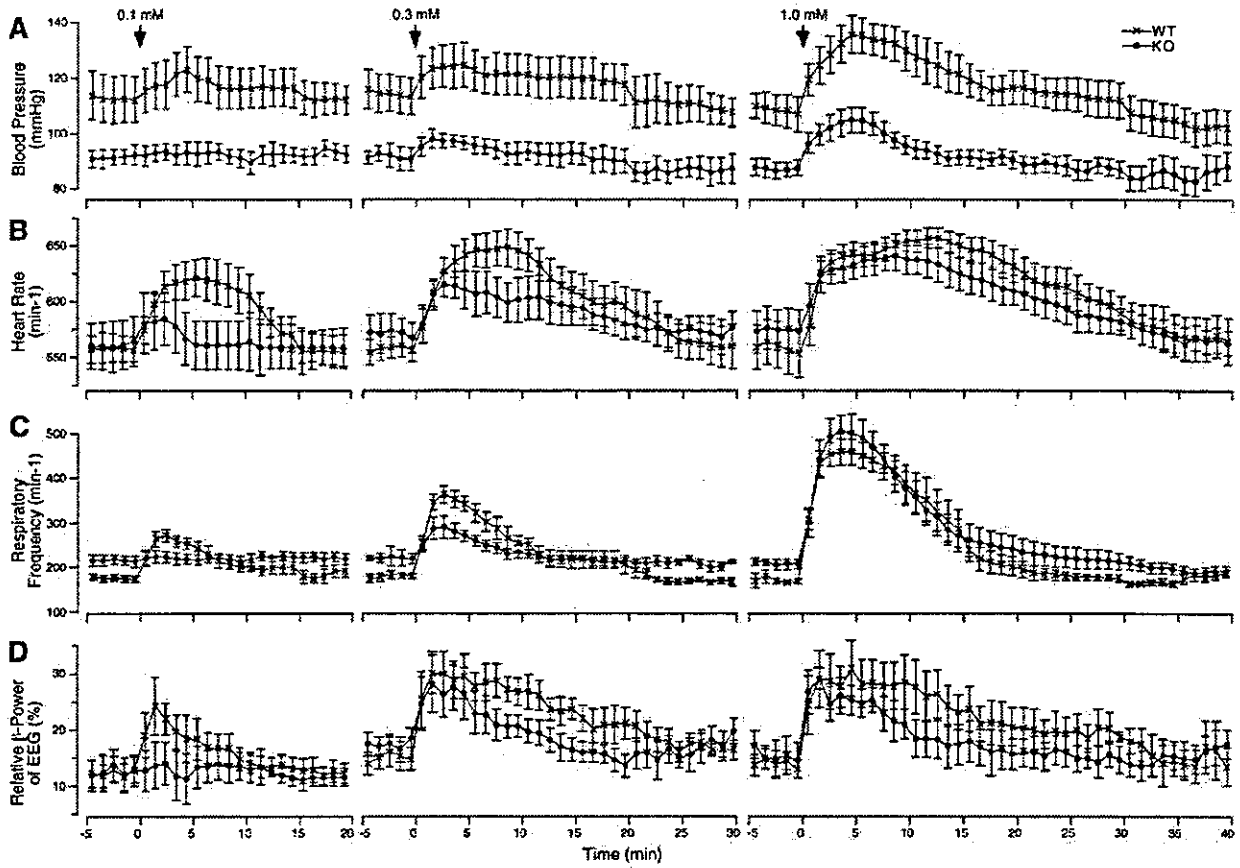
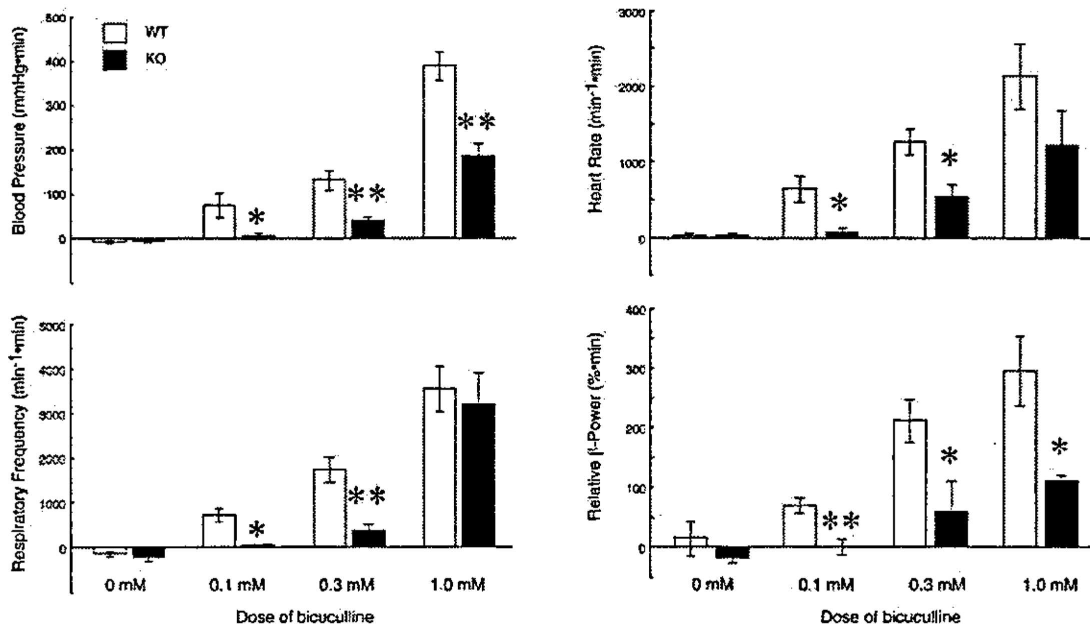

A
B
C
D
0.1 mM
0.3 mM
1.0 mM
WT
KO
Blood Pressure (mmHg)
Heart Rate (min-1)
Respiratory Frequency (min-1)
Relative θ-Power of EEG (%)
Time (min)


E Changes expressed as AUC
WT
KO
Blood Pressure (mmHg•min)
Heart Rate (min-1•min)
Respiratory Frequency (min-1•min)
Relative θ-Power (%•min)
0 mM
0.1 mM
0.3 mM
1.0 mM
Dose of bicuculline

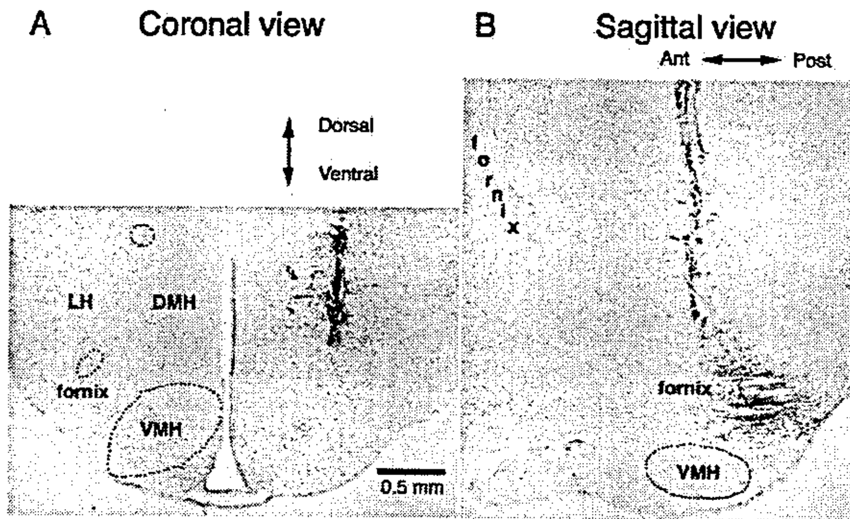


Fig. 3. Photomicrographs of coronal (*A*, at the level of 2 mm caudal to the bregma) and sagittal (*B*, at the level of 0.65 mm lateral to the midline) sections through the hypothalamus prepared after the experiment. Injection site of bicuculline was marked with Evans blue. Each section was stained with 1% neutral red. Ant, anterior; Post, posterior.

respiratory responses to those in wild-type mice (Fig. 2). In detailed analysis, however, there was a significant quantitative difference between knockout mice and wild-type mice. Whereas 0.1 mM of bicuculline was effective to elicit significant changes in MAP, HR, and Rf in wild-type mice, the same dose of bicuculline did not cause any significant changes in the knockout mice (Fig. 2, *A–D*, *left*), except for a small and shortlasting increase in HR. At the dose of 0.3 mM, cardiorespiratory responses in the knockout mice were shorter lasting than those in wild-type mice (Fig. 2, *A–D*, *middle*, and Table 1). At the dose of 1.0 mM, responses in AP and EEG were still shorter lasting although recovery time of HR and Rf responses was not statistically different from the wild-type mice (Fig. 2, *A–D*, *right*, and Table 1). Consequently, response magnitudes as calculated by AUC were significantly smaller in knockout mice for all the parameters (Fig. 2*E*) at least for 0.1 and 0.3 mM of bicuculline.

By histological examination, there was no difference in the dye distribution between wild-type mice and orexin knockout mice, and all the injections were successfully made in dorsal part of the perifornical area (Fig. 3).

