In Vitro Analysis of SERCA2 Gene Regulation in Hypertrophic Cardiomyocytes and Increasing Transfection Efficiency by Gene-Gun Biolistics[a]

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ABSTRACT: The transcriptional downregulation of the SERCA2 gene is studied using neonatal rat cardiomyocytes stimulated with endothelin-1 to induce hypertrophy. Liposome-based transfection of cells with a 1.9 kb SERCA2 promoter fragment directed expression of a reporter gene identical to the downregulation of genomic SERCA2 expression by endothelin-1. Results of a new gene gun technology for transient transfection of cardiomyocytes with a RSV-β-galactosidase construct are reported. This new method for propelling DNA-coated gold beads into cardiomyocytes is extremely suitable for directly testing promoter/reporter gene DNA constructs since the transfection efficiency (approximately 10%) appears to be higher than traditional transfection methods.

INTRODUCTION

In both humans and animal models, sustained increased cardiac workload, as a result, for example, of myocardial infarction, chronic hypertension or valvular insufficiency, elicits a hypertrophic response of the heart, i.e., an increase in the size of individual myocytes. The hypertrophic response is characterized by a period of compensation during which remodeling of the heart normalizes systolic wall stress and basal parameters of contractile function. However, this adaptation carries a price. This apparent salutory response to excess load is accompanied by reversion to a fetal program of cardiac gene expression. The upregulation of cardiac neurohumoral hormones, including angiotensin II, catecholamine, endothelin-1 (ET-1), and atrial natriuretic factor (ANF), is partially involved in mediating the hypertrophic and gene expression responses. The period of adaptation is followed by a transition to cardiac failure. The underlying cause of the negative outcome is believed to reside partially in the reprogramming of gene expression.[1–5] This hypothesis is consistent with the observed relative downregulation of key proteins (indirectly) involved in regulation

[a]This work was supported by Grants M 93.004 and 95.109 from the Netherlands Heart Foundation.

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of the uptake and release of Ca^{2+} from the cardiac sarcoplasmic reticulum: the β-adrenergic receptor, Ca^{2+} ATPase (SERCA2), and phospholamban (PL).[2–9] On the other hand, the genes encoding ANF, β-myosin heavy chain, α-skeletal actin, for example, are found to be relatively upregulated during hypertrophy.[1,5,10,11] The nuclear mechanisms involved in coordinately regulating these cardiac genes during hypertrophy are fully unknown, although binding sites for several transcription factors, including serum response factor (SRF), transcription enhancer factor (TEF1), AP-1 (a heterodimer of jun/fos), and transcription factor Sp1, were shown to be important for activation of fetal cardiac genes in response to hypertrophy.[12–15]

As to the upstream signaling events, there is abundant evidence that the G_q-coupled phospholipase C-β, which produces Ca^{2+}-releasing inositol 1,4,5-triphosphate and protein kinase C activating 1,2-diacylglycerol, are linked to the receptor-mediated Ras and mitogen-activated (MAP) kinase pathways as transducers of hypertrophic signaling processes.[5,10,14,16] A very recent study of mice, transgenic for dominant-negative G_q-mediated signaling, convincingly demonstrated that receptor-mediated PLC-β is partially involved in pressure overload–induced hypertrophy *in vivo*.[17] The known details of the phosphatidylinositol signaling pathway were reviewed extensively by us.[18]

About the same time, there was a report of the discovery of a Ca^{2+}-calmodulin-dependent pathway via calcineurin in the myocardium during hypertrophy that activated the transcription factor NF-AT3 which, by cooperation with the transcription factor GATA4, induces fetal cardiac gene transcription.[19] In the latter study it was hypothesized that the Ca^{2+} release induced by inositol-1,4,5-triphosphate mainly causes calmodulin-calcineurin complex activation.

