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Biochemical and Biophysical Research Communications 353 (2007) 67–73
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NRSF regulates the developmental and hypertrophic changes of HCN4 transcription in rat cardiac myocytes

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Received 13 November 2006
Available online 4 December 2006

Abstract

The HCN4 channel shows differential expression patterns during the embryonic development and hypertrophy of hearts. Briefly, HCN4 expression is maximally activated in embryonic hearts and quickly diminishes after birth. However, it is reactivated during cardiac hypertrophy. The sequence analysis of HCN4 gene revealed the presence of a conserved NRSE motif, which is known to bind the transcriptional factor neuron-restrictive silencing factor (NRSF). A promoter analysis of HCN4 with rat cardiac myocytes identified the region inducing a basal transcriptional activity. This region drove a high activity in embryonic myocytes, but not in neonatal myocytes treated with hypertrophic agents. After confirming that NRSF protein binds to the NRSE, HCN4 promoter activities modified by NRSE were evaluated. With wild-type NRSE, the promoter activity correlated well with the developmental and hypertrophic changes of HCN4 expression, whereas mutant NRSE constructs failed. We conclude that the NRSE–NRSF system was implicated in HCN4 expression in cardiac myocytes.

Keywords: HCN4 channel; NRSE; NRSF; Transcription; Cardiac myocyte; Development; Hypertrophy

Hyperpolarization-activated, cyclic nucleotide-gated cation channels (HCN channels) are expressed in a variety of cardiac cells and neurons. These channels generate an inward current termed I_f, which plays a crucial role in the autonomous rhythmic activity of excitable cells [1]. To date, four mammalian HCN isoforms (HCN1–4) have been identified, three of which (HCN1, HCN2, and HCN4) are present in the heart with varying expression levels in different cardiac regions [2,3]. Among these isoforms, HCN4 is best known as the predominant isoform in the pacemaker region located in the sinoatrial (SA) node [2,4]. The HCN4 channel gene is also a member of embryonic cardiac genes. The HCN4 channel is widely expressed in the heart in the early developmental stage of an embryo, but its expression diminishes with embryonic differentiation [5]. In most forms of cardiac hypertrophy, there is an increase in the expression of embryonic genes, including ion channels. The upregulation of HCN4 expression in the heart is considered to underlie arrhythmogenesis during cardiac hypertrophy and heart failure [6,7]. Therefore, the transcriptional regulation of the HCN4 appears to be important for understanding the mechanisms of differentiation of the electrophysiological properties of cardiac myocytes and the mechanisms underlying the electrical remodeling of diseased hearts [8].

The neuron-restrictive silencer element (NRSE [9]) has been identified as a negative regulatory element that silences neuronal gene expression in nonneuronal cells [10]. The repression is induced through the binding of the zinc finger

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0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.119

transcriptional factor neuron-restrictive silencer factor (NRSF [11]). Using transgenic mice expressing dominant negative NRSF in their hearts, we previously reported that NRSF plays a critical role in the structural and functional alterations that occur during heart failure [12]. These transgenic mice exhibit increased vulnerability to arrhythmias and the upregulation of HCN4 channel expression in their hearts.

The objective of the present study was to characterize the transcriptional regulation through the NRSE–NRSF system in HCN4 expression during the development and hypertrophy of cardiac myocytes. Here, we identified a functional NRSE regulatory DNA element located within the first intron of the HCN4 gene and demonstrated that the NRSE–NRSF system regulated the HCN4 gene promoter during cardiac myocyte development and hypertrophy.

Materials and methods

Rapid amplification of cDNA ends (RACE). RNA ligase-mediated 5′-RACE was performed using the GeneRacer kit (Invitrogen) by using the additional reverse primers; HCN4-specific primer (5′-CAT GGC ACC GAA CTG GCG CTG CAT GAA G-3′) and HCN4-specific nested primer (5′-GCC GCG CCT CCC TCC ACT TTG ATA-3′). The amplified products were sequenced to determine the transcription start site.

Construction of a promoter reporter plasmid. Various fragments of the promoter of the mouse HCN4 gene were isolated from a mouse genomic bacterial artificial chromosome (BAC) and cloned into the luciferase reporter plasmid pGL3-Basic (Promega). The intronic fragments containing mouse HCN4 NRSE at its center were prepared by performing PCR. All constructs were verified by DNA sequencing.

