Mechanisms of Diastolic Dysfunction in Cardiovascular Disease

Baroreflex failure increases the risk of pulmonary edema in conscious rats with normal left ventricular function

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According to the 2014 statistics, approximately 0.8 million persons annually develop heart failure and the financial burden reaches over 3 billion dollars in the United States (13). The increase in heart failure with preserved ejection fraction (HFpEF) has become a global concern. In particular, in contrast to heart failure with reduced ejection fraction (HFrEF), no effective treatments have been established for HFpEF. Treatments such as beta blockers, angiotensin converting enzyme inhibitors, angiotensin type 1 receptor blockers, and/or aldosterone antagonists, which have been shown to work for HFrEF, failed to benefit patients with HFpEF (21, 26, 30, 39). Although epidemiological studies have identified the characteristics of HFpEF (age, women, hypertension, and atrial fibrillation), there remains no clue to effective treatment (2, 28, 29). The complexity of the pathophysiology of HFpEF may hinder the discovery of effective therapeutic options. HFpEF was characterized by left ventricular diastolic dysfunction with high filling pressure. However, recent investigations have indicated that HFpEF results from the complex interplay of multiple impairments in ventricular inotropy, lusitropy and chronotropy, atrial function and vascular function (4, 5, 12, 19, 34, 38). What primarily causes HFpEF remains to be elucidated.

Clinical observations revealed that pulmonary edema could develop in HFpEF within a few hours in the absence of weight gain. The rapidity of drastic changes in hemodynamics strongly suggests that the neural mechanism plays an important role in the pathogenesis of HFpEF (3, 23). Among several physiological reflexes, the arterial baroreflex system is a very powerful and fast acting mechanism in regulating the cardiovascular system. Previous studies reported that the arterial baroreflex regulates arterial pressure by changing vascular properties and cardiac function (6, 32, 35). In addition, we have recently clarified that vascular effects such as stressed blood volume (effective blood volume that generates the mean systemic filling pressure under no-flow conditions) and resistance are by far greater than the cardiac effects (33). Moreover, we demonstrated that baroreflex failure markedly impairs the volume load tolerance and predisposes to pulmonary edema in rats without left ventricular dysfunction (10). HFpEF patients are known to have reduced baroreflex sensitivity and impaired heart rate recovery (4, 31). As HFpEF patients have multiple arterioscle-
rotic risk factors, it is conceivable that stiffened arterial walls in the baroreceptor regions impair baroreflex function. Hence we hypothesized that impaired baroreflex caused by the stiffened arterial wall in HFpEF plays a critical role in the decompensation of heart failure.

The purpose of this study was to investigate whether sino-aortic denervation (SAD) in rats, which is an established model of baroreflex failure, destabilizes left atrial pressure (LAP) and increases the risk of pulmonary edema even in the absence of left ventricular dysfunction.

MATERIALS AND METHODS

Animal preparations and experimental protocols. Experiments and animal care were approved by the Committee on Ethics of Animal Experiment, Kyushu University Graduate School of Medical Sciences, and performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The protocol consists of two parts. The first part investigated the impact of baroreflex failure using SAD rats fed a normal-salt diet (0.5% NaCl, NS). The second part examined the effect of SAD under a condition mimicking dietary salt loading in HFpEF patients, by switching the NS to a high-salt diet (8% NaCl, HS).

Fifteen-week Sprague-Dawley male rats (SAD n = 6, Sham n = 9) were used. In SAD rats, bilateral SAD was performed according to the procedure described by Krieger (22). In sham-operated (Sham) rats, cervical incision and exposure of carotid sinuses were performed without denervation. As shown in Fig. 1, 1 wk after SAD or Sham operation, we implanted a radio transmitter (Data Sciences International) with a pair of catheters; one for arterial pressure (AP) recording via the left femoral artery and the other for LAP recording directly via the left atrial appendage (LAA). Anesthesia was induced by 2.5% isoflurane inhalation and intubation, and maintained at an appropriate level with 1.0% isoflurane using a mechanical ventilator. The telemeter was implanted in a subcutaneous pocket created through a skin incision in the midabdominal region. The catheters were pulled to appropriate positions through subcutaneous tunnels. For AP recording, a skin incision was made in the inguinal region, and the arterial catheter was inserted into the femoral artery and advanced to the abdominal aorta. The catheter was immobilized by a ligature around the femoral artery and catheter. For LAP recording, a left thoracotomy was made at the 4th costal level and the LAA was exposed. The LAA was elevated gently using two small clips (S&T Vascular Clamps, Fine Science Tools) and the mid-portion of LAA was ligated loosely with a 3-0 silk. A minimum cut was made in the LAA for insertion of the catheter tip. The catheter was secured by tightening the ligature around the LAA and catheter. After tying the costal bone and closing the skin, isoflurane inhalation was discontinued and the rats were allowed to recover. After the rats were awakened, they were returned to the original cages and observed carefully.