Experiment 3: measurement of cardiovascular parameters and activity by radiotelemetry. Attenuation of defense response in orexin knockout mice was further confirmed by radiotelemetric measurement of behavioral and cardiovascular parameters in unanesthetized freely moving mice with a natural stressor (Fig. 4). At the baseline before stress, low AP and similar HR in orexin knockout mice were observed as was the case in anesthetized condition. When the test animal was confronted with an intruder, increases in AP, HR, and activity were significantly smaller in orexin knockout mice than in wild-type mice (Fig. 4*A*). The same was true when the test animal intruded into a resident's home cage (Fig. 4*B*). On the other hand, noxious stimuli of the tail pinch elicited similar increases in AP and HR in both genotypes, irrespective of stimulus intensity (Fig. 5).

We next examined circadian rhythm of AP, HR, and activity in the orexin knockout mice, because deficiency of orexin has been shown to induce disturbance in sleep-awake cycle and thus may induce abnormality in circadian rhythm of cardiovascular parameters. Activity during dark phase but not during light phase was significantly lower in orexin knockout mice and thus amplitude of 24-h fluctuation in activity was significantly lower in the mutant mice (Fig. 6, Table 2). Contrary to our expectation, however, circadian fluctuation of AP and HR was similar between knockout and wild-type mice except that AP in knockout mice was consistently lower during both dark phase and light phase. Although amplitude of AP in orexin knockout mice tended to be smaller, the difference did not reach statistical significance ($P = 0.34$).

To exclude the possibility that basal hypotension in orexin knockout mice in *experiment 2* was due to a difference in sensitivity to anesthetic, we next examined the effect of urethane administration on AP and HR in radiotelemeter-indwelling mice. As expected, urethane lowered AP in a similar manner in mutant (before urethane, 108 ± 3 mmHg and after, 83 ± 5 mmHg, −23.5 ± 3.5%) and wild-type mice (before urethane, 127 ± 6 mmHg and after, 102 ± 7 mmHg, −19.7 ± 3.6%). HR was not different between knockout

Fig. 2. Effects of microinjection of bicuculline methiodide to the perifornical area in urethane-anesthetized WT mice and orexin knockout mice (KO) on arterial blood pressure (*A*), HR (*B*), Rf (*C*), and relative β-band power of EEG (*D*), an indicator of arousal. Arrowheads indicate timing of microinjection of bicuculline (20 nl). *E*: changes in above parameters expressed as area under the curve (AUC). Data are presented as means ± SE of 5 WT mice and 5 KO mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. WT mice.

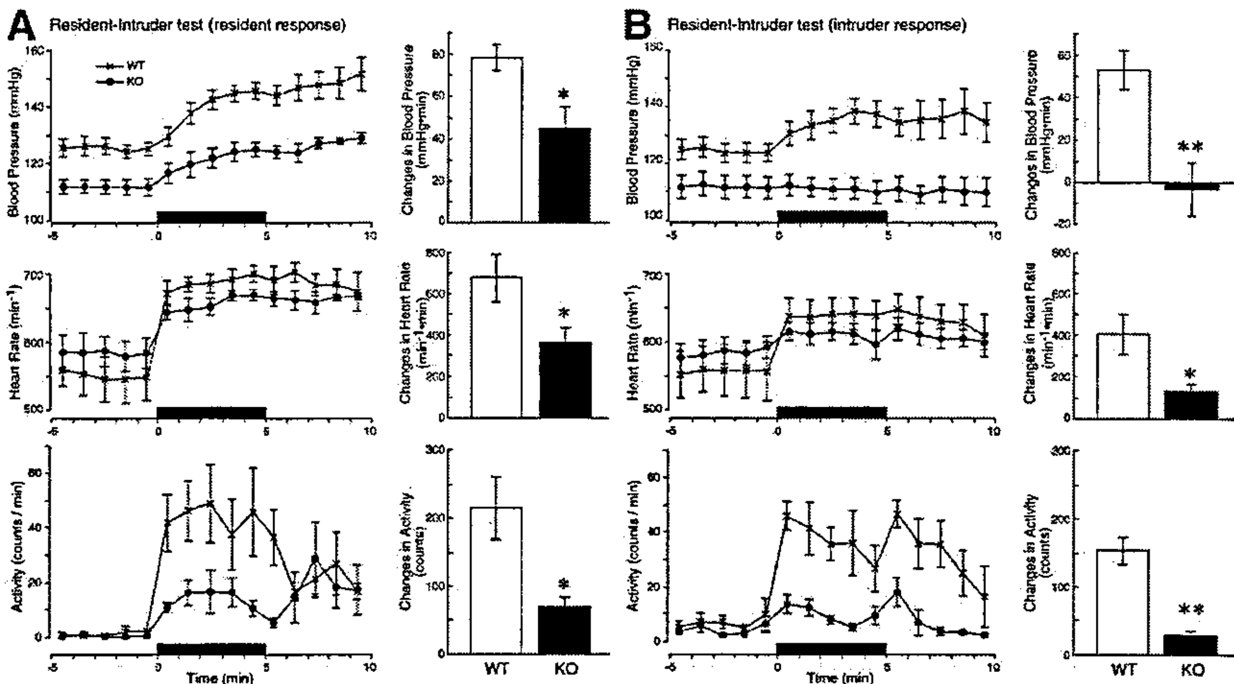


Fig. 4. Cardiovascular and behavioral responses during the resident-intruder test in radiotelemeter-indwelled freely moving WT mice and orexin KO mice. *A*: resident response when the test animal was confronted with an intruder. *B*: intruder response when the test animal intruded into a resident's home cage. The presence of an intruder (or resident) is indicated by the horizontal solid bar. Right side panels are the changes in blood pressure, HR, and activity expressed as AUC during 5 min when an intruder or resident was present in the same cage. Data are presented as means ± SE of 6 WT mice and 6 KO mice. *$P < 0.05$, **$P < 0.01$ vs. WT mice.

mice and wild-type mice either before or after injection of urethane (data not shown).

Experiment 4: measurement of cardiovascular parameters with indwelling catheter and pharmacological interventions. Lower basal AP in orexin knockout mice was again confirmed in this experiment (Fig. 7*A*). Basal HR of both genotypes (Fig. 7*B*) was higher than that in *experiment 3* where cardiovascular parameters were measured with radiotelemeter (Table 2). Nevertheless, there was no difference in HR between the knockout mice and wild-type mice as was the case in *experiment 3*.

To examine why basal AP was lower in orexin knockout mice, possible abnormalities of the renin-angiotensin system, vasoconstriction through vasopressin V1 receptor, or autonomic nervous system were evaluated using captopril, V1 antagonist, hexamethonium, and prazosin. Administration of captopril lowered AP in both mutant ($-12.0 \pm 1.7\%$, $n = 3$) and wild-type mice ($-11.9 \pm 2.7\%$, $n = 3$) in a similar manner ($P > 0.05$). Resultant AP was still lower in knockout mice than in wild-type mice, although the difference did not reach statistical significance presumably because of small numbers of the animals. V1 antagonist did not affect AP in either mutant ($-4.6 \pm 2.5\%$, $n = 5$) or wild-type mice ($-2.6 \pm 1.1\%$, $n = 5$). Thus resultant AP was still significantly lower in knockout mice (Fig. 7*A*). On the other hand, hexamethonium lowered AP more in wild-type ($-32.1 \pm 2.1\%$, $n = 13$) than in mutant mice ($-23.4 \pm 2.6\%$, $n = 6$; $P < 0.05$). Resulting AP was not different between the two. In a similar manner, prazosin lowered AP greater in wild-type ($-62.2 \pm 3.1\%$, $n = 5$) than in mutant mice ($-51.2 \pm 2.5\%$, $n = 5$; $P < 0.05$). Difference in AP was canceled after the treatment with prazosin (Fig. 7*A*). There was no difference in HR between the two strains with any drugs used in the current experiment (Fig. 7*B*).

Experiment 5: measurement of echocardiography. To examine the possibility that cardiac abnormality contributed to the lower AP in orexin knockout mice, we measured echocardiography in another set of the animals. Stroke volume and other basal contractile parameters were not significantly different between the two groups (Table 3).

