

*Current Perspective***Crucial Interactions Between Selective Serotonin Uptake Inhibitors and Sigma-1 Receptor in Heart Failure**Md. Shenuarin Bhuiyan<sup>1</sup>, Hideaki Tagashira<sup>2</sup>, and Kohji Fukunaga<sup>2</sup><sup>1</sup>*Division of Molecular Cardiovascular Biology, Department of Pediatrics, The Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA*<sup>2</sup>*Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan**Received October 14, 2012; Accepted December 27, 2012*

**Abstract.** Depression is associated with a substantial increase in the risk of developing heart failure and is independently associated with increased cardiovascular morbidity and mortality. Inversely, cardiovascular disease can lead to severe depression. Thus, therapy with selective serotonin reuptake inhibitors (SSRIs) is strongly recommended to reduce cardiovascular disease-induced morbidity and mortality. However, molecular mechanisms to support evidence-based SSRI treatment of cardiovascular disease have not been elucidated. We recently found very high expression of the sigma-1 receptor, an orphan receptor, in rat heart tissue and defined the cardiac sigma-1 receptor as a direct SSRI target in eliciting cardioprotection in both pressure overload (PO)-induced and transverse aortic constriction (TAC)-induced myocardial hypertrophy models in rodents. Our findings suggest that SSRIs such as fluvoxamine protect against PO- and TAC-induced cardiac dysfunction by upregulating sigma-1 receptor expression and stimulating sigma-1 receptor-mediated Akt-eNOS signaling. Here, we discuss the association of depression and cardiovascular diseases, the protective mechanism of SSRIs in heart failure patients, and the pathophysiological relevance of sigma-1 receptors to progression of heart failure. These findings should promote development of clinical therapeutics targeting the sigma-1 receptor in cardiovascular diseases.

**Keywords:** cardiovascular disease, SSRI, hypertrophy, sigma-1 receptor, endothelial NOS

**1. Introduction**

According to the American Heart Association's heart disease and stroke 2010 statistical update, cardiovascular diseases have been the major cause of death every year since 1900 and account for 34.3% of all deaths in 2006 in the United States (1). Among cardiovascular diseases, heart failure (HF) is a complex syndrome characterized by functional and structural changes in the heart and resulting from several chronic and acute conditions. Moreover, HF is often accompanied by a range of comorbidities requiring complex clinical management influencing prognosis, health outcomes, and mortality, all of which impose an increased burden on the health

care system. In 2010, the estimated direct and indirect cost of HF in the United States was 39.2 billion dollars (1). In recent years, the prevalence of depression in HF patients has drawn attention, as numerous publications have suggested poorer clinical outcomes for HF patients reporting symptoms of depression (2).

The presence of depressive symptoms is widely considered a major risk factor in patients with coronary heart disease (3). Such symptoms are associated with adverse cardiovascular outcomes, independent of traditional risk factors, and cardiac disease severity (3). Numerous studies indicate significant association of depressive symptoms with age, gender, employment status, past history of depression, and functional severity of illness in hospitalized HF patients, as well as in outpatients (4). Major depression is prevalent in nearly 20% of patients with coronary heart disease and minor depression is seen in approximately 27% (5). Moreover, recently the New York Heart Association (NYHA)

\*Corresponding author. kfukunaga@m.tohoku.ac.jp

Published online in J-STAGE on February 22, 2013

doi: 10.1254/jphs.12R13CP

reported on depression prevalence based on HF Functional Class, noting that incidence increased steadily from 11% in patients with NYHA class I, 20% in those with class II, 38% in those with class III, and 42% in those with class IV heart failure. Current American College of Cardiology / American Heart Association guidelines for coronary artery bypass graft (CABG) surgery, acute myocardial infarction (MI), and chronic angina all recommend evaluation for symptoms of depression and consideration of treatment (6). Here, we focus on the current literature related to longitudinal associations between depression and clinical outcomes in HF, including HF incidence, mortality, and cardiovascular events. We also discuss the effect of pharmacological intervention with selective serotonin reuptake inhibitors (SSRIs) on depression among patients with HF and possible mechanisms of cardioprotective action by SSRIs based on our animal studies.

