

# Overexpression of the Sarcoplasmic Reticulum $\text{Ca}^{2+}$ -ATPase Improves Myocardial Contractility in Diabetic Cardiomyopathy

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Diabetic cardiomyopathy is characterized by reduced cardiac contractility due to direct changes in heart muscle function independent of vascular disease. An important contributor to contractile dysfunction in the diabetic state is an impaired sarcoplasmic reticulum (SR) function, leading to disturbed intracellular calcium handling. We investigated whether overexpression of the SR calcium pump (SERCA2a) in transgenic mice could reduce the impact of diabetes on the development of cardiomyopathy. Diabetes was induced by streptozotocin injection (200 mg/kg), and left ventricular (LV) function was analyzed in isolated hearts 3 weeks later. In diabetic hearts systolic LV pressure was decreased by 15% and maximum speed of relaxation ( $-\text{dP}/\text{dt}$ ) by 34%. Functional changes were also assessed in isolated papillary muscles. Active force was reduced by 61% and maximum speed of relaxation by 65% in the diabetic state. The contractile impairment was accompanied by a 30% decrease in SERCA2a protein in diabetic mice. We investigated whether increased SERCA2a expression in transgenic SERCA2a-overexpressing mice could compensate for the diabetes-induced decrease in cardiac function. Under normal conditions, SERCA2a overexpressors show improved contractile performance relative to wild-type (WT) mice ( $-\text{dP}/\text{dt}$ : 3,169 vs. 2,559 mmHg/s, respectively). Measurement of LV function in hearts from diabetic SERCA2a mice revealed systolic and diastolic functions that were similar to WT control mice and markedly improved relative to diabetic WT mice ( $-\text{dP}/\text{dt}$ : 2,534 vs. 1,690 mmHg/s in diabetic SERCA2a vs. diabetic WT mice, respectively). Similarly, the contractile behavior of isolated papillary muscles from diabetic SERCA2a mice was not different from that of control mice. SERCA2a protein expression was higher (60%) in diabetic SERCA2a mice than WT diabetic mice. These results indicate that overexpression of SERCA2a can protect diabetic hearts from severe contractile dysfunction, presumably by improving the calcium sequestration of the SR. *Diabetes* 51: 1166–1171, 2002

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LV, left ventricular; SR, sarcoplasmic reticulum; STZ, streptozotocin; WT, wild type.

Heart disease is the leading cause of death in diabetic patients. Although coronary artery disease is accelerated in the diabetic state, it has been recognized for a number of years that diabetes independently impairs myocardial performance by decreasing cardiac muscle function (1,2). This disease is termed diabetic cardiomyopathy and can progress toward overt heart failure, with increased mortality (3–5).

Various animal models of diabetes have been used to study the causes and underlying subcellular events of diabetic cardiomyopathy (6,7). Numerous studies suggest a dysfunctional sarcoplasmic reticulum (SR), leading to altered intracellular calcium handling in cardiac myocytes, might be involved in the development of this disease. A reduced sequestration of calcium into the SR could readily explain the prolonged cardiac relaxation observed in diabetic cardiomyopathy (8). As a consequence, the SR calcium content declines, leading to a reduced systolic calcium release and therefore a weaker cardiac contraction (9,10). At the molecular level, the impairment of SR function was shown to be caused by a reduced activity of the SR calcium pump (SERCA2a), which induced diastolic relaxation by sequestering calcium from the cytoplasm (11). SERCA2a activity depends on the amount of SERCA2a protein and is further regulated by its inhibitory protein phospholamban. SERCA2a is the major determinant of the beat-to-beat regulation of cardiac contraction, and overexpression of this protein in mice has been shown to enhance myocardial contractility (12,13). In recent studies, we have demonstrated that depressed levels of SERCA2a can be reconstituted by adenoviral gene transfer in isolated cardiac myocytes, or in SERCA2a transgenic mice submitted to hypothyroidism (14,15).

