Calcium (Ca^{2+}) is known to be a key messenger in cardiac excitation-contraction (E-C) coupling. Alterations in E-C coupling reportedly play a crucial role in the pathogenesis of heart failure and lethal arrhythmias, such as catecholaminergic polymorphic ventricular tachycardia (CPVT).[1,2] The common pathophysiologic phenomenon occurring in the 2 abovementioned heart diseases is termed as a "diastolic Ca^{2+} leak," which involves an aberrant release of Ca^{2+} during diastole via the cardiac ryanodine receptor (RyR2).[3]

In heart failure, diastolic Ca^{2+} leak through RyR2 results in a decrease in the sarcoplasmic reticulum (SR) Ca^{2+} content, along with a decrease in Ca^{2+} uptake by SR Ca^{2+}-ATPase 2a (SERCA2a).[1] However, in cases of CPVT, wherein >150 mutation sites in RyR2 have been identified, a Ca^{2+} leak occurs on beta-adrenergic stimulation without a decrease in SR Ca^{2+} content, because SERCA2a activity is normally restored in such cases. In cases of either heart failure or CPVT, the Ca^{2+} leak commonly provides a substrate for delayed after-depolarization, which leads to life-threatening arrhythmia.[2,3] Four hypotheses have been proposed to explain the mechanism underlying the Ca^{2+} leak observed in cases of heart failure and CPVT. The first mechanism involves posttranslational modifications such as oxidation, S-nitrosylation, and phosphorylation of RyR2.[1-4] RyR2 function is modulated by protein kinase A (PKA)-mediated phosphorylation and the subsequent dissociation of FKBP12.6 from RyR2.[4] The depletion of FKBP12.6, caused by the hyperphosphorylation of RyR2 by PKA upon beta-adrenergic stimulation, destabilizes RyR2; however, this hypothesis remains highly controversial.[5] The second mechanism involves direct activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) by excessive catecholamine.[5] Phosphorylation of RyR2 by CaMKII is another candidate for modulation of RyR2 function,

which can subsequently cause Ca^{2+} leak.[5] The third mechanism involves defective inter-domain interaction between the N-terminal (amino acids 1–600) and central domain (amino acids 2000–2500) of RyR2.[3] In a normal state, the N-terminal and central domains interact with each other to act as a regulatory switch for channel-gating activity, with a tight zipping of the interacting domains serving to stabilize RyR2. The weakness of the inter-domain interaction results in an increasing tendency towards unzipping, leading to leakiness of RyR2 in the diseased state. In the canine pacing-induced heart failure model, both K201, which binds to the central domain of RyR2,[6] and dantrolene, which binds to the N-terminal domain,[7] prevented the progression of heart failure. The fourth mechanism involves a store overload-induced Ca^{2+} release.[8] The threshold of SR Ca^{2+} load for channel activation, regulated by luminal [Ca^{2+}], but not by cytosolic [Ca^{2+}] in the SR, is markedly reduced in the mutated RyR2.

Several knock-in (KI) mouse models with CPVT-linked mutations of RyR2—such as R4496C[9] and R2474S[10]—have been established, and these KI mouse models have all clearly indicated that only a single point mutation in RyR2 causes lethal arrhythmia, although the severity (frequency of ventricular tachycardia [VT] upon beta-adrenergic stimulation) differs according to the mutation site in RyR2. Studies of such KI mouse models provided several important points on the pathological mechanisms of CPVT, such as: 1) bidirectional VT originates through alternative spontaneous activation of either the right or left ventricular Purkinje fibers[11]; and 2) overactivation of CaMKII appears to be an essential factor for promoting progression towards heart failure and ventricular arrhythmia.[12]

In this issue of the *Journal of American College of Cardiology*, Sedej et al.[13] stated an important finding using a R4496C KI mouse model wherein a Ca^{2+} leak—mediated through

the mutated RyR2—promoted pressure overload-induced myocardial remodeling and inhibition of Ca^{2+} leak by the RyR2 stabilizer K201, thus preventing the progression towards cardiac hypertrophy and heart failure. The most important finding of that study is that even a small Ca^{2+} leak through RyR2 is directly linked to the pathogenesis of cardiac hypertrophy and failure, and the specific inhibition of the Ca^{2+} leak reversed the abnormal pathogenic process towards heart failure and resulted in improved prognosis.