Most of the previous studies on nuclear factors concern upregulation of cardiac genes during hypertrophy.[10,12–15,19] To initiate studies on the nuclear factors involved in transcriptional downregulation of the SERCA2 gene during hypertrophy, we employed the model of cultured neonatal rat ventricular myocytes as used successfully by many other groups.[9,19–21] In this model, we were previously able to show that ET-1 is a strong inducer of hypertrophy by its increasing effect on the rate of [^{3}H]leucine incorporation in total protein and protein/DNA ratio.[21,22] Recently, we have isolated, characterized, and tested the activity (by liposome-based transfection) of an isolated SERCA2 promoter fragment as the first step in the search for the *cis*-acting elements and *trans*-acting factors responsible for downregulation of this gene during cardiomyocyte hypertrophy. That study[22] confirmed the general experience of other investigators[12–15,19] that the use of traditional methods (liposomes) for transfection of cardiomyocytes can be of value for studying promoter regulation. However, transfection efficiencies obtained by these methods are still extremely low, generally no more than one percent, and therefore not suitable for functional testing of the effects of overexpression of cardiac proteins. Other transfection methods, including cationic lipid/plasmid DNA complexes, incubations with "naked" plasmid DNA, and calcium phosphate ($CaPO_4$) precipitation, do not achieve higher efficiencies in cardiac cells.[23] A recent report on a new transfection system combined the convenience of plasmid DNA with the unique targeting properties of adenovirus vectors.[24] However, this "component system" gave variable results in our laboratory. In the mean time, it has been demonstrated by several authors that recombinant replication-defective human adenovirus can transfect primary cardiac cultures with close

to 100% efficiency.[25–27] These authors could show alterations in cellular Ca^{2+} transients after infecting cardiomyocytes with adenovirus overexpressing SERCA2 (Ad.RSV.SERCA2a[27] or Ad.CMV.SERCA2a[26]) and PL (Ad.RSV.PL[25]). Since recombinant adenoviruses overexpressing cardiac Ca^{2+} handling proteins are time-consuming to prepare, there is a need for methods with high-efficiency transfection of cardiomyocytes with plasmid DNA. Recently, a new product for transfection Fugene6 (Boehringer), and a gold particle–based transfer technology, Helios Gene Gun (Biorad), have become available.[28,29] The Helios Gene Gun uses helium pressure to propel DNA-coated gold particles into cells (biolistics[30]), originally developed for *in vivo* gene transfer into somatic tissues of live animals. Elemental gold has been chosen for mammalian gene transfer because pure gold is chemically inert and does not produce cytotoxicity. The high density of gold also permits greater momentum allowing deeper penetration into target cells. Gene guns have also been used for plant cells and bacteria because these cells have a hard cell wall, whereas other methods require the production of protoplasts before gene introduction (reviewed by Yang and colleagues[28]). So far, several successful applications using the gene gun to transfect primary cultures have been reported.[28] However, to our knowledge no one has reported on adapting this method for use with primary cultures of neonatal rat cardiomyocytes. In this report, we present the first results with the Helios Gene Gun system in cardiomyocytes using a reporter plasmid harboring the β-galactosidase (β-Gal) gene under the control of the Rous sarcoma virus (RSV) promoter. We demonstrate that this method is applicable for transfecting beating rat cardiomyocytes and gives superior results when compared to other—traditional and new—transfection reagents.

METHODS

DNA Constructs

The SERCA2 5′ upstream regulatory region (1.9 kb, including 0.4 kb of the 5′ UTR of the mRNA) was obtained by screening of a rat genomic library with a probe derived by PCR based on published rat SERCA2 promoter sequences, as described in detail elsewhere.[22] After restriction mapping of the obtained genomic fragment (13 kb), the *Bam*HI-*Not*I fragment (1.9 kb) that was promoter-positive in Southern analysis was subcloned in a chloramphenicol acetyltransferase (CAT) reporter vector (pCAT-Basic, Promega) (FIG. 1). The RSV-β-Gal construct was a kind gift from our Department of Endocrinology and Reproduction. The DNAs were isolated and purified with the Wizard Midiprep Kit (Promega) in the study aimed at SERCA2 promoter regulation and purified with CsCl, Wizard, or Qiagen (Endofree Plasmid Maxi Kit; Qiagen, Westburg) for the study aimed at gene gun versus other vehicle-mediated gene transfer.

