Transcoronary gene transfer of SERCA2a increases coronary blood flow and decreases cardiomyocyte size in a Type 2 diabetic rat model

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THE OTSUKA LONG-EVANS TOKUSHIMA fatty (OLETF) rat was used. DM rats (70–80 wk old) with hypercholesterolemic rabbit and mouse aorta smooth muscle (2, 3), the abnormal Ca2+ handling due to the SERCA2 protein reduction contributes to the impaired relaxation.

Our group (6, 10) has previously shown that global cardiac gene transfer of SERCA2a improves contractile function and energetic state (as measured by phosphocreatine-to-ATP ratio) in aortic-banded rats. Moreover, SERCA2a overexpression improved anterior wall thickening and reduced ventricular arrhythmias in a rat model of ischemia (5). Most recently, our group (15) has demonstrated that, in OLETF DM hearts, the high oxygen cost of LV contractility can be restored to non-DM levels by SERCA2a gene transfer. The aim of this study was to examine how transcoronary gene transfer of SERCA2a can influence coronary blood flow (CBF) and cardiomyocyte diameter (CMD) in the DM rat model. Thus, in the present study, we analyzed both CBF in response to LV mechanical loading and histological data [CMD and collagen area percentage (CAP)] in 15 of the same hearts that our group recently studied (15) and one DM heart with adenoviral β-galactosidase transfer. We report here that, in DM failing hearts, SERCA2a gene transfer can increase CBF and decrease CMD without reduction in collagen production.

MATERIALS AND METHODS

Animals and adenoviral vectors. All animal procedures were performed with the approval of the Animal Care Committee of Massachusetts General Hospital and in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals. Twelve male DM OLETF and four normal non-DM Long-Evans Tokushima Otsuka rats were used. DM rats (70–80 wk old) with ~300 mg/dl of blood glucose concentration were randomized into three groups [group without gene transfer (DM), group with SERCA2a transfer (DM+SERCA), and group with β-galactosidase transfer (DM+βGal); n = 4 in each group]. Recombinant adenoviral vectors were used with cytomegalovirus-driven expression cassettes for SERCA2a (Ad.SERCA2a) or β-galactosidase (Ad.βGal) (5, 6, 10, 15). Ad.SERCA2a and Ad.βGal had concentrations of 6.2 × 10^10 and 4.8 × 10^9 pfu/ml, respectively.

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**Transcoronary gene transfer.** The transcoronary adenoviral delivery system has been described previously by our group (5, 6, 10, 15). Briefly, after the rats were anesthetized and a thoracotomy was performed, a 22-gauge catheter containing 200 μl of adenovirus and 50 μl of adenosine (3 mg/ml) was advanced from the apex of the LV to the aortic root. The aorta and main pulmonary artery were clamped for 30 s distal to the site of the catheter, and the solution was injected. The chest was then closed, and the animals were allowed to recover. The rats underwent CBF analysis in a cross-circulated excised heart 3 days after the adenoviral gene transfer.

**Cross-circulated heart preparation.** The cross-circulated, blood-perfused, excised heart preparation has been described previously by our group (12). Briefly, in each experiment, two male (500–650 g) Wistar rats (blood supplier and metabolic supporter) and one DM or non-DM rat (heart donor) were anesthetized with pentobarbital sodium (50 mg/kg ip) and intubated and heparinized (1,000 U iv). In the rat used as the metabolic supporter, the common carotid arteries and the right external jugular vein were cannulated and connected to the arterial and venous cross-circulation tubing, respectively. The arterial and venous cross-circulation tubes from the support rat were cannulated into the brachiocephalic artery and the right ventricle (RV) via the superior vena cava, respectively, of the heart donor rat. In the excised beating heart maintained at 37°C, a thin latex balloon, connected to a pressure transducer for measuring LV pressure, was inserted into the LV and primed with water. Intraplanal arterial blood volume was changed from 0 to 0.1 ml by 0.025 ml. Heart rate was maintained constant at 300 beats/min by electrical pacing of the right atrium. Total CBF was continuously measured with an ultrasonic flowmeter (model T206; Transonic System), in which the inline tubing from the RV. LV thebesian flow was negligible. Systemic arterial blood pressure of the metabolic supporter rat served as a reference for calculating consumption (V˙O2) was obtained as the product of CBF and arteriovenous oxygen content difference (AVOD). Arterial pH, PO2, and PCO2 of the LV specimens, obtained after the cross-circulation studies, were fixed with 10% formalin and embedded in paraffin. Sections (3 μm thick) were stained with hematoxylin-eosin to determine CMD or with Azan-Mallory to assess CAP. In longitudinally oriented cardiomyocytes, transmural width was measured as CMD. Digital photographs were taken at six sites on each Azan-Mallory section. Interstitial/perivascular collagen area and myocyte area were determined separately by counting the computerized pixels with the use of NIH Image.

**Western blot for SERCA2a protein.** Lysates from the hearts were matched for protein concentration and then separated by SDS-PAGE and transferred to nitrocellulose membranes. For immunoreaction, the blots were incubated with SERCA2a antibodies followed by detection with enhanced chemiluminescence.

**Statistics.** Data are expressed as means ± SD. Multiple comparisons were performed by ANOVA, followed by a Student-Newman-Keuls post hoc test. Statistical significance was accepted at the level of 0.05 vs. DM, #P < 0.05 vs. DM group, and DM+βGal. Symbols represent data from individual animals, and each horizontal bar with vertical bar represents means ± SD. *P < 0.05 vs. non-DM, †P < 0.05 vs. DM, #P < 0.05 vs. DM+βGal.
cantly higher than those shown in non-DM (Fig. 1C). These results suggest that lowered LV/BW in DM+SERCA animals is mainly due to reduced size of cardiomyocytes.

