Decreased brain sigma-1 receptor contributes to the relationship between heart failure and depression

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Aims
Depression often coexists with cardiovascular disease, such as hypertension and heart failure, in which sympathetic hyperactivation is critically involved. Reduction in the brain sigma-1 receptor (S1R) functions in depression pathogenesis via neuronal activity modulation. We hypothesized that reduced brain S1R exacerbates heart failure, especially with pressure overload via sympathetic hyperactivation and worsening depression.

Methods and results
Male Institute of Cancer Research mice were treated with aortic banding and, 4 weeks thereafter, fed a high-salt diet for an additional 4 weeks to accelerate cardiac dysfunction (AB-H). Compared with sham-operated controls (Sham), AB-H showed augmented sympathetic activity, decreased per cent fractional shortening, increased left ventricular dimensions, and significantly lower brain S1R expression. Intracerebroventricular (ICV) infusion of S1R agonist PRE084 increased brain S1R expression, lowered sympathetic activity, and improved cardiac function in AB-H. ICV infusion of S1R antagonist BD1063 increased sympathetic activity and decreased cardiac function in Sham. Tail suspension test was used to evaluate the index of depression-like behaviour, with immobility time and strain amplitude recorded as markers of struggle activity using a force transducer. Immobility time increased and strain amplitude decreased in AB-H compared with Sham, and these changes were attenuated by ICV infusion of PRE084.

Conclusion
These results indicate that decreased brain S1R contributes to the relationship between heart failure and depression in a mouse model of pressure overload.

Keywords
Sympathetic nervous system • Heart failure • Depression • Brain sigma-1 receptor • Pressure overload

1. Introduction
Numerous studies have demonstrated that heart failure and depression often coexist, and that depression is linked to the severity of heart failure symptoms.¹,² Heart failure and depression are both individually associated with poor health outcomes, and depression also adversely affects heart failure outcomes.³,⁴ Importantly, hypertension is associated with the risk of heart failure among patients with hypertension.⁵ Furthermore, hypertensive heart disease is well recognized as the major cause of heart failure.⁶ One of the physiological mechanisms involved in both depression and heart failure is high sympathetic tone caused by cardiovascular autonomic dysregulation.⁷,⁸ The central nervous system contributes to the worsening of both heart failure⁹ and hypertension.¹⁰ The detailed mechanisms involved in this process remain unclear, but these findings suggest the presence of a common pathway for heart failure and depression in the brain.

Recently, a reduction in brain sigma-1 receptor (S1R) expression has been shown to play a key role in the pathogenesis of depression.¹¹ S1R ligands have been reported to have antidepressant activity in behavioural models of depression.¹² The S1R has been shown to modulate neuronal intracellular calcium levels¹³ and N-methyl-D-aspartate-mediated response.¹⁴,¹⁵ The results of these studies strongly suggest that the S1R contributes to the regulation of neuronal activity. Neurosteroids such as dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS) are recognized as endogenous S1R agonists.¹⁶
and DHEAS was reported to decrease in heart failure.\textsuperscript{17} Therefore, we hypothesized that expression of brain S1R is reduced in heart failure via decreased DHEAS, and that this reduced expression contributes to the exacerbation of heart failure via enhanced sympathetic activity and worsening of depression. Therefore, the aim of the present study was to assess the role of the brain S1R in the relationship between heart failure and depression.

Recently, we found that a pressure overload model with salt loading is a model of hypertensive heart disease leading to heart failure.\textsuperscript{19} The relationship between hypertension and depression has been well recognized, and depression is associated with the risk of heart failure among patients with hypertension.\textsuperscript{5} In addition, the pressure overload model was reported to decrease S1R expression in the left ventricle.\textsuperscript{16,19} Therefore, the pressure overload model was used in the present study. Aortic banding (AB) was performed in mice then fed a high-salt (HS) diet to accelerate cardiac dysfunction.\textsuperscript{18} We investigated (i) sympathetic activity by 24-h urinary norepinephrine (U-NE) excretion\textsuperscript{18,20} and cardiac function by echocardiography\textsuperscript{18,20}, (ii) brain S1R expression; (iii) the index of depression-like behaviour; (iv) the effects of intracerebroventricular (ICV) infusion of PRE084, a selective S1R agonist,\textsuperscript{24,25} and BD1063, a selective S1R antagonist,\textsuperscript{21,22} on sympathetic activity, cardiac function, brain S1R expression, and the index of depression-like behaviour and (v) the concentration of serum and brain DHEAS.