Two weeks after SAD or Sham operation, we recorded ambulatory AP and LAP simultaneously for 24 h under NS. After recording, we switched the diet from NS to HS. One week after the dietary switch, we recorded AP and LAP again in the same manner as for NS diet. After completing the protocol, the rats were euthanized by isoflurane anesthesia, and the heart and lungs were excised and weighed.

Echocardiographic studies. After each 24-h recording, we performed transthoracic echocardiography (SSA-380A, TOSHIBA) to measure left ventricular wall thickness and left ventricular ejection fraction.

Data analysis. We recorded pressure waveforms at 500 Hz sampling frequency and averaged data using 1-s time bins. This resulted in 86,400 data points per day. We depicted the histograms of AP and LAP at intervals of 1 and 0.1 mmHg, respectively. To compare the histograms of SAD and Sham groups, we averaged 9 histograms of SAD and 6 of Sham. We calculated the average and standard deviation (SD) of AP and LAP and used the SD as the index of lability. To evaluate susceptibility to pulmonary edema, we defined the total duration in which LAP was higher than 18 mmHg as “the pulmonary edema risk duration.” LAP higher than 18 mmHg was considered to be pulmonary edema risk based on the Forrester classification used in clinical practice, in which the presence of edema is defined as a PCWP > 18 mmHg (11). All pressure values, organ weight, and echocardiographic parameters are expressed as means ± SD.

Statistics. We compared hemodynamic parameters and anatomical characteristics among SAD + NS, Sham + NS, SAD + HS, and Sham + HS using analysis of variance with post hoc Steel-Dwass test and Student’s unpaired t-test. JMP software version 11 (SAS Institute, NC) was used in statistical analyses. We defined the statistical difference as P < 0.05.

RESULTS

Simultaneous AP and LAP recording. Representative 24-h recordings of AP are shown in Fig. 2. SAD did not change mean AP but increased the variability, compared with Sham. In contrast, HS increased mean AP in both Sham and SAD.

Similarly, 24-h recordings of LAP are shown in Fig. 3. SAD did not change mean LAP but increased the variability. HS appeared to augment the variability of LAP especially in SAD, as shown by the standard deviation of measurement in each group (Fig. 3).

Histograms of AP and LAP. Figure 4 summarizes the histograms obtained from 9 Sham and 6 SAD rats. In Sham rats, HS increased mean AP without changing the range of distribution (variability) compared with NS. Under NS, SAD had little effect on mean AP but increased the variability of AP compared with Sham. Under HS, SAD also had little effect on mean AP but markedly increased the variability compared with Sham.

In Sham, HS did not increase mean LAP but increased the variability compared with NS. Under NS, SAD had no effect on mean LAP and on the variability compared with Sham. Under HS, SAD remarkably increased mean LAP and also increased the variability compared with Sham.

Impact of SAD and HS on AP and LAP. Shown in Fig. 5 are the impact of SAD and HS on AP and LAP. SAD did not change mean AP compared with Sham, under both NS and HS.
(SAD + NS: 97.2 ± 5.4 mmHg vs. Sham + NS: 97.4 ± 6.4 mmHg, \( P = 0.99 \); SAD + HS: 115.0 ± 8.2 mmHg vs. Sham + HS: 113.8 ± 4.1 mmHg, \( P = 0.99 \)). On the other hand, HS significantly elevated mean AP compared with NS, irrespective of baroreflex function (Sham + HS vs. Sham + NS, \( P = 0.008 \); SAD + HS vs. SAD + NS, \( P = 0.04 \)). In contrast, SAD significantly increased AP lability compared with Sham, under both NS and HS (SAD + NS: 17.9 ± 3.3 mmHg vs. Sham + NS: 7.5 ± 1.0 mmHg, \( P = 0.001 \); SAD + HS: 23.0 ± 3.2 mmHg vs. Sham + HS: 9.5 ± 1.5 mmHg, \( P = 0.001 \)).