Diabetic cardiomyopathy has been extensively studied in a variety of animal models through the use of streptozotocin (STZ) to destroy the pancreatic islet  $\beta$ -cells and induce an insulin-dependent diabetic state. Although STZ-induced diabetic cardiomyopathy has been examined in larger animals, direct measurement of cardiac mechanical function in mouse models has not been reported. Our results indicate that the STZ model is a valuable tool to study diabetic cardiomyopathy, using mice as an animal model. Similar to studies in larger animals, we observed a depression in cardiac function in the hearts of diabetic mice accompanied by a significant decrease of SERCA2a

protein levels. To investigate whether SERCA2a overexpression could ameliorate the diabetes-induced contractile failure, we used a transgenic SERCA2a mouse model. Our results indicate that overexpression of SERCA2a can prevent the severe contractile dysfunction observed in diabetic cardiomyopathy.

## RESEARCH DESIGN AND METHODS

All animal experiments were approved by the local animal subject committee and were performed in accordance with federal guidelines for the use of animals in biomedical research.

**Induction of diabetes in mice.** Mice (12–20 weeks old) were made diabetic by injection with a single dose of freshly prepared STZ solution (200 mg/kg body wt i.p. in citrate saline, pH 4.2) after overnight fasting. The mice were housed in cages of four mice each and had unlimited access to water and standard mouse chow (Teklad, Madison, WI). The urine glucose was assessed with glucostix 2 days after STZ injection. Mice with urine glucose >400 mg/dl were presumed to be diabetic. The diabetic status was confirmed by blood glucose measurement at the time of the contractile studies. All mice with high urine glucose had blood glucose levels >400 mg/dl.

Changes in SERCA2a expression were confirmed in a group of mice with less severe diabetes. In this group mice, which appeared severely diabetic 2 days after STZ injection, the animals were treated with limited insulin therapy. Insulin replacement consisted of 40 mg/kg subcutaneous daily ultralente human insulin (Eli Lilly, Indianapolis, IN). SERCA2a mRNA levels were compared with nondiabetic levels on a Northern blot.

To rule out toxic effects of STZ on cardiac myocytes, STZ-injected mice were intensely treated with insulin; wild-type (WT) mice were made diabetic with 200 mg/kg STZ according to the protocol used previously. Starting 2 days after STZ injection, mice were treated with 60 units/kg subcutaneous b.i.d. NPH human insulin (HumulinN; Eli Lilly, Indianapolis, IN). SERCA2a mRNA levels were compared with nondiabetic WT levels on a Northern blot.

**SERCA2a transgenic mouse model.** The SERCA2a transgenic mice are described in detail in a previous study (13). Briefly, the rat SERCA2a construct included the first intron, second exon, second intron, and part of the third exon derived from a genomic SERCA2a sequence. The remainder of the sequence was taken from a rat SERCA2a cDNA sequence (16). This construct is driven by the cytomegalovirus immediate early enhancer followed by the chicken  $\beta$ -actin promoter. The heterozygous SERCA2a mice were identified by Southern blot analysis.

**Isolated perfused hearts.** Hearts were isolated and transferred to a miniaturized Langendorff set-up for contractile studies in isolated mouse hearts as previously described (17). In brief, hearts were removed from anesthetized mice and immersed in cold cardioplegic solution. After cannulation of the aorta, the hearts were perfused retrograde at 37°C with a modified Krebs-Henseleit buffer (in mmol/l: 118 NaCl; 4.7 KCl; 2.25 CaCl<sub>2</sub>; 1.2 MgSO<sub>4</sub>; 1.2 KH<sub>2</sub>PO<sub>4</sub>; 25.0 NaHCO<sub>3</sub>; 0.5 Na<sub>2</sub>EDTA; and 5.5 glucose). A small fluid-filled balloon was inserted into the left ventricular (LV) cavity and coupled to a pressure transducer (Millar Instruments, Houston, TX). The balloon was inflated until the diastolic pressure reached 10 mmHg. Platinum wires were placed on the surface of the right atrium and used to pace the hearts at 380 beats/min. Hearts were perfused and paced for 15 min to reach a stable cardiac function. Digitized recordings of the ventricular pressure and its first derivative were captured on an IBM-compatible 486 computer. Peak systolic pressure (LV pressure), end-diastolic pressure, maximum speed of contraction (+dP/dt), and maximum speed of relaxation (−dP/dt) were analyzed from the recordings.