Cell preparation and culture. Rat cardiac myocytes were cultured as previously described [13]. In neonatal myocyte assays, the cardiac ventricles were excised from 2- to 4-day-old Wistar rats. For embryonic myocytes, embryonic rat whole hearts were isolated from anesthetized pregnant rats (postcoitum day 12.5). After enzymatic digestion of their tissues, a cardiac myocyte fraction was prepared by Percoll gradient centrifugation (GE Healthcare). After 24 h of cell plating, the cells were cultured in serum-free media. In hypertrophy-inducing assays, the media were replaced by media containing hypertrophy-inducing agents (50 μM phenylephrine (PE) or 50 nM endothelin (ET)-1) 24 h after the first media change.

Transient transfection and luciferase reporter assay. Cardiac myocytes grown in 24-well plates were transfected by using Lipofectamine (Invitrogen). The cells were cotransfected with 0.6 μg of the luciferase reporter construct and 0.2 μg of the pRL-TK vector (Promega) expressing *Renilla* luciferase. Following a 72-h culture, the cell lysate was harvested, and the luciferase activity was measured using Promega's Dual-Luciferase Reporter Assay System. All luciferase activities were normalized with the *Renilla* luciferase activities.

Electrophoretic mobility shift assays (EMSA). Nuclear extracts were prepared from cultured neonatal rat cardiac myocytes by using NE-PER kit (Pierce). The radiolabeled probe was prepared using 22-bp oligonucleotides containing the intronic NRSE of HCN4 gene. The binding reaction was performed in a 20-μl final volume of a reaction buffer containing 20 mM Hepes (pH 7.6), 150 mM KCl, 2.5 mM $MgCl_2$, 0.1% Nonidet P-40, 10% glycerol, 1 mM dithiothreitol, and 10 μg poly(dI–dC) per ml. The nuclear extract (5 μg of protein) was added to the reaction buffer and incubated with 10 fmol of a probe for 30 min on ice. In the competition experiments, 10- or 100-fold molar excess competitors were coincubated. For EMSA-antibody assays, the nuclear extracts were preincubated with 1 μg of antibodies. The samples were separated by electrophoresis on 5% polyacrylamide gels in 0.25× TBE buffer.

Chromatin immunoprecipitation (ChIP). Chromatin from neonatal rat primary cardiac myocytes was prepared using the ChIP assay kit (Upstate). The purified chromatin was immunoprecipitated using NRSF antibody (Upstate). The immunoprecipitated product was analyzed by PCR using the following primer pairs: NRSE ChIP primers 5′-AGA GGG TGG TAT ACA CTG GAG AAG-3′ (forward) and 5′-ACT ACA CTG GGA AGA TGA GAG GAT-3′ (reverse) and control ChIP primers 5′-AAT GGG ACT CCT CTT ACT CAT TTC T-3′ (forward) and 5′-AAA GTC CCT GAT GAC ACA CTA GTT C-3′ (reverse).

RT-PCR and quantitative real-time RT-PCR analysis. Conventional RT-PCR was performed using Platinum PCR SuperMix (Invitrogen). To avoid the amplification of genomic DNA, each pair of primers was designed to reside in the exons separated by introns. Quantitative real-time RT-PCR was conducted for HCN4 and GAPDH by using predesigned TaqMan Gene Expression Assays (Applied Biosystems). The reaction was performed on ABI Prism 7700 System (Applied Biosystems). The mRNA levels of HCN4 were normalized to the endogenous GAPDH.

Western blotting analysis. Nuclear proteins were prepared from $\sim 10^7$ cultured neonatal rat cardiac myocytes by using an NE-PER kit (Pierce). The proteins were separated on 6% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk and incubated with a NRSF antibody (1:500; Upstate). Signals were detected using ECL system (GE Healthcare).

Statistical analysis. Data were expressed as means ± SE values. Statistical analysis was performed using Student's *t*-test. The *P* values less than 0.05 were interpreted to represent statistically significant differences.

Results

Identification of the mouse HCN4 promoter region and the NRSE within the intron region

To determine whether NRSF participates in the transcriptional regulation of HCN4, we searched for an NRSE-like sequence in the mouse HCN4 genomic sequence. The computer search revealed that an NRSE-like sequence was located ~2 kb upstream of exon 2 of the HCN4 gene (Fig. 1, upper panel). As shown in the inset of Fig. 1, the NRSE-like sequence identified within the intron of the HCN4 gene is highly conserved among several species and is homologous to the consensus NRSE, suggesting that it might mediate an important regulatory function in the HCN4 transcription. We next attempted to identify the promoter region. The mouse HCN4 gene consists of eight exons, and the translational initiation site (ATG) is located within exon 1. The transcription start site (+1) in the heart was determined by a 5′-RACE reaction performed with several primers anchored in exon 1 to amplify the total RNA isolated from mouse hearts. Cloning and sequencing of the 5′-RACE product revealed the start site located 401 bp upstream from the ATG. Several restriction sites used in the subsequent promoter truncation assays are shown in the promoter region (Fig. 1, lower panel).