## 2. Clinical importance of anti-depression therapy for HF

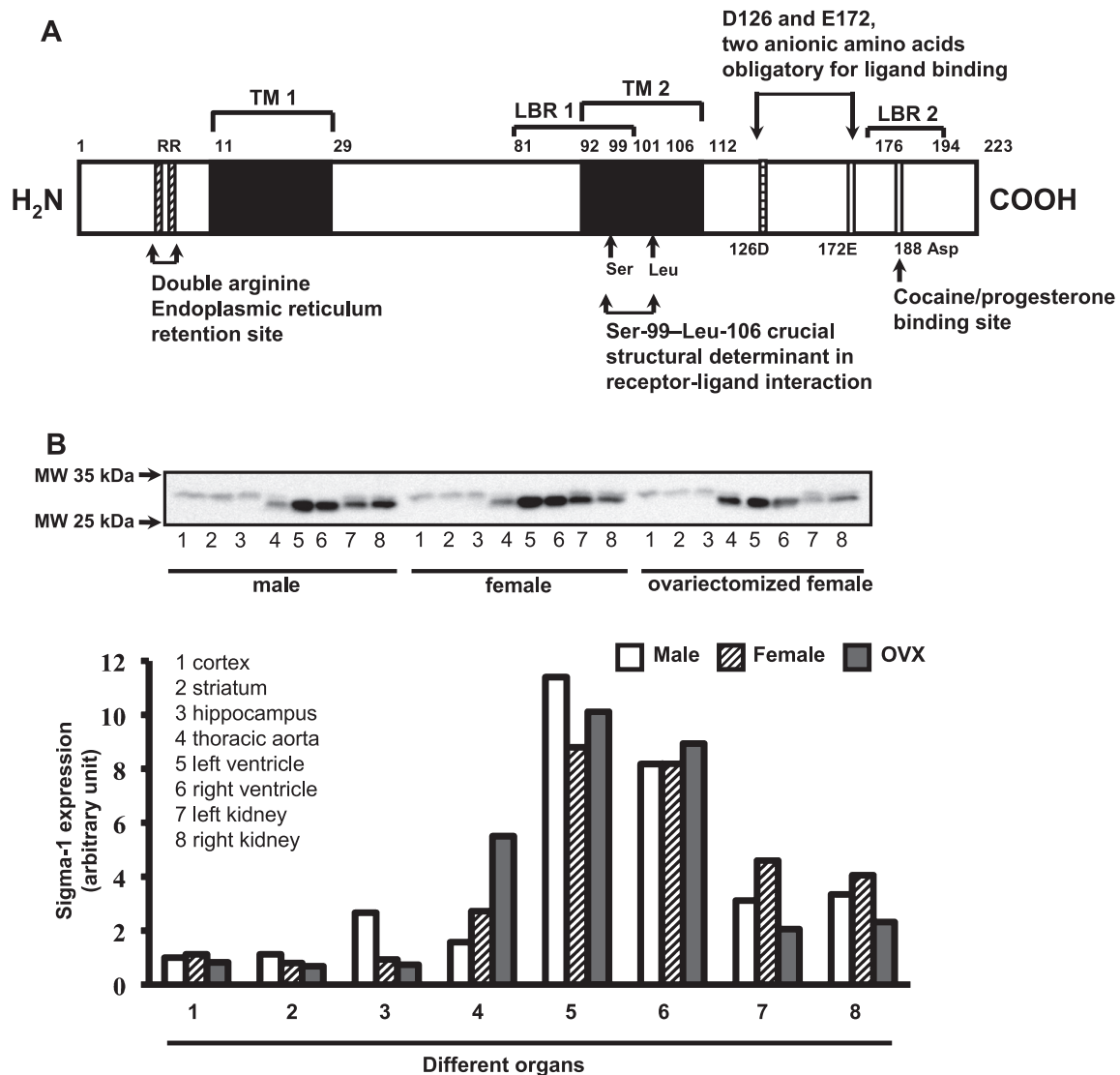
A recent meta-analysis reported a moderate to high prevalence of depression among patients with HF and an increased risk of mortality and clinical events among HF patients with depressive symptoms. Randomized clinical trials have demonstrated that SSRI antidepressants (sertraline and citalopram) are safe for patients with coronary heart disease and effective to treat moderate, severe, or recurrent depression (7). SSRIs have become an alternative choice when tricyclic antidepressants produce side effects such as pro-arrhythmic effects. Moreover, the Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) study revealed that patients treated with an SSRI, whether assigned to receive cognitive behavioral therapy or usual care, showed a 42% reduction in mortality or recurrent MI compared with depressed patients not receiving an antidepressant (8). SSRI treatment administered soon after acute MI improves mood and quality of life, and treatment of depressive symptoms may improve medication adherence in post-MI patients. Notably, SSRIs such as fluoxetine are generally safe when administered to patients with cardiovascular disease and do not slow cardiac conduction, cause orthostatic hypotension, decrease heart rate variability, or change QT variability measures (9). Thus, overall SSRIs appear to improve patients' depressive symptoms and quality of life (6, 7). SSRIs increase brain monoaminergic levels and apparently reverse many physiological derangements associated with depression, as evidenced by normalization of urinary cortisol excretion, improved heart rate variability, reduced platelet activation, and reduced expression of inflammatory markers (6, 7). Some studies indicate that in non-depressive individuals,

SSRIs decrease sympathetic nervous system activity at rest (as indicated by reduced plasma norepinephrine appearance rates) and during mental stress tasks (as measured by lower levels of heart rate, blood pressure, and plasma catecholamine concentrations). Moreover, SSRIs have also been shown to decrease platelet activation both in patients treated for depression and in healthy volunteers. Sauer et al. (10) reported a significantly reduced risk of MI in SSRI-treated smokers, while in the Sertraline Antidepressant Heart Attack Randomized Trial (SADHART), the incidence of severe cardiac events was only 14.5% in the sertraline group compared to 22.4% in the placebo group (7). Likewise, when 457 fatal and nonfatal cardiovascular events were followed by SADHART for 29 months, the risk of death or recurrent MI was significantly lower in patients taking SSRIs (8). Current American College of Cardiology / American Heart Association guidelines for CABG surgery, acute MI, and chronic angina all recommend evaluation for symptoms of depression and consideration of SSRI treatment (6). Although follow-up studies should reveal further links between depression and cardiovascular disease, currently the mechanism underlying improvement of cardiac dysfunction by SSRIs is largely unknown.