**Isolated papillary muscle experiments.** The contractile behavior of papillary muscles was studied as previously described (13). Briefly, hearts were removed from deeply anesthetized mice, and papillary muscles were prepared under cardioprotective 2,3-butanedione-monoxime containing Tyrode solution. The muscle strips were transferred to a muscle chamber, stimulated at 2 Hz, and stretched to the length of maximum force development ( $l_{max}$ ). The muscles in the chamber were superfused with a Tyrode solution (2.5 mmol/l calcium, 37°C). The developed isometrical force at 2- and 6-Hz stimulation frequency was recorded on a chart recorder. Maximum force, maximum speed of contraction ( $dF/dt_{max}$ ), and maximum speed of relaxation ( $dF/dt_{min}$ ) were obtained from the recordings. The papillary muscles did not differ in cross-sectional area ( $0.41 \pm 0.04$  mm<sup>2</sup> for muscles from diabetic mice,  $0.33 \pm 0.05$  mm<sup>2</sup> for muscles from WT mice).

**Western blot analysis.** Ventricular tissue was homogenized with a Polytron Homogenizer. Protein content in the samples was measured and adjusted for equal loading. Next, 20  $\mu$ g protein was resolved on a 4–20% Tris-glycine polyacrylamide gel (Novex, San Diego, CA). After the gel was electro-

TABLE 1  
Body weight and heart weight data

	WT mice	WT diabetic mice
Body weight (g)	28.3 $\pm$ 1.2	24.1 $\pm$ 1.2*
Heart weight (mg)	151 $\pm$ 11	116 $\pm$ 11*
HW/BW ratio	5.18 $\pm$ 0.14	5.05 $\pm$ 0.11

Data are means  $\pm$  SD. Body weight and heart weight data from nondiabetic WT mice ( $n = 8$ ) and diabetic mice WT ( $n = 7$ ). HW/BW, heart weight/body weight. \*Values significantly different ( $P < 0.05$ ) from nondiabetic WT mice.

transferred to a nitrocellulose membrane, the blot was cut in two pieces at a ~70-kd marker band. The upper part of the blot was incubated with a polyclonal antibody directed against SERCA2a and the lower part with a mouse monoclonal  $\alpha$ -actin antibody and a mouse monoclonal phospholamban antibody (Affinity BioReagents, Golden, CO) (18,19). The two parts of the blot were exposed to secondary antibodies carrying horseradish peroxidase (Amersham, Arlington Heights, IL) to drive a chemiluminescence reaction (ECL; Amersham, Little Chalfont, U.K.), which was captured on film. Signals on the films were digitized on a 600-dots per inch scanner. Band densities were quantified with the Scion Image Software for PC (Scion, Frederick, MD). The SERCA2a signals were normalized to actin. SERCA2a protein levels were compared in diabetic and nondiabetic WT mice, as well as diabetic and nondiabetic SERCA2a transgenics.

**Northern blot analysis.** For the analysis of mRNA of SERCA2a, tissue RNA was extracted from myocardial tissue by a guanidine thiocyanate method (20). The mRNA was separated on a 1% agarose gel and transferred to a nylon membrane. A 1.6-kb fragment corresponding to the 5' end of the rat-SERCA2a-cDNA was used to generate <sup>32</sup>P-labeled probes (Multiprime DNA labeling systems; Amersham), which were used for the hybridization. Radioactivity was assessed on film (Kodak, Rochester, NY), and the resulting image was quantified with Image 1.61 software. The SERCA2a signals were normalized to GAPDH. SERCA2a mRNA levels were compared in the following groups: 1) diabetic and nondiabetic WT mice; 2) diabetic and nondiabetic SERCA2a mice; 3) diabetic WT mice with limited insulin therapy and nondiabetic WT mice; and 4) diabetic mice with intensive insulin treatment, diabetic mice, and WT mice.

**Statistical analysis.** An unpaired Student's *t* test ( $P < 0.05$ ) was used to make comparisons between diabetic mice and their respective nondiabetic controls. ANOVA followed by a Student-Neuman-Keuls test was used to determine differences among the nondiabetic and diabetic WT and SERCA2a groups.