The finding that another RyR2 stabilizer, dantrolene, which has been shown to fix the defective inter-domain interaction,[7] reduces the frequency of Ca^{2+} sparks in KI cardiomyocytes strongly supports the idea that RyR2 dysfunction in KI mice with thoracic aortic constriction (TAC) may be largely influenced by defective inter-domain interaction between the N-terminal and central domain of RyR2. Thus, the Ca^{2+} leak caused by the defective inter-domain interaction of RyR2 may have a direct effect on the progression of cardiac hypertrophy and heart failure.

However, several concerns exist that remain to be clarified. In a R4496C KI mouse model with TAC, the phosphorylation level was not altered at either Ser2808 or Ser2814 in RyR2. Moreover, in cardiomyocytes from the hearts of a R4496C KI mouse model with TAC, the CaMKII inhibitor KN93 had no significant effect on Ca^{2+} sparks in cardiomyocytes, suggesting that CaMKII has only a minor role in the pathogenesis of abnormal Ca^{2+} handling and cardiac hypertrophy. These findings appear to be inconsistent with those of previous studies, which showed that 1) phosphorylation of RyR2 by CaMKII—but not by PKA—plays a crucial role in the Ca^{2+} leak and the development of heart failure in a mouse model of TAC[14] and 2) overexpression of CaMKIIdeltac in R4496C KI increased the PKA- and CaMKII-mediated phosphorylation of RyR2 with progressive heart failure and ventricular

arrhythmia.[12]

The reasons for such discordance remain unclear. The mechanistic relationship between PKA- and CaMKII-mediated phosphorylation of RyR2, diastolic Ca^{2+} leak, and development of cardiac hypertrophy and heart failure remains to be clarified.

With regard to the factor affecting channel gating of RyR2 in diseased hearts, a nitroso-redox imbalance has been proposed to cause RyR2 oxidation, hyponitrosylation, and SR Ca^{2+} leak.[15] Moreover, recent reports have indicated that that nitric oxide is produced upon beta-adrenergic stimulation, and subsequently, CaMKII is activated, resulting in a surge of diastolic Ca^{2+} sparks and increased wave propensity.[16]

However, the precise mechanism for Ca^{2+} leak and its pathogenic role in post-translational signal transduction towards cardiac hypertrophy remains uncertain. Although further investigation is required to resolve many questions on Ca^{2+} leak, stabilization of RyR2 appears to provide a new gateway for the treatment of cardiac hypertrophy and heart failure.