Functional analysis of the HCN4 promoter region

We next evaluated the transcriptional activity of the fragment comprising bp −3075 to +400 of the region of the HCN4 gene in cardiac myocytes. A series of HCN4

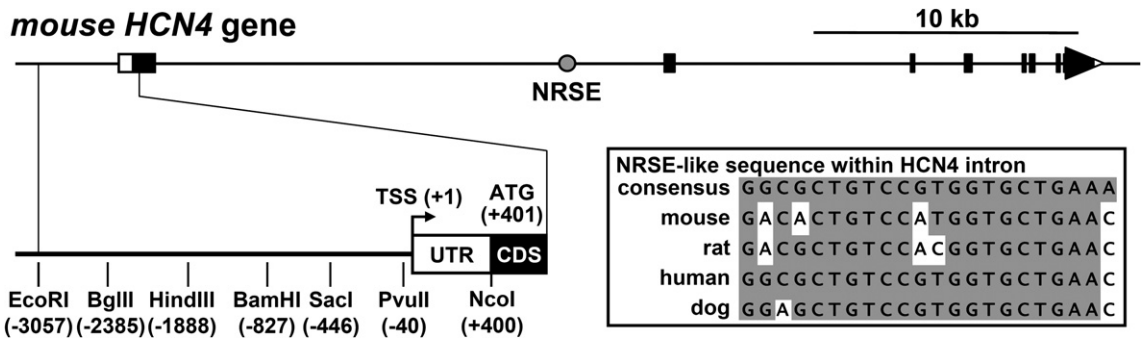

Fig. 1. Genomic structure of the mouse HCN4 gene and identification of the conserved NRSE. The boxes represent exons, including the coding sequence (CDS) and the untranslated region (UTR). The arrows indicate the transcription start site (+1). The inset shows the consensus sequence of NRSE and the conserved region of the intronic NRSE-like sequences in HCN4 among four species. The shadowed areas indicate the sequence that is homologous to the consensus NRSE.

promoter deletion luciferase reporter constructs were generated and transiently transfected into the primary cultures of neonatal rat cardiac myocytes (Fig. 2A). Serial truncations from −3057 to –446 bp regions resulted in an increase in transcriptional activity, suggesting the presence of potential negative regulatory elements in this region. Further deletion of the promoter region led to a decrease in reporter activity. Therefore, we concluded that the −446/+400 promoter region was responsible for the basal transcriptional activity of HCN4, and this fragment was used as the basic HCN4 promoter in the following experiments.