2. Methods

2.1 Animals

The study was reviewed and approved by the Committee on Ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Male Institute of Cancer Research (ICR) mice (10 weeks old; SLC, Fukuoka, Japan) were used. Sodium pentobarbital was used as anaesthetic, and the adequacy of anaesthesia was confirmed by the absence of a withdrawal response to hindpaw nociceptive stimulation.

2.2 Mouse heart failure model

Mice under sodium pentobarbital (25–40 mg/kg intraperitoneal (ip)) anaesthesia were treated with AB at the suprarenal abdominal aorta\textsuperscript{18,20} with 5–0 silk sutures guided by a blunted 27-gauge needle, which was withdrawn as quickly as possible. Sham-operated mice (Sham) served as controls. Four weeks later, both AB mice and Sham mice were fed a HS (8% NaCl) diet for 4 weeks (Sham-H or AB-H).\textsuperscript{18,20} The survival rate after heart failure model preparation were as follows: 4 weeks after AB, 97%; 8 weeks after AB with HS diet (AB-H), 83%.

2.3 Evaluation of cardiac function

Cardiac function was evaluated by echocardiography\textsuperscript{18,20} under light sodium pentobarbital anaesthesia with spontaneous respiration. An echocardiography system (SSD5000; Aloka, Tokyo, Japan) with a dynamically focused 7.5-MHz linear array transducer was used, and M-mode tracings from the short-axis view at the level of the papillary muscle were recorded. Left ventricle (LV) end-diastolic diameter (LVDD), LV end-systolic diameter (LVSD), and LV wall thickness (LVWT), calculated as the mean thickness of the interventricular septum and the posterior LV wall, were measured, and per cent fractional shortening (%FS) was calculated as follows:

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%FS = \frac{(LVDD - LVSD)}{LVDD} \times 100.
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2.4 Evaluation of blood pressure, heart rate, and sympathetic activity

In acute experiments, sympathetic activity was evaluated by power spectral analysis.\textsuperscript{24,25} Blood pressure (BP) and the heart rate were measured by right carotid artery cannulation with a stretched polyethylene tube (PE50). Data were recorded using the Powerlab system and Chart 5 software (AD Instruments), and the power spectrum of the beat-by-beat systolic blood pressure (SBP) time series and the beat-by-beat pulse interval time series was calculated using the maximum entropy method with MemCalc software (Suwa Trust Co., Ltd).\textsuperscript{23} Two zones of interest for autonomic control of BP and the heart rate were observed. The first zone, covering the 0.15–0.6 Hz range of the SBP spectrum, was used as a low-frequency zone reflecting sympathetic control in mice. The second zone, covering the 2.5–5.0 Hz range of the pulse interval spectrum, was used as a high-frequency zone reflecting vagal control in mice.\textsuperscript{22} In chronic experiments, sympathetic activity was evaluated by measuring 24-h U-NE excretion using high-performance liquid chromatography.\textsuperscript{18,20}