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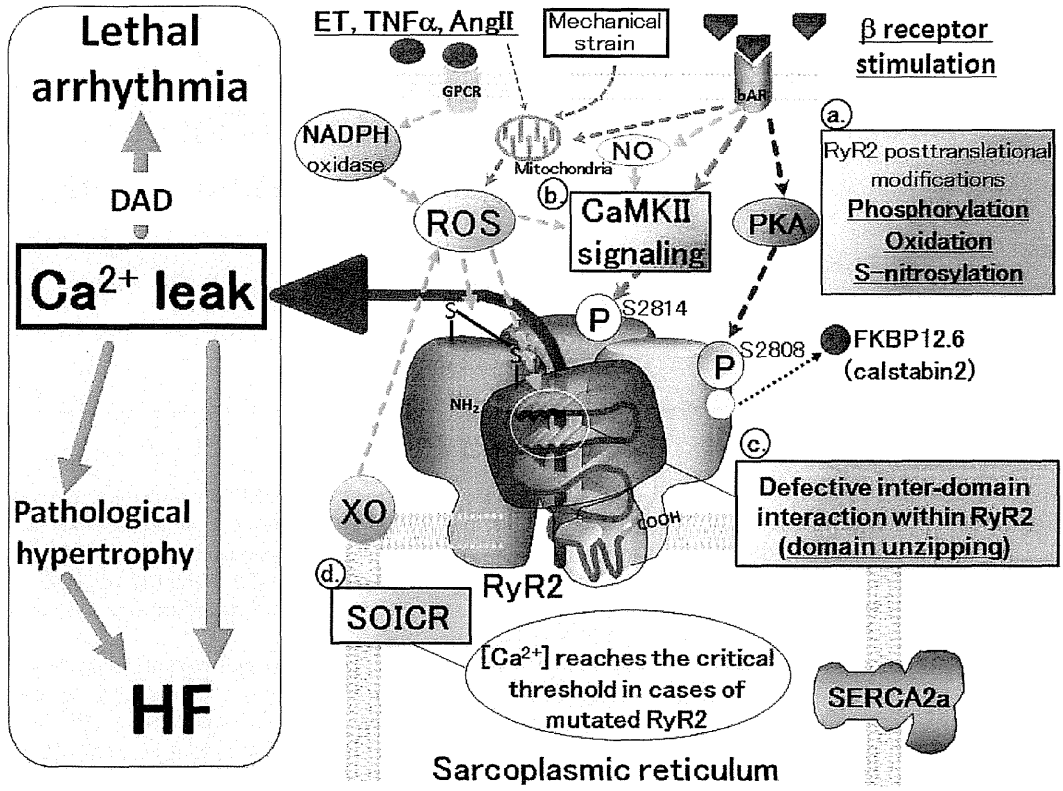
Figure: Schematic diagram showing how a diastolic Ca^{2+} leak predisposes to heart failure and lethal arrhythmia. Four hypotheses have been proposed to explain a Ca^{2+} leak: a) posttranslational modifications such as oxidation, S-nitrosylation, and phosphorylation of RyR2; b) direct activation of CaMKII by excessive catecholamine via NO production, c) defective inter-domain interaction within RyR2, and d) store overload-induced Ca^{2+} release. The Ca^{2+} leak may subsequently lead to lethal arrhythmia, with pathological hypertrophy in some cases, and heart failure.

RyR2: cardiac ryanodine receptor 2, SERCA2a: SR Ca^{2+}-ATPase 2a, PKA: protein kinase A, CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II, ROS: reactive oxygen species, FKBP12.6: FK506 binding protein 12.6, NO: nitric oxide, XO: xanthine oxidase, SOICR: store overload-induced Ca^{2+} release, DAD: delayed after-depolarization, P: phosphorylation, $[Ca^{2+}]$: free calcium concentration, bAR: beta-adrenergic receptor, GPCR: G protein coupled receptor, HF: heart failure, ET: endothelin, TNFα: tumor necrosis factor α, AngII: angiotensin II, NADPH oxidase: nicotinamide adenine dinucleotide phosphate-oxidase


Lethal arrhythmia
DAD
Ca2+ leak
Pathological hypertrophy
HF
ET, TNFα, AngII
Mechanical strain
β receptor stimulation
GPCR
βAR
NADPH oxidase
Mitochondria
NO
ROS
b.
CaMKII signaling
PKA
a.
RyR2 posttranslational modifications
Phosphorylation
Oxidation
S-nitrosylation
P
S2814
P
S2808
FKBP12.6
(calstabin2)
NH2
c.
Defective inter-domain interaction within RyR2 (domain unzipping)
XO
COOH
RyR2
d.
SOICR
[Ca2+] reaches the critical threshold in cases of mutated RyR2
SERCA2a
Sarcoplasmic reticulum

Basic Science and Experimental Studies

Striking Volume Intolerance Is Induced by Mimicking Arterial Baroreflex Failure in Normal Left Ventricular Function

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ABSTRACT

Background: Patients with heart failure and preserved ejection fraction (HFpEF) are supersensitive to volume overload, and a striking increase in left atrial pressure (LAP) often occurs transiently and is rapidly resolved by intravascular volume reduction. The arterial baroreflex is a powerful regulator of intravascular stressed blood volume. We examined whether arterial baroreflex failure (FAIL) mimicked by constant carotid sinus pressure (CSP) causes a striking increase in LAP and systemic arterial pressure (AP) by volume loading in rats with normal left ventricular (LV) function.