Increase in CBF by SERCA2a overexpression. Although mean coronary perfusion pressure was almost constant in all hearts, DM+SERCA rats showed a sustained increase in basal CBF under mechanically unloaded (i.e., free of intraballoons) contraction without any inotropic interventions, compared with other groups (Fig. 1D), and thus a significant decrease in coronary vascular resistance. The increase either in the LV volume in control volume-loading run or in the Ca2+ infusions in Ca2+ inotropism run induced not only increased mechanical performance [upward-shifted end-systolic pressure-volume relation or increased end-systolic pressure at 0.05 ml of intraballoons, as previously reported in the same hearts (15)] but also increased AVOD with no change in CBF (Fig. 2A) and hence increased myocardial VO2 (Fig. 2B). Therefore, the VO2 could be determined primarily by AVOD (Fig. 2C). Thus the CBF in all groups remained unchanged, even in control volume-loading run and Ca2+ inotropism run. DM+SERCA rats showed a significantly higher CBF in both runs (P < 0.05), as well as higher basal CBF, compared with other groups [mean CBF in control volume-loading run (n = 4 in each group) as follows: for non-DM, 2.41 ± 0.10; for DM, 2.55 ± 0.39; for DM+βGal, 2.56 ± 0.43; and for DM+SERCA, 3.68 ± 0.58 ml·min⁻¹·g⁻¹; mean CBF in Ca2+ inotropism run (n = 4 in each group) as follows: for non-DM, 2.49 ± 0.11; for DM, 2.49 ± 0.44; for DM+βGal, 2.47 ± 0.45; and for DM+SERCA, 3.59 ± 0.40 ml·min⁻¹·g⁻¹]. The unchanged CBF in all hearts suggests that the vascular tone is preserved irrespective of the increase in LV mechanical or Ca2+ loading, and the sustained high CBF is a specific response in SERCA2a-overexpressed hearts. Finally, SERCA2a protein expression, which was decreased in DM and DM+βGal rats, was restored to non-DM levels by SERCA2a gene transfer (Fig. 3).

DISCUSSION

In this study, we found that transcoronary gene transfer of SERCA2a can increase CBF and reduce cardiomyocyte size in DM hearts.

![Picture 1](http://ajpheart.physiology.org/figure3.jpg) Fig. 3. SERCA2a protein expression in the 4 experimental groups (n = 3 in each group). The density of bands in immunoblots is presented in arbitrary units. *P < 0.05 vs. DM and DM+βGal.

Our group (6, 10, 16) has previously shown that gene transfer of SERCA2a improves both systolic and diastolic function in failing hearts (due to pressure overload) and diastolic function in aging rats. However, in these studies, it was not clear whether the improvement in contractile function was accompanied by changes in cardiomyocyte size and resolution of fibrosis. In the present study, the lowered LV/BW by SERCA2a overexpression in DM hearts (which have combined systolic and diastolic dysfunction) appears to be mainly due to reduced size of cardiomyocytes but not to reduced CAP. Likewise, lowered LV/BW and reduced CMD were found in a postinfarct rat model following gene transfer of phospholamban mutant, which must have activated the function of SERCA2a (7). Although many intracellular signaling pathways may contribute to CMD reduction by SERCA2a overexpression, one possible explanation is as follows: increased SERCA2a activity achieves the decrease in diastolic intracel-
lular Ca\(^{2+}\) concentration by increasing uptake into the SR, thereby possibly inducing inactivation of a Ca\(^{2+}\)-regulated phosphatase, calcineurin, which plays a central role in transducing environmental signals that control gene expression and hypertrophic growth in cardiac muscles (13).

One possible explanation for increased CBF by SERCA2a overexpression is as follows. The DM+SERCA2a group, as recently reported by our group (15), showed systolic/diastolic LV function superior to normal LV function observed in non-DM rats (i.e., the increased end-systolic pressure at 0.1 ml of intraballoon water and the decreased end-diastolic pressure at 0.1 ml of intraballoon water), although the DM and DM+βGal groups showed LV dysfunction. This superior LV relaxation induced by SERCA2a transfer may increase CBF via well-dilated coronary vessels due to a more efficient relaxation because CBF occurs primarily during the diastolic phase. This is supported by the recent finding that impaired coronary hemodynamics is associated with ventricular dysfunction in salt-loaded spontaneously hypertensive rats (17). However, we cannot rule out another possibility that exogenous SERCA2a gene may be expressed not only in cardiac but in vascular cells and induces relaxation of coronary vascular smooth muscle, resulting in increased CBF. In fact, nitric oxide physiologically stimulated vasodilation by activation of SERCA (4), which was induced by S-glutathiolation of SERCA (2, 3).

In conclusion, from the data presented here, targeting for SERCA2a may be a potential therapeutic strategy for chronic DM-induced heart failure. The overexpression of SERCA2a clearly decreased cardiomyocyte size but not the amount of fibrosis. It is interesting to contrast our results with those clearly decreased cardiomyocyte size but not the amount of fibrosis. It is interesting to contrast our results with those of a Ca\(^{2+}\)-ATPase in a rat model of heart failure. Circulation 104: 1424–1429, 2001.


REFERENCES