2.5 ICV infusion

Under sodium pentobarbital anaesthesia (25–40 mg/kg, i.p.), mice were placed in a stereotaxic frame, and the skin overlying the midline of the skull was incised. A small hole was made with a dental drill at 0.3 mm posterior and 1 mm lateral to bregma.\textsuperscript{18,20} An infusion cannula (Alzet brain infusion kit 3; DURECT Corporation, CA, USA) was inserted and fixed to the skull surface with tissue adhesive (the tip of the cannula located 3 mm below the skull surface).\textsuperscript{18,20} In acute experiments, the infusion cannula was connected to a syringe filled with PRE084, a specific S1R agonist (1 mM), and the agent was infused using a microsyringe pump (infusion rate 1.0 µL/min for 10 min) while measuring BP and the heart rate. In chronic experiments, the infusion cannula was connected to an osmotic minipump (Alzet model 1004; DURECT) inserted subcutaneously into the back for infusion of PRE084 into AB-H (2 mM; infusion rate 0.11 µL/h for 4 weeks initiated concomitantly with HS intake) or BD1063, a specific S1R antagonist, into Sham (2 mM; infusion rate 0.11 µL/h for 4 weeks initiated from 4 weeks after sham operation).

2.6 Oral drug administration

Fluvoxamine maleate (Sigma Aldrich Co., St Louis, MO, USA) mixed in powdered chow was administered orally to AB-H for 4 weeks initiated concomitantly with HS intake for an estimated oral administered fluvoxamine dose of ~1.0 mg/kg/day.\textsuperscript{19}

2.7 Measurement of organ weight

After completion of the experiments, mice were killed with an overdose of sodium pentobarbital, the heart and lungs were removed, and organ weight was measured.

2.8 Evaluation of S1R expression in the brain

2.8.1 Western blot analysis

Animals were killed with an overdose of sodium pentobarbital. Tissues obtained from the circumventricular tissues including the hypothalamus were homogenized in lysis buffer containing 40 mmol/L HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid), 1% Triton X-100, 10% glycerol, 1 mmol/L sodium orthovanadate, and 1 mmol/L phenylmethylsulfonyl fluoride. Protein concentration was determined with a bicinchoninic acid protein assay kit (Pierce Chemical Co., Rockford, IL, USA). A 15 µg protein aliquot from each sample was separated on a polyacrylamide gel with 10% sodium dodecyl sulfate. The proteins were subsequently transferred onto polyvinylidene difluoride membranes (Immobilon®-P membranes; Millipore, Billerica, MA, USA). A rabbit immunoglobulin G (IgG) polyclonal antibody against S1R (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as primary antibody.\textsuperscript{26} Membranes were then incubated with horseradish peroxidase-
conjugated horse anti-rabbit IgG antibody (1:10 000). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control for the brain tissues. Immunoreactivity was detected by enhanced chemiluminescence autoradiography (ECL,Amersham Pharmacia Biotech, Uppsala, Sweden), and the film was analysed using the public domain software NIH Image (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

2.8.2 Reverse transcriptase PCR
Total RNA was prepared from the circumventricular tissues including hypothalamus using RNAlater solution (Ambion, Austin, TX, USA). Complementary DNAs were synthesized by standard techniques using a ReverTra Ace qPCR RT Kit (TOYOBO, Osaka, Japan). Real-time PCR was performed, recorded, and analysed using a thermal cycler dice real-time system (Takara Bio, Shiga, Japan) with SYBR Green I detection. cDNA was amplified using a SYBR Premix Ex Taq (Perfect Real Time) PCR kit (Takara Bio) with specific primers (Sigma R1, forward: 5′-CTC GCT GTA GAT GTG 3′; reverse: 5′-AAC TTT CGT GCT AGT GCA A-3′; HPRT1, forward: 5′-GGC TCG TGA TTA GCG 3′; reverse: 5′-CCT CCC ATC TCC TTT AGC TCT-3′; HPRT1 was used as a reference to normalize the amount of total RNA amplified in each reaction. Relative gene expression data were analysed using the 2 (−ΔΔCT) method.27

2.9 Evaluation of the depression-like behaviour index
Depression was evaluated by the tail suspension test, which uses increased immobility time as an index of depression-like behaviour in mice.28 Tail suspension was conducted in a force transducer, and strain amplitude data were also recorded using the Powerlab system as a marker of struggle activity. The test was performed in the afternoon, and immobility time and struggle activity were determined during 6-min recordings. Additionally, locomotor activity was evaluated by a digital actophotometer.29 Two mice were placed in the actophotometer apparatus cage, and the total number of ambulatory movements was scored over 24 h. The daily variation of locomotor activity was also evaluated by the ratio of movement scores from night to day.