DISCUSSION

We demonstrated here that electrical stimulation and disinhibition of GABAergic input to the perifornical area elicited the cardiovascular and respiratory defense responses and increased arousal level in urethane-anesthetized mice in a similar manner as in cats (1), rabbits (49), and rats (13, 57). A recent report that spontaneous activity of identified orexin-containing neuron in slice preparation was inhibited by the GABA-A receptor agonist muscimol supports the rele-

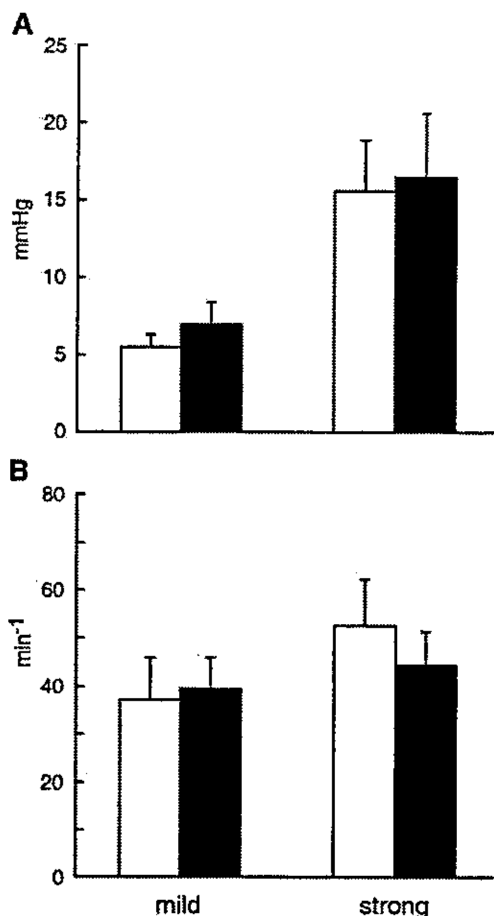


Fig. 5. Cardiovascular responses during noxious stimuli. Peak changes in blood pressure (*A*) and HR (*B*) within 30 s after the start of stimulation are summarized for WT mice (open bar, n = 6) and orexin KO mice (solid bar, n = 6). Mild and strong stimulations were applied by attaching two strengths (60 and 125 g, respectively) of vascular clamps to the tail for 10 s.

vance of our experimental setup (16, 32). The increase in AP during electrical stimulation of the hypothalamic regions seemed to be due to increasing resistance, because HR did not change much (20 beats/min, Fig. 1). It is likely that baroreflex was suppressing major increases in HR in these conditions. We did not examine the blood flow redistribution to skeletal muscle from visceral vascular beds, another characteristic of the defense response. However, mice made movement of limbs that resembled running by microinjections of bicuculline and electrical stimulations (data not shown), indicative of defense response. Cardiovascular, respiratory, and arousal components of the defense response seem to be mediated, at least in part, by orexin-containing neurons. Moreover, intrinsic orexin participates in blood pressure maintenance at basal conditions probably through activation of the sympathetic vasoconstrictor outflow. To the best of our knowledge, this is the first report demonstrating that endogenous orexins play a role in cardiovascular and respiratory regulations in the central nervous system.

Present results confirmed the proposal of possible contribution of orexin in cardiovascular regulation (3, 7, 15, 26, 36, 47, 50) and in stress-induced behavior (27, 55). In other words, our present results using knockout mice were generally in accordance with the earlier reports using exogenous application of orexin and determination of orexin content in the brain. Moreover, we successfully linked cardiovascular and stress regulatory roles of orexin by showing attenuation of defense response in orexin knockout mice in both anesthetized and freely moving conditions.

We demonstrated here diminished defense response in orexin knockout mice, but the response was not completely abolished. Preserved responses, namely short-lasting cardiorespiratory excitation at low doses of bicuculline and even similar responses in HR and Rf at high dose of bicuculline (Fig. 2), should be mediated by other transmitter(s)/modulator(s) than orexin. Existence of other factors than orexin is further suggested by incomplete disappearance of cardiovascular and behavioral responses in resident-intruder test (Fig. 4).

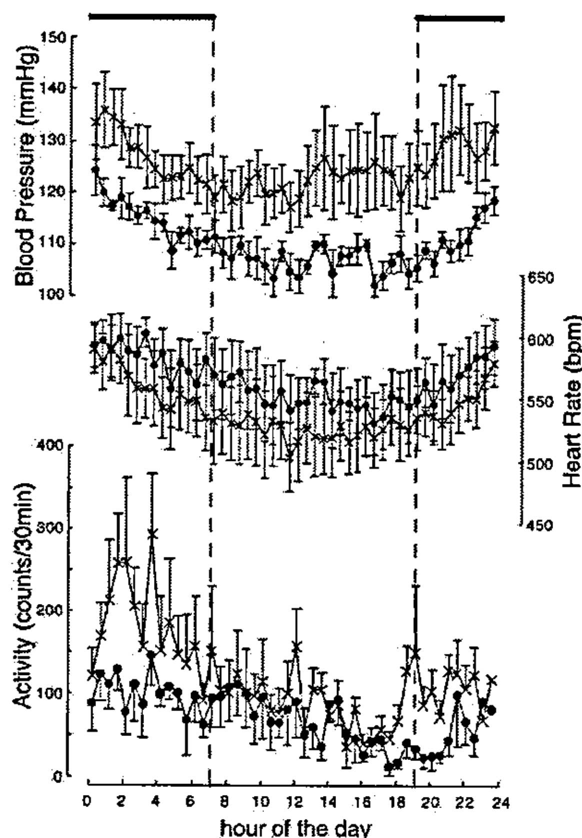


Fig. 6. Circadian patterns of blood pressure, HR, and activity. Symbols of filled circles and cross are average values from orexin KO mice (n = 6) and WT mice (n = 6), respectively. Bars indicate ±1 SE. Filled bars along the top horizontal axis indicate the dark phase of the 12:12-h light-dark cycle. bpm, Beats/min.

Table 2. *Circadian statistics and values for light and dark phases*

Parameter	Wild-Type Mice	Knockout Mice
Blood pressure, mmHg		
MESOR	125±6	109±2*
Amplitude	9±2	6±1
Acrophase (h:min)	0:03±0:56	1:20±0:46
Light phase mean	122±6	107±2*
Dark phase mean	128±6	113±2*
Heart rate, min^{-1}		
MESOR	529±41	565±22
Amplitude	35±8	34±4
Acrophase (h:min)	0:48±0:42	2:50±0:49
Light phase mean	527±28	553±23
Dark phase mean	554±38	575±21
Activity, counts/30 min		
MESOR	135±24	84±17
Amplitude	79±20	25±4*
Acrophase (h:min)	2:09±0:43	2:48±0:13
Light phase mean	98±17	78±17
Dark phase mean	162±26	91±17*

Values are means ± SE of 6 mice in each genotype. MESOR, midline estimating statistic of rhythm. *$P < 0.05$ vs. wild-type mice (*t*-test).

Perhaps glutamate may be one of such transmitters, since bilateral microinjection into the RVLM of an ionotropic excitatory amino acid receptor antagonist kynurenic acid attenuated an increases in AP and renal sympathetic nerve activity evoked by air jet stress (37) or by direct hypothalamic stimulation (52).

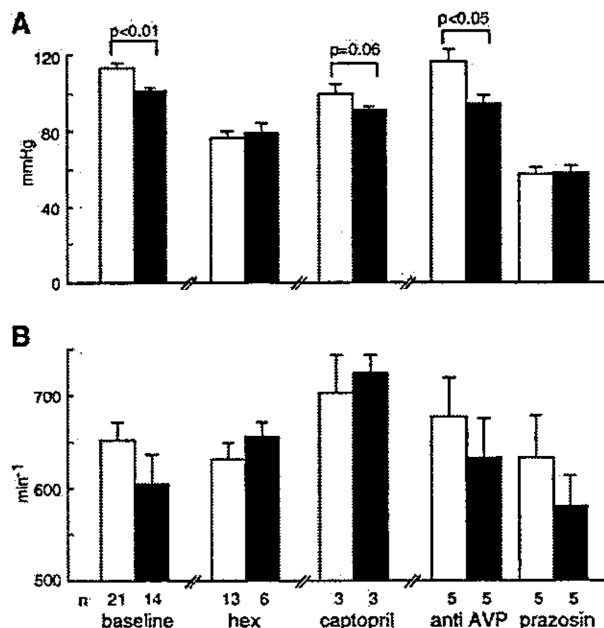


Fig. 7. Cardiovascular effects of intra-arterial injection of hexamethonium (hex), captopril, vasopressin V1 receptor antagonist (anti-AVP), and prazosin. MAP (*A*) and HR (*B*) were measured through indwelling catheter during pre- and postinjection periods in orexin null mutated mice (filled bars) and in WT mice (open bars). Numbers below horizontal axis indicate numbers of animals used in the measurement.

Table 3. *Echocardiography parameters*

Parameter	Wild-Type Mice	Knockout Mice
Body weight, g	30.3±0.7	29.0±1.1
LV diastolic diameter, mm	3.54±0.06	3.45±0.13
LV systolic diameter, mm	1.99±0.06	1.99±0.08
LV end-diastolic volume, μl	46.6±2.2	43.7±4.9
LV end-systolic volume, μl	8.29±0.69	8.42±1.14
Stroke volume, μl	38.3±1.7	35.3±3.9
Fractional shortening, %	43.9±1.1	42.4±0.8
Ejection fraction, %	82.3±1.0	80.8±0.8

Values are means ± SE of 6 mice in each genotype. No parameters were significantly different between orexin-knockout and wild-type mice. LV, left ventricular.

Orexin was able to increase pre- and postsynaptic activity of the glutamate-releasing neurons (54). Thus orexin may act as a modulator of glutamatergic input into the RVLM. A toxin-induced orexin-neuron specific degenerative animal model (21) may help to study other factors, especially cotransmitters contained in the orexin neuron, involved in defense response. On the other hand, above-cited reports dealt with only the cardiovascular components of the defense response. There is little information about neurotransmitter(s) that convey respiratory or behavioral component of the defense response. In this respect, this is the first report suggesting a possible neurotransmitter/modulator that contributes to the simultaneous and coordinated changes in cardiovascular, respiratory, EEG, and behavioral components of the defense response.