## RESULTS

**Myocardial contractility in diabetic mice.** After 3 weeks of diabetes, diabetic WT mice had a 15% lower body weight and a 23% lower heart weight than normal WT mice. The ratio of heart weight to body weight was not significantly changed (Table 1).

Contractile performance of isolated perfused hearts (Table 2) showed a profound decrease in LV function in WT diabetic mice ( $n = 15$ ) when compared with hearts from normal WT mice ( $n = 7$ ). In hearts from diabetic mice, the LV systolic pressure was 15% lower compared with hearts from control mice. The maximum speed of contraction (+dP/dt) as an indicator of SR calcium release was reduced by 34% in diabetic mice. Maximum speed of relaxation (−dP/dt), which mostly reflects calcium re-uptake into the SR, was depressed by 34%.

A significant decrease in myocardial contractility was also observed in papillary muscle strips from diabetic WT mice compared with nondiabetic WT mice (Table 2). At a stimulation frequency of 2 Hz, papillary muscles from diabetic mice showed a 61% reduced active force. The indicators for calcium uptake in the SR and the calcium release from it, maximum speed of contraction ( $dF/dt_{max}$ ), and maximum speed of relaxation ( $dF/dt_{min}$ ) were also decreased by 60 and 65%, respectively. To exclude that

TABLE 2  
Functional data for isolated hearts and papillary muscle strips

	10-mmHg preload		2-Hz stimulation frequency		6-Hz stimulation frequency	
	WT	WT diabetic	WT	WT diabetic	WT	WT diabetic
<b>Isolated hearts</b>						
Pressure (mmHg)	96 ± 4	82 ± 4*	—	—	—	—
+dP/dt (mmHg/s)	3,436 ± 139	2,272 ± 197*	—	—	—	—
-dP/dt (mmHg/s)	2,559 ± 124	1,690 ± 134*	—	—	—	—
<b>Papillary muscle</b>						
Force (mN/mm <sup>2</sup> )	—	—	6.1 ± 1.4	2.3 ± 0.5*	4.0 ± 0.8	1.5 ± 0.3*
+dF/dt (mN/s)	—	—	169 ± 37	67 ± 17*	168 ± 30	60 ± 13*
-dF/dt (mN/s)	—	—	106 ± 25	37 ± 9*	100 ± 18	31 ± 6*

Data are means ± SD. Functional data from nondiabetic WT mice and diabetic WT mice. Pressure development data for isolated Langendorff perfused hearts for nondiabetic WT mice (n = 7) and diabetic WT mice (n = 15). Force production from isolated papillary muscles was obtained from (n = 8) WT mice and (n = 7) WT diabetic mice. \*Values significantly different (P < 0.05) from nondiabetic WT mice.

these changes in contractility are frequency-dependent (frequency is known to have strong effects on cardiac contractility), a second set of data was obtained at a stimulation frequency of 6 Hz. Again, the principal differences between the two groups were conserved. Papillary muscle strips from diabetic mice generated 62% less force (1.5 ± 0.3 mN/mm<sup>2</sup> in WT diabetic mice vs. 4.0 ± 0.8 mN/mm<sup>2</sup> in WT mice, P < 0.05). In addition, the force development-related parameters dF/dt<sub>max</sub> and dF/dt<sub>min</sub> were reduced by 64 and 69%, respectively (60 ± 13 and 31 ± 6 mN/s in WT diabetic mice compared with 168 ± 30 and 100 ± 18 mN/s in WT mice, respectively; P < 0.01).

**Messenger RNA and protein levels of SERCA2a in diabetic mice.** Northern blot analysis of heart tissue from WT diabetic mice showed a decrease of SERCA2a mRNA when compared with nondiabetic WT mice (Fig. 1). To confirm that SERCA2a mRNA levels were depressed as a direct result of the diabetic state, mice made diabetic with STZ received insulin replacement therapy. These mice, which received limited insulin replacement, showed only a small decrease in body weight (1 g body wt) compared with age- and sex-matched nondiabetic mice. Under these conditions, insulin prevented the decrease in SERCA2a mRNA levels (Fig. 1).

