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Carvedilol effectively blocks oxidative stress-mediated downregulation of sarcoplasmic reticulum Ca^{2+}-ATPase 2 gene transcription through modification of Sp1 binding

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Abstract

Carvedilol is a β-adrenoceptor blocker and a potent antioxidant that improves cardiac function in patients with heart failure. The restoration of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) gene expression may be an underlying mechanism of its beneficial effects on cardiac function. In primary cultured neonatal rat cardiac myocytes, treatment with either carvedilol or its β-receptor inactive metabolite, BM910228, attenuated the hydrogen peroxide-mediated decrease in SERCA2 mRNA and protein levels, while metoprolol, a pure β-blocker, had no effect. Moreover, carvedilol itself significantly enhanced SERCA2 gene transcription, suggesting that carvedilol specifically restores SERCA2 gene transcription. Site-directed mutagenesis revealed that two Sp1 sites in the SERCA2 gene promoter region mediated the response to carvedilol under oxidative stress. Further, electrophoretic mobility shift assays revealed that Sp1 and Sp3 transcription factors correlated with carvedilol-mediated changes in the promoter assays. These studies may provide a mechanistic explanation for the beneficial effects of carvedilol in heart failure.

Keywords: Heart failure; Sarcoplasmic reticulum; β-Adrenergic receptor; Gene expression; Oxidative stress

β-Adrenoceptor blocker therapy (e.g., carvedilol, metoprolol) decreases mortality and improves cardiac performance in patients with chronic heart failure [1–3]. Normalization of β-adrenoceptor function and downstream signaling pathways may account for the therapeutic efficacy of these agents. Although metoprolol has been shown to increase the β-adrenoceptor receptor density and restore function of the β-adrenoceptor/adenylate cyclase signal transduction pathway, carvedilol does not display similar effects [3,4]. Therefore, other mechanisms may underlie the clinical utility of carvedilol in the treatment of heart failure.

The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase pumps Ca^{2+} from the cytosol back into the SR after myocardial contraction, thereby coordinating contractile tension and relaxation kinetics [5]. SR Ca^{2+}-ATPase protein in the heart is encoded by the sarco(endo)-plasmic reticulum Ca^{2+}-ATPase 2 (SERCA2) gene. Studies have shown that decreased SERCA2 expression in the failing myocardium results in an abnormal force-frequency relationship [5–7]. We recently demonstrated

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that decreases in SERCA2 mRNA levels resulted from downregulation of SERCA2 gene transcription in pressure-overloaded rat hearts and that the binding sites of Sp1 transcription factor in the proximal region of SERCA2 promoter are important for its transcriptional regulation [8].

Recent studies have demonstrated that treatment of heart failure patients with a β-adrenoceptor blocker results in normalization of SERCA2 expression [9,10] and that this effect might underlie the clinical utility of these agents. However, it is not clear whether increased SERCA2 expression is a primary result of β-adrenoceptor blockade or whether it is a general secondary effect of improved cardiac function. Therefore, we investigated the hypothesis that carvedilol increases the SERCA2 gene expression in failing hearts.

Oxidative stress is implicated in several forms of cardiac dysfunction, including doxorubicin-induced cardiomyopathy [11], myocardial ischemia–reperfusion injury [12], and dilated cardiomyopathy [13,14]. Hydrogen peroxide is cell membrane permeable and is often used to induce oxidative stress in cardiac myocytes as a model of cardiac injury in vitro [15,16], where it induces a decrease in SERCA2 proteins levels [13]. Thus, the goal of the present study was to examine the effect of carvedilol on the transcriptional activation of the SERCA2 gene and expression of SERCA2 mRNA and protein in the presence or absence of hydrogen peroxide in cultured cardiac myocytes.

Materials and methods

Reagents. Carvedilol and BM910228, kindly provided by Daiichi Pharmaceutical (Tokyo, Japan), were solubilized in a small volume of *N*,*N*-dimethylformamide (DMF) and diluted with 0.1% acetic acid. The final concentration of DMF was less than 0.1% and had no effect on measured activities. U0126, SB203580, and SP600125 were purchased from Cell Signaling Technology (Beverly, MA), CALBIOCHEM (San Diego, CA), and BIOMOL Research Laboratories (Plymouth Meeting, PA), respectively. Other chemicals were purchased from Sigma–Aldrich Japan (Tokyo, Japan) unless otherwise indicated.

Cell culture. Primary cultures of neonatal rat cardiac ventricular myocytes were prepared as described previously [17], with minor modifications. Myocytes were plated at a density of $5 \times 10^4/cm^2$ on 35 or 10 mm dishes for 24 h in Dulbecco's modified Eagle's (DME) medium containing 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin–streptomycin. The culture medium was changed to serum free DME medium for 24 h before exposure to pharmacologic agents.

This investigation was performed according to the *Guide for the Care and Use of Laboratory Animals* published by U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996), and the study protocol was approved by the Animal Research Committee of the Gunma University Graduate School of Medicine.

