryonic heart fibers demonstrated two phases, an initial phase of high compliance and a subsequent stiff phase.

No significant differences could be seen among the three embryonic groups when Ca^{2+} sensitivity of isometric force generation was analyzed. In contrast, all three sets of embryonic fibers showed a higher Ca^{2+} sensitivity in comparison with wild-type adult ventricular fibers (Fig. 3). Normal adult fibers developed a pCa_{50} of isometric force generation of 5.66 ± 0.10, whereas embryonic ventricular fibers reached values between 5.94 ± 0.03 (wild-type), 6.10 ± 0.12 (heterozygous), and 6.09 ± 0.05 (homozygous mutant; Fig. 4). Furthermore, we analyzed the cooperativity of the pCa force curves of adult and embryonic demembranated fibers. The Hill coeficient (n_H) was more than twofold higher in the adult demembranated fibers (n_H = 3.0 ± 0.1) in comparison with wild-type embryonic fibers (n_H = 1.5 ± 0.2). However, no significant differences ($P > 0.05$) were detected between homozygous mutant (n_H = 1.4 ± 0.2) and heterozygous (n_H = 1.5 ± 0.1) fibers.

When measuring shortening velocity at near-zero load, we found higher levels in normal adult fibers (V_{max} = 1.88 ± 0.01 muscle lengths per second; ML/sec) than in wild-type embryonic fibers (V_{max} = 1.42 ± 0.08 ML/sec; Fig. 5B). In Figure 5A, one representative experiment is shown per age group. No significant difference between homozygous mutant (V_{max} = 1.31 ± 0.20 ML/sec) and heterozygous (V_{max} = 1.42 ± 0.12 ML/sec; Fig. 5B) fibers was found.

Tension generation at maximal Ca^{2+} concentration (pCa 4.5) activation per cross-section was higher in the adult fibers (F = 37.00 ± 1.6 mN/mm^2) than in the embryonic wild-type ones (F = 9.66 ± 1.8 mN/mm^2; Fig. 6). The difference in force generation seen in homozygous mutant embryos (F = 7.70 ± 1.5 mN/mm^2) in comparison with controls (F = 9.66 ± 1.8 mN/mm^2 in wild-type and F = 9.98 ± 2.0 mN/mm^2 in heterozygous embryos) was not distinguishable from random variation (Fig. 5). Thus, neither calcium sensitivity, V_{max}, and tension production

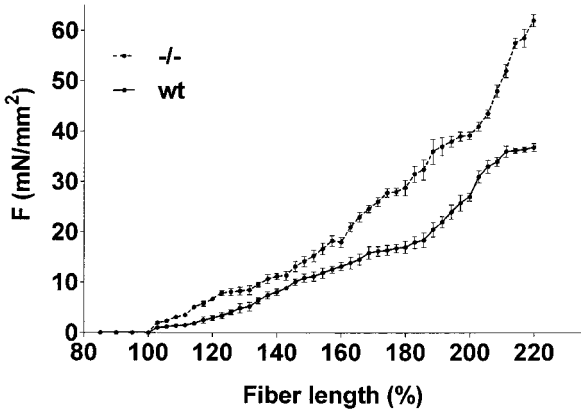

Fig. 2. Passive stress–strain curves of demembranated multicellular ventricular fibers of a plakoglobin *null* mutant (−/−) and a wild-type (wt) embryonic mouse. Fiber length is expressed as a percentage; slack length (i.e., length where passive force is just threshold) is given as 100%.

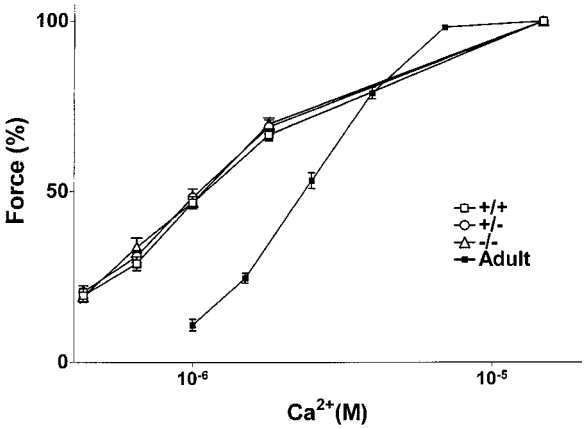

Fig. 3. Ca^{2+} sensitivity of demembranated ventricular fibers from 7-week-old adult mice and from 12–13-day-old embryos. Six fibers from an adult animal and from six animals per embryonic group and one fiber per embryo were analyzed. Adult, fibers from adult mice; +/+, +/− and −/− represent embryonic fibers from wild-type, heterozygous, and homozygous mutant plakoglobin animals, respectively.