2.10 Measurement of serum and brain DHEAS
Under anaesthesia with an overdose of sodium pentobarbital, a blood sample was collected from the right ventricle, and the mice were perfused with distilled H2O. After adequate perfusion to remove blood, the brain circumventricular tissues and hypothalamus were dissected out. The tissues (0.10 g) were homogenized in 200 μL distilled H2O, rapidly centrifuged, and the supernatant was collected.10 DHEAS concentration was measured by enzyme-linked immunosorbent assay (ELISA).

2.11 Statistical analysis
All values are expressed as mean ± SE. Analysis of variance was used to compare U-NE, organ weight, LVDD, LVSVD, LVVWT, %FS, immobility time, strain amplitude, DHEAS concentration, mRNA levels, and protein levels between groups. An unpaired t-test was used to compare locomotor activity between Sham and AB-H and changes in protein levels between mice treated with and without S1R ligands. Differences were considered significant at P < 0.05.

3. Results
3.1 Characteristics of AB-H
Both relative heart weight (heart weight/body weight) and absolute heart weight were greater in AB-H than Sham (relative heart weight: Sham, 4.87 ± 0.05 mg/g; AB-H, 6.45 ± 0.08 mg/g; absolute heart weight: Sham, 0.23 ± 0.02 g; AB-H, 0.26 ± 0.05 g; P < 0.05, n = 8 per group). Relative lung weight (lung weight/body weight) tended to increase in AB-H compared with Sham (Sham, 5.81 ± 0.15mg/g; AB-H, 6.20 ± 0.09 mg/g; n = 8 per group). The body weight of AB-H was significantly lower than that of Sham (Sham, 47.4 ± 0.8 g; AB-H, 40.6 ± 1.1 g; P < 0.05, n = 8 per group). Echocardiography revealed that LV dimensions and LVVWT were greater in AB-H than Sham, and %FS was significantly lower in AB-H than Sham (Figure 1). Sympathetic activity evaluated by U-NE excretion was increased in AB-H compared with Sham (Sham, 350 ± 44 ng/day; AB-H, 731 ± 26 ng/day; P < 0.05, n = 8 per group). In Sham, HS intake did not alter body weight, organ weight, cardiac function, or sympathetic activity (Sham vs. Sham-H). Mean BP was lower and the heart rate was higher in AB-H than Sham (mean BP: Sham, 422 ± 16 b.p.m.; AB-H, 477 ± 14 b.p.m.; P < 0.05, n = 5 per group). Tail suspension test revealed increased immobility time and decreased strain amplitude in AB-H compared with Sham (Figure 1A–C). Locomotor activity (24 h) was lower in AB-H than Sham (Sham, 7387 ± 459 counts; AB-H, 3877 ± 864 counts; P < 0.05, n = 6 per group), and the ratio of locomotor activity during night to day, a marker of daily variation, was smaller in AB-H than Sham (Sham, 3.2 ± 0.6; AB-H, 1.6 ± 0.1; P < 0.05, n = 6 per group).

3.2 S1R expression in the brain
The protein levels of brain S1R were decreased in AB-H compared with Sham, in AB-H than Sham (Figure 2A–C). Chronic ICV infusion of PRE084 decreased immobility time and strain amplitude (AB-H, 459 counts; AB-H with PRE, 26 ng/day; AB-H with PRE, 571 ± 43 ng/day; P < 0.05, n = 5 per group). Furthermore, sympathetic activity evaluated by power spectral analysis of SBP was decreased significantly only in Sham (Figure 2B).

3.3 Effects of acute PRE084 ICV infusion on cardiovascular regulation
ICV infusion of PRE084 lowered heart rate in both AB-H and Sham. However, changes in the heart rate were significantly smaller in AB-H than in Sham (Figure 3). The mRNA levels of brain S1R did not significantly alter between Sham and AB-H (Sham, 1.2 ± 0.1; AB-H, 0.9 ± 0.1; n = 5 per group).