Transfection and luciferase assays. Promoter deletion constructs within the 5′ upstream region of the SERCA2 gene (nucleotides −1810 to +350 bp relative to the transcription initiation site [18]) were used to test the transcriptional activity of the gene. To determine the promoter region that is necessary for the carvedilol-response, serial deletion constructs were produced within the SERCA2 gene promoter. Furthermore, mutations in the four Sp1 sites were generated using an Altered Sites II in vitro mutagenesis kit (Promega, Madison, WI) to eliminate Sp1 binding sites. Second and third guanine or cytosine nucleotides were replaced with two thymidine nucleotides in each Sp1 consensus sequence. Transfection of neonatal rat myocytes was performed by the lipofection method using tfx-50 (Promega) according to manufacturer's protocol, as previously reported [19]. After transfection, cells were incubated with the experimental pharmacologic agents for 24 h and then harvested for assay and further analysis.

Northern blot analysis. Total cellular RNA was isolated using the ISOGEN reagent (Nippongene, Tokyo, Japan) in accordance with the manufacturer's instruction. The cDNA fragment for rabbit SERCA2 cDNA [20] was used as hybridization probes and labeled with [α-^{32}P]dCTP (Amersham Biosciences, Piscataway, NJ) using a random primer DNA labeling kit (Roche Diagnostics, Indianapolis, IN).

Western blot analysis. Twenty-four hours after stimulation with hydrogen peroxide, cells were harvested and lysed by adding ice-cold lysis buffer [150 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L NaF, 20 mmol/L Tris–HCl (pH 7.5), 10% glycerol, 1% Triton X-100, 1 mmol/L Na3VO4, 1 mmol/L PMSF, 10 μg/mL leupeptin, and 10 μg/ml aprotinin] for Western blot analyses of SERCA2. The cell lysates were centrifuged, and supernatants were subjected to electrophoresis on a SDS–polyacrylamide gel (8% for SERCA2 or 12% for α-sarcomeric actin detection) and transferred to nitrocellulose membranes. Membranes were then blocked in TBST (0.1% Tween 20, 10 mmol/L Tris, pH 7.6, and 150 mmol/L NaCl) containing 5% bovine serum albumin (BSA), followed by overnight incubation with rabbit SERCA2 antibody [21] or α-sarcomeric actin (Sigma, St. Louis, MO) at 4 °C. Chemiluminescent detection was performed with the enhanced chemiluminescence protocol (Amersham Biosciences).

Electrophoretic mobility shift assay. Nuclear extracts were prepared from neonatal rat cardiac myocytes as described previously [22]. The protein concentration of the nuclear extract was determined using a BCA protein assay kit (Amersham). The sequences of the sense strands of double-stranded oligonucleotides used as probes or competitors in the electrophoretic mobility shift assay (EMSA) were as follows (consensus motif underlined and mutations of wild-type sequences in boldface): Sp1(I), 5′-GTTCCTGGGGGCGGTGCGCGG-3′; Sp1(II), 5′-CGGGAGGGGGCGGGGCCTGCG-3′; Sp1(III), 5′-GGGGGAGGGGGCGGGGCCGCG-3′; Sp1(IV), 5′-GGGGCCGCGCCGCCCGCGCCG-3′; mSp1(I), 5′-GTTCCTGG**GTTCGG**TGCGCGG-3′; mSp1(II), 5′-CGGGAGG**GTTCGG**GGCCTGCG-3′; mSp1(III), 5′-GGGGGAGG**GTTCGG**GGCCGCG-3′; and mSp1(IV), 5′-GGGGCCGCG**CCGAAC**GCGCCG-3′.

In supershift reactions, 2 μL of antibody (anti-Sp1, anti-Sp3, anti-Egr1, anti-AP2, or anti-c-*jun* at 200 μg/mL, Santa Cruz Biotechnology, Santa Cruz, CA) was added and incubated on ice for 60 min prior to probe addition.

Data analysis. Data are expressed as means ± SD Overall differences within groups were determined by one-way analysis of variance. When this test indicated that differences existed, individual experimental groups were compared by Bonferroni's test. A level of $P < 0.05$ was defined as statistically significant.

Results

Carvedilol restores SERCA2 promoter activity in hydrogen peroxide-treated myocytes

To determine whether carvedilol can restore the transcription of the SERCA2 gene under oxidative stress, neonatal cardiac myocytes were exposed to hydrogen peroxide, and the effects of carvedilol on the SERCA2

and β-actin genes were assessed. Since carvedilol is a nonselective β-adrenoceptor blocker with an α-adrenoceptor antagonist and strong antioxidant activity [23], we compared its effects to those of: (1) metoprolol, a selective β1-adrenoceptor blocker that has much lower antioxidant capacity than carvedilol, (2) BM910228, which has a 400-fold lower β-adrenoceptor affinity and a 30-fold higher antioxidant capacity than carvedilol [1,23,24], (3) *N*-acetylcysteine (NAC), a pure antioxidant, and (4) prazosin, an α-adrenoceptor blocker.