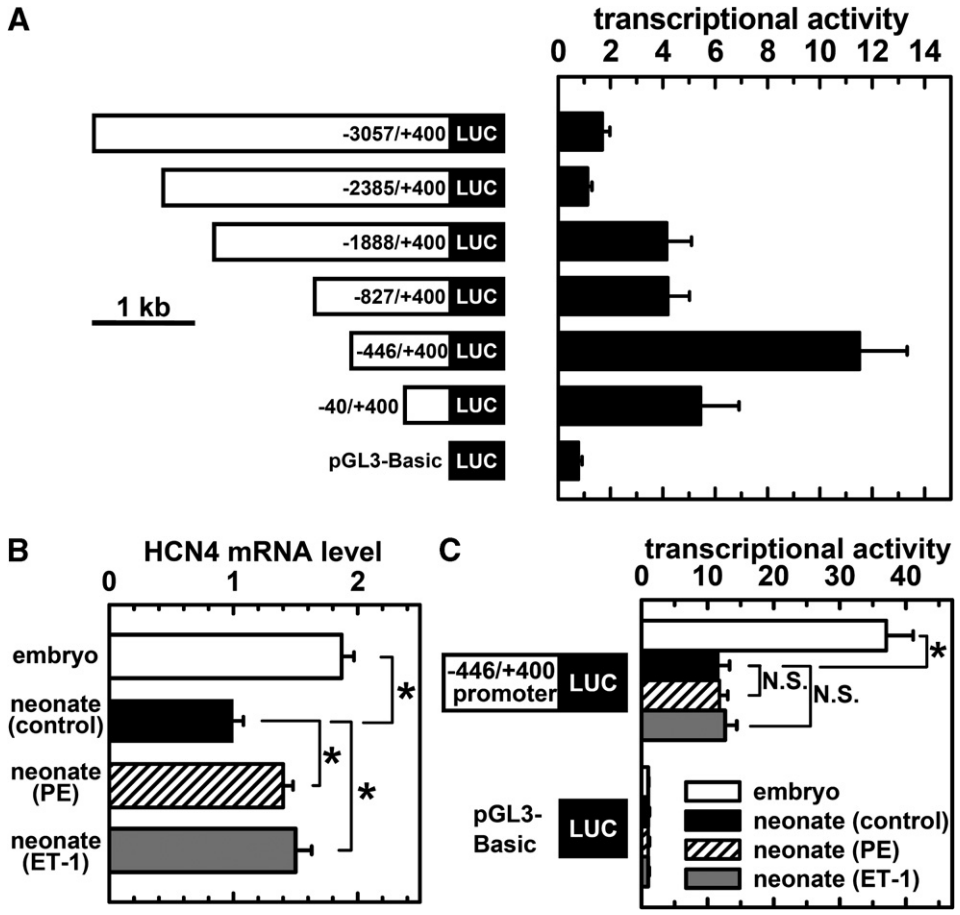

Fig. 2. Transcriptional activity of the mouse HCN4 promoter region. (A) Various 5′ truncated promoter reporter constructs were transfected into the neonatal rat primary cardiac myocytes. (B) Quantification of mRNA with real-time PCR using the primary cultures of embryonic, neonatal control, neonatal PE-treated, and neonatal ET-1-treated cardiac myocytes. The mRNA levels are expressed as normalized values relative to the neonatal control myocytes. (C) Transcriptional activity of the −446/+400 fragment in the embryonic, neonatal control, neonatal PE-treated, and neonatal ET-1-treated cardiac myocytes. All data are expressed as means ± SE values obtained from at least three separate assays carried out in quadruplicate. $^{*}P < 0.05$.

The HCN4 expression in cardiac myocytes is maximally activated in the early embryonic stage and diminishes in the course of embryonic development. However, HCN4 expression is reactivated during cardiac myocyte hypertrophy [6,7]. To assess whether the −446/+400 promoter activity quantitatively mimics the above pattern of HCN4 expression, we prepared several types of primary cultures of cardiac myocytes. We first compared the mRNA level of HCN4 in embryonic and neonatal myocytes. As shown in Fig. 2B, the mRNA level of HCN4 was higher in embryonic myocytes. When neonatal cardiac myocytes were stimulated with PE and ET-1 (commonly used hypertrophy-inducing agents) the HCN4 mRNA was also upregulated.

We then performed reporter assays with the −446/+400 promoter construct in these myocytes. In this assay, a pGL3-Basic vector was used as control. As shown in Fig. 2C, embryonic myocytes expressed ~3-fold higher activity compared to neonatal control myocytes, whereas no significant increases in the reporter activity were detected in neonatal myocytes stimulated with PE and ET-1. These results appear to indicate that −446/+400 promoter activity results in the high expression level of HCN4 in embryonic myocytes, but is not involved in HCN4 expression induced by PE and ET-1. The HCN4 gene appeared to contain other regulatory elements associated with the changes in HCN4 expression, and the intronic NRSE may be a putative important regulatory element.

NRSF represses the HCN4 promoter activity via the NRSE of the intron

To determine whether *cis*-regulatory functions are encoded in the intronic NRSE-like sequence of the HCN4 gene, we inserted the ~3-kb intron fragment containing the NRSE sequence at its center into the −446/+400 HCN4 promoter construct and performed reporter assays by using the neonatal rat cardiac myocytes (Fig. 3A). Compared to the transcriptional activity of the −446/+400 HCN4 promoter, that of the wild-type NRSE construct was attenuated to less than 18%. This supported the hypothesis that the inserted fragment encoded repression activity, and this activity was possibly mediated by the NRSE-like sequence. To confirm this, we prepared the construct of mutated NRSE [9,13]. When the mutant NRSE construct was transfected, the repression by the wild-type NRSE almost disappeared. These findings indicated that the intact NRSE sequence was necessary for the repression activity.