The decrease in SERCA2a mRNA was paralleled by a decrease in SERCA2a protein (Fig. 2A and B). Western blots showed that the SERCA2a protein levels are decreased by 30 ± 4% (P < 0.05) in the WT diabetic group when compared with WT mice. No change in the SERCA2a inhibitory protein phospholamban was observed, indicat-

ing that a decrease in SERCA2a protein likely contributes to disturbed diastolic SR function. However, the possibility of a change in the extent of phospholamban phosphorylation and its effect on SERCA2a cannot be discounted.

Diabetic SERCA2a transgenic mice showed elevations of glucose levels in urine and blood after STZ injection that were not different from WT diabetic mice. After 3 weeks of untreated diabetes, body weights, heart weights, and the heart weight-to-body weight ratios were similar to those

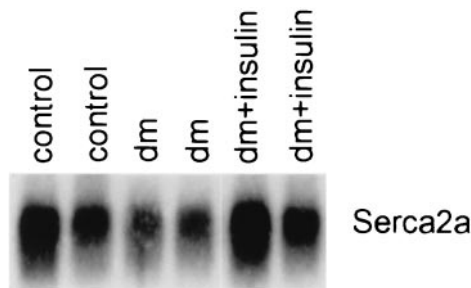


FIG. 1. Northern blot probed for SERCA2a in cardiac tissue from control mice, diabetic mice, and mice which were treated with insulin after STZ injection. Insulin replacement could prevent the decrease of SERCA2a mRNA levels, which was observed in insulin-deficient diabetic mice. dm, diabetic mice; dm+insulin, diabetic mice treated with insulin.

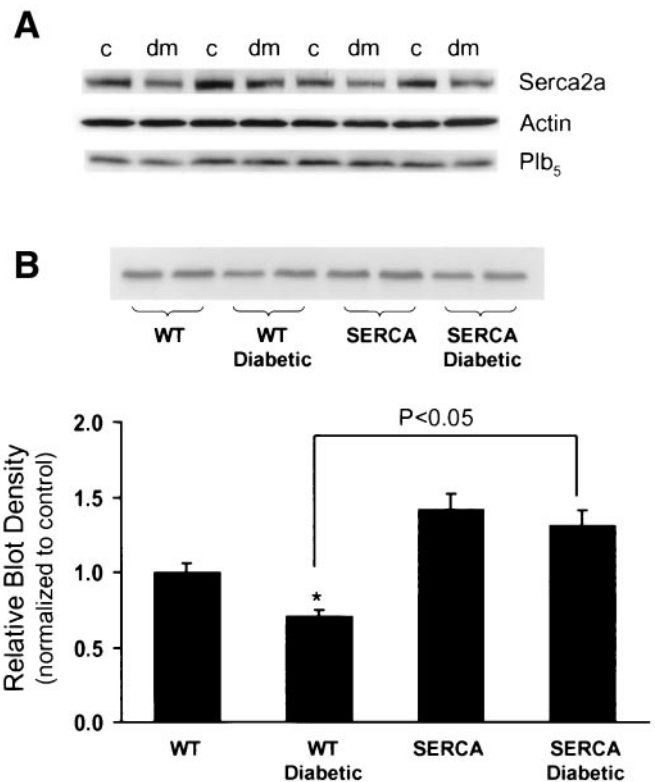
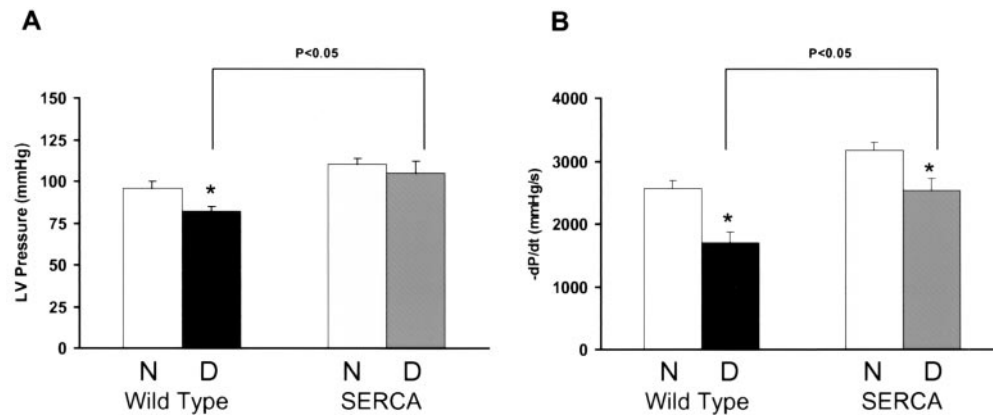