Stimulation with hydrogen peroxide at 50 μmol/L for 24 h resulted in a significant decrease in SERCA2 gene promoter activity levels (Fig. 1). Carvedilol at 10 μmol/L antagonized this effect and completely restored SERCA2 gene promoter activity to normal levels. Interestingly, the antagonist effect for SERCA2 gene transcription was stronger for carvedilol than for NAC. Similarly, although BM910228 antagonized the hydrogen peroxide-induced effects, its effect was lower than that of carvedilol. In contrast with carvedilol, metoprolol, and prazosin did not restore the diminished SERCA2 gene transcription induced by hydrogen peroxide.

Carvedilol restores SERCA2 mRNA levels in hydrogen peroxide-treated myocytes

As shown in Fig. 2A, carvedilol dose-dependently reversed the downregulation of SERCA2 mRNA levels induced by hydrogen peroxide. Similar to changes in gene transcriptional activity, BM910228, metoprolol or prazosin did not restore SERCA2 mRNA expression. However, NAC treatment restored SERCA2 mRNA levels following hydrogen peroxide administration. Changes in SERCA2 protein levels paralleled those changes in mRNA levels, as demonstrated by Western blot analysis (Fig. 2B).

Carvedilol increases SERCA2 promoter activity in the absence of hydrogen peroxide

Because carvedilol restored transcription of the SERCA2 gene and SERCA2 mRNA levels following hydrogen peroxide exposure, experiments were conducted to determine whether carvedilol directly enhances SERCA2 gene transcription in the absence of hydrogen peroxide. Carvedilol at 10 μmol/L significantly increased SERCA2 transcription to 5.4-fold that of basal levels (Fig. 3). Although NAC restored SERCA2 transcription and mRNA levels following hydrogen peroxide administration, NAC did not upregulate SERCA2 gene transcription in the absence of hydrogen peroxide. This difference between carvedilol and NAC may be derived from additional pharmacological properties of carvedilol, which are more than simple antioxidant activity. BM910228, metoprolol or prazosin had no effect on SERCA2 gene transcription.

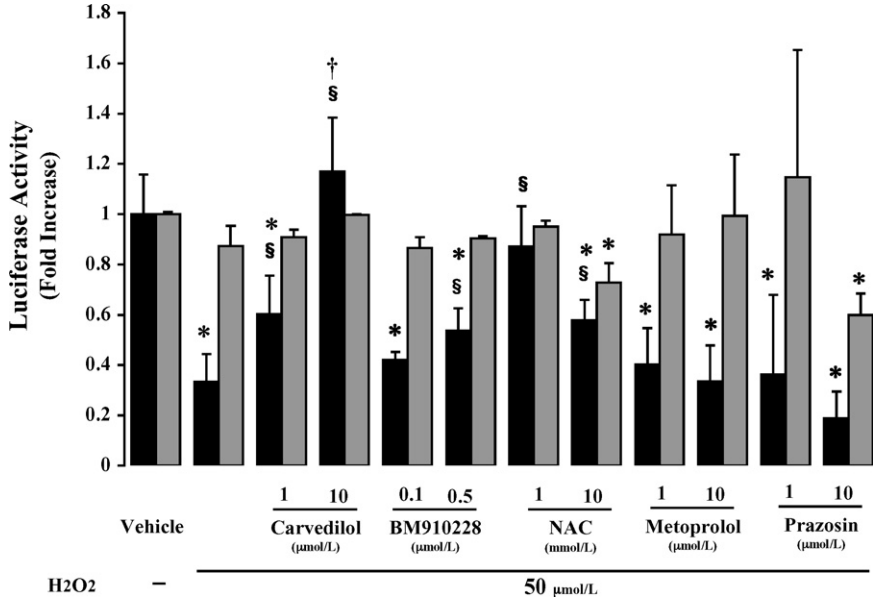

Fig. 1. Effect of carvedilol on oxidative stress-induced changes in SERCA2 promoter activity in comparison with pure antioxidants and adrenoceptor antagonists. Cultured neonatal rat cardiac myocytes transfected with luciferase reporter plasmid harboring the 5′ upstream region of the SERCA2 gene (nucleotides −1810 to +350 bp) were stimulated with 50 μmol/L hydrogen peroxide (H_2O_2) in the presence of either carvedilol, BM910228 (β-receptor inactive metabolite of carvedilol), *N*-acetylcysteine (NAC) (pure antioxidants), metoprolol (β-blocker) or prazosin (α-blocker) for 24 h. Luciferase activity of the SERCA2 gene was measured 24 h after exposure to H_2O_2 and each reagent (solid bar). The transcription level of the β-actin gene (gray bar) was co-examined under the same conditions to verify the specificity of the transcriptional change. Luciferase activities are shown as fold increase in average from four independent experiments compared with controls stimulated by vehicle (solvent of carvedilol). NAC; *N*-acetylcysteine. $^{*}P < 0.01$ vs. vehicle; $^{\S}P < 0.05$ vs. H_2O_2 only group; and $^{\dagger}P < 0.05$ vs. NAC at 1 mmol/L group.