Transfection and Induction of Hypertrophy by ET-1

Rat neonatal ventricular cardiomyocytes were isolated as described previously,[20] preplated, and cultured in 20 cm^2 (7.5×10^4 cells/cm^2 (SERCA2 promoter regulation)), or 1.8 cm^2 dishes (1.5×10^5 cells/cm^2 (gene gun–mediated gene transfer)) up

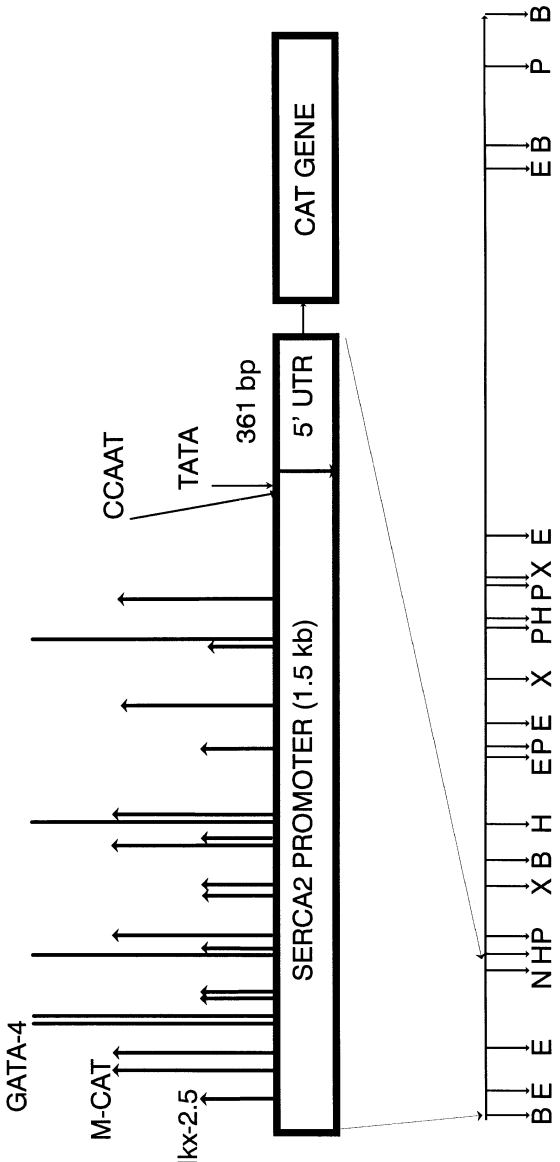

FIGURE 1. Graphical representation of the rat SERCA2 promoter-reporter gene construct. The putative CAAT and TATA sequences are indicated.[22] The rat SERCA2 promoter sequence was analyzed for putative transcription factor binding sites (GATA4, M-CAT, and Nkx2.5) with the Transfac database using the MatInspector program (*Top*). (*Bottom*) The obtained genomic DNA fragment with the locations of the *Bam*HI-*Not*I promoter positive region indicated. Abbreviations: B = *Bam*HI, E = *Eco*RI, H = *Hin*dII, N = *Not*I, P = *Pst*I, X = *Xba*I.

to 24 h in DMEM/M199 (4:1) supplemented with 5% fetal calf serum and 5% horse serum (HS). Thereafter, the medium was changed to DMEM/M199 (4:1) with only 5% HS. After 64 h, the medium was changed to serum-free DMEM/M199. Transfection was performed in this serum-free medium by standard $CaPO_4$ (0.5–3 μg DNA per well, HEPES buffer, pH 7.05, overnight incubation), Fugene6, and DOTAP methodologies (according to instructions provided by Boehringer Mannheim, Germany) (Fugene6/DNA ratio: 1.5 μl/0.5 μg DNA or 3.6 μl/1.44 μg DNA) (DOTAP/DNA ratio: 15 μg/5 μg DNA, 18 h) or by gene gun biolistics (see below). Subsequently, cells were washed with serum-free DMEM/M199 (4:1) and fresh medium was added. After 3 h, ET-1 (10 nM) was added for 48 h to induce hypertrophy.[9,21,22]