To investigate whether the nuclei of the cardiac myocytes bind to the NRSE of the HCN4 gene and to establish whether this binding activity is conferred by NRSF protein, we conducted an EMSA. A radiolabeled 22-bp oligonucleotide containing the HCN4 NRSE sequence was used as a probe. As shown by the arrow in Fig. 3B, a prominent slow-migrating DNA–protein complex was visualized with nuclear extracts. This complex was also formed with the consensus NRSE probe (data not shown). We next performed a competition experiments by using an unlabeled oligonucleotide containing wild-type or mutated NRSE. The signal for the prominent complex was abolished by the addition of wild-type NRSE oligonucleotide, whereas the mutated NRSE oligonucleotide was much less effective. This demonstrates that the major band was NRSE sequence-specific. We further examined whether the NRSE sequence-specific complex is related to the NRSF protein. The EMSA was performed with an NRSF antibody (Fig. 3C). The major complex was specifically eliminated by the NRSF antibody, but not by nonspecific antibodies. The interaction between the NRSF protein and the NRSE of HCN4 was also confirmed by the ChIP assay (Fig. 3D). The DNA immunoprecipitated with the NRSF antibody was amplified by the NRSE ChIP primers specifically recognizing the intronic NRSE region of HCN4, but the sample with the control IgG was not. Furthermore, coimmunoprecipitation was not observed with control ChIP primers, which recognized the first intron region of HCN4 but was located away from NRSE. Altogether, these findings indicated that NRSF directly binds to the NRSE of the HCN4 intron and represses HCN4 promoter activity in cardiac myocytes.

The NRSE–NRSF system is functionally important for cardiac HCN4 transcriptional regulation

Having confirmed that the NRSE–NRSF regulatory system is involved in the transcription of HCN4, we next investigated the functional role of NRSE–NRSF in the HCN4 expression in developmental and hypertrophic changes of cardiac myocytes. Because the −446/+400 promoter construct failed to mimic the HCN4 expression pattern (Fig. 2B and C), we conducted the reporter assays using the NRSE constructs with the primary cultures of embryonic, neonatal control, neonatal PE-treated, and neonatal ET-1-treated cardiac myocytes. To evaluate the NRSE–NRSF-specific transcriptional function, the reporter gene activities were normalized to the −446/+400 promoter activity expressed in the respective cells, and the activity in neonatal control myocytes was assigned a value of one (Fig. 4A). The embryonic myocytes expressed ~3.5-fold higher promoter activity compared to neonatal control cells, whereas the myocytes stimulated with PE and ET-1 displayed ~2-fold increases in the reporter activity. These data appeared to correlate closely with the HCN4 mRNA expression demonstrated in Fig. 2B. In contrast, mutant NRSE construct demonstrated no significant differences. These notable results indicate that the NRSE–NRSF system mediates an important regulatory function in the HCN4 expression of the embryonic myocytes and in the HCN4 upregulation induced by PE and ET-1.

Finally, we investigated whether NRSF could regulate the developmental change in HCN4 expression. We performed an RT-PCR to examine the mRNA levels of HCN4 and NRSF by using the total RNA isolated from rat hearts in the embryonic and adult stages. As shown