## 3. Structure and expression of sigma-1 receptor

The sigma-1 receptor, which is composed of 223 amino acids, was first purified from guinea-pig liver using radiolabeled ligands (11). Several publications indicate that the molecular weight of the receptor ranges from 26–30 kDa (Fig. 1A) (12). Sigma-1 receptors exhibit more than 90% sequence homology across mammalian species such as guinea pigs, humans, rats, and mice (12). Despite exhibiting 30% homology to a sequence found in the fungal sterol C8-C7 isomerase (11), the sigma-1 receptor lacks sterol isomerase activity (13). The murine sigma-1 receptor gene (approximately 7 kb) is made up of 4 exons and 3 introns and is TATA-less, containing CCAATC and GC boxes immediately upstream of the transcription start site (12). Southern blot analysis of genomic DNA indicates that the gene is located on human chromosome 9, band p13, a region associated with different psychiatric disorders (14).

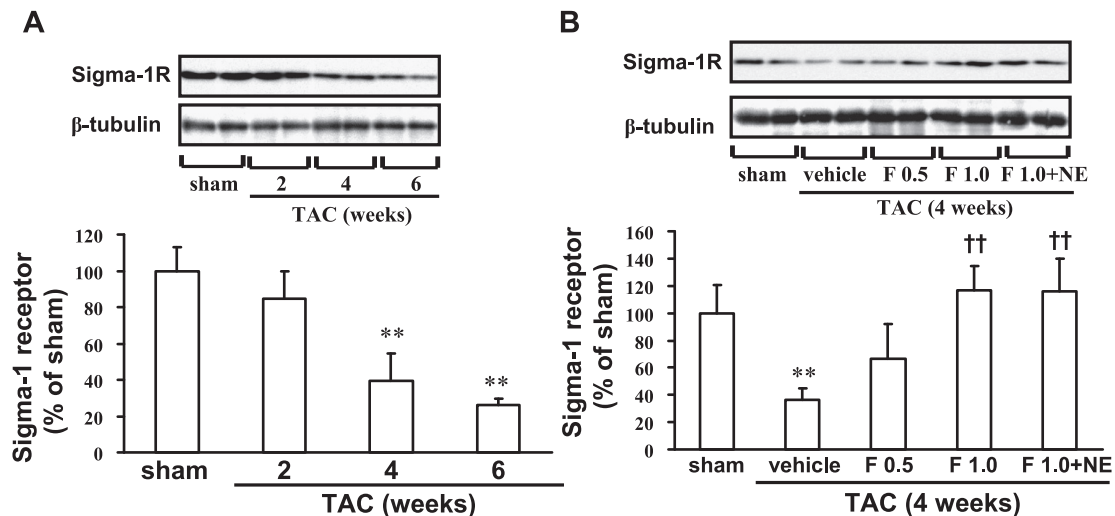
Structural models of the sigma-1 receptor based on the original cloning study predicted that its N-terminal hydrophobic domain (amino acids 11–29) could serve as a potential transmembrane domain (TM 1) (11). Other studies suggest a hydrophobic domain in the middle of the protein could function as another transmembrane domain (TM 2) (Fig. 1A) (12). Moreover, the sigma-1 receptor expressed in *Xenopus laevis* oocytes has two



**Fig. 1.** Structure and tissue distribution of the sigma-1 receptor. A: Schematic diagram of the sigma-1 receptor. TM, transmembrane domain; LBR, ligand binding region. B: Western blot analysis and densitometry quantification of sigma-1 receptor expression in different tissues of male, female, and ovariectomized female rats (1, cortex; 2, striatum; 3, hippocampus; 4, thoracic aorta; 5, left ventricle; 6, right ventricle; 7, left kidney; 8, right kidney). Data are expressed as fold expression relative to the cortex. Modified from Ref. 20.

transmembrane segments with both the NH<sub>2</sub> (amino acids 11 – 29) and COOH termini (amino acids 80 – 100) on the cytoplasmic face of the membrane (15). Studies also proposed two additional hydrophobic segments (one partially overlapping the second proposed transmembrane domain) termed as steroid binding-like domains as these regions of the sigma-1 receptor aligns favorably with the sequence of the steroid binding domain of the yeast and fungal sterol isomerases (16). A radiolabeled photoreactive ligand study indicated two possible ligand binding sites (LBR) in the sigma-1 receptor comprising LBR I (amino acids 91 – 109) and

LBR II (amino acids 176 – 194) (16) (Fig. 1A). Studies also suggest the existence of another sigma-1 receptor ligand binding site outside the LBR I region composed in part of TM 1 (amino acids 11 – 29) or of part of TM 2 (amino acids 80 – 89) in close apposition to LBR II domain (Fig. 1A). Amino acid residues Ser-99-Leu-106 in the putative transmembrane domain (TM2) of sigma-1 receptor serves as a crucial structural determinant in receptor-ligand interaction (17) (Fig. 1A). Cyanogen bromide cleavage of the 3-iodo-4-azidococaine photolabeled sigma-1 receptor followed by radiosequencing identified Asp188 located in the LBR II as the cocaine