Gene Gun–Mediated Biolistics Transfection

The most recent hand-held version of the pulse gene gun (Biorad), which uses helium gas–driven force to propel the gold particles into cultured cells, was employed. DNA capsules were 1.2-cm pieces of Tefzel® tubing inner-coated with gold particles and loaded with CsCl-purified DNA. These capsules were then inserted into gene gun cartridges, which hold 12 capsules, allowing 12 transfections per single loading. The cartridges were prepared according to the manufacturer's protocol with slight but essential modifications.[29] Briefly, 200 mg gold particles (0.6 μm diameter) were suspended and sonicated in 1 ml analytically pure (100% is essential) ethanol to facilitate the accurate determination of the amount of gold particles needed per preparation. The exact amount of gold needed (4 mg for 40 cartridges) was transferred to a new tube and dried. Gold particles, DNA, spermidine, and $CaCl_2$ were mixed as described by the manufacturer (Biorad manual), incubated for 10 minutes, centrifuged, washed twice with 100% ethanol, and dried. The DNA-coated particles were suspended in the appropriate amount of 100% ethanol containing 0.015 mg/ml polyvinyl pyrrolidone in a 10-ml glass vial. Thereafter, the solution was transferred into the Tefzel® tubing, which was already inserted into the tubing prep station, by attaching a syringe to the nitrogen inlet. The solution was carefully sucked in just before the O-ring. The solution was left for 10 min to let the gold settle, followed by removal of the ethanol using the still-attached syringe. Immediately thereafter, the tubing chamber was turned by hand and then automatically directed by the apparatus until rings of precipitation of gold particles around the tube became visible (most clearly at the beginning of the tubing). A nitrogen flow (0.5 ml/min) was then applied to dry the DNA-coated gold particles for 15 minutes. After visual examination of the tubing, the parts exhibiting evenly distributed DNA-coated gold particles were cut into 1.2-cm cartridges and stored at 4°C. Per shot 0.5 μg DNA was delivered, which was coated on 0.1 mg gold particles (DLR: 0.5, MLQ: 0.1). Loading of the cartridge was checked by dissociation of the DNA from the gold by adding 100 μl Tris-HCl (10 mM)/EDTA (1 mM) (pH 7.5) buffer to one cartridge followed by vigorous mixing and sonication. The optical density was determined and the concentration of the DNA calculated. On average, within one complete coating of tubing a standard deviation of less than 15% was obtained. A special delivery device on top of the gene gun was developed at our laboratory to specifically enable high efficiency bombardment of 3×10^5 cardiomyocytes in a 1.8-cm^2 well. Medium was first aspirated from the cells followed by immediate bombardment with the Helios Gene Gun system (Biorad) at 100 psi helium pressure, then new serum-free medium was carefully layered back on the cells.

Northern Blotting

Total RNA was isolated by the guanidinium isothiocyanate method, quantified by spectrometry, separated on 1% denaturing formaldehyde-agarose gels, and blotted onto Hybond (Amersham). Probes described below were labeled by random priming using [α-^{32}P]dCTP (Amersham). The CAT probe was excised from the CAT reporter plasmid.[22] The glyceraldehyde phosphate dehydrogenase (GAPDH), PL, and ANF probes were developed by RT-PCR on rat heart RNA based on published sequences. A SERCA2 cDNA (RHCa-117) was a kind gift from Lompre.[31] Hybridization was performed as described.[22] The hybridization signal was quantified by the Molecular Imager (Biorad).

Analysis of β*-Gal Gene Expression*

Quantification of the β-Gal protein was performed by ELISA according to the manufacturer's protocol (Boehringer Mannheim). Histochemical staining of β-Gal protein was performed overnight according to standard protocols. After staining, cells were refixed and photographed. Cells staining positive for β-Gal were counted per well. Transfection efficiencies were based on this visual examination, as well as by comparing the obtained ELISA results to the amount of β-Gal measured (by ELISA) after 100% infection of cardiomyocytes with an adenovirus encoding β-Gal under the control of a Cowpea Mosaic virus promoter (results not shown).

Statistical Analysis

Significance was set at $p < 0.05$, Student-Newman-Keuls test, for at least four independent experiments.

RESULTS

Changes in Gene Expression in Cardiomyocytes Stimulated by ET-1

Previously, we demonstrated that 24 to 48 h ET-1 stimulation of serum-free cultured cardiomyocytes led to an increase of the rate of protein synthesis and protein/DNA mass ratio, both reminiscent of hypertrophy.[9,21,22] To determine whether ET-1–induced changes in cardiac gene expression are characteristic of hypertrophy and heart failure *in vivo*, we measured the changes in expression of ANF, SERCA2, and PL relative to GAPDH (TABLE 1). The increase in GAPDH mRNA is in accordance with the general increase of total RNA content seen 48 h after stimulation with ET-1.[22] As expected, ANF and SERCA2 (including PL) expression were respectively up- and downregulated. However, it should be noted that the absolute amounts of PL and SERCA2 mRNA levels, i.e., uncorrected for GAPDH levels, did not change significantly when compared to control.