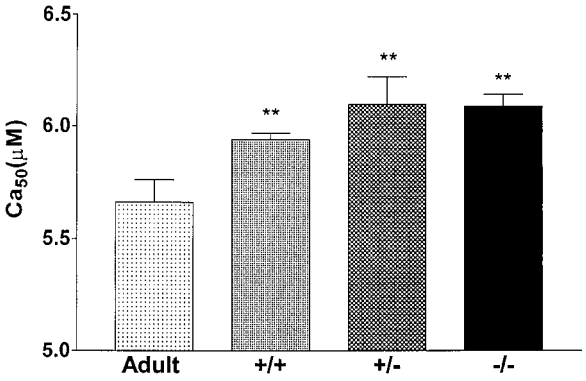

Fig. 4. pCa of isometric force generation at half maximal activation for adult and embryonic ventricular demembranated fibers. Adult, fibers from adult mice; +/+, +/− and −/− represent embryonic fibers from wild-type, heterozygous, and homozygous mutant plakoglobin animals, respectively.

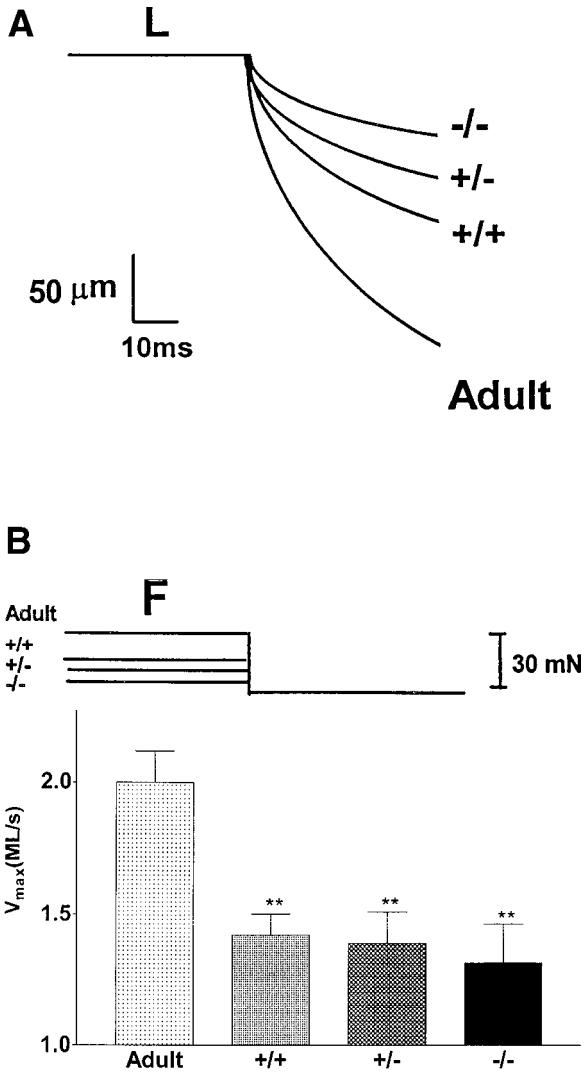

Fig. 5. Shortening velocity at near-zero load from demembranated ventricular fibers. **A:** Overlayed original registration (redrawn from the oscilloscope) of load-clamped force–velocity signals. L, length signal; F, force signal (100% corresponds to maximal isometric force obtained at maximal Ca^{2+} activation). Adult: L = 2,240 μm length, F = 37.14 mN/mm^2; plakoglobin (PG) +/+: L = 1,260 μm length, F = 12.25 mN/mm^2; PG +/−: L = 1,120 μm length, F = 10.03 mN/mm^2; PG −/−: L = 840 μm length, F = 8.06 mN/mm^2. **B:** Shortening velocity at near-zero load of demembranated fibers expressed in muscle length per second (ML/s). Adult, fibers from adult mice; +/+, +/−, and −/− represent embryonic fibers from wild-type, heterozygous, and homozygous mutant plakoglobin animals, respectively.

at maximal Ca^{2+} were affected by the abscence of plakoglobin.

DISCUSSION

We report the functional analysis of the involvement of plakoglobin in the myofibrillar apparatus of the heart. Plakoglobin was found to be essential for both the formation of cardiac

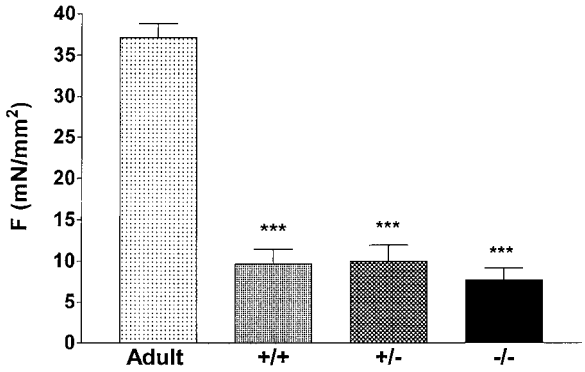

Fig. 6. Force per cross-sectional area of demembranated fibers (mN/mm^2). The steady-state tension developed by the adult fibers was significantly higher than that developed by the embryonic fibers, whereas there was no significant difference within the embryonic groups ($P > 0.05$). Adult, fibers from adult mice; +/+, +/−, and −/− represent embryonic fibers from wild-type, heterozygous, and homozygous mutant plakoglobin animals, respectively.