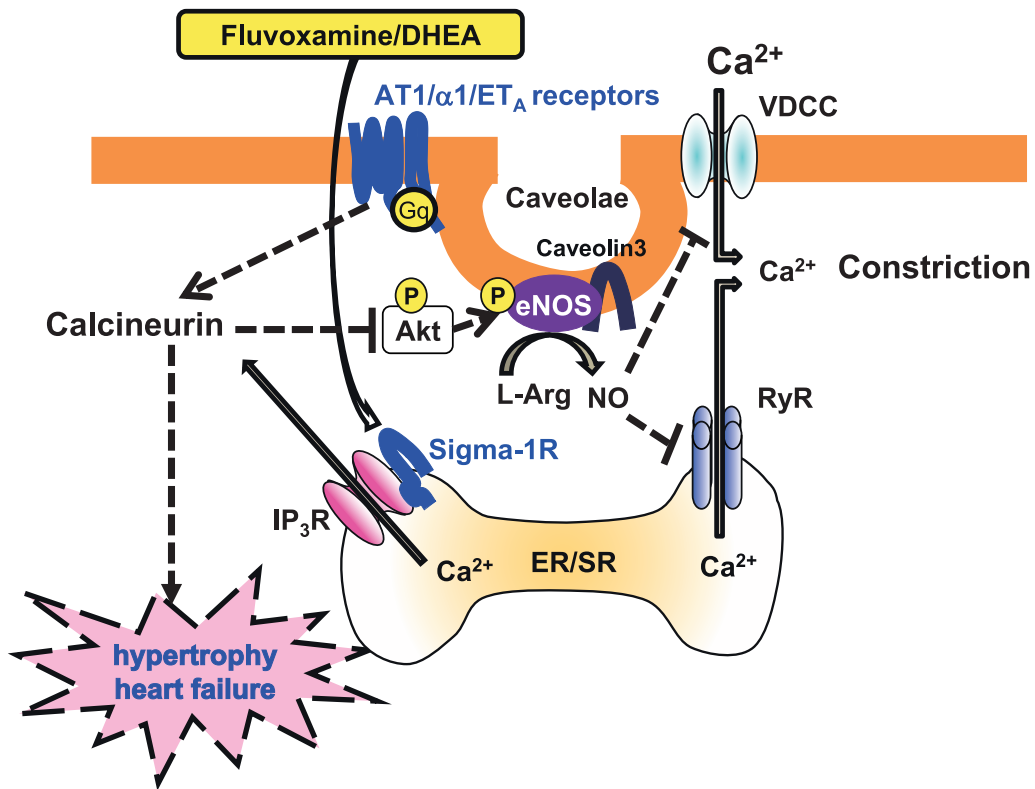
**Fig. 2.** Reduction of sigma-1 receptor expression following TAC and its restoration by fluvoxamine treatment. A: Temporal changes in sigma-1 receptor expression. Western blot analysis and densitometry quantification of temporal changes in sigma-1 receptor expression and  $\beta$ -tubulin in the LV using cell extracts from mice before and after 2–6 weeks of TAC. B: Effects of fluvoxamine (F) (0.5 and 1.0 mg/kg, oral) and NE-100 (NE, 1 mg/kg oral) on sigma-1 receptor expression. Western blot analysis and densitometry quantification of sigma-1 receptor expression and  $\beta$ -tubulin in the LV using cell extracts from mice with and without drug treatment. Data are expressed as percentages of the value of sham mice. Each column represents the mean  $\pm$  S.E.M. \*\* $P < 0.01$  versus the sham group, †† $P < 0.01$  versus the TAC-vehicle treated group. Modified from Ref. 22.

binding site (16). In addition, chemical modification of anionic amino acid residues and site-directed mutagenesis identify the two anionic amino acid residues, D126 and E172, in the ligand-binding domain of sigma-1 receptor critical for ligand binding (18). The deduced amino acid sequence of the receptor also reveals the presence of a double-arginine endoplasmic reticulum retention signal at its N-terminus (Fig. 1A), suggesting that the receptor resides on membranes of the endoplasmic reticulum (11, 12).

We previously reported ubiquitous expression of sigma-1 receptor in different tissues (brain, heart, and kidney) using male, female, and ovariectomized rats. Western blot analysis using whole cell extracts from cortex, striatum, hippocampus, thoracic aorta, left ventricle (LV), right ventricle (RV), and left and right kidney showed that the sigma-1 receptor is most abundantly expressed in heart LV and RV compared to brain extracts (Fig. 1B) (19, 20). We did not observe significant changes in sigma-1 receptor expression in hearts of male, female, or ovariectomized female rats. Western blot analyses also indicated that the brain- and heart-type sigma-1 receptors exhibit different molecular weights (Fig. 1B), suggesting different molecular subtypes, which should be confirmed with further studies.

Despite numerous studies analyzing sigma-1 receptor ligands in heart tissues, we still do not fully understand the pathophysiological role of the sigma-1 receptor (21).