The present study did not clarify the site at which orexin acted in the central nervous system and the mechanisms of cardiovascular and respiratory responses induced by disinhibition of GABAergic neurotransmission in the perifornical area. However, previous studies enabled us to suppose key sites where orexin acted as a mediator of defense response. The most apparent site seems to be the RVLM, since inhibition of RVLM or subjacent ventral surface of the medulla (glycine-sensitive area) attenuated sympathetic and defense responses evoked by stimulation to the lateral hypothalamic area (12, 24, 52). A double-virus transneuronal labeling technique revealed that not only perifornical neurons but also RVLM neurons provided a dual input to the sympathetic outflow systems that regulate cardiac and adrenal medullary functions, indicating these neurons were the "central command neurons" of defense response (28). Actually, orexin immunoreactive fibers were seen in the RVLM (44), and exogenous applied orexin into the RVLM elicited AP and sympathetic excitation (7, 36). Present results with hexamethonium and prazosin also support the view since RVLM is the major source of sympathetic outflow (8). Nevertheless, we cannot exclude the possible involvement of sites other than RVLM, such as periaqueductal gray, nucleus tractus solitarius, or intermediolateral cell column of the spinal cord (2, 26), as active sites of orexin in the defense response. This subject needs further experimentation.

In clear contrast to attenuated cardiovascular responses to socioemotional stress by resident-intruder

test, AP and HR responses to noxious stimuli by tail pinch were not different between orexin knockout mice and wild-type mice (Fig. 5). This may be because stimulus duration was shorter in tail pinch (10 s) than in resident-intruder test (5 min). Another explanation may be that cardiovascular responses to noxious stimuli by tail pinch do not always require supraspinal structures (48) and hence the responses are independent from hypothalamic orexin. On the other hand, intrathecal administration of orexin induced analgesia in rats (56). Analgesia but not exaggerated pain response may be advantageous to defense response. Thus orexin may also participate in stress-induced analgesia. Although this hypothesis should be clarified in future experiments, we can say, at present, that the orexin system may be activated by some but not all kinds of stressors. We cannot exclude the possibility that absence of orexin resulted in some deficit in sensory system for socioemotional stress but not for noxious stressor. Nevertheless, results from urethane-anesthetized mice with direct hypothalamic stimulation point to a probable deficit in effecter pathway.

Circadian rhythm of AP and HR, calculated as phase and amplitude, was not significantly different between orexin knockout mice and wild-type mice although activity during dark phase was less in the mutant mice (Fig. 6, Table 2). The latter observation was consistent with previous studies showing reduced awake time and increased sleep time during dark phase in orexin knockout mice (5) and reduced activity during dark phase in orexin neuron-ablated mice (21). It is not clear from the present experiment why amplitudes of circadian fluctuations of AP and HR were not different between the two strains while that of activity was smaller in orexin knockout mice. Activity may not be the sole determinant of AP and HR. Whatever may be the reason, an important point is that AP in orexin knockout mice was smaller than wild-type mice even during the light phase when both animals were at rest and activities were not different between the two. The difference in AP was also independent of HR or cardiac contractility (Table 3). The cause of the difference in AP seemed to be attenuated sympathetic outflow that regulates peripheral vascular resistance in orexin knockout mice (Fig. 7). Basal cardiac sympathetic nerve activity seems not influenced by the absence of orexin. Contribution of the renin-angiotensin system and vasopressin V1 receptor seemed minimal, although small numbers of the animals prevented us from making conclusive remarks about the former. Our conclusion is in line with sympathetic excitation by exogenously administered orexin (3, 15, 50) and extended possible contribution of orexin to basal determination of AP through tonic excitation of sympathetic nervous system.

Perspectives

The difference in AP between two genotypes was maintained after urethane anesthesia and attenuated defense response was observed in both awake and anesthetized conditions. This suggests that the difference in basal AP cannot be explained by possible attenuation of defense response in orexin knockout mice because defense response is not always activated in daily life or under anesthesia. It is possible that there may be two subgroups of orexin-containing neurons: one group contributes to determination of basal AP and another participates in defense response, since orexin-containing neurons widely distribute in lateral hypothalamus and dorsomedial hypothalamus (39) and subpopulations of orexin neurons have been proposed in respect to responses to psychotic drugs (18). However, orexin neurons may be activated only by arousal without any particular stress (17). Therefore, it is also possible that the same orexin system contributes to both basal AP determination by mild activation and to defense response when further activated by stress. We do not have the answer at present on this issue.

There are only a few reports describing autonomic regulation in narcolepsy patients. Sachs and Kaijser (45) reported that never-medicated narcoleptic patients showed attenuated autonomic reflexes (changes in AP and HR) in handgrip test and Valsalva's maneuver, but not in face immersion test or orthostatic standing. Because some but not all reflexes had been disturbed, they proposed intact peripheral nerves and a localization of the defect to the central nervous system. Our findings of attenuated defense response and preserved pain-induced response in orexin-deficient mice are in accordance with their findings in human narcolepsy. Basal AP in narcolepsy patients is rather controversial. The same authors reported normal AP and HR at rest before the autonomic testing (45). However, Guilleminault (20) reported that withdrawal of medication with amphetamine for 4 wk significantly decreased AP in narcoleptic patients, indicating low AP, otherwise taking a central stimulant (20). To the best of our knowledge, 24-h AP and HR have not been reported in narcoleptic patients. We feel that systematic reinvestigation about autonomic regulation in narcoleptic patients is needed, since these reports cited here appeared before 1999 when deficiency of orexin had been revealed as the cause of narcolepsy.

In summary, we found that defense response could be elicited in mice as in the other experimental animals by stimulation to the perifornical region of the posterior hypothalamus. Anesthetized prepro-orexin knockout mice showed lower blood pressure and faster respiratory frequency than those in the wild-type mice and attenuated defense response evoked by microinjection of bicuculline. Attenuated defense response and hypotension was also reproduced in unanesthetized freely behaving mutant mice. The hypotension observed might be due to an attenuated sympathetic outflow. The present study suggests that orexin-containing neurons in the perifornical area play a role for one of the efferent pathways of defense response. Moreover, intrinsic orexin contributes to the maintenance of basal blood pressure.

We thank C. Kumagai and W. Nakamura for excellent technical assistance. We also thank Dr. M. Shimoyama for valuable discussion.

DISCLOSURES

Part of this work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (14570037, 14770270) and by grants from the Shimadzu Science Foundation and the Yamanouchi Foundation for Research on Metabolic Disorders.

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This Review is part of a thematic series on **Gene Expression in Hypertrophy and Stress,** which includes the following articles:

Gene Expression in Fibroblasts and Fibrosis: Involvement in Cardiac Hypertrophy

Roles of Cardiac Transcription Factors in Cardiac Hypertrophy

Ras, Akt, and Mechanotransduction in the Cardiac Myocyte

G Protein–Coupled Signaling and Gene Expression

Genetic Models and Mechanisms of Transcription in Cardiac Hypertrophy

Ryozo Nagai, Guest Editor

Roles of Cardiac Transcription Factors in Cardiac Hypertrophy

Hiroshi Akazawa, Issei Komuro

Abstract—Different cell types, equipped with unique structure and function, synthesize different sets of proteins on the basis of different patterns of gene expression, even though their genomes are identical. Cardiac transcription factors have been reported to control a cardiac gene program and thus to play a crucial role in transcriptional regulation during embryogenesis. Recently, postnatal roles of cardiac transcription factors have been extensively investigated. Consistent with the direct transactivation of numerous cardiac genes reactivated in response to hypertrophic stimulation, cardiac transcription factors are profoundly involved in the generation of cardiac hypertrophy or in cardioprotection from cytotoxic stress in the adult heart. In this review, the regulation of a cardiac gene program by cardiac transcription factors is summarized, with an emphasis on their potential role in the generation of cardiac hypertrophy. (*Circ Res.* **2003;92: 1079-1088.**)

Key Words: cardiac transcription factors ■ gene expression ■ cardiac hypertrophy ■ cardiogenesis

Cardiomyocytes are terminally differentiated and lose their ability to proliferate soon after birth. Thereafter, cardiomyocytes grow in cell size without cell division to adapt to a demand for an increased workload. In a number of pathological conditions (eg, hypertension, valvular disease, myocardial infarction, and cardiomyopathy) that impose overwork on the heart, postnatal cardiomyocytes undergo cardiac hypertrophy. Although cardiac hypertrophy is initially compensatory for an increased workload, prolongation of this process leads to congestive heart failure, arrhythmia, and sudden death.[1,2] At the cellular level, cardiac hypertrophy is characterized by an increase in cell size and protein synthesis and reactivation of the fetal gene program.[3–5] In addition, recent large-scale expression analyses have identified numerous genes other than fetal genes or immediate-early genes that were upregulated in hypertrophied hearts, including genes encoding proteins involved in signaling pathways and energy metabolism.[6,7] The points at issue are how extracellular hypertrophic stimulation is perceived and converted into intracellular signals and how these signals change the transcriptional program that eventually leads to cardiac hypertrophy. With regard to the differential gene expression induced by hypertrophic stimulation, it is reasonable to assume that cardiac transcription factors play the leading part, because they directly regulate a number of cardiac genes that are upregulated in hypertrophied myocardium.