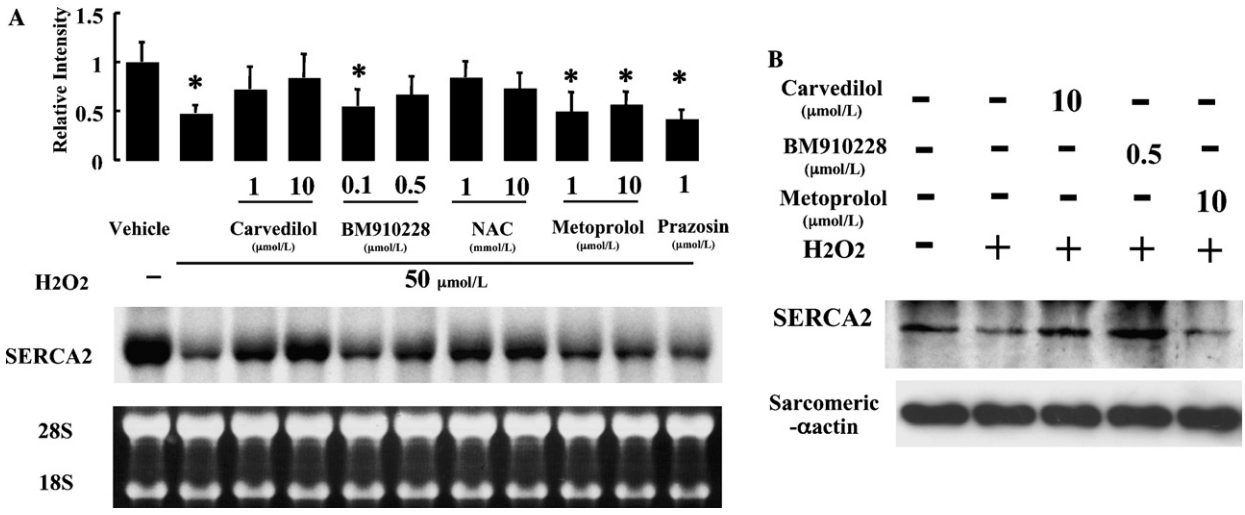

Fig. 2. Effect of carvedilol on oxidative stress-induced changes in SERCA2 mRNA expression (A) and protein (B) levels in comparison with pure antioxidants and adrenoceptor antagonists. (A) Northern blot analysis of SERCA2 mRNA levels. The amount of mRNA was quantified by densitometry and was normalized against the amount of 28S ribosomal RNA ($n = 4$). The ordinate indicates the ratio of each value relative to control (vehicle, a solvent of carvedilol, only). *$P < 0.01$ vs. vehicle. (B) The amount of SERCA2 protein of cultured rat cardiac myocytes stimulated by carvedilol, BM910228, and metoprolol. Sarcomeric-α-actin is shown as an internal control for the loading variation of protein sample.

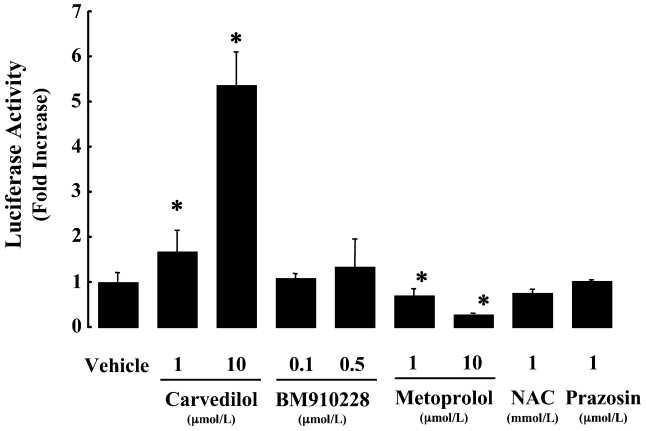

Fig. 3. The effects of carvedilol on the transcription of SERCA2 gene in comparison with pure antioxidants and adrenoceptor antagonists in the absence of hydrogen peroxide. Luciferase activities are shown as fold increase in average from four independent experiments compared with controls stimulated by vehicle. *$P < 0.05$ vs. vehicle.

Sp1 elements in the proximal SERCA2 gene promoter mediate the response to carvedilol

To define the promoter elements within the 5′ upstream transcription regulatory sites of the SERCA2 gene that mediate the response to carvedilol, deletion fragments were produced, and the transcriptional activity in the presence or absence of carvedilol was measured following hydrogen peroxide administration. The protective effect of carvedilol was obviated by deletion of the −284 to +350 bp region but not by deletion of the −72 to +350 bp region, suggesting that the −284 to −72 bp region of the SERCA2 promoter contains a site for carvedilol-response following hydrogen peroxide administration (Fig. 4). Since this region has multiple Sp1 sites, we examined whether Sp1 sites were responsible for the effect of carvedilol on SERCA2 gene transcription. The effect of carvedilol was abolished by mutating the two proximal Sp1 sites (Sp1 sites III and IV), suggesting that Sp1 sites III and IV, but not sites I and II, were responsible for the carvedilol-mediated restoration of SERCA2 gene transcription following hydrogen peroxide administration (Fig. 5).