To define its function in heart, we investigated temporal changes in receptor expression following cardiac hypertrophy and HF. Along with progression of LV hypertrophy, sigma-1 receptor expression was significantly reduced in the LV of ovariectomized rats (Fig. 2A) (20) and showed a significant negative linear correlation with heart function in mice with transverse aortic constriction (TAC) (22). We also confirmed decreased expression of sigma-1 receptor in LV following cardiac hypertrophy and failure by an immunohistochemical study (19). Interestingly, Ito et al. reported that cardiac dysfunction induced by pressure-overload (PO) with high-salt intake is associated with reduced expression of sigma-1 receptor protein in the brain concomitant with increased depression-like behavior in mice. Intracerebroventricular infusion of the sigma-1 receptor agonist PRE084 significantly improved cardiac dysfunction (23). This group speculated that inhibition of sympathetic hyperactivation by sigma-1 receptor stimulation in the central nervous system mediates improved cardiac dysfunction seen in this study. We conclude that improvement of hypertrophy-induced cardiac dysfunction is rescued by reduced sympathetic nerve activity following stimulation of the central nervous sigma-1 receptor and direct stimulation of the cardiac sigma-1 receptor by fluvoxamine.



**Fig. 3.** Schematic representation of the mechanism underlying sigma-1 receptor-mediated cardioprotection. Myocardial hypertrophy and heart failure causes decreased expression of sigma-1 receptor and deactivates Akt signaling pathways via activation of calcineurin in the heart. Treatment with sigma-1 receptor agonists protects the heart from myocardial hypertrophy and tissue injury via upregulation of the sigma-1 receptor and stimulation of receptor-mediated Akt-eNOS signaling.

#### 4. Possible mechanism of SSRI-mediated cardioprotection

SSRIs such as sertraline and fluvoxamine are potent sigma-1 receptor agonists and inhibitors of serotonin uptake (24). The order of affinity of SSRIs for the sigma-1 receptor is: fluvoxamine ( $K_i = 36$  nM) > sertraline ( $K_i = 57$  nM) > fluoxetine ( $K_i = 120$  nM) > citalopram ( $K_i = 292$  nM) > paroxetine ( $K_i = 1,893$  nM) (24). Thus, cardiac sigma-1 receptors are likely potential physiological targets of SSRIs. Sigma-1 receptors are suggested to regulate the cardiovascular system, as evidenced by the fact that several receptor ligands influence cardiovascular function and that cardiomyocytes exhibit sigma receptor ligand binding sites (21). Sigma receptor ligands such as (+)-3-PPP, (+)-pentazocine and haloperidol alter contractility,  $\text{Ca}^{2+}$  influx, and rhythmic activity of cultured cardiomyocytes, although these activities are complex and some of these findings remain controversial (21).

One study from another laboratory showed that fluvoxamine administration at a dose used in our experiments (22) attenuated sympathetic hyperactivation and improved

depression-like behavior (23). Although these mechanisms remain unclear, our work suggests that the cardiac sigma-1 receptor and its downstream signaling protect the heart against TAC (22) and PO-induced cardiomyopathy (21, 25). Previously, we also reported in several studies that the neurosteroid dehydroepiandrosterone (DHEA) serves as an endogenous ligand for the sigma-1 receptor and that treatment with DHEA ameliorated PO-induced cardiac injury by up-regulating the cardiac sigma-1 receptor in heart and activating downstream signaling (21, 26, 27). In support of our hypothesis, a recent study demonstrated significant reduction in DHEA-sulfate concentrations in brain circumventricular and hypothalamic tissue after aortic banding (23). Our studies of DHEA and fluvoxamine as sigma-1 receptor ligands also suggest that restored Akt activity and amelioration of impaired eNOS expression and phosphorylation are signals downstream of the receptor that modulates cardioprotection (21, 22, 25 – 28).

Upregulation of the sigma-1 receptor following fluvoxamine and DHEA treatments may also function in its cardioprotective action. We currently do not know how chronic fluvoxamine or DHEA treatment upregu-



lates sigma-1 receptor expression. Our results showing sigma-1 receptor upregulation by fluvoxamine reveal complexity (22). Although fluvoxamine-induced Akt and eNOS phosphorylation was completely abolished by pre-administration of the sigma-1 receptor antagonist NE-100, the fluvoxamine-induced sigma-1 receptor upregulation was not inhibited by NE-100 (Fig. 2B) (22). Other groups reported that chronic treatment of rats with the sigma-1 receptor antagonist E-5842 increased its receptor mRNA expression in the brain (29), whereas chronic imipramine (a sigma-1 receptor agonist) treatment decreased levels of sigma binding sites in the rat brain (30). Moreover, chronic haloperidol (a sigma-1 receptor antagonist) treatment promoted a reduction of sigma-1 receptor binding sites (31). Apparent discrepancies in these reports are likely due to use of different methodologies, including in vivo versus in vitro tests and binding assays versus immunodetection. Our study indicates that treatment with the sigma-1 receptor antagonist NE-100 does not alter sigma-1 receptor expression in sham-operated mice (22). Similarly, combining NE-100 with fluvoxamine had no effect on fluvoxamine-induced upregulation of sigma-1 receptors in the LV but nullified fluvoxamine-mediated antihypertrophic effects (22). These data suggest that sigma-1 receptor stimulation has antihypertrophic effects possibly through Akt signaling but that stabilization or upregulation of the receptor by fluvoxamine is not mediated by sigma-1 receptor-stimulated signaling.