Analysis of Transcriptional Regulation of the SERCA2 Gene

Our observation that the SERCA2 gene was downregulated in hypertrophied cardiomyocytes is consistent with the numerous previous reports (including our own

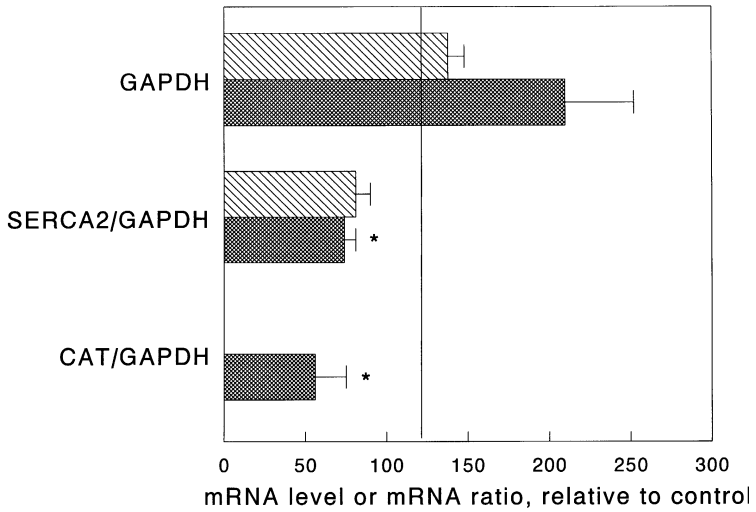

FIGURE 2. Transcriptional regulation of SERCA2 gene in rat cardiomyocytes. The CATSpro$_{1.9}$ DNA construct was transfected into rat cardiomyocytes using DOTAP and hypertrophy was induced by stimulation of the cells with 10^{-8} M ET-1 for 48 h. Subsequently, mRNA levels were quantified as described in *Methods*. Hybridization signals are represented relative to unstimulated control cells for GAPDH mRNA, while the (endogenous) SERCA2 and CAT mRNA signals were first corrected for increase in total RNA by expressing the ratio of these mRNAs relative to GAPDH mRNA before calculating the increase or decrease in expression relative to unstimulated control. GAPDH and SERCA2 expression are assessed in untransfected (*striped bars*) versus transfected cardiomyocytes (*cross-hatched bars*). Data represent mean ± SEM for 4–8 independent experiments. $^{*}p < 0.05$.

reports[32]) on reduced Ca^{2+} pump activity in the hypertrophied (failing) human and animal heart *in vivo* (reviews[1–4]). To perform detailed analysis of SERCA2 promoter regulation and identify *cis*-regulatory elements involved, a pure culture of cardiomyocytes (e.g., free of coronary smooth muscle cells) expressing the splice variant SERCA2a[3] is essential. Furthermore, we have isolated a genomic fragment of the SERCA2 gene containing 1.5 kb of the promoter region and 0.4 kb of the 5′ UTR, which was subcloned into the CAT reporter plasmid (nucleotide sequences were published by van Heugten and colleagues[22]). The sequence of this promoter was analyzed with the Transfac database using the MatInspector program[33] to detect any putative recognition sites for transcription factors, which might be important for the regulation of the SERCA2 promoter. FIGURE 1 represents the SERCA2 promoter-reporter gene construct with indicated recognition sites of putative interacting transcription factors. Indeed many transcription factors (Nkx-2.5, M-CAT, and GATA-4) known to be important for cardiac development and/or hypertrophic responses have putative recognition sites in this promoter.[34–36] To analyze the response of this promoter fragment, we transfected the reporter plasmid into cardiomyocytes that were subsequently stimulated by ET-1 to become hypertrophied. FIGURE 2 illustrates that, also in this separate series of experiments, ET-1 induces a decrease of SERCA2/GAPDH mRNA ratio and an increase in GAPDH mRNA level. Although only a mi-

TABLE 1. Changes in gene expression in cardiomyocytes stimulated by ET-1 for 48 h

	mRNA Level (%)	mRNA Ratio (%)
GAPDH mRNA	170 ± 18	
ANF/GAPDH		247 ± 48
PL/GAPDH		51 ± 8
SERCA2/GAPDH		74 ± 7