SAD did not change mean LAP compared with Sham under NS. The combination of SAD and HS increased LAP slightly \( (P = 0.09) \). In contrast, SAD significantly increased LAP lability compared with Sham, under both NS and HS (SAD + NS: 2.57 ± 0.43 mmHg vs. Sham + NS: 1.73 ± 0.30 mmHg, \( P = 0.01 \); SAD + HS: 4.13 ± 1.18 mmHg vs. Sham + HS: 2.45 ± 0.33 mmHg, \( P = 0.02 \)). In addition, HS also increased LAP lability compared with NS, in both SAD and Sham rats (Sham + HS vs. Sham + NS, \( P = 0.003 \); SAD + HS vs. SAD + NS, \( P = 0.06 \)). As a result, SAD + HS markedly increased LAP lability and strikingly prolonged the pulmonary edema risk duration (SAD + NS: 261 ± 148 s vs. Sham + NS: 17 ± 20 s, \( P = 0.001 \); SAD + HS: 2,831 ± 2,366 s vs. Sham + HS: 148 ± 248 s, \( P = 0.01 \)).

**Organ weight and cardiac function.** SAD slightly but significantly reduced body weight (Table 1). SAD did not affect the whole heart and lung weights normalized by body weight. Left ventricular wall thickness and left ventricular ejection fraction were similar between SAD and Sham, both after NS and HS periods.

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**Organ weight and cardiac function.** SAD slightly but significantly reduced body weight (Table 1). SAD did not affect the whole heart and lung weights normalized by body weight. Left ventricular wall thickness and left ventricular ejection fraction were similar between SAD and Sham, both after NS and HS periods.
In the present study, we showed that SAD destabilized LAP as well as AP and increased the frequency of high LAP in conscious rats with normal left ventricular function. Salt loading further enhanced these impacts. Therefore, salt loading combined with loss of baroreflex increased the risk of pulmonary edema irrespective of left ventricular function.

Impact of SAD and salt loading on LAP. To the best of our knowledge, this is the first experimental observation using 24-h simultaneous monitoring in conscious animals which demonstrates that SAD destabilizes both AP and LAP. SAD increased AP and LAP lability, but did not change mean AP or LAP. Although many studies have reported that SAD increased AP lability (8, 14, 18, 24, 27, 36, 37), the impact of SAD on LAP regulation was not known. However, previous studies reported the impact of the baroreflex on the cardiac preload (6, 32, 35). In addition, a recent quantitative analysis indicated that baroreflex-induced changes in vascular resistance and stressed blood contributed to AP regulation far greater than changes in contractility and heart rate (33). Therefore, it is not surprising that baroreflex failure fails to regulate the stressed blood volume, which in turn predisposes to pulmonary edema. This notion is further supported by the experiment conducted by Funakoshi and colleagues (10), in which we demonstrated that baroreflex failure induced striking volume intolerance and predisposed to pulmonary edema even without left ventricular dysfunction. Taken together, we hypothesize that baroreflex failure destabilizes LAP as well as AP leading to repetitive pulmonary congestion and predisposition to pulmonary edema.

Many studies have shown the impact of HS on AP in baroreflex failure models. DiBona and Sawin (9) reported that arterial baroreceptor denervation, which increased renal sodium retention during HS and elevated AP, impaired the ability to establish sodium balance. Similar sodium dynamism was observed in salt-sensitive rats (7). The result of this study suggests that the interaction between HS and SAD affects natriuresis as reported previously.

Baroreflex failure and pulmonary edema in HFpEF. HS markedly enhanced LAP lability in SAD rats and prolonged the duration in which LAP was higher than 18 mmHg (pulmonary edema risk duration) to 47 min (2,831 s) per day (Fig. 5). The pulmonary edema risk duration was rarely observed in normal rats. Therefore, the whole 47 min of high LAP may be a risk factor of pulmonary edema. Although baroreflex failure-induced volume intolerance alone may not lead to pulmonary edema, the presence of comorbid renal dysfunction and/or excessive sodium intake, and diastolic dysfunction may predispose to pulmonary edema in HFpEF.

It is conceivable that baroreflex failure could also play a role in the pathogenesis of HFrEF, because baroreflex failure-induced volume intolerance could be a risk factor for HFrEF.
**Table 1. Organ weight and cardiac function**