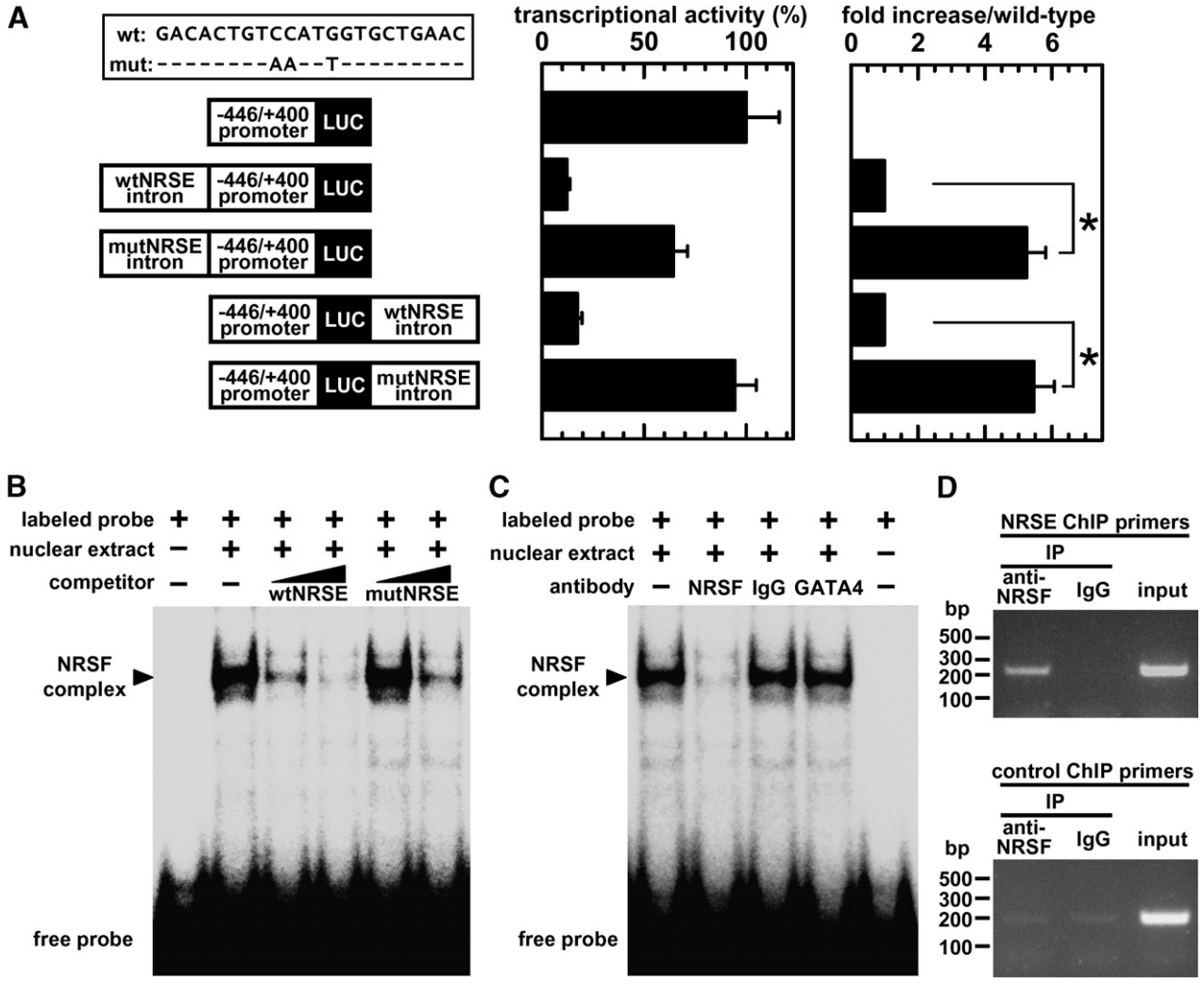

Fig. 3. Transcriptional activity of the intronic NRSE and NRSF binding to the HCN4 NRSE. (A) The inset shows the wild-type and mutated HCN4 NRSE. The constructs illustrated in the left were used in reporter assays with the control culture of neonatal rat cardiac myocytes. The bar graph in the right side demonstrates the fold increases by mutagenesis. The results are expressed as means ± SE $^{*}P < 0.05$. (B) EMSA with labeled HCN4 NRSE probe performed using the nuclear extracts from the neonatal cardiac myocytes. The binding activity of NRSF complex was competed by adding unlabeled wild-type NRSE oligonucleotide (wtNRSE) but not mutated NRSE (mutNRSE). (C) EMSA was performed using a NRSF antibody and nonspecific antibodies (normal IgG, GATA4 antibody). (D) The ChIP assay was performed by using a NRSF antibody and control IgG. The immunoprecipitates were analyzed using PCR with two sets of primers—NRSE ChIP and control ChIP primers. The 230-bp (upper panel) and 200-bp (lower panel) products correspond to the NRSE and nonNRSE regions, respectively.

in Fig. 4B, the expression of HCN4 mRNA decreases with development; conversely, the mRNA of NRSF increases. This inverse correlation between HCN4 and NRSF is consistent with our findings that NRSF negatively regulates HCN4 expression, implying that the increase in NRSF might repress HCN4 expression during the embryonic development of hearts. In contrast, our previous study has demonstrated that the mRNA level of NRSF did not decrease with hypertrophic change in cardiac myocytes [13]. Therefore, we assessed another mechanism of the downregulation of NRSE repression activity during cardiac myocyte hypertrophy. To examine whether the NRSE-specific DNA binding activity of nuclei was modulated with hypertrophic agents, EMSA was performed using the neonatal cardiac myocytes treated with PE and ET-1 for up to 24 h. As shown in Fig. 4C, the treatment with hypertrophic agents decreased the DNA binding activity in a time-dependent manner up to 3 h, and its activity was recovered at 8 h after stimulation. Western blot performed in parallel with corresponding nuclear extracts detected no clear differences in the NRSF protein levels (Fig. 4D). Therefore, the stimulus-induced change detected by EMSA appeared to correlate with the modulation of NRSF binding activity, rather than with the decrease in the NRSF protein levels. These results suggest that the upregulation of HCN4 expression induced by PE and ET-1 could be mediated by the downregulation of the NRSF bindings to the NRSE. In summary, we concluded that the NRSE–NRSF system was involved in the expression of HCN4 in the cardiac myocytes, although its mech-