Note: Hybridization signals are represented relative to unstimulated control cells for GAPDH mRNA, while the ANF, PL, and SERCA2 signals were first corrected for increase in total RNA by expressing the ratio relative to GAPDH mRNA before calculating the increase or decrease in expression relative to unstimulated control. Data represent mean ± SEM for at least four independent experiments. All values are significantly different from 100%.

nority of cells are transfected by the SERCA2 promoter-reporter plasmid pCAT-Spro$_{1.9}$, the latter result proves that introduction of the SERCA2 promoter fragment did not influence endogenous GAPDH and SERCA2 gene expression. Transfection of pCAT-Spro$_{1.9}$ to cultured cardiomyocytes followed by 48 h of hypertrophy induction with 10^{-8} M ET-1 resulted in decreased reporter CAT mRNA level when compared to unstimulated transfected cells. Absolute CAT mRNA levels (i.e., uncorrected for GAPDH data) remained unchanged under these hypertrophic conditions when compared to unstimulated cardiomyocytes.

Gene Gun–Mediated Biolistic Transfection

The results in TABLE 1 and FIGURE 2 demonstrate that, as expected, it is possible to investigate SERCA2 promoter regulation in neonatal cardiomyocytes using "traditional" transfection methods. However, these methods (liposome- and $CaPO_4$-based methods) are known to have very low transfection efficiencies (less than 1%, see below). Firstly, studying promoters with very low activities may be difficult using these transfection methods. Secondly, high transfection efficiencies are required to study physiological changes of, e.g., overexpressed SERCA2 gene expression or PL synthesis disabled by antisense oligonucleotides, by exogenously introduced DNA constructs in these cardiomyocytes. Therefore, we set out to develop a biolistic technology using the recently commercially available Helios Gene Gun for *in situ* bombardment of tissue. We optimized the biolistics transfection for use with a primary culture of cardiomyocytes and analyzed its efficacy by using a plasmid containing the β-Gal reporter under control of the RSV promoter. Bombarded into the cells was 0.5 μg of DNA coated onto 0.1 mg gold particles (0.6 μm diameter). Forty eight hours after bombardment, cells were stained for β-Gal and the cellular morphology examined (FIG. 3). Transfection efficiencies of maximally up to 10% of the bombarded cells were obtained. Although we occasionally observe some cell death in the center of the bombarded well, this is usually very limited, as checked by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bizomid (Sigma) test assay for viable cells) (unpublished results). Next, we examined β-Gal expression in bombarded cells quantitatively by ELISA and compared these results with other traditional transfection reagents (note that in the traditional methods the same low amount of 0.5 μg DNA was also used). FIGURE 4 depicts the comparison of transfec-