Carvedilol restores Sp1/Sp3 binding

To determine whether transcription factor(s) bind to the four Sp1-consensus sites and to confirm that sites III and IV mediate the carvedilol-mediated restoration of the SERCA2 gene transcription following hydrogen peroxide exposure, EMSAs were performed using specific antibodies for putative transcription factors. Fig. 6 shows protein–DNA binding in the Sp1-consensus site III. Three specifically shifted bands can be identified, including one fast and two slower migrating bands. The lowest mobility band was supershifted by preincubation with an anti-Sp1 antibody (Fig. 6A, lanes 8–11), suggesting that this complex is composed primarily of Sp1. The other two higher mobility bands were supershifted by preincubation with an anti-Sp3 antibody (Figs. 6A, lane 12 and 7B, lanes 1–4), suggesting that this complex is composed primarily of Sp3. There was no apparent interaction of Egr-1, AP2, or c-*jun* antibodies with any specific complexes (Fig. 6B, lanes 5–7). Three complexes were also bound to Sp1-consensus sites I, II, and IV (Fig. 7, supershift data are not shown).

Specific bindings of Sp1 and Sp3 transcription factors to Sp1-consensus site III were reduced following hydro-

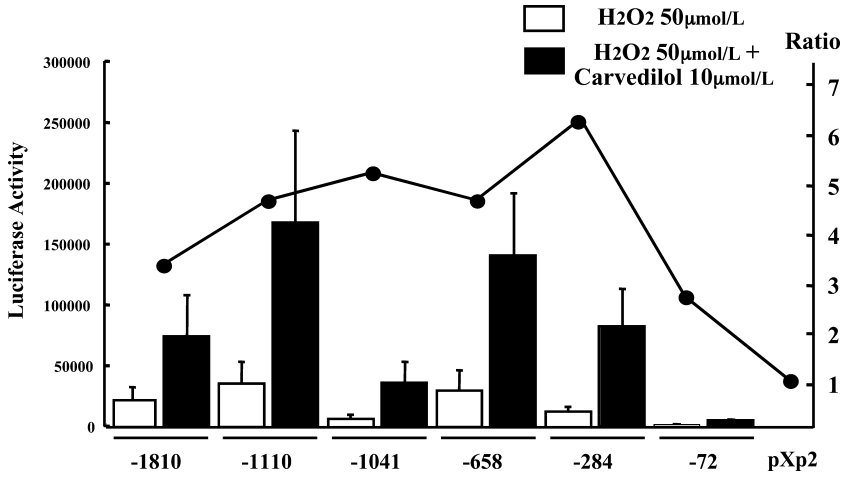

Fig. 4. Effect of carvedilol on the transcription of the serially deleted SERCA2 gene promoter. Serial deletion constructs (−1810 to +350 bp, −1110 to +350 bp, −685 to +350 bp, −284 to +350 bp, and −72 to +350 bp) created within the SERCA2 promoter region from −1810 to +350 bp relative to the transcription site were used to examine the effect of carvedilol on the transcriptional activity of the SERCA2 gene following exposure to 50 μmol/L hydrogen peroxide (H_2O_2). Right ordinate indicates the ratio of transcriptional activity of each construct when 10 μmol/L carvedilol was added to the H_2O_2-exposed cells.