Cardiac transcription factors are defined, in this context, as essential transcriptional activators that are expressed predominantly in the myocardium and that regulate the expression of the

Original received July 23, 2002; resubmission received October 23, 2002; revised resubmission received April 9, 2003; accepted April 10, 2003.
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DOI: 10.1161/01.RES.0000072977.86706.23

TABLE 1. Transgenic Overexpression of Wild-Types or Dominant-Negative Mutants

Cardiac Transcription Factor	Effects	References
GATA4		
Wild-type	Hypertrophic myocardial cell growth with hypertrophic gene expression	20
MEF2		
Dominant-negative	Attenuated postnatal growth of the myocardium	67
Csx/Nkx2-5		
Wild-type	Normal cell size of myocardium with hypertrophic gene expression	133
Dominant-negative	Impaired cardiac function	139

cardiac genes encoding structural proteins or regulatory proteins characteristic of cardiomyocytes. Recent studies have established the notion that cardiac transcription factors govern the intricate process of cardiogenesis by regulating cardiac-specific gene expression.[8] Cardiac transcription factors are represented by the GATA family transcription factors, myocyte enhancer factor 2 (MEF2) transcription factors, and the homeobox transcription factor Csx/Nkx2-5. Accumulating data have suggested the significant role of these transcription factors in postnatal myocardium as well. In the present review, transcriptional regulation by cardiac transcription factors is summarized, with an emphasis on the potential mechanisms involved in cardiac hypertrophy.

GATA Transcription Factors

GATA transcription factors are characterized by the conserved double zinc fingers that are required for binding to the specific consensus DNA sequence (A/T)GATA(A/G).[9] Among 6 GATA transcription factors in vertebrates, GATA4, GATA5, and GATA6 are expressed in the heart.[10] Especially, targeting disruption of *GATA4* in mice resulted in embryonic lethality that was due to failure in the formation of a ventrally fused heart tube, indicating an important role of GATA4 in transcriptional regulation during cardiac morphogenesis.[11,12] Indeed, functional analysis of the *cis*-regulatory elements has revealed that GATA4 directly regulates basal expression of a spectrum of cardiac-specific genes, such as *α-myosin heavy chain (α-MHC), myosin light chain 1/3 (MLC1/3), cardiac troponin C, cardiac troponin I, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), cardiac-restricted ankyrin repeat protein (CARP), cardiac sodium-calcium exchanger (NCX1), cardiac m2 muscarinic acetylcholine receptor,* A_1 *adenosine receptor,* and *carnitine palmitoyl transferase I β*.[10,13]

Besides supporting the basal transcription levels of these cardiac genes and thus conferring tissue specificity on cardiomyocytes, GATA4 is critically involved in inducible gene expression evoked by a variety of hypertrophic stimulations. For example, GATA-binding elements are required for the upregulation of *β-MHC* or *angiotensin II type 1a receptor* in response to aortic constriction.[14,15] In addition, GATA-binding elements are responsible for inducible gene expression of *BNP* in the hearts of bilaterally nephrectomized rats.[16] Furthermore, in cultured cardiomyocytes, upregulation of *NCX1* or *BNP* by adrenergic stimulation is mediated by GATA-binding elements within the regulatory regions of the individual genes.[17,18]

Consistent with the essential role of GATA4 in activating the gene program in response to hypertrophic stimulation, the overexpression of GATA4 generated cardiac hypertrophy both in cultured cardiomyocytes[19,20] and in the hearts of mice[20] (Table 1). These results suggest that GATA4 is a sufficient transcriptional regulator for the generation of cardiac hypertrophy. Moreover, the overexpression of a dominant-negative GATA4 by adenoviral gene transfer inhibited an agonist-induced increase in protein synthesis and hypertrophic gene expression in cultured cardiomyocytes.[20] Although electrical stimulation upregulates *GATA4* expression,[21] the expression levels of *GATA4* are not affected by

TABLE 2. Expression Levels or Activities of Cardiac Transcription Factors in Hypertrophied Hearts

Cardiac Transcription Factor	State	Stimulation	References
GATA4	Enhanced DNA binding	Pressure overload	14, 15, 22
		α-Adrenergic agonist	20, 23
		β-Adrenergic agonist	18
		ET-1	25, 26
		Ang II	27
MEF2	Enhanced DNA binding	Pressure overload	70
		Volume overload	70
Csx/Nkx2-5	Upregulated expression	Pressure overload	131
		α-Adrenergic agonist	132
		β-Adrenergic agonist	132
HAND	Downregulated expression	Pressure overload	146
		α-Adrenergic agonist	146

TABLE 3. Posttranslational Modification by Kinases During Cardiac Hypertrophy

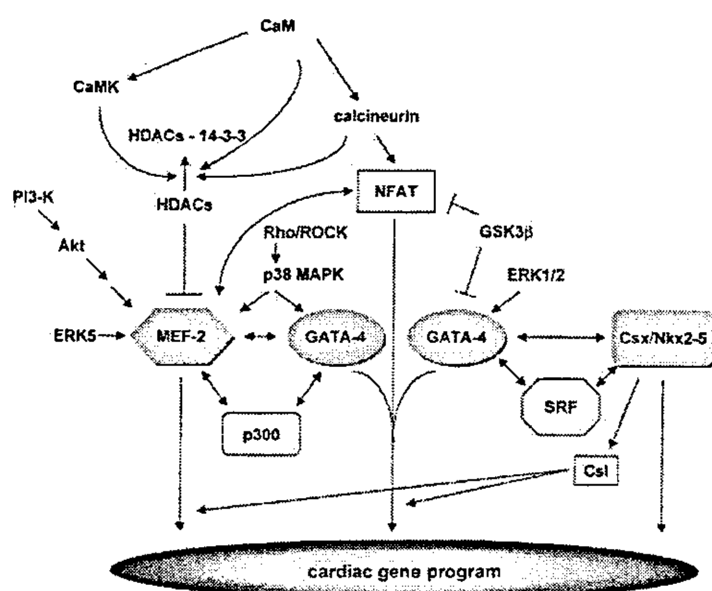
Cardiac Transcription Factor	State	Stimulation	References
GATA4	ERK	Activation	23, 24
	p38 MAPK	Activation	19, 26
	GSK3β	Nuclear export	44
MEF2	p38 MAPK	Activation?	70–73
	ERK5	Activation?	75, 76, 78
Csx/Nkx2-5	Unknown		
HAND	Unknown		

hypertrophic stimulation induced by pressure overload,[22] α-adrenergic agonists,[20,23–25] or endothelin-1 (ET-1).[25] On the basis of an increase in DNA-binding activity of GATA4 in response to pressure overload[14,15,22] or neurohumoral stimulation by α-adrenergic agonists,[20,23] β-adrenergic agonists,[18] ET-1,[25,26] or angiotensin II[27] (Table 2), it is reasonable to postulate that GATA4 is activated through posttranslational modification by hypertrophic stimulation (Table 3).

Indeed, recent studies have demonstrated that GATA4 activation induced by phenylephrine (PE) stimulation is coupled with serine phosphorylation of GATA4.[23,24] Extracellular signal–regulated kinase 2 (ERK2) directly phosphorylates GATA4 in vitro, and PE-induced phosphorylation and activation of GATA4 are inhibited either by incubation with an ERK kinase (MEK1) inhibitor or by adenoviral transfection of dominant-negative MEK1, indicating an essential role of the ERK pathway in GATA4 activation. The ERK pathway, one of the ternary branches of the mitogen-activated protein kinase (MAPK) cascades, is a key biochemical signal that mediates hypertrophic responses.[28,29] In this respect, GATA4 may function as a transcriptional effector acting downstream from the ERK signaling pathway activated by hypertrophic stimulation, because dominant-negative GATA4 inhibited MEK1-induced hypertrophic responses in cultured cardiomyocytes.[24] GATA4 is also activated through direct serine phosphorylation by the p38 MAPK pathway,[19,26] which is another branch of the MAPK cascades and mediates hypertrophic growth in cultured cardiomyocytes.[30–32] Pharmacological inhibition of p38 MAPK attenuated ET-1–induced protein synthesis in addition to DNA binding and phosphorylation of GATA4.[26]

A recent report has suggested that Rho and ROCK, a target of Rho, are linked to PE-induced GATA4 activation through the ERK pathway.[33] Moreover, the potentiation of GATA4 transcriptional activity through p38 MAPK is induced by RhoA,[19] a member of the Rho family of GTPases, which regulate diverse cellular events such as transcriptional regulation, cell growth control, and membrane trafficking as well as cytoskeletal organization.[34] In cardiomyocytes, Rho is critically involved in mediating hypertrophic features[35] induced by mechanical stress[36] and G-protein–coupled receptor agonists such as PE,[37–40] angiotensin II,[41,42] and ET-1.[43] Collectively, these observations highlight the role of GATA4 as an essential transcriptional effector by which divergent protein phosphorylation pathways integrate during the generation of cardiac hypertrophy.