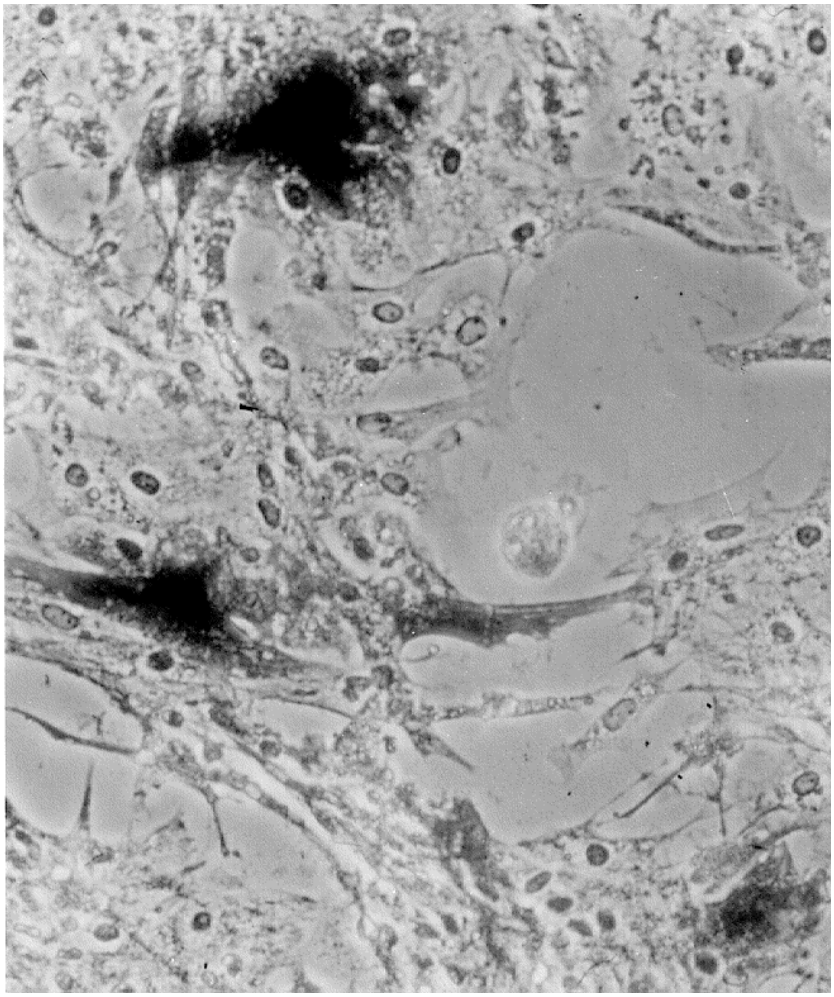

FIGURE 3. β-Gal protein expression after transfection of rat cardiomyocytes with RSV-β-Gal plasmid using gene gun biolistics. Cardiomyocytes were transfected with the biolistic transfection methodology using the RSV-β-Gal plasmid. After 48 h cells were fixed and stained histochemically for β-galactosidase activity (dark staining) to analyze the efficacy of transfection. Up to 10% of the cells are transfected using the gene gun and no gross morphology changes could be observed. Magnification = 200×.

tion methods using the RSV β-Gal DNA construct as measured by ELISA. It is clear that the biolistic method of transfection gives superior results compared to the traditional methods. Using more DNA for transfections with Fugene6 and $CaPO_4$ raises the efficiencies obtained with these methods for only the $CaPO_4$ method, although the amount of β-Gal measured after $CaPO_4$ transfection is still considerably lower compared to the biolistics results (FIG. 4). Recently, Boerhinger, the manufacturer of

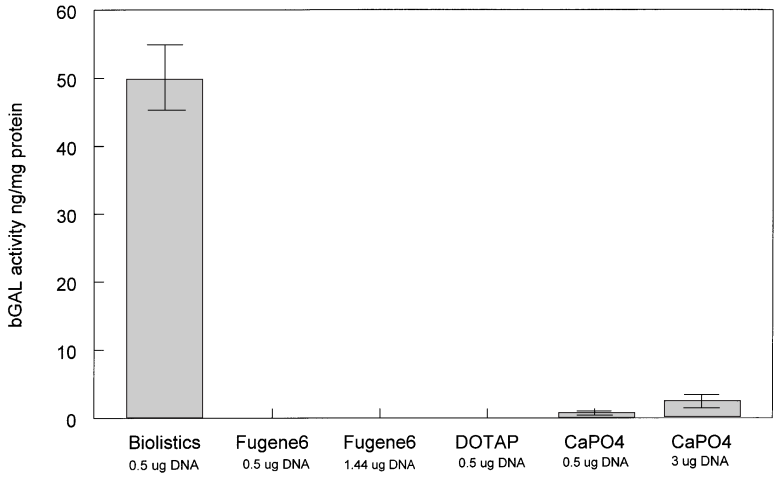

FIGURE 4. Comparison of gene gun versus other vehicle-mediated gene transfer methodologies. In all experiments the same plasmid (RSV-β-Gal) was used. Forty eight hours after transfection cells are homogenized and protein expression measured by ELISA. Data are represented as ng β-Gal/mg protein content±SEM. Cells were subjected to biolistic bombardment using 0.1 mg of gold particles coated with 0.5 μg of DNA (biolistics), or transfected using Fugene6, DOTAP, or $CaPO_4$. The amount of DNA used per 1.8 cm^2 well in the transfection experiments is indicated.

Fugene6, reported that, using mouse cardiomyocytes, approximately 1% of the cells could be transfected. The new Fugene6 transfection reagent did not give any measurable transfection in our test conditions (FIG. 4).