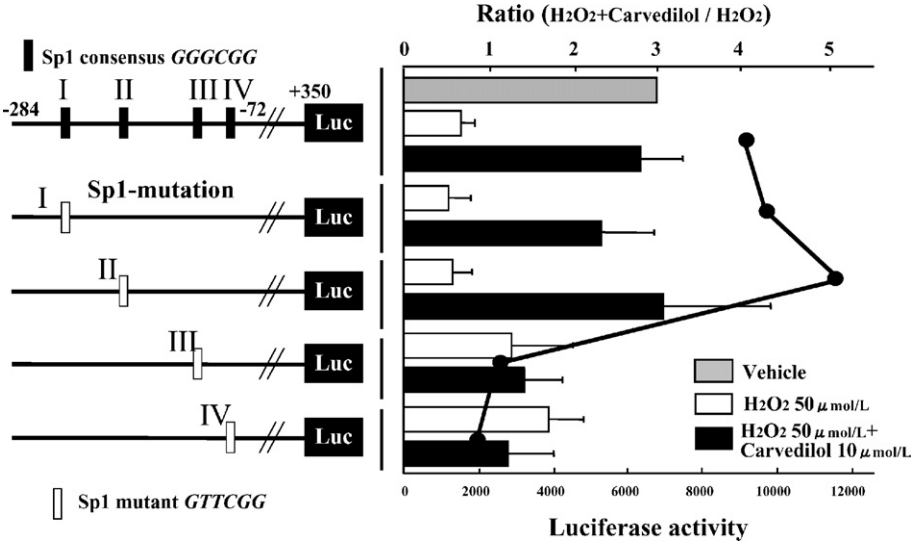

Fig. 5. Effect of individual mutation of the four Sp1 sites on the transcriptional activation of SERCA2 gene by carvedilol. Sp1 consensus sites 1–3 (GGGCGG) and site 4 (CCGCCC) in the −284 to −72 bp region were switched to GTTCGG and CTTCCC, respectively. The effect of each Sp1 mutation on SERCA2 transcription in hydrogen peroxide (H_2O_2) treated cells was assessed in the absence or presence of carvedilol. Luciferase activity without H_2O_2 exposure (vehicle group) in each Sp1 mutant was not represented in this figure in order to avoid complexity. Luciferase activities were significantly lower in the H_2O_2 group and in the H_2O_2 + carvedilol group when compared to the vehicle group in each Sp1 mutant. Upper abscissa indicates the ratio of the carvedilol-treated to the non-treated group.

gen peroxide stimulation, and restored, or rather increased by carvedilol (Fig. 6, Sp1-SS and Sp3-SS, open arrows). Similar changes in DNA–protein complexes were observed in the Sp1-consensus site IV (Fig. 7C), but not in sites I (Fig. 7A) or II (Fig. 7B).

Discussion

Recent clinical studies reported that SERCA2 mRNA [9] and protein levels [10] were normalized in heart failure patients following treatment with a β-adrenoceptor blocker. However, these studies did not characterize whether changes in SERCA2 mRNA and protein levels were primary events that lead to improvements in cardiac function or whether they were secondary effects of improved cardiac function that were induced through some other mechanism. The present study clearly demonstrated that carvedilol maintained the transcription of the SERCA2 gene under oxidative stress and thus, restored SERCA2 mRNA and protein levels. These observations suggest a mechanistic expla-