3.4 Effects of chronic PRE084 ICV infusion
Chronic ICV infusion of PRE084 (PRE) increased protein levels of brain S1R in AB-H compared with no treatment (Figure 4A). Chronic PRE084 ICV infusion lowered the enhanced sympathetic activity (AB-H, 731 ± 26 ng/day; AB-H with PRE, 571 ± 43 ng/day; P < 0.05, n = 8 and 5, respectively) and improved cardiac function in AB-H compared with no treatment (Figure 4B). Furthermore, PRE084 also decreased both relative heart weight (heart weight/body weight) and absolute heart weight compared with no treatment (relative heart weight: AB-H, 6.45 ± 0.08 mg/g; AB-H with PRE, 4.50 ± 0.15 mg/g, absolute heart weight: AB-H, 0.26 ± 0.05 g; AB-H with PRE, 0.19 ± 0.07 g; P < 0.05, n = 8 and 5, respectively). Chronic ICV infusion of PRE084 decreased immobility time and increased strain amplitude in AB-H compared with no treatment (Figure 5C).
3.5 Effects of chronic oral administration of fluvoxamine

Chronic oral administration of fluvoxamine lowered enhanced sympathetic activity (AB-H, 731 ± 26 ng/day; AB-H with fluvoxamine, 561 ± 20 ng/day; P < 0.05, n = 8 and 5, respectively) and improved cardiac function compared with no treatment (%FS: AB-H, 27 ± 2%; AB-H with fluvoxamine 37 ± 3%; P < 0.05, n = 8 and 5, respectively). Chronic oral administration of fluvoxamine decreased immobility time and increased strain amplitude compared with no treatment (immobility time: AB-H, 162 ± 9 s; AB-H with fluvoxamine, 93 ± 10 s; strain amplitude: AB-H, 0.12 ± 0.01; AB-H with fluvoxamine, 0.19 ± 0.02; both P < 0.05, n = 15 and 5, respectively).

3.6 Effects of chronic BD1063 ICV infusion on characteristics of Sham mice

Chronic ICV infusion of BD1063 (BD)-enhanced sympathetic activity (24-h U-NE excretion: Sham, 350 ± 44 ng/day; Sham with BD, 576 ± 25 ng/day; P < 0.05, n = 8 and 5, respectively) and tended to impair cardiac function compared with no treatment (%FS: Sham, 47 ± 2%; Sham with BD, 40 ± 3%; P < 0.05, n = 8 and 5, respectively).

3.7 DHEAS concentration

Serum DHEAS concentrations were lower in AB-H compared with Sham or Sham-H (Sham, 0.039 ± 0.009 μg/mL; Sham-H, 0.040 ± 0.006 μg/mL; AB-H, 0.013 ± 0.008 μg/mL; P < 0.05, n = 5 per group). Brain DHEAS concentrations were also lower in AB-H compared with Sham or Sham-H (Sham, 0.047 ± 0.001 μg/mL; Sham-H, 0.046 ± 0.002 μg/mL; AB-H, 0.033 ± 0.005 μg/mL; P < 0.05, n = 5 per group).

4. Discussion

The present study demonstrates that in AB-H (i) cardiac function decreased with enhanced sympathetic activity; (ii) brain S1R expression decreased; (iii) the index of depression-like behaviour was higher compared with Sham; (iv) the decrease in sympathetic activity and the heart rate in response to acute ICV infusion of PRE084, a selective S1R agonist, was smaller compared with Sham; and (v) chronic ICV infusion of PRE084 increased brain S1R expression, lowered enhanced sympathetic activity, and improved cardiac function and the index of depression-like behaviour. These findings indicate that the reduction in brain S1R expression in AB-H contributed to both the exacerbation of cardiac dysfunction via enhanced sympathetic activity and the worsening of depression.
Figure 2  (A) Representative recordings of strain amplitude in the tail suspension test from Sham (upper) and AB-H (lower). (B and C) Grouped data of immobility time (B) and strain amplitude (C) in Sham and AB-H. *P < 0.05 vs. Sham (Sham and Sham-H, n = 13; AB-H, n = 15).