desmosomes and the architectural stability and functioning of the embryonic mouse heart [Ruiz et al., 1996]. To investigate the relevance of the myofibrillar apparatus in the pathological features of plakoglobin *null* mouse embryos, we analyzed contractile parameters on demembranated embryonic ventricular fibers. We found that Ca^{2+} sensitivity, force generation, and shortening velocity were not significantly altered in plakoglobin-deficient embryos in comparison with controls. However, passive stress–strain curves were impaired in null embryos. Altogether, we conclude that plakoglobin seems to be important not for anchorage of the myofibrillar apparatus to adherens junctions but for myofibrillar compliance. Thus, we suggest that cardiomyocyte junction disarrangement and cardiac dysfunction in plakoglobin-deficient embryos are mainly related to the loss of function of desmosomes and to a higher degree of stiffness of cardiac tissue.

During embryonic development, contractile proteins rearrange within myofibers into a well-organized, precise apparatus necessary for guaranteeing mechanical continuity between cardiac cells and heart function [Lyons et al., 1990]. When we analyzed contractile parameters, we found that shortening velocity of embryonic ventricular fibers was significantly reduced in comparison with that of adult fibers. The predominance of the β-MHC isoform in the embryonic mouse hearts in contrast to the α-MHC isoform in the adult organism [Lyons et al., 1990] may explain this effect because α-MHC

confers a higher shortening velocity than does the β-MHC isoform [Ebrecht et al., 1982]. However, the predominance of either MHC isoform has been described as not influencing the force–pCa relation [Ebrecht et al., 1982] and thus cannot explain the difference in Ca^{2+} sensitivity seen between the embryonic and the adult ventricular fibers. Rather, the expression of embryo-specific regulatory proteins (i.e., slow skeletal troponin I and the atrial myosin light chain) may serve as a rational explanation for the enhanced Ca^{2+} sensitivity seen in the embryonic fibers [Martin et al., 1991; Morano et al., 1997]. Our observation of a reduced generation of isometric force per cross-section may be due to the immature sarcomeres of the embryonic tissue [Wang et al., 1988] and to the reduced expression of the motor molecule myosin in the embryonic heart (Table 1).

When active force generation was analyzed in plakoglobin mutant embryos, force per cross-section, Ca^{2+} sensitivity of isometric force, and shortening velocity at near-zero load were found to be comparable in all samples. In contrast, we found that passive stress–strain ratios were strikingly different between *null* and control embryonic heart fibers: plakoglobin-deficient fibers were less compliant than wild-type fibers in the normal working range of the heart; at 120% extension, compliance was 2.5-fold higher in the wild-type group than in the plakoglobin-deficient group. This observation suggests the generation of higher end diastolic pressure on ventricular filling in the *null* embryos as compared with control littermates. Thus, it is reasonable to speculate that the active systolic force generation required to reach after-load pressure (i.e., the isovolumetric force required for valve opening and blood ejection) may be diminished in plakoglobin-deficient embryos. The amplitude of ventricular contraction during blood ejection therefore may also be reduced. Alternatively, diastolic filling of the stiffened ventricle may be impaired. Ruiz et al. [1996] reported a decreased contraction amplitude during ejection of plakoglobin *null* embryos when using a high-resolution ultrasonic echo-tracking device [Girerd et al., 1994]. Such a decreased ejection volume with the subsequent fall in blood pressure could lead to tachycardia [see Maloney et al., 1977] on baroreceptor reflex mechanisms that operate during early fetal development [Maloney et al., 1977]. Wall stress per cardiac cycle may be unchanged in *null* mutant embryos, but over longer time periods (e.g., per minute) and with an increased cardiac frequency, total wall stress may be higher in plakoglobin-deficient ventricles. Enhanced total wall stress could exhaust and finally rupture the heart of plakoglobin null embryos.

Because myofibrils anchor into adherens junctions, the present shortening velocity and force generation analyses may reflect the involvement of plakoglobin in myofibrillar adherens junction assembly only. An additional explanation for the insignificant differences on contractile parameters between normal and plakoglobin-deficient fibers is that β-catenin is expressed normally in the mutant junctions [Ruiz et al., 1996] and therefore may compensate for the absence of plakoglobin. We conclude that the cardiac dysfunction characteristic of the plakoglobin-deficient embryos seems to be related mainly to the loss of function of cardiac desmosomes and to the profound disarrangement of junctional components. This disturbance seems to be associated with a decreased passive compliance of the cardiac tissue and eventually with a decreased amplitude of ventricular contraction during blood ejection. Thus, plakoglobin seems to be essential for mechanical continuity in heart tissue. In the present study, we provide new mechanistic and conceptual information for the better understanding of the involvement of plakoglobin in myocardial function.

ACKNOWLEDGMENTS

We thank Dr. Michael Schneider for comments and suggestions on the manuscript and Renate Franke for excellent technical assistance. P. Ruiz is a recipient of a TMR fellowship of the European Commission.

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