The transcriptional activity of GATA4 is regulated through its nucleocytoplasmic shuttling mechanism. Glycogen synthase kinase 3β (GSK3β) directly phosphorylates GATA4 and thereby decreases basal and β-adrenergic–stimulated GATA4 expression in the nucleus by activating the nuclear export system.[44] Phosphorylation of GATA4 by GSK3β negatively regulates GATA4 transcriptional activity, in con-



Roles of cardiac transcription factors in regulation of cardiac gene program during cardiac hypertrophy. GATA4 transcriptional activity is stimulated through phosphorylation by ERK1/2 and p38 MAPK, although phosphorylation by GSK3β negatively regulates GATA4 activity. In addition, the transcriptional activity of GATA4 is regulated through physical interaction with NFAT, MEF2, SRF, or a coactivator, p300. Most important, in response to hypertrophic stimulation, NFAT is dephosphorylated by calcineurin and translocates into the nucleus, where it activates gene expression partly through forming a complex with GATA4. MEF2 transcriptional activity is enhanced through phosphorylation by p38 MAPK and ERK5 and physical interaction with GATA4, NFAT, and coactivator p300. In addition, MEF2 might be involved in the PI3-K/Akt-mediated hypertrophic signal. Most important, MEF2 factors function as important effectors of Ca^{2+} signaling. MEF2 activity is stimulated by constitutively active calcineurin or CaMK in vivo. Activation of MEF2 is dependent on dissociation from class II HDACs. Signal-mediated phosphorylation of HDACs recruits chaperones 14-3-3 to dissociate the HDAC-MEF2 formation, although the endogenous HDAC kinase has not been determined. Csx/Nkx2-5 might regulate cardiac gene expression (1) directly, (2) via association with GATA4 or SRF, and (3) via upregulation of Csl, which activates transcriptional activities of GATA4 and MEF2. Contribution of Csx/Nkx2-5 transcriptional activity to pathophysiological hypertrophic responses remains undefined.

trast to phosphorylation by ERK or p38 MAPK. Interestingly, recent studies have indicated an inhibitory role of GSK3β in the stimulation of cardiac hypertrophy.[45-47] Therefore, GSK3β is supposed to inhibit cardiac hypertrophy in part by interfering with GATA4 transcriptional activity.

In addition to phosphorylation, the transcriptional activity of GATA4 is regulated through interaction with cofactors such as p300[48] and other transcription regulators such as serum response factor (SRF),[49] Csx/Nkx2-5,[50-52] MEF2,[53] nuclear factor of activated T cells (NFAT),[54] dHAND/HAND2,[55] FOG-2,[56,57] YY1,[58] and peroxisome proliferator-activated receptor binding protein.[59] p300 interacts with GATA transcription factors to enhance the promoter activation of the *ANP*[48,60] and *β-MHC* genes,[48] which is dependent on the histone acetyltransferase (HAT) activity of p300. With respect to protein-protein interaction with other transcription factors, the interaction between GATA4 and NFAT is particularly noteworthy because NFAT plays a critical role in activating the hypertrophic gene program. In response to hypertrophic stimuli, NFAT is dephosphorylated by calcineurin and translocates into the nucleus, where it activates gene expression partly through forming a complex with GATA4.[54,61] SRF is a transcriptional regulator of a wide variety of cardiac-specific genes, and cardiac overexpression of SRF induces hypertrophic features in mice.[62] MEF2 is another important transcription factor regulating the cardiac gene program during myocardial cell hypertrophy. Transcriptional synergy based on protein-protein interaction involving GATA4 and these transcriptional factors may be implicated in the generation of cardiac hypertrophy.

Taken together, GATA4 transcriptional activity is positively regulated by multiple signaling pathways in response to hypertrophic stimulation. GATA4 plays an essential role in transcriptional regulation during the generation of cardiac hypertrophy (Figure).

MEF2 Transcription Factors

MEF2 transcription factors contain a MADS (indicating MCM1, agamous, deficiens, and SRF) domain and an adjacent MEF2-specific domain in the N-terminus, which together direct dimerization and binding to their cognate DNA sequence CAT(A/T)4TAG/A.[63,64] In vertebrates, 4 members (MEF2-A, MEF2-B, MEF2-C, and MEF2-D) have been identified. Although MEF2A through MEF2-D are expressed in many types of cells, their specific functions are assigned to transcriptional regulation in the immune system, neurons, and striated muscle. Especially, targeted disruption of *MEF2C* has been shown to lead to arrested cardiac looping and right ventricular formation during embryogenesis, and several cardiac genes have been shown to be downregulated in *MEF2C*-null embryos,[65,66] indicating an essential role of MEF2 in myocardial cell differentiation. MEF2C is involved in transcriptional regulation in postnatal hearts as well, inasmuch as transgenic mice expressing a dominant-negative MEF2C have displayed attenuated postnatal growth of the myocardium[67] (Table 1). Consistently, the MEF2-binding A/T-rich DNA sequences have been identified within the promoter regions of a number of cardiac genes, (eg, *muscle creatine kinase* gene, *α-MHC, MLC1/3, MLC2v, skeletal α-actin, sarcoplasmic reticulum* Ca^{2+}*-ATPase, cardiac troponin T, cardiac troponin C, cardiac troponin I, desmin,* and *dystrophin*).[63,68]

In addition, MEF2 transcription factors are critically involved in the regulation of inducible gene expression during myocardial cell hypertrophy, inasmuch as the MEF2-binding site within the *MLC2* promoter is required during PE-mediated and ET-1–mediated hypertrophy,[69] and MEF2 DNA-binding activity is increased in the hearts of rats subjected to pressure overload or volume overload[70] (Table 2). Recent studies have elucidated complex signaling pathways that link hypertrophic stimulation and MEF2 activation (Table 3). First, MEF2 is phosphorylated by p38 MAPK.[67,71-73] Specifically, p38 MAPK-MEF2 signaling is implicated in the regulation of skeletal muscle cell differentiation[74] and immune response.[71] Although activation of p38 MAPK induces hypertrophic growth in cultured cardiomyocytes[30-32] and p38 MAPK phosphorylates MEF2 in hypertrophied heart, the pathophysiological significance of the p38 MAPK–MEF2 pathway during cardiac hypertrophy has not been fully determined. Second, MEF2 is activated through phosphorylation by ERK5, also known as big MAPK 1.[75,76] The ERK5-MEF2 pathway participates in inducible gene expression of an immediate-early gene *c-fos* in response to growth stimulation such as serum[75] or G-protein–coupled receptor agonists.[77] A recent study has demonstrated that ERK5 is activated by hypertrophy-stimulating factors such as PE, leukemia inhibitory factor, and oxidative and osmotic stress in cultured cardiomyocytes.[78] Additionally, dominant-negative MEK5, the MAPK kinase for ERK5, inhibited leukemia inhibitory factor–induced hypertrophic features, and transgenic overexpression of constitutively active MEK5 in the heart resulted in eccentric hypertrophy.[78] Collectively, these results suggest a role of the ERK5-MEF2 pathway in the generation of cardiac hypertrophy, although it is not determined whether MEF2 is an essential downstream effector of ERK5-induced cardiac hypertrophy.

During the skeletal muscle differentiation evoked by insulin-like growth factor-1 (IGF-1), the transcriptional activity of MEF2 is activated through the phosphoinositide 3-kinase (PI3-K)–Akt pathway.[79,80] Interestingly, transgenic mice overexpressing the constitutively active form of either PI3-K or Akt exhibit physiological cardiac hypertrophy characterized by proportional myocardial cell growth without interstitial fibrosis or deterioration of cardiac function.[81,82] Although the transcriptional activity of MEF2 has not been examined in these transgenic mice, it may be possible that MEF2 is involved in PI3-K/Akt–mediated hypertrophic growth of cardiomyocytes.

Most important, the MEF2 factors function as important effectors that converge in the binary downstream pathway of the Ca^{2+} signaling. A growing body of evidence has suggested that Ca^{2+} signaling plays a critical role in the generation of cardiac hypertrophy.[83] Increased intracellular Ca^{2+} binds to and activates Ca^{2+}-binding proteins, including calmodulin (CaM), which regulates several downstream effectors, such as calcineurin and Ca^{2+}/CaM-dependent protein kinases (CaMKs). Activation of either calcineurin[54] or CaMKs[84,85] induces cardiac hypertrophy both in cultured

cardiomyocytes and in murine hearts. The MEF2 activity is stimulated by CaMK, as indicated by LacZ expression in the hearts of double transgenic animals harboring activated CaMKIV and a MEF2-dependent *LacZ* reporter.[85] Although CaMKs directly phosphorylate MEF2D in vitro,[86] the activation of MEF2 by CaMK is mediated mainly through the phosphorylation of transcriptional repressors, the histone deacetylases (HDACs).[87] Especially, class II HDACs (HDAC-4, HDAC-5, HDAC-7, and HDAC-9) associate with MEF2 to repress MEF2-induced gene expression.[88–92] In general, transcriptional activity is controlled by the state of histone acetylation, the balance of which is maintained through opposing activities of HDACs and HATs.[93] HDACs repress gene expression through intrinsic deacetylase activity and recruitment of a transcriptional corepressor COOH-terminal–binding protein.[94] Recent studies have demonstrated that phosphorylation of HDACs by CaMKs results in the recruitment of intracellular chaperones 14-3-3 to dissociate the HDAC-MEF2 formation.[95–97] Consequently, HDACs are sequestered in the cytoplasm by the nucleocytoplasmic shuttling mechanism,[95,98,99] and MEF2 is released from HDACs in the nucleus and transcriptionally activated through binding to coactivators harboring intrinsic HAT activity, such as p300[100–102] and CBP.[103] HDAC4 has a CaM-binding domain that overlaps the MEF2-binding domain, and dissociation of MEF2 from HDACs is also regulated by CaM,[101] indicating that the HDAC-MEF2 complex is controlled by a series of mediators in the Ca^{2+} signaling pathway.