FIG. 2. A: Western blot showing SERCA2a and phospholamban (Plb<sub>5</sub>) proteins detected and analyzed (normalized to actin) in cardiac tissue from diabetic WT mice (dm) and nondiabetic WT mice (c). The SERCA2a inhibitor protein phospholamban was not changed. B: Western blot showing SERCA2a for nondiabetic and diabetic WT and SERCA2a-overexpressing mice (SERCA). The bar diagram represents the relative density of SERCA2a measured for mice (n = 4) from the various groups. \*Values in the diabetic groups that are significantly lower than their nondiabetic counterparts. Note that SERCA2a expression in the WT diabetic group is 60% lower than that observed in the diabetic SERCA2a-overexpressing group.





**FIG. 3.** Pressure (A) and maximum rate of relaxation (B) in isolated perfused hearts from nondiabetic (N) and diabetic (D) mice from both the WT and SERCA2a-overexpressing groups. Values correspond to the mean  $\pm$  SE for  $n = 7$  and 15 nondiabetic and diabetic WT mice, respectively; and  $n = 9$  and 11 nondiabetic and diabetic SERCA2a-overexpressing mice, respectively. \*Values in the diabetic (D) groups that are significantly lower than their nondiabetic (N) counterparts. Note that for both pressure development and the rate of relaxation, the values obtained for the diabetic SERCA group are significantly higher than those obtained for the WT diabetic group.

from WT diabetic mice (body weight  $22.8 \pm 1.1$  g, heart weight  $112 \pm 8$  mg, and heart weight-to-body weight ratio  $5.19 \pm 0.12$ ;  $n = 10$ ). Western blots of hearts from diabetic SERCA2a transgenic mice revealed a 60% increase ( $P < 0.05$ ) in SERCA2a protein compared with WT diabetic mice (Fig. 2). In comparison, SERCA2a levels in normal SERCA2a-overexpressing transgenic mice were only 40% higher than levels observed in normal WT mice, indicating that even under diabetic conditions, SERCA2a-overexpressing mice retain their high level of SERCA2a expression. SERCA2a expression was only slightly depressed in diabetic SERCA2a-overexpressing mice relative to nondiabetic SERCA2a-overexpressing mice.

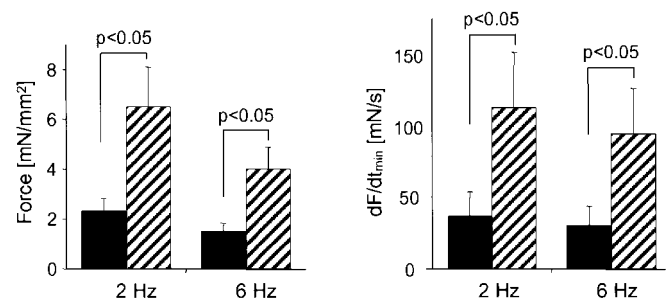
**Preservation of contractile function in diabetic SERCA2a mice.** When LV pressure and mechanical function were measured in isolated hearts using an intraventricular balloon, the values obtained for pressure development and rate of relaxation for normal SERCA2a mice ( $110 \pm 3$  mmHg and  $3,169 \pm 180$  mmHg/s, respectively) were significantly higher ( $P < 0.05$ ) than the corresponding values obtained from normal WT mice ( $95 \pm 4$  mmHg and  $2,559 \pm 124$  mmHg/s, respectively), confirming our previous observation that increased SERCA2a expression improves myocardial performance (13). Isolated perfused hearts from diabetic transgenic mice ( $n = 11$ ) developed higher LV pressure ( $105 \pm 7$  mmHg) compared with hearts from WT diabetic mice ( $82 \pm 4$  mmHg,  $P < 0.05$ ) (Fig. 3). As expected from the increased expression of SERCA2a protein in the diabetic SERCA2a group, the maximum speed of relaxation ( $-dP/dt$ ) was significantly improved ( $2,534 \pm 180$  mmHg/s in diabetic SERCA2a mice vs.  $1,690 \pm 134$  mmHg/s in WT diabetic mice,  $P < 0.05$ ).