<table>
<thead>
<tr>
<th></th>
<th>Sham + NS (n = 9)</th>
<th>SAD + NS (n = 6)</th>
<th>Sham + HS (n = 9)</th>
<th>SAD + HS (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>444 ± 8</td>
<td>428 ± 9*</td>
<td>457 ± 9</td>
<td>437 ± 10*</td>
</tr>
<tr>
<td>Whole heart weight, g/kg</td>
<td>2.72 ± 0.09</td>
<td>2.62 ± 0.13</td>
<td>2.81 ± 0.11</td>
<td>2.76 ± 0.11</td>
</tr>
<tr>
<td>Lung weight, g/kg</td>
<td>3.13 ± 0.17</td>
<td>3.46 ± 0.32</td>
<td>3.15 ± 0.16</td>
<td>3.32 ± 0.24</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>UCG PW, mm</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>EF, %</td>
<td>72.1 ± 2.4</td>
<td>72.7 ± 1.2</td>
<td>73.9 ± 3.3</td>
<td>75.1 ± 3.7</td>
</tr>
<tr>
<td>Mean heart rate, beats/min</td>
<td>330 ± 15</td>
<td>321 ± 10</td>
<td>321 ± 28</td>
<td>311 ± 15</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Heart weight and lung weight were normalized to body weight. SAD, sinoaortic-denervated rats; Sham, sham-operated rat; NS, normal-salt diet; HS, high-salt diet; UCG, ultrasonic cardiogram; IVS, interventricular septum thickness; PW, posterior wall thickness; EF, ejection fraction. *P < 0.05, SAD vs. Sham.

**Limitations.** First, SAD is an extremely unusual condition in humans. However, patients with HfPEF have multiple cardiovascular risk factors including hypertension, dyslipidemia, and diabetes. These risk factors are known to accelerate arteriosclerosis and stiffen arterial walls including the aortic wall and carotid artery wall. Since the baroreceptors are embedded in the arterial wall and sense blood pressure-induced changes in the arterial wall strain, stiffening of the arterial wall inevitably deteriorates arterial baroreflex function (1). In this regard, SAD mimics the essence of baroreflex failure induced by physiological arteriosclerosis.

Second, it is not clear whether patients with HfPEF have baroreflex failure, and if they have, how much the baroreflex impairment contributes to the pathophysiology in HfPEF. Borlaug and associates (4) reported lowered baroreflex sensitivity in patients with HfPEF. Therefore, it is fair to say that baroreflex failure appears to be a common feature in HfPEF and contributes to worsening pulmonary congestion. However, as we found no evidence of pulmonary edema in this study, we have to validate that SAD causes pulmonary edema in a more suitable HfPEF model.

Third, we did not investigate the impact of low pressure baroreflex on HfPEF. Besides arterial baroreflex, the low pressure control system plays a pivotal role in the regulation of heart rate, vascular resistance, and diuresis (15). Especially, Henry et al. (16) found that left atrial pressure was associated with diuresis via neural reflex. We need to develop other experimental protocols to investigate the role of the low pressure control system in the pathogenesis of HfPEF.

Furthermore, several issues remain unsolved in this study. Periodic left ventricular dysfunction and diastolic dysfunction could affect LAP. Moreover, venous tone as well as arterial resistance theoretically could fluctuate LAP because locomotion and emotion change the resistance and the stressed blood volume. In SAD, the abolished buffering function of AP may coexist with the fluctuation of LAP.
**Clinical perspectives.** In previous studies, several mechanisms of baroreflex failure in heart failure have been reported, such as reduced arterial compliance, impaired central reflex integration, and decrease in end-organ responsiveness (20, 25). Moreover, Borlaug et al. (4) have reported impaired chronotropic and vasodilator reservoir in HFrEF patients. In these patients, an alternative electrophysiological option would be to artificially restore the baroreflex function. We reported that incorporating an artificial bionic baroreflex system in rats with baroreflex failure restored volume tolerance to a similar extent as the native baroreflex in acute experiments (10, 17). Therefore, the potential of artificial bionic baroreflex system as a novel therapeutic tool for heart failure should be considered in the near future.

**Conclusions.** This study demonstrated that baroreflex failure destabilized LAP in addition to AP in freely moving conscious rats with normal heart function. Furthermore, salt loading markedly enhanced the fluctuation of LAP induced by baroreflex failure and increased the risk of pulmonary edema. We conclude that baroreflex failure may contribute in part to the pathogenesis of HFrEF and prevention of baroreflex failure or restoration of baroreflex function could be a novel therapeutic target in HFrEF.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: K. Sakamoto conception and design of research; K. Sakamoto and K. Saku performed experiments; K. Sakamoto, K.H., and K. Saku analyzed data; K. Sakamoto, T.T., and Y.O. interpreted results of Sakamoto and K. Saku performed experiments; K. Sakamoto, K.H., and K. Saku performed experiments; K. Sakamoto, T.T., and Y.O. interpreted results of experiments; K. Sakamoto prepared figures; K. Sakamoto drafted manuscript; K.H., K. Saku, T.S., T.T., Y.O., T.K., T.I., and K. Sunagawa edited and revised manuscript; K. Sunagawa approved final version of manuscript.

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