Although the mechanisms responsible for Akt dephosphorylation following HF remain unclear, it has recently been shown that the  $\text{Ca}^{2+}$ -dependent protein phosphatase PP2B, known as calcineurin, can dephosphorylate Akt (32) (Fig. 3). We previously documented that stimulation with angiotensin II, endothelin-1, and phenylephrine markedly increase calcineurin expression following hypertrophy in cultured rat cardiomyocytes (33). The transcriptional upregulation of calcineurin  $A\beta$  by endothelin-1 is partially mediated by activation of myocyte enhancer factor-2 (MEF2) by  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II $\delta$  in rat cardiomyocytes (34). Our speculation is supported by the fact that transient ischemia-induced calcineurin activation reduced Akt phosphorylation in rat retina (32). Our hypothesis that activation of the sigma-1 receptor enhances Akt activity is supported by observations by others that receptor antagonists, such as rimcazole (BW 234U), IPAG, reduced haloperidol, BD-1047, and BD-1063, promote calcium-independent inhibition of PI3K signaling and inhibit Akt phosphorylation in tumor cell lines. Moreover, sigma-1 receptor knockdown in lens cells via small interference RNA (siRNA) inhibits thrombin-stimulated Akt phosphorylation and increases cell death (35).

However, currently we do not know whether calcineurin activation directly downregulates the sigma-1 receptor in cardiomyopathies, thereby inhibiting Akt signaling. Future studies should address basic mechanisms underlying Akt dephosphorylation and potential crosstalk between calcineurin and the sigma-1 receptor.

Our novel findings indicate that fluvoxamine prevents development of TAC-induced LV hypertrophy in mice (22), pressure overload-induced hypertrophy in ovariectomized rats (25) in vivo, and angiotensin II-induced cardiomyocyte hypertrophy in vitro (22). We also found that fluvoxamine-mediated activation of Akt and eNOS signaling via sigma-1 receptors likely mediates anti-hypertrophic effects (22, 25, 28). Interestingly, our studies show that continuous administration of fluvoxamine for 4 weeks not only increases Akt-mediated eNOS phosphorylation on Ser<sup>1177</sup> but also enhances eNOS protein expression in the LV (Fig. 3). Treatment with the sigma-1 receptor antagonist NE-100 significantly nullified fluvoxamine-mediated eNOS upregulation and Akt-mediated eNOS phosphorylation, confirming that the sigma-1 receptor modulates eNOS activity in the heart. Our study indicated that the fluvoxamine-mediated cardioprotective effect is partly mediated by increased receptor expression and that the sigma-1 receptor stimulates both increased eNOS expression and increased Akt-mediated eNOS phosphorylation at Ser<sup>1177</sup> in the heart (Fig. 3) (22, 25, 28).

The affinity ( $K_i$ ) for sigma-1 receptor of fluvoxamine and paroxetine are 36 nM (potent) and 1,893 nM (weak), respectively (24). To confirm whether fluvoxamine-mediated cardioprotective action is associated with SSRI activity, we tested the effects of paroxetine on hypertrophy because paroxetine at the doses used clinically lacks significant sigma-1 receptor binding affinity. Paroxetine administration failed to inhibit hypertrophy (22). A positron emission tomography study in humans demonstrated that fluvoxamine (50–200 mg/body weight) binds to sigma-1 receptors but that a paroxetine dose of 20 mg/body weight has no effect on the intact human brain, suggesting that the sigma-1 receptor functions in the pharmacological activity of fluvoxamine (24). Importantly, we found that fluvoxamine (0.5–1 mg/kg) significantly rescued cardiac dysfunction following TAC in mice, whereas paroxetine (0.2–0.4 mg/kg) failed to rescue cardiac dysfunction and inhibit hypertrophy following TAC (22). Notably, SSRIs generally do not slow cardiac conduction, cause orthostatic hypotension, decrease heart rate variability, or alter QT variability measures (9). SSRIs also appear to improve both depressive symptoms and patient quality of life (6, 7). Nonetheless, further epidemiological investigation is required to define cardioprotec-

tive effects of subgroups of SSRIs with or without sigma-1 agonistic effects.

Taken together, we propose a working hypothesis of sigma-1 receptor-mediated protective mechanisms in cardiomyocytes as shown in Fig. 3. The hypertrophic stimuli through AT<sub>1</sub>,  $\alpha_1$ , and ET<sub>A</sub> receptors by prolonged exposure to angiotensin II, noradrenaline, and endothelin-1 cause elevation of calcineurin activity through persistent Ca<sup>2+</sup> elevation by IP<sub>3</sub> receptors and other Ca<sup>2+</sup> channels. Calcineurin-mediated dephosphorylation of Akt leads to eNOS dephosphorylation, thereby decreasing nitric oxide (NO) production. The reduced NO mediates overload of Ca<sup>2+</sup> in cardiomyocytes because sarcoplasmic and plasma membranes lack the physiological feedback mechanism to inhibit ryanodine receptor (RyR) and voltage-dependent calcium channels (VDCCs), respectively (36). Since the sigma-1 receptor is predominantly expressed in endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) in smooth and striated muscles, and regulates IP<sub>3</sub> receptor functions (37), we speculate that fluvoxamine and DHEA likely inhibit Ca<sup>2+</sup> release from the ER. Finally, the suppression of the basal Ca<sup>2+</sup> concentration mediates sigma-1 receptor agonist-induced anti-hypertrophic effects. However, we should address whether sigma-1 receptor agonists suppress calcineurin activity through inhibition of Ca<sup>2+</sup> leakage from ER/SR or through transcriptional regulation in future studies.