Figure 3  Representative western blots demonstrating the expression of S1R in the brain. The graph shows the means for the quantification of three separate experiments. Data are expressed as the relative ratio of GAPDH expression. *P < 0.05 vs. Sham and Sham-H. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase.
4.1 Reduction of brain S1R in AB-H mice

We demonstrated that the expression of brain S1R was decreased in AB-H and that this reduction was involved in the enhanced sympathetic activity. In AB-H, LV dimensions and LVWT increased, and %FS decreased with the enhanced sympathetic activity. We have previously confirmed the elevated LV end-diastolic pressure observed in this model. Therefore, AB-H was used as a model for pressure-overload-induced heart failure. The protein levels of brain S1R were significantly decreased in AB-H, and ICV infusion of PRE084 decreased sympathetic activity and the heart rate to a greater extent in Sham than in AB-H. These results indicate that the protein levels of brain S1R and the S1R response to the receptor agonist were decreased in AB-H. The mRNA levels of brain S1R were also examined in the present study and found not to differ between Sham and AB-H, suggesting a higher turnover rate of S1R in AB-H.

4.2 Depressive status in AB-H

We demonstrated that immobility time was increased in AB-H compared with Sham. In addition, a decrease in strain amplitude was confirmed as a marker of struggle activity in AB-H. AB-H had lower cardiac function compared with Sham, and the results of the tail suspension test may reflect exercise intolerance caused by cardiac dysfunction. However, short-term momentum in the home cage measured immediately after the test did not differ between AB-H and Sham (data not shown), suggesting that the results of the tail suspension test reflect the greater depressive status in AB-H, rather than exercise intolerance caused by cardiac dysfunction. Furthermore, the decreased 24-h locomotor activity and disappearance of daily variation of locomotor activity were confirmed in AB-H.

4.3 Effects of brain S1R stimulation

To clarify the importance of the reduction in brain S1R expression in this heart failure model, chronic ICV infusion of PRE084 in AB-H and BD1063 in Sham was performed. Chronic PRE084 ICV infusion increased brain S1R expression in AB-H. Our findings are compatible with those of previous studies, in which expression of the S1R was reported to increase in response to chronic agonist stimulation. In addition, chronic PRE084 ICV infusion attenuated the enhanced sympathetic activity and improved cardiac function in AB-H. In contrast, chronic BD1063 ICV infusion enhanced sympathetic activity and tended to decrease cardiac function in Sham. ICV infusion of PRE084 also improved the index of depression-like behaviour in AB-H.

To our knowledge, no previous studies have reported chronic PRE084 or BD1063 infusion into the cerebrospinal fluid (CSF). PRE084 has an IC50 of 44 nM in the S1R and IC50 > 10 000 nM in a variety of other receptors. BD1063 has nanomolar affinity for S1R and is 30-fold more selective for S1R than the sigma-2 receptor. In the present study, the estimated concentration of both chemicals in the CSF was considered to be in the nanomolar range. Therefore, the doses of both chemicals in the present study were adequate for use as specific S1R ligands. Using these doses, we were able to successfully show that the brain S1R plays an important role in modulating sympathetic activity and depressive status.

To clarify the effects of systemic treatment with S1R agonist, fluvoxamine, a potent agonist of S1R in addition to its function as a selective serotonin reuptake inhibitor, was orally administered. The dose of fluvoxamine was determined according to a previous report. Systemic administration of S1R agonist also attenuated the

Figure 4 (A) Grouped data of MBP and HR response to ICV infusion of PRE084 in Sham and AB-H mice. X-axis (10, 20, 30 min) indicates time after initiating PRE084 ICV infusion. *P < 0.05 vs. AB-H (n = 5 per group). (B) Grouped data of LF/HF, as a marker of sympathetic activity, and HF/total power, as a marker of parasympathetic activity, in response to PRE084 ICV infusion. *P < 0.05 vs. AB-H (n = 5 per group). MBP, mean blood pressure; HR, heart rate.
sympathetic hyperactivation in addition to improving depression-like behaviour.