The implication of class II HDACs during cardiac hypertrophy is underscored by a recent report demonstrating that *HDAC9*-deficient mice display spontaneous cardiac hypertrophy and are predisposed to more severe hypertrophic growth after banding of the thoracic aorta.[104] In cultured cardiomyocytes, overexpression of class II HDACs with mutations of 2 conserved CaMK phosphorylation sites blocks hypertrophic features, including agonist-induced gene expression of *ANP* and *β-MHC* and histone acetylation of the promoter regions of these genes.[104] These data indicate the repressive role of class II HDACs in the generation of cardiac hypertrophy. Although the HDAC kinase activity is enhanced in cardiac extracts from hypertrophied hearts of mice and although CaMKs are capable of phosphorylating HDACs, it remains unclear whether CaMKs are the functional HDAC kinases that are responsive to hypertrophic stimulation, because HDAC kinase activity in in vitro kinase assays is only partially blocked by CaMK inhibitors.[104] Instead, HDAC kinase activity is enhanced by calcineurin signaling, inasmuch as the hypertrophic features in *HDAC9*-deficient mice harboring the activated calcineurin transgene are more prominent with increased transcriptional activity of MEF2.[104] In T lymphocytes or skeletal muscle, activated calcineurin promotes complex formation between MEF2 and NFAT to synergistically transactivate downstream target genes.[86,105–107] Calcineurin-mediated dephosphorylation of MEF2 is observed in skeletal muscle and neurons.[107–109] The precise role of calcineurin in the activation of MEF2 during cardiac hypertrophy remains to be determined.

The transcriptional activity of MEF2, like that of GATA4, is regulated through protein-protein interaction with other transcription factors, such as MyoD,[110] GATA4,[53] NFAT,[86,105,106,109] thyroid hormone receptor,[111] and Smad proteins.[112] Especially, MEF2 and GATA4 synergistically activate the transcription of several cardiac genes, such as *ANP, BNP, α-MHC,* and *cardiac α-actin,* indicating a significant cooperative role of MEF2 and GATA4 in the transcriptional regulation of these cardiac genes. However, it remains to be determined whether the transcriptional synergy between MEF2 and GATA4 is implicated in the generation of cardiac hypertrophy.

Recent reports have demonstrated that a novel cardiac helicase, CHAMP, is activated by MEF2 protein[66] and acts as a suppressor of cardiac hypertrophy.[113] *CHAMP* was originally identified by differential array analysis as a cardiac-specific gene downregulated in *MEF2C*-deficient embryos.[66] Overexpression of CHAMP in cultured cardiomyocytes impairs PE- and serum-induced hypertrophic gene expression.[113] These data appear contradictory to the notion that the MEF2 factors are important in regulation of hypertrophic gene expression. However, *CHAMP* expression is downregulated in the hearts of transgenic mice overexpressing activated calcineurin. On the basis of the enhanced activity of MEF2 in these mice,[104] it is plausible that *CHAMP* expression is not dependent on MEF2 in the postnatal heart.

Collectively, MEF2 activity is enhanced in response to hypertrophic stimulation, and MEF2 functions as an essential effector of divergent intracellular signaling pathways mediating hypertrophic features (Figure).

Cardiac Homeobox Transcription Factor Csx/Nkx2-5

Csx/Nkx2-5 is a homeodomain-containing transcriptional activator, originally identified as a potential homologue of *Drosophila tinman.*[114,115] The homeodomain of Csx/Nkx2-5 has a helix-turn-helix motif that binds to the specific consensus DNA sequence T(C/T)AAGTG.[116] Targeted disruption of *Csx/Nkx2-5* in mice caused embryonic lethality due to the arrested looping morphogenesis of the heart tube and growth retardation.[117,118] The expression of several cardiac genes in the heart of *Csx/Nkx2-5*–deficient embryos (including *MLC2v, ANP, BNP, CARP, MEF2-C, eHAND/HAND1, N-myc, Iroquois homeobox gene 4,* and *HOP*) was reduced.[117–122] In addition, direct downstream targets for Csx/Nkx2-5 (such as *ANP,*[52,123] *cardiac α-actin,*[124] *A_1 adenosine receptor,*[125] *calreticulin,*[126] *connexin40,*[127] and *NCX1*) have been identified.[128] These results indicate a functional role of *Csx/Nkx2-5* in the transcriptional regulation of a cardiac gene program.

In contrast to the essential role of Csx/Nkx2-5 during embryogenesis, its functional role in the postnatal heart has not been fully determined. *Csx/Nkx2-5* is expressed in the adult heart,[114,129,130] and notably, its expression is upregulated in hypertrophied hearts (Table 2). Banding of the feline pulmonary artery induces right ventricular hypertrophy with increased expression of *Csx/Nkx2-5* and its downstream target genes, *ANP* and *cardiac α-actin.*[131] In PE- or isoproterenol-mediated hypertrophic hearts, expression of *Csx/Nkx2-5* is stimulated as well as the expression of fetal genes, such as *ANP* and *β-MHC,* and immediate-early genes,

such as *c-fos*, *c-jun*, and *Egr-1*.[132] The upregulation of *Csx/Nkx2-5* expression in pressure overload–induced and agonist-induced hypertrophic hearts indicates a potential role of *Csx/Nkx2-5* in the process of cardiac hypertrophy in general.

However, transgenic mice overexpressing Csx/Nkx2-5 under the control of the cytomegalovirus enhancer/chicken *β-actin* promoter exhibit normal-sized hearts[133] (Table 1). The expression levels of cardiac genes such as *ANP*, *BNP*, *CARP*, and *MLC2v* are upregulated in the hearts of *Csx/Nkx2-5* transgenic mice. These gain-of-function studies suggest that Csx/Nkx2-5 is not sufficient for the generation of cardiac hypertrophy but that Csx/Nkx2-5 functions to control cardiac gene program in adult hearts as well as in embryonic hearts. Csx/Nkx2-5 interacts with other cardiac transcription factors. Transcriptional activity of Csx/Nkx2-5 is modulated through physical interaction with other transcription factors such as GATA4,[50–52,134] SRF,[124] T-box–containing transcription factor Tbx5,[127,135] Tbx2,[136] and eHAND/HAND1.[137] We have identified several factors that interact with Csx/Nkx2-5 and modulate Csx/Nkx2-5–induced gene expression. One of the coactivating factors potentiates Csx/Nkx2-5–induced promoter activation in response to a signal evoked by hypertrophic stimulation (authors' unpublished data, 2003). Therefore, combinatorial regulation involving Csx/Nkx2-5 and its coactivators might be necessary for the generation of cardiac hypertrophy, although it is still speculative.

A novel muscle-specific gene, *Chisel* (*Csl*), was identified by a differential screening as a target gene downregulated in *Csx/Nkx2-5*–null embryonic hearts.[138] Overexpression of Csl in C2C12 myoblasts induced lamellipodia formation and differentiation into large myosacs in the presence of IGF-1 as a result of enhanced cell fusion. Interestingly, Csl augmented transcriptional activities of MEF2 and NFAT in an IGF-1 signaling-dependent manner. Both MEF2 and NFAT are important in the differentiation and hypertrophy of cardiac muscle as well as skeletal muscle. Although the activation of NFAT by Csl in the presence of IGF-1 is not dependent on the calcineurin pathway, it is intriguing that the downstream target of Csx/Nkx2-5 might operate in connection with the NFAT and MEF-2 transcription factors, which are involved in the generation of cardiac hypertrophy.

Recently, transgenic mice overexpressing dominant-negative mutant of Csx/Nkx2-5 under control of *α-MHC* promoter have been generated[139] (Table 1). These mice show impaired cardiac function with the degeneration of cardiomyocytes. Furthermore, in response to doxorubicin, dominant-negative Csx/Nkx2-5 transgenic mice show more severe cardiac dysfunction accompanied by a larger number of apoptotic myocardial cells, although doxorubicin-induced myocardial damage is mild in transgenic mice overexpressing the wild-type of Csx/Nkx2-5. These results indicate a cardioprotective role of Csx/Nkx2-5 in postnatal hearts.

Taken together, Csx/Nkx2-5 is upregulated in response to hypertrophic stimulation and may have implications in the transcriptional regulation of the cardiac gene program in hypertrophied hearts (Figure). In the adult heart, Csx/Nkx2- 5 also plays an important role in protecting the myocardium against cytotoxic damage.

HAND Transcription Factors

dHAND/HAND2 and eHAND/HAND1 are basic helix-loop-helix transcription factors that have distinctive roles in cardiac and extraembryonic development.[140] The expression of *eHAND* is predominant in the left ventricle and is excluded from the right ventricle. Analysis of *eHAND*-null mice defined an essential role of *eHAND* in myocardial differentiation of the left ventricle.[141–143] In contrast, the expression of *dHAND* is restricted to the right ventricle, and development of the right ventricle has been shown to be selectively compromised in *dHAND*-null embryos.[144]

Initial insight into postnatal HAND function was provided by a report showing that the expression of *dHAND* and *eHAND* is detectable in human adult hearts and that the cardiac expression of *eHAND* is significantly downregulated in patients with cardiomyopathies.[145] Likewise, in a PE-induced hypertrophic mouse model, a chamber-specific downregulation of *eHAND* in the left ventricle and *dHAND* in the right ventricle was observed[146] (Table 2). In addition, in abdominal aorta–banded rats, the expression of *dHAND* and of *eHAND* was shown to be downregulated in both the ventricles. The reduced expression of *HAND* genes may indicate a role in the inhibition of myocardial cell growth. At present, a limited number of direct downstream target genes of the HAND transcription factors have been identified. Through binding to p300, dHAND interacts with GATA4 to induce synergistic transactivation of the promoters of *ANP*, *BNP*, and *α-MHC*.[55] Similarly, eHAND interacts with Csx/Nkx2-5 to synergistically transactivate the *ANP* promoter.[137] Elucidation of the molecular basis of the HAND transcription factors will be required to understand the postnatal roles relevant to their reduced expression in hypertrophied hearts.