## 5. Future directions

The benefits of SSRIs to patients with cardiovascular diseases have not been fully established because the molecular mechanism underlying SSRI-induced cardioprotection is largely unknown. Our studies have focused on the cardiac sigma-1 receptor as part of an underlying molecular mechanism of SSRI-mediated cardioprotection. We documented that both TAC- and PO-induced hypertrophy downregulates sigma-1 receptor expression in the rat LV. We should conduct studies to measure pathological changes in mRNA and protein expression levels of sigma-1 receptor in cardiovascular disease patients developing to HF. Sigma-1 receptor is highly expressed not only in the heart but also in many peripheral organs including aorta, kidney, and lung (25). To define the pharmacological relevance of the sigma-1 receptor in the peripheral organs, we are now investigating the pharmacological actions using specific ligands without SSRI actions such as SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride). SA4503 has recently been tested in humans in a stroke and depression clinical phase II trial (NCT00639249). In addition, molecular genetic

approaches such as cardiac-specific knockdown of the sigma-1 receptor or creation of transgenic animals are appropriate for studies to define potential physiological and pathological relevance of sigma-1 receptors in the heart.

## Acknowledgment

This work was supported in part by Kakenhi 22390109 (to K.F.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References

- 1 Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–e215.
- 2 Rumsfeld JS, Havranek E, Masoudi FA, Peterson ED, Jones P, Tooley JF, et al. Depressive symptoms are the strongest predictors of short-term declines in health status in patients with heart failure. *J Am Coll Cardiol*. 2003;42:1811–1817.
- 3 Whooley MA, de Jonge P, Vittinghoff E, Otte C, Moos R, Carney RM, et al. Depressive symptoms, health behaviors, and risk of cardiovascular events in patients with coronary heart disease. *JAMA*. 2008;300:2379–2388.
- 4 Gottlieb SS, Khatta M, Friedmann E, Einbinder L, Katzen S, Baker B, et al. The influence of age, gender, and race on the prevalence of depression in heart failure patients. *J Am Coll Cardiol*. 2004;43:1542–1549.
- 5 Schleifer SJ, Macari-Hinson MM, Coyle DA, Slater WR, Kahn M, Gorlin R, et al. The nature and course of depression following myocardial infarction. *Arch Intern Med*. 1989;149:1785–1789.
- 6 Rumsfeld JS, Ho PM. Depression and cardiovascular disease: a call for recognition. *Circulation*. 2005;111:250–253.
- 7 Glassman AH, O'Connor CM, Califf RM, Swedberg K, Schwartz P, Bigger JT Jr, et al. Sertraline treatment of major depression in patients with acute MI or unstable angina. *JAMA*. 2002;288:701–709.
- 8 Taylor CB, Youngblood ME, Catellier D, Veith RC, Carney RM, Burg MM, et al. Effects of antidepressant medication on morbidity and mortality in depressed patients after myocardial infarction. *Arch Gen Psychiatry*. 2005;62:792–798.
- 9 Fisch C. Effect of fluoxetine on the electrocardiogram. *J Clin Psychiatry*. 1985;46:42–44.
- 10 Sauer WH, Berlin JA, Kimmel SE. Selective serotonin reuptake inhibitors and myocardial infarction. *Circulation*. 2001;104:1894–1898.
- 11 Hanner M, Moebius FF, Flandorfer A, Knaus HG, Striessnig J, Kempner E, et al. Purification, molecular cloning, and expression of the mammalian sigma-1-binding site. *Proc Natl Acad Sci U S A*. 1996;93:8072–8077.
- 12 Seth P, Leibach FH, Ganapathy V. Cloning and structural analysis of the cDNA and the gene encoding the murine type 1 sigma receptor. *Biochem Biophys Res Comm*. 1997;241:535–540.
- 13 Labit-Le Bouteiller C, Jamme MF, David M, Silve S, Lanau C, Dhers C, et al. Antiproliferative effects of SR31747A in animal cell lines are mediated by inhibition of cholesterol biosynthesis at the sterol isomerase step. *Eur J Biochem*. 1998;256:342–349.