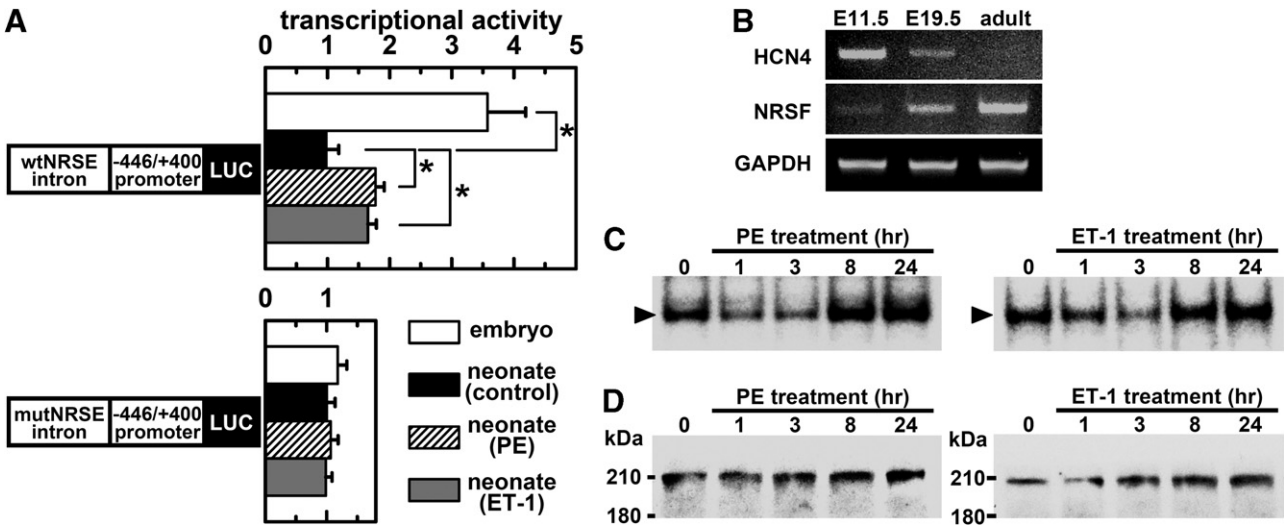

Fig. 4. (A) Transcriptional responses of the NRSE. Each experiment was performed at least three times in quadruplicate. The results are expressed as means ± SE $^{*}P < 0.05$. (B) RT-PCR analysis for HCN4, NRSF, and GAPDH. The RNA isolated form rat hearts in the embryonic (embryonic day 11.5, 19.5) and adult stages were used. (C) The modulation of NRSF DNA binding activity was analyzed by EMSA. The arrow denotes the NRSF–DNA complexes. (D) NRSF protein was analyzed by Western blotting.

anism may be different in the development and hypertrophy of cardiac myocytes.

Discussion

In the present study, we have focused on the transcriptional mechanisms that control the expression of the HCN4 in the embryonic development and hypertrophy of cardiac myocytes. Although previous studies have demonstrated changes in current density and mRNA levels during cardiac development, hypertrophy, and heart failure [6,7], this is the first analysis demonstrating that these related to the regulation of the transcriptional activity of the HCN4 gene.

The HCN gene family encodes proteins responsible for the ionic conductance termed I_f. Among the ion currents expressed by the heart, I_f is known to possess unique properties [1]. I_f is activated by membrane hyperpolarization, not by depolarization, and is carried by both Na^+ and K^+. Early electrophysiological studies suggested that I_f plays an important role in the ionic conductance during cardiac pacemaker depolarization, thus determining the heart rate and rhythm generated by the SA node cells [14]. Embryonic cardiac myocytes also show spontaneous rhythmic activity, and it has been proposed that I_f plays a pivotal role in this electrical function [5,15]. Therefore, the HCN4 gene regulatory mechanism appears to be important for understanding the mechanism behind cardiac cells acquiring spontaneous activity. The present study demonstrated the functional evidences that NRSF was associated with the expression of HCN4 in embryonic hearts. Additionally, our preliminary results demonstrate that the mRNA levels of HCN4 and NRSF display an inverse expression pattern between the SA node and the ventricle of adult rat hearts (unpublished data). This finding may suggest that cardiac cells without automatic rhythmic activities used the NRSF silencing system to repress HCN4 expression.

Heart failure patients experience a number of changes in the electrical function of the heart that lead to potentially lethal cardiac arrhythmias. Arrhythmias associated with cardiac hypertrophy and heart failure are likely to involve multiple pathophysiological mechanisms. Action potential prolongation, increase in intracellular Na^+, and altered Ca^{2+} handling are consistently reported in failing hearts [16]. These changes are possibly due, at least in part, to the decreases in the number of K^+ channels and the reexpressions of fetal-type ion channels, including HCN4. Nevertheless, their gene regulatory mechanisms remain poorly understood. In this regard, our present study has provided a new insight that the NRSE–NRSF gene regulatory system might participate in cardiac electrical remodeling. The elucidation of the transcriptional mechanisms of ion channels and transporters may provide a clue to prevent the electrical remodeling in diseased hearts.