Future Issues

Functional roles of the cardiac transcription factors during cardiogenesis have been considerably deciphered. Many downstream target genes are identified, and transcriptional regulatory mechanisms whereby protein-protein interactions with other cardiac transcription factors allow fine-tuned gene expression have been clarified. Recent studies have demonstrated that GATA4 and MEF2 are involved in reactivation of the fetal gene program in response to a variety of hypertrophic stimulation and that these factors function as important effectors during the generation of cardiac hypertrophy. Furthermore, mechanistic insights have been provided into the signaling pathways that enhance the transcriptional activities of these transcription factors. In contrast to GATA4 and MEF2, Csx/Nkx2-5 participates in the activation of the hypertrophic gene program but does not have the ability to induce hypertrophic myocardial cell growth. In this respect, a challenging problem (ie, how the cardiac transcription factors influence an increase in protein synthesis and myocardial cell size) remains unsolved. An increased capacity of protein synthesis underlying hypertrophic growth is facilitated not by increased translational efficiency but by ribosome accumulation resulting from increased transcription of ribosomal DNA by the nucleolar factor UBF.[147,148] Interestingly, adenoviral introduction of UBF antisense RNA into cultured cardiomyocytes abolished an increase in general protein synthesis and

hypertrophic cell growth in response to α-adrenergic and contraction stimulation but had little effect on fetal gene expression.[148] It has not been clarified whether the UBF activity is influenced by cardiac transcription factors. Comprehensive analyses of target genes regulated by cardiac transcription factors during cardiac hypertrophy will provide a clue toward solving this problem.

Transcriptional regulation by multiple cardiac transcription factors such as GATA4, MEF2, and Csx/Nkx2-5 is interrelated. It is conceivable that combinations of the ubiquitous and tissue-specific transcription factors execute regulatory decisions under a spectrum of hypertrophic conditions as well as during embryogenesis. Although transcriptional synergy has been reported to be significant in controlling the expression of several cardiac genes, an important issue (ie, how much the cooperative transcriptional regulation weighs with the generation and progression of cardiac hypertrophy) remains unsolved. It is also undetermined how the mutual interaction is regulated in response to hypertrophic stimulation. Functional analysis of the individual cardiac transcription factors and clarification of their interactive roles will be required.

Finally, whether the cardiac transcription factors may be potential therapeutic targets in cardiovascular diseases is a challenging problem. Although compensatory cardiac hypertrophy is beneficial in some pathological conditions, evidence-based studies have suggested that the regression of cardiac hypertrophy in patients leads to better prognosis.[2] It is an ideal adaptation to excessive workload to enhance myocardial contractility without a pathological increase in left ventricular mass, which may be feasible, as exemplified by a rat model of N^G-nitro-L-arginine methyl ester–induced hypertension.[149] Cardiac transcription factors are the potential candidates, because it is now clear that they orchestrate inducible gene expression in postnatal cardiomyocytes. Further investigation will be required to understand the molecular basis of the gene expression program directing cardiac hypertrophy and to target this for therapeutic purposes.

Acknowledgments

This review was supported in part by grants from the Japanese Ministry of Education, Science, Sports, and Culture; Japan Health Sciences Foundation; Takeda Medical Research Foundation; Takeda Science Foundation; Uehara Memorial Foundation; Kato Memorial Trust for Nambyo Research; Japan Medical Association (to Dr Komuro); Japanese Heart Foundation; and Kanae Foundation for Life and Sociomedical Science (to Dr Akazawa). Dr Akazawa is a Research Resident for Research on Human Genome, Tissue Engineering Food Biotechnology of the Japan Health Sciences Foundation.

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Myocardial Fibrosis in Fabry Disease Demonstrated by Multislice Computed Tomography

Comparison With Biopsy Findings

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A 54-year-old man presented with dyspnea on effort. Echocardiogram revealed reduced apical wall motion of the left ventricle (LV) with extreme hypertrophy of the interventricular septum (IVS). Conventional coronary angiogram showed normal coronary arteries. Endomyocardial-biopsy specimens obtained from the IVS revealed extensive vacuolization of cardiac myocytes and mild fibrosis on light microscopy, and typical lysosomal inclusions with a concentric lamellar configuration were seen with electron microscopy (Figure 1). With these findings and low plasma α-galactosidase activity, he was diagnosed as having Fabry disease. To evaluate the characteristics of the LV, ECG-gated enhanced multislice computed tomography (CT) (Light Speed Ultra, General Electric) was performed with a 1.25-mm slice thickness, helical pitch 3.25. After intravenous injection of 100 mL of iodinated contrast material (350 mgI/mL), CT scanning was performed with retrospective ECG-gated reconstruction at 30 seconds and 8 minutes after injection. In the axial source images, extreme hypertrophy of the IVS and the posterior wall of the LV compared with the apical and lateral walls of the LV could be observed (Figure 2). The apical and lateral portions of the LV revealed lower CT intensity than the IVS in the early phase (arrows), and in the late phase they were abnormally enhanced compared with the IVS, suggesting fibrotic changes in the apical and lateral myocardium. Therefore, we concluded that despite the IVS biopsy results, more fibrotic changes occurred in the apical and lateral portions of the LV rather than in the IVS.

Acknowledgments

This work was supported by Grant from Takeda Science Foundation.

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(*Circulation.* 2003;107:2519-2520.)

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Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000062036.35852.01

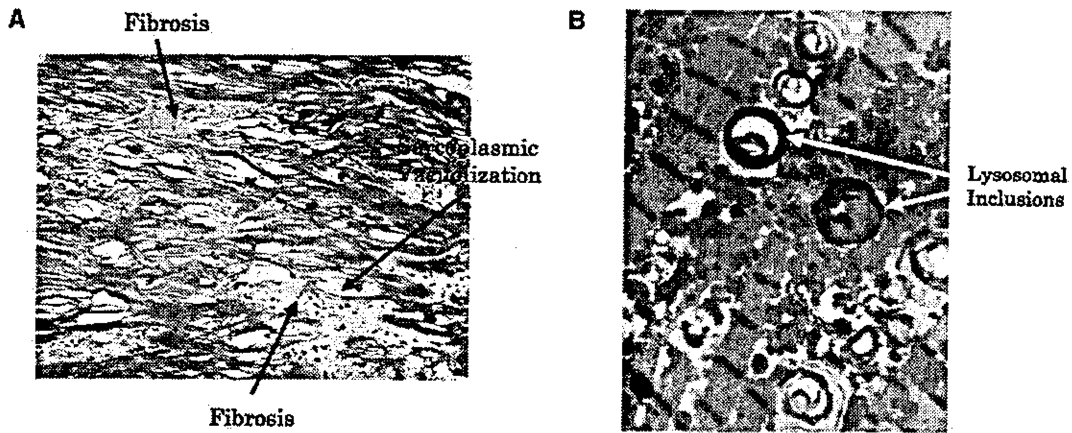

Figure 1. Histological findings of endomyocardial-biopsy specimens. A, Photomicrograph shows sarcoplasmic vacuolization of cardiac myocytes and fibrosis (hematoxylin and eosin staining). B, Electron micrograph shows typical lysosomal inclusions with a concentric lamellar configuration.

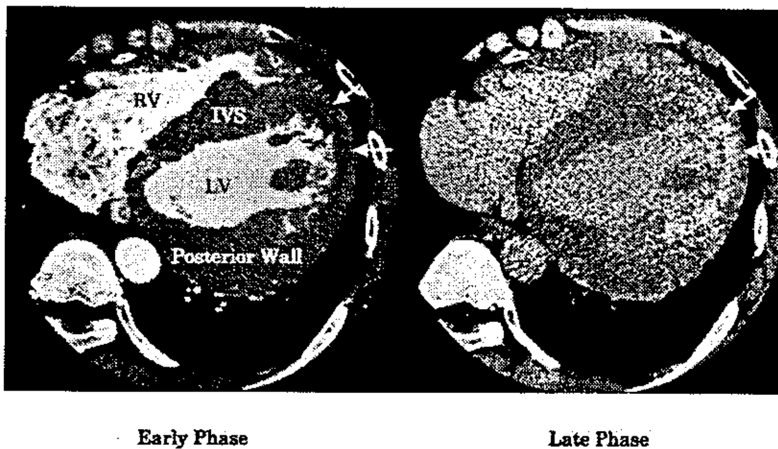

Figure 2. Axial source images of enhanced multislice computed tomography acquired 30 seconds (early phase) and 8 minutes (late phase) after the injection of the contrast material. Images show extreme hypertrophy of the interventricular septum (IVS) and posterior wall compared with the apical and lateral walls of the left ventricle (LV). The apical and lateral portions revealed lower computed tomography intensity than the IVS in the early phase (arrows). Conversely, in the late phase, the apical and lateral portions of the LV (arrows) were abnormally enhanced compared with the extremely hypertrophic IVS, suggesting more fibrotic changes in the apical and lateral myocardium. RV indicates right ventricle.