DISCUSSION

We isolated and determined the sequence of the rat SERCA2 gene 5′-regulatory region and analyzed its function in the presence or absence of a hypertrophy inducing stimulus (ET-1). This promoter region appears to contain several recognition sites for cardiac myogenic regulatory factors, e.g., Nkx-2.5, M-CAT, and GATA-4, which could be involved in the observed downregulation of transcription induced by ET-1 (FIGS. 1 and 2). Transfection of the SERCA2 promoter fragment in cultured cardiomyocytes did not change normal downregulation of the endogenous SERCA2 mRNA level during hypertrophy, showing transcription factors not becoming limited by the introduced SERCA2 promoter fragment. A 1.5 kb 5′ regulatory region together with 360 bp 5′ UTR dictated downregulation of a reporter gene similar to that observed for the SERCA2 mRNA (FIG. 2). This suggests that (most) *cis*-acting elements responsible for hypertrophy-associated downregulation of SERCA2 expression are confined to this regulatory region. Expression of the Ca^{2+} pump during hypertrophy was only downregulated when compared to (increased levels of) GAPDH mRNA, but absolute SERCA2 mRNA amounts remained unchanged. Therefore,

the downregulation of the SERCA2 promoter may be interpreted as not responding to the general increase in transcription that accompanies hypertrophy. Alterations in mRNA stability could also be an additional regulator of mRNA levels as demonstrated by actinomycin-D mRNA stability assays of cardiomyocytes, treated with phorbol-12-myristate-13-acetate (PMA) to induce hypertrophy, which revealed a marked destabilization of the SERCA2 transcript by PMA treatment.[38] We showed previously that during ET-1 stimulation only PKC-ε becomes redistributed intracellularly, whereas PMA caused redistribution of PKC-α, ε, and δ.[39] Therefore, PMA might act at more cellular sites compared to the physiological stimulus ET-1.

Recently Baker and coworkers,[40] using the rabbit SERCA2 promoter, demonstrated that an E/AT Box located at −1115 bp (corresponding to −1510 in the rat SERCA2 promoter[22,40]) was important for muscle-specific activation of the SERCA2 gene. However, when comparing the sequences of the rat and rabbit SERCA2 promoters it is clear that this E/AT box is not conserved in the rat SERCA2 gene promoter, making this box a less likely candidate for transcriptional regulation of the rat promoter.[22] Whether this E/AT box is important in the response to hypertrophic stimuli has not been investigated so far in the rabbit SERCA2 promoter.

The homology regions previously identified by comparing the rat promoter sequence with the human and rabbit promoter sequences[22] (nt −1520 to −1310, and nt −220 to +363 of the rat sequence) were recently also identified in the mouse SERCA2 promoter[41] (and personal communication, T.D. Reed). Transgenic mice harboring different parts of the mouse SERCA2 promoter clearly demonstrate an important role for these homology regions[41] (and personal communication, T.D. Reed). Future studies will include the role of the homology regions in the transcriptional regulation of the rat SERCA2 promoter and the preparation of deletion constructs of the rat SERCA2 promoter to elucidate the importance of the identified putative transcription factor binding sites.

In this paper we also present the first results using the gene gun technology for transient transfection of cardiomyocytes with a RSV-β-Gal construct. Using helium pressure to propel DNA-coated gold beads into the cardiomyocytes is extremely suitable for directly testing promoter/reporter gene DNA constructs since the transfection efficiency appears to be appreciably higher compared to more traditional transfection methods (FIG. 4). A transfection efficiency of approximately 10% of the cells in the bombarded area can be maximally achieved. This high transfection efficiency opens the possibility to test DNA constructs aimed at overexpressing or oligonucleotide antisense inhibition of specific cardiac Ca^{2+} handling proteins (e.g., SR-Ca^{2+} pump or PL). Up to now this was only feasible using recombinant adenoviruses.[25–27] Several parameters had to be optimized before these results were obtained. The amount of gold used per shot is limited since too much gold bombarded into the cells causes extensive cell damage in the center of the well. The newly developed diffusion screen (Biorad) prevents cell damage in the center of the bombarded area enabling possibly higher amounts of gold to be used. The helium pressure used is relatively low compared to applications using other cell types, due to the fragility of the cardiomyocytes. The most critical factor in achieving reproducible results turned out to be the production of the cartridges, we had to adapt the standard procedure on some essential points (see *Methods*) and used CsCl-purified DNA, which gave remarkably better results compared to DNA purified with the Wizard or Qiagen kits (results not shown).

Taken together, we demonstrate for the first time that the hand-held gene gun is a quick and reliable method to achieve high transfection efficiencies with primary rat neonatal cardiomyocytes as target cells. Experiments on the 5′-flanking regions of SERCA2 and PL genes using the gene gun transfection methodology are currently in progress.

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