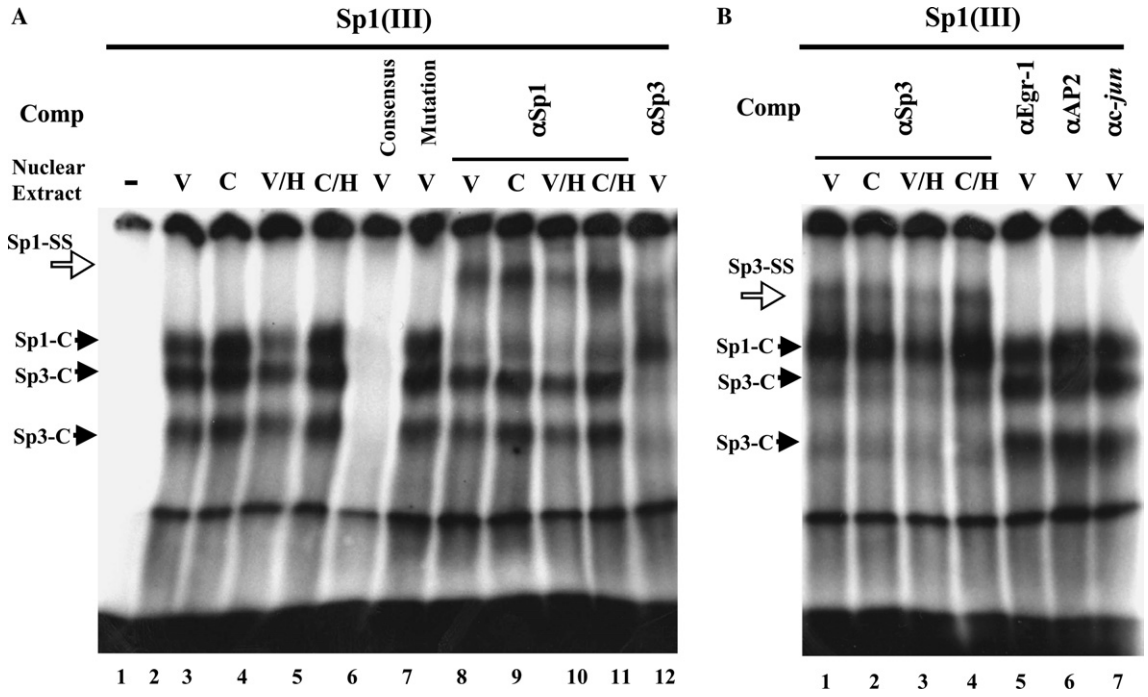

Fig. 6. Effect of carvedilol on the binding of Sp1 and Sp3 transcription factors to the 5′-regulatory region of the SERCA2 gene. Double-stranded oligonucleotide probe for Sp1-consensus site III in this figure was incubated with 10 μg nuclear extract prepared from neonatal rat cardiac myocytes stimulated with 50 μmol/L hydrogen peroxide (H_2O_2) in the presence or absence of 10 μmol/L carvedilol or with vehicle alone for 8 h. V, vehicle; C, carvedilol; V/H, vehicle and H_2O_2; and C/H, carvedilol and H_2O_2. Competitions with 100-fold excess unlabeled consensus probe (A, lane 6) and mutation probe (A, lane 7) are shown. Arrows indicate specific DNA–protein binding complexes composed of Sp1 (Sp1-C) and Sp3 (Sp3-C) transcription factors. Supershifted bands using anti-Sp1 antibody (αSp1) and anti-Sp3 antibody (αSp3) are indicated by open arrows (Sp1-SS and Sp3-SS, respectively). Anti-Egr1 antibody (αEgr-1), anti-AP2 antibody (αAP2), and anti-c-jun antibody (αc-jun) did not interact with any of the complexes. Experiments were performed in triplicate.

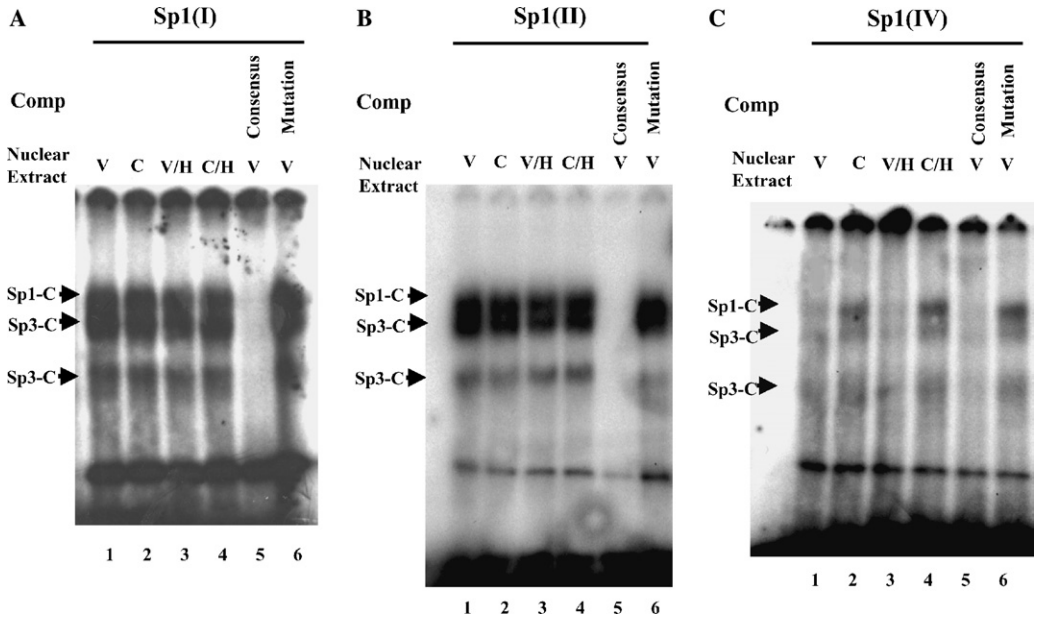

Fig. 7. Site-specific differences in the effect of carvedilol on the binding of Sp1 and Sp3 transcription factors to the 5′-regulatory region of SERCA2 gene. Conditions are identical to those described in this figure. EMSAs were conducted using probes for Sp1-consensus site I (A), Sp1-consensus site II (B), and Sp1-consensus site III (C). Competitions with 100-fold excess unlabeled consensus probe (lane 5) and mutation probe (lane 6) are shown in each panel. Arrows at the left of each panel indicate specific DNA–protein complexes composed of Sp1 (Sp1-C) and Sp3 (Sp3C) transcription factors.

nation for the ability of carvedilol to improve cardiac function in patients with heart failure.

Chronic heart failure arises from a complex interaction between impaired ventricular performance and extrinsic stimuli, i.e., neurohormonal factors, cytokines, mechanical-stretch, and oxidative stress [25]. Of these stimuli, oxidative stress plays a major role in cardiac hypertrophy, cardiac remodeling, and contractile dysfunction [13,26]. Plasma biochemical markers of oxidative stress are increased in heart failure patients and are directly associated with the severity of cardiac contractile dysfunction [13,14]. Hydrogen peroxide was used to induce dysfunction in cultured cardiac myocytes in the present study because it is one of the major reactive oxygen intermediates produced in the failing myocardium and because it is cell membrane-permeable [13,15,16].