Acknowledgments

We thank Prof. Kawakami and Prof. Endo (Jichi Medical University) for valuable discussions and Ms. Y. Ohori for excellent technical assistance. This work was supported by Grants-in-Aid from Takeda Science Foundation, the Vehicle Racing Commemorative Foundation, and the Japan Medical Association.

References

[1] R.B. Robinson, S.A. Siegelbaum, Hyperpolarization-activated cation currents: from molecules to physiological function, Annu. Rev. Physiol. 65 (2003) 453–480.

[2] W. Shi, R. Wymore, H. Yu, J. Wu, R.T. Wymore, Z. Pan, R.B. Robinson, J.E. Dixon, D. McKinnon, I.S. Cohen, Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues, Circ. Res. 85 (1999) e1–e6.
[3] C. Marionneau, B. Couette, J. Liu, H. Li, M.E. Mangoni, J. Nargeot, M. Lei, D. Escande, S. Demolombe, Specific pattern of ionic channel gene expression associated with pacemaker activity in the mouse heart, J. Physiol. 562 (2005) 223–234.
[4] T.M. Ishii, M. Takano, L.H. Xie, A. Noma, H. Ohmori, Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node, J. Biol. Chem. 274 (1999) 12835–12839.
[5] K. Yasui, W. Liu, T. Opthof, K. Kada, J.K. Lee, K. Kamiya, I. Kodama, *I*(f) current and spontaneous activity in mouse embryonic ventricular myocytes, Circ. Res. 88 (2001) 536–542.
[6] M. Fernandez-Velasco, N. Goren, G. Benito, J. Blanco-Rivero, L. Bosca, C. Delgado, Regional distribution of hyperpolarization-activated current (I_f) and hyperpolarization-activated cyclic nucleotide-gated channel mRNA expression in ventricular cells from control and hypertrophied rat hearts, J. Physiol. 553 (2003) 395–405.
[7] U.C. Hoppe, E. Jansen, M. Sudkamp, D.J. Beuckelmann, Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts, Circulation 97 (1998) 55–65.
[8] B. Rosati, D. McKinnon, Regulation of ion channel expression, Circ. Res. 94 (2004) 874–883.
[9] N. Mori, C. Schoenherr, D.J. Vandenbergh, D.J. Anderson, A common silencer element in the SCG10 and type II Na^+ channel genes binds a factor present in nonneuronal cells but not in neuronal cells, Neuron 9 (1992) 45–54.
[10] C.J. Schoenherr, A.J. Paquette, D.J. Anderson, Identification of potential target genes for the neuron-restrictive silencer factor, Proc. Natl. Acad. Sci. USA 93 (1996) 9881–9886.
[11] C.J. Schoenherr, D.J. Anderson, The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes, Science 267 (1995) 1360–1363.
[12] K. Kuwahara, Y. Saito, M. Takano, Y. Arai, S. Yasuno, Y. Nakagawa, N. Takahashi, Y. Adachi, G. Takemura, M. Horie, Y. Miyamoto, T. Morisaki, S. Kuratomi, A. Noma, H. Fujiwara, Y. Yoshimasa, H. Kinoshita, R. Kawakami, I. Kishimoto, M. Nakanishi, S. Usami, M. Harada, K. Nakao, NRSF regulates the fetal cardiac gene program and maintains normal cardiac structure and function, EMBO J. 22 (2003) 6310–6321.
[13] K. Kuwahara, Y. Saito, E. Ogawa, N. Takahashi, Y. Nakagawa, Y. Naruse, M. Harada, I. Hamanaka, T. Izumi, Y. Miyamoto, I. Kishimoto, R. Kawakami, M. Nakanishi, N. Mori, K. Nakao, The neuron-restrictive silencer element-neuron-restrictive silencer factor system regulates basal and endothelin 1-inducible atrial natriuretic peptide gene expression in ventricular myocytes, Mol. Cell. Biol. 21 (2001) 2085–2097.
[14] K. Yanagihara, H. Irisawa, Inward current activated during hyperpolarization in the rabbit sinoatrial node cell, Pflugers Arch. 385 (1980) 11–19.
[15] J. Stieber, S. Herrmann, S. Feil, J. Loster, R. Feil, M. Biel, F. Hofmann, A. Ludwig, The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart, Proc. Natl. Acad. Sci. USA 100 (2003) 15235–15240.
[16] G.F. Tomaselli, D.P. Zipes, What causes sudden death in heart failure? Circ. Res. 95 (2004) 754–763.