A similar preservation of myocardial function in the diabetic state was observed in muscle strip preparations (Fig. 4). At 2 Hz, muscle strips from diabetic SERCA2a transgenic mice ( $n = 8$ ) showed a 1.8-fold increase in developed force in SERCA2a diabetic mice ( $6.5 \pm 1.6$  mN/mm<sup>2</sup>) versus in WT diabetic mice ( $2.3 \pm 0.5$  mN/mm<sup>2</sup>,  $P < 0.05$ ). The twitch parameter  $dF/dt_{\max}$  was increased 1.6-fold ( $174 \pm 38$  mN/s in diabetic SERCA2a mice vs.  $67 \pm 17$  mN/s in diabetic WT mice,  $P < 0.05$ ) and  $dF/dt_{\min}$  was increased 2.1-fold ( $113 \pm 28$  mN/s in diabetic SERCA2a

mice vs.  $37 \pm 9$  mN/s in diabetic WT mice). A similar compensation was also observed at the 6-Hz stimulation frequency: compared with muscle strips from diabetic WT mice, force developed in muscle strips from diabetic SERCA2a mice was increased-1.6 fold,  $dF/dt_{\max}$  1.5-fold, and  $dF/dt_{\min}$  2.0-fold.

#### DISCUSSION

Decreased contractility in diabetic cardiomyopathy is known to be associated with changes in calcium homeostasis at the level of cardiac myocytes (21). These changes have been attributed to a reduced ability to sequester calcium into the SR (8,22), which primarily determines the speed of cardiac relaxation and has several consequences on other parameters of cardiac contraction (23). A diminished calcium uptake into the SR leads to a reduction of calcium stored within this organelle. Consecutively, less calcium is available to initiate the following systolic stroke, which leads to a reduction of cardiac contractility. Because the speed of the calcium release is also determined by calcium load of the SR (reduced calcium load leads to slowed calcium release), the speed of the contraction is also prolonged. The importance of SERCA2a in the control of the dynamic calcium homeostasis in cardiac myocytes has been demonstrated in vitro and in vivo. In transgenic mice overexpressing SERCA2a, cardiac performance was found to improve the speed of



**FIG. 4.** Force in isolated muscle strips from diabetic WT (■) and diabetic SERCA2a-overexpressing (▨) mice. SERCA2a-overexpressing diabetic mice ( $n = 8$ ) showed better force development than WT diabetic mice ( $n = 7$ ).

cardiac contraction and relaxation *in vivo* and in isolated muscle strips. However, it did not lead to a significant increase in systolic pressure or force *in vivo* (12,13), and adenoviral-mediated transfer of SERCA2a in isolated neonatal myocytes with depressed levels of SERCA2a led to greatly abbreviated calcium transients (14).

In diabetic cardiomyopathy, the activity of the calcium pump at the SR (SERCA2a) is reduced (11). Our study provides direct evidence that a reduced amount of SERCA2a protein might account for the observed changes in calcium uptake activities. The decrease in SERCA2a protein appears to be mediated by SERCA2a gene expression (24), since the mRNA levels of SERCA2a were also reduced in our model. In previous studies (24–26) using cardiac tissue from diabetic rats, no consistent changes in SERCA2a mRNA and protein levels were found. Although these studies used similar animal models of diabetic cardiomyopathy induced by STZ, two studies reported a decrease in SERCA2a mRNA levels and SERCA2a protein, whereas another study found no more than a trend to lower SERCA2a mRNA levels and no changes in SERCA2a protein. This difference could imply that changes in SERCA2a mRNA and protein levels depend on the severity of diabetes, similar to changes observed in heart failure, which become more pronounced with advanced stages. Therefore, our data showing decreased SERCA2a mRNA and protein levels in a mouse model of severe diabetes are in line with those previous studies. In general, SERCA2a protein has higher importance in calcium handling in rodents than in larger animals. However, in comparison with rats, mice (which are an order of magnitude smaller in body mass) have a higher metabolic rate and consequently require a higher dose of STZ to induce diabetes. Mice, with their lower body mass and less body fat, may be more susceptible to the contractile effects of diabetes.