Since carvedilol is a nonselective β-adrenoceptor blocker with an α-adrenoceptor blocking and strong antioxidant activity [23], this study investigated which pharmacological property mediates the restoration of SERCA2 transcriptional activity in the presence and the absence of hydrogen peroxide by comparing the effect on the SERCA2 gene to those of metoprolol, BM910228, NAC, and prazosin. NAC, a pure strong antioxidant, at a concentration of 1 mmol/L reversed the downregulation of the SERCA2 gene to the basal level. Importantly, carvedilol at 10 μmol/L restored SERCA2 transcription to a significantly greater degree than did NAC (Fig. 1). In addition, although NAC did not increase SERCA2 transcription in the absence of hydrogen peroxide, carvedilol significantly increased SERCA2 level up to 5.4-fold that of basal level. These data suggest that carvedilol upregulates SERCA2 gene transcription by an undefined mechanism as well as by its antioxidant property. As shown in Figs. 1–3, neither metoprolol nor prazosin alone was enough to rescue or activate SERCA2 gene transcription both in the presence and the absence of hydrogen peroxide, suggesting that α-blocking and β-blocking actions are not involved in such undefined mechanism. Since carvedilol is a highly lipophilic compound, this separate action may involve diffusion of carvedilol across the plasma membrane and action inside the cell. Further study to test this hypothesis would be of benefit.

The present study demonstrated that carvedilol activated transcription of the SERCA2 gene in the cardiac myocytes. SERCA2 gene has putative binding sites for GATA-1, CArG, CREB, MCAT, Sp1, Egr-1, and AP2 in the 5′ upstream regulatory sites [18]. By examining the transcriptional activity of serial deletion constructs of the SERCA2 gene promoter, we characterized the −284 to −72 bp region as critical for the response to carvedilol. Muscle specific genes such as cardiac and skeletal α-actin [27,28], β-myosin heavy chain [29], and SERCA2 [18] require Sp1 for efficient gene expression, making Sp1 binding sites attractive candidates for carvedilol-mediated restoration of the SERCA2 gene. Mutation of four Sp1 sites revealed that the two proximal Sp1 sites (sites III and IV) mediate carvedilol-induced restoration of SERCA2 gene transcription following hydrogen peroxide administration (Fig. 6).

To identify which transcription factor(s) regulate SERCA2 gene transcription following hydrogen peroxide exposure and/or carvedilol administration, EMSA was performed. Several Sp family transcription factors, including Sp1, Sp3, and Sp4, and Egr-1, may bind to the Sp1-consensus DNA sequence. EMSAs revealed binding of Sp1 and Sp3 to the two proximal Sp1-consensus sites (sites III and IV) in the SERCA2 gene promoter. We previously demonstrated that expression of Sp1 in cardiac myocytes resulted in enhancement of SERCA2 gene transcription (e.g., 5-fold above basal levels) [19]. These data, taken together with the present observation that restoration of SERCA2 transcription was prevented by mutation of sites III and IV, suggest that the binding of Sp1 and Sp3 transcription factors with Sp1-consensus sites III and IV mediates transcriptional restoration of the SERCA2 gene by carvedilol following hydrogen peroxide exposure.

EMSAs demonstrated that carvedilol increased the binding of Sp1 and Sp3 following hydrogen peroxide exposure, which is consistent with previous reports that suggest a role for Sp1 and Sp3 in the oxidative stress-mediated changes in the expression of other genes. Zhang et al. [30] demonstrated that the expression of the hepatocyte growth factor (HGF) receptor gene was maintained by Sp1 and that its sequestration by Egr-1 was the mechanism by which HGF receptor gene is suppressed under hydrogen peroxide exposure in mouse inner medullary collecting duct epithelial cells. Schafer et al. [31] demonstrated that enhanced binding of Sp1 and Sp3 to two proximal GC-boxes mediated hydrogen peroxide-triggered vascular endothelial growth factor (VEGF)-A gene activation in gastric adenocarcinoma cells. Further, the authors reported that posttranscriptional modification of Sp1, but not Sp3, represented an additional mechanism of VEGF-A gene regulation under oxidative stress and that the Ras-Raf-MEK1-ERK1/2 pathway transduced the effects of oxidative stress to regulate the VEGF-A gene. In contrast to the study by Zhang, Egr-1 did not participate in the regulation of the SERCA2 gene under hydrogen peroxide and carvedilol administration in our experimental model. However, the present study did demonstrate that Sp1 and Sp3 mediate the signal of hydrogen peroxide and carvedilol via MEK-ERK1/2 pathway (data not shown), which is consistent with Schafer's results. However, it should be noted that hydrogen peroxide increased the transcription of the VEGF-A gene but decreased the transcription of the SERCA2 gene. These

reports and our data illustrate the complex gene- and tissue-dependent nature of the transcriptional regulation in response to oxidative stress.

In summary, our data suggest that carvedilol can block oxidative stress-mediated down-regulation of SERCA2 gene expression in neonatal rat cardiac myocytes independent of its β-blocking activity. Sp1- and Sp3-mediated activation of the SERCA2 gene plays a significant role in the effect of carvedilol under oxidative stress. We propose that carvedilol may induce a favorable "genetic remodeling" in failing hearts exposed to oxidative stress. This study provides a novel therapeutic rationale for carvedilol in the treatment of heart failure patients.

Acknowledgments

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