- 14 Prasad PD, Li HW, Fei YJ, Ganapathy ME, Fujita T, Plumley LH, et al. Exon-intron structure, analysis of promoter region, and chromosomal localization of the human type 1 sigma receptor gene. *J Neurochem*. 1998;70:443–451.
- 15 Aydar E, Palmer CP, Klyachko VA, Jackson MB. The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. *Neuron*. 2002;34:399–410.
- 16 Chen Y, Hajipour AR, Sievert MK, Arbabian M, Ruoho AE. Characterization of the cocaine binding site on the sigma-1 receptor. *Biochemistry*. 2007;46:3532–3542.
- 17 Yamamoto H, Miura R, Yamamoto T, Shinohara K, Watanabe M, Okuyama S, et al. Amino acid residues in the transmembrane domain of the type 1 sigma receptor critical for ligand binding. *FEBS letters*. 1999;445:19–22.
- 18 Seth P, Ganapathy ME, Conway SJ, Bridges CD, Smith SB, Casellas P, et al. Expression pattern of the type 1 sigma receptor in the brain and identity of critical anionic amino acid residues in the ligand-binding domain of the receptor. *Biochem Biophys Acta*. 2011;1540:59–67.
- 19 Bhuiyan MS, Fukunaga K. Targeting sigma-1 receptor signaling by endogenous ligands for cardioprotection. *Expert Opin Ther Targets*. 2011;15:145–155.
- 20 Bhuiyan MS, Fukunaga K. Stimulation of sigma-1 receptor signaling by dehydroepiandrosterone ameliorates pressure overload-induced hypertrophy and dysfunctions in ovariectomized rats. *Expert Opin Ther Targets*. 2009;13:1253–1265.
- 21 Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y. Sigma receptor ligands modulate contractility, Ca<sup>++</sup> influx and beating rate in cultured cardiac myocytes. *The Journal of pharmacology and experimental therapeutics*. 1994;269:1300–1309.
- 22 Tagashira H, Bhuiyan S, Shioda N, Hasegawa H, Kanai H, Fukunaga K. Sigma-1-receptor stimulation with fluvoxamine ameliorates transverse aortic constriction-induced myocardial hypertrophy and dysfunction in mice. *Am J Physiol Heart Circ Physiol*. 2010;299:H1535–H1545.
- 23 Ito K, Hirooka Y, Matsukawa R, Nakano M, Sunagawa K. Decreased brain sigma-1 receptor contributes to the relationship between heart failure and depression. *Cardiovasc Res*. 2012;93:33–40.
- 24 Narita N, Hashimoto K, Tomitaka S, Minabe Y. Interactions of selective serotonin reuptake inhibitors with subtypes of sigma receptors in rat brain. *Eur J Pharmacol*. 1996;307:117–119.
- 25 Bhuiyan MS, Tagashira H, Shioda N, Fukunaga K. Targeting sigma-1 receptor with fluvoxamine ameliorates pressure-overload-induced hypertrophy and dysfunctions. *Expert Opin Ther Targets*. 2010;14:1009–1022.
- 26 Bhuiyan MS, Tagashira H, Fukunaga K. Dehydroepiandrosterone-mediated stimulation of sigma-1 receptor activates Akt-eNOS signaling in the thoracic aorta of ovariectomized rats with abdominal aortic banding. *Cardiovasc Ther*. 2011;29:219–230.
- 27 Tagashira H, Bhuiyan S, Shioda N, Fukunaga K. Distinct cardio-protective effects of 17beta-estradiol and dehydroepiandrosterone on pressure overload-induced hypertrophy in ovariectomized female rats. *Menopause*. 2011;18:1317–1326.
- 28 Bhuiyan MS, Tagashira H, Fukunaga K. Sigma-1 receptor stimulation with fluvoxamine activates Akt-eNOS signaling in the thoracic aorta of ovariectomized rats with abdominal aortic banding. *Eur J Pharmacol*. 2011;650:621–628.
- 29 Zamanillo D, Andreu F, Ovalle S, Perez MP, Romero G, Farre AJ, et al. Up-regulation of sigma(1) receptor mRNA in rat brain by a putative atypical antipsychotic and sigma receptor ligand. *Neurosci Lett*. 2000;282:169–172.
- 30 Shirayama Y, Nishikawa T, Umino A, Takahashi K. p-chlorophenylalanine-reversible reduction of sigma binding sites by chronic imipramine treatment in rat brain. *Eur J Pharmacol*. 1993;237:117–126.
- 31 Inoue A, Sugita S, Shoji H, Ichimoto H, Hide I, Nakata Y. Repeated haloperidol treatment decreases sigma(1) receptor binding but does not affect its mRNA levels in the guinea pig or rat brain. *Eur J Pharmacol*. 2000;401:307–316.
- 32 Park CH, Kim YS, Kim YH, Choi MY, Yoo JM, Kang SS, et al. Calcineurin mediates AKT dephosphorylation in the ischemic rat retina. *Brain research*. 2008;1234:148–157.
- 33 Lu YM, Shioda N, Han F, Moriguchi S, Kasahara J, Shirasaki Y, et al. Imbalance between CaM kinase II and calcineurin activities impairs caffeine-induced calcium release in hypertrophic cardiomyocytes. *Biochem Pharmacol*. 2007;74:1727–1737.
- 34 Lu YM, Shioda N, Yamamoto Y, Han F, Fukunaga K. Transcriptional upregulation of calcineurin A $\beta$  by endothelin-1 is partially mediated by calcium/calmodulin-dependent protein kinase II $\delta$ 3 in rat cardiomyocytes. *Biochim Biophys Acta*. 2010;1799:429–441.
- 35 Wang L, Duncan G. Silencing of sigma-1 receptor induces cell death in human lens cells. *Exp Cell Res*. 2006;312:1439–1446.
- 36 Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature*. 2002;416:337–339.
- 37 Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell*. 2007;131:596–610.