We used STZ to induce diabetes, which is an established animal model to generate diabetic cardiomyopathy (7,26). Although STZ-induced diabetes is an established model to generate diabetic cardiomyopathy (7,27), it has not been used to directly quantitate diabetic cardiomyopathy in mice, although it has been used to study the effect of diabetes on skeletal muscle (28). We confirm that STZ-induced diabetes in mice leads to a pronounced diabetic cardiomyopathy similar to that observed in larger animals (6,28,29). The contractile parameters were assessed *ex vivo* in a whole-heart preparation and in strips of isolated papillary muscles. These approaches to assess cardiac function allowed us to directly determine the contractile alterations caused by diabetes, without the need to consider diabetes-related vascular disease or neuropathy, which also affect the cardiovascular system.

Having established the existence of depressed cardiac function in the diabetic state, and linking this to changes in SERCA2a protein, it was of interest to study the effects of diabetes on a mouse model of SERCA2a overexpression. In this study, we demonstrated that cardiac contractility in diabetic cardiomyopathy can be improved by expressing increased amounts of SERCA2a. Diabetic SERCA2a transgenic mice had SERCA2a protein levels that were 60% higher than in WT diabetic mice. Although the diabetic SERCA2a transgenic mice did show a depression in the rate of relaxation relative to SERCA2a transgenic mice,

pressure development and the rates of pressure change (+dP/dt and -dP/dt) were equivalent to that observed in normal WT mice. This suggests that SERCA2a overexpression provides sufficient contractile reserve to ameliorate the detrimental effects of diabetes on cardiac contractility. Some of the decrease in contractility in SERCA2a diabetic mice may be caused by additional subcellular changes, like the diabetes-induced switch in myosin isoforms, which leads to a predominant expression of a slower myosin isoform (30,31). Metabolic changes in the diabetic heart, including a lesser uptake of glucose in the absence of insulin or an enhanced dependence on free fatty acids, could also affect cardiac activities.

Impaired SR function and decreased SERCA2a levels are not unique to diabetic cardiomyopathy and play a role in end-stage heart failure due to ischemic and dilated cardiomyopathy (32–34), hypertrophic cardiomyopathy (35), and aging hearts (36). In fact, a recent study on heart failure in rats demonstrated an improvement in contractile function after gene transfer of SERCA2a using adenoviral vectors (37). The underlying mechanisms leading to a reduced expression of SERCA2a are poorly understood. SERCA2a transcription is known to be thyroid hormone-sensitive (16), and reduced SERCA2a protein levels are found in hearts from hypothyroid animals. However, although diabetic animal models exhibit lowered thyroid hormone levels, it was shown that correction of the thyroid status did not reverse molecular changes (38), indicating that subcellular changes in the myocytes cannot be attributed to subnormal thyroid hormone levels. An additional explanation for our results could be an increased amount of the SERCA2a inhibitory protein phospholamban in our model of diabetic cardiomyopathy. Because no difference in protein levels was found in diabetic versus nondiabetic WT mice, this could be excluded as a reason for the observed changes in contractility.

Although we did not measure calcium transients in diabetic mouse myocytes, a recent study using diabetic rat cardiac myocytes demonstrated a decrease in both the peak amplitude and the rate of decay of calcium transients, suggesting a depression in SR calcium handling (39). Interestingly, they observed that the beat-to-beat fraction of calcium recirculating through the SR was similar in both diabetic and control myocytes, despite the difference in calcium transients. They suggest that this phenomenon was due to an overall reduction in beat-to-beat calcium efflux from the myocyte. Whether a similar effect occurs in the diabetic mouse myocyte and is affected by SERCA2a overexpression remains to be examined.

In summary, this study underlines the important role of SERCA2a expression in diabetic cardiomyopathy and indicates that increased SERCA2a protein levels can be beneficial in preserving cardiac contractility in this disease.

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