The mechanisms involved in the modulation of sympathetic activity via the brain S1R remain unclear. The S1R is known to modulate neuronal intracellular calcium levels and N-methyl-D-aspartate-mediated response. Furthermore, S1R was reported to enhance brain plasticity and functional recovery after stroke. These results suggest that the brain S1R affects neuronal activity and that the S1R in the central cardiovascular control region contributes to the regulation of sympathetic activity. Western blotting for brain S1R detection in circumventricular brain tissue including hypothalamus was performed. Wide distribution of the S1R has been reported in the brain, most abundantly in the hippocampus, facial nucleus, thalamus, and hypothalamus. Therefore, the hypothalamus, one of the central cardiovascular control regions, is a promising candidate for the target nucleus involved in modulating sympathetic activity via the S1R. However, the S1R has also been reported in the brainstem, where the other cardiovascular control centre is located. ICV infusion of PRE084 or BD1063 possibly affected the S1R in the brainstem.

In addition, microglia releasing pro-inflammatory mediators such as cytokines and reactive oxygen species also expresses high levels of S1R, and S1R suppresses microglial activation. Such pro-inflammatory mediators in the brain were reported to cause sympathetic hyperactivation in models of heart failure. Therefore, both neuronal and microglial S1R may be involved in the modulation of sympathetic activity.

4.4 Involvement of DHEAS in brain S1R reduction

The mechanisms involved in the reduction in brain S1R expression in this heart failure model also remain unclear. Neurosteroids, such as DHEA and its sulfate conjugate DHEAS, are recognized as endogenous S1R agonists. DHEAS is produced mainly in adrenal tissues, and its serum concentration was reported to decrease in heart failure patients. In the present study confirmed that both serum and brain DHEAS concentrations decreased in AB-H compared with Sham mice. Furthermore, a recent study demonstrated that DHEAS deficiencies predicted the severity of depression in heart failure. Therefore, a decrease in serum DHEAS concentration may have been responsible for the reduction in brain S1R expression observed in this heart failure model.

4.5 Limitations

The present study revealed that brain S1R expression decreased in AB-H, and the reduction in brain S1R may be involved in depression-like behaviour in this model. However, depression is a clinical syndrome that may have multi-pathogenetic causes. Therefore, other mechanisms may contribute to depression-like behaviour in this model. In fact, brain angiotensin type 1 receptor (AT1R) was reported to be a novel therapeutic approach for treatment of mood disorders via anti-inflammatory effects. In previous studies, we confirmed that the brain AT1R increased in AB-H. Further studies are needed to
clarify the contribution of AT1R to depression-like behaviour and the interaction between AT1R and S1R.

A strong association with depression has been well known in both heart failure and hypertension. Therefore, the pressure overload model, a mimic of hypertensive heart disease, was used as the experimental heart disease model in the present study. Other experimental heart failure models, such as that induced by myocardial ischaemia, have also been used widely. Recently, patients with coronary disease who screened as positive on depression screening test had a greater risk for adverse cardiovascular outcomes. Therefore, the experimental heart failure model induced by myocardial ischaemia should also be used to test our hypothesis in the future.

4.6 Conclusion

In conclusion, these findings indicate that brain S1R expression was decreased in AB-H, and that this reduction in brain S1R expression contributed to both the exacerbation of cardiac dysfunction via enhanced sympathetic activity and the worsening of depression.

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References

26. Rohl DH, Kim HW, Yoon ST, Seo HS, Kwon YB, Kim K et al. Intrathecal injection of the r1 receptor antagonist BD-1047 blocks both alcohol mydriasis and increases in spinal N1R expression during the induction phase of rodent neuropathic pain. Anesthesiology 2008;109:879–889.