Methods and Results: In anesthetized Sprague-Dawley rats, we isolated bilateral carotid sinuses and controlled CSP by a servo-controlled piston pump. We mimicked the normal arterial baroreflex by matching CSP to instantaneous AP and FAIL by maintaining CSP at a constant value regardless of AP. We infused dextran stepwise (infused volume [Vi]) until LAP reached 15 mm Hg and obtained the LAP-Vi relationship. We estimated the critical Vi as the Vi at which LAP reached 20 mm Hg. In FAIL, critical Vi decreased markedly from 19.4 ± 1.6 mL/kg to 15.6 ± 1.6 mL/kg ($P < .01$), whereas AP at the critical Vi increased (194 ± 6 mm Hg vs 163 ± 6 mm Hg; $P < .01$). We demonstrated that an artificial arterial baroreflex system we recently developed could fully restore the physiologic volume intolerance in the absence of native arterial baroreflex.

Conclusions: Arterial baroreflex failure induces striking volume intolerance in the absence of LV dysfunction and may play an important role in the pathogenesis of acute heart failure, especially in states of HFpEF. (*J Cardiac Fail 2014;20:53–59*)

Key Words: Heart failure, arterial baroreflex, volume intolerance.

A subgroup of patients with acute decompensated heart failure often experience sudden onset of pulmonary edema with a striking increase in left atrial pressure (LAP). Epidemiologic studies revealed that approximately one-half of these patients have preserved left ventricular (LV) ejection fraction (LVEF), a condition known as heart failure with preserved LVEF (HFpEF).[1–4] These patients have marked hypertension at admission[1–4] and rapid onset of symptoms,[5] though they have few symptoms in the chronic phase.[6] Moreover, the striking increase in LAP may occur only transiently and is rapidly resolved with mild volume reduction by diuresis.[7] In other words, patients with acute decompensated heart failure are supersensitive to volume overload and have volume intolerance regardless of LVEF.

Many studies have shown that patients with HFpEF have structural and functional changes consistent with concentric remodeling and dominant abnormalities in LV diastolic function, whereas patients with reduced LVEF have eccentric remodeling and LV systolic dysfunction.[8–15] The most common causes of HFpEF include chronic hypertension and age-associated cardiovascular changes.[7] However, therapies with unequivocal benefits for heart failure with

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reduced LVEF (HFrEF) or hypertension have not shown consistent benefits for HFpEF.[16] At present, the treatment for acute decompensated heart failure is largely empirical, focusing on blood pressure control and treatment or avoidance of intravascular volume overload.[7] There is a need to focus on the precise mechanisms of volume intolerance in acute decompensated heart failure.

The stressed blood volume and systemic blood pressure are controlled by several systems. Among them, the arterial baroreflex system is an important and powerful regulator.[17–19] Arterial baroreceptors are stretch receptors located within the arterial wall of elastic vessels such as the aortic arch and carotid sinuses. The arterial baroreflex is impaired in patients with both HFrEF and HFpEF.[20] LV diastolic dysfunction is induced by arterial baroreflex failure (FAIL).[21] Many studies have revealed that major risk factors of HFpEF, such as aging, hypertension, diabetes, renal insufficiency, and atherosclerosis, are closely associated with FAIL.[5,22–26]

Therefore, we hypothesized that FAIL plays a pivotal role in the pathogenesis of volume intolerance in acute decompensated heart failure regardless of LVEF. To test this hypothesis, we developed a FAIL model in rats with normal LV function and assessed the LAP and systemic arterial pressure (AP) responses to volume overload. Furthermore, to study the reversibility of impaired volume tolerance in the FAIL model, we tested a novel artificial (bionic) arterial baroreflex system that we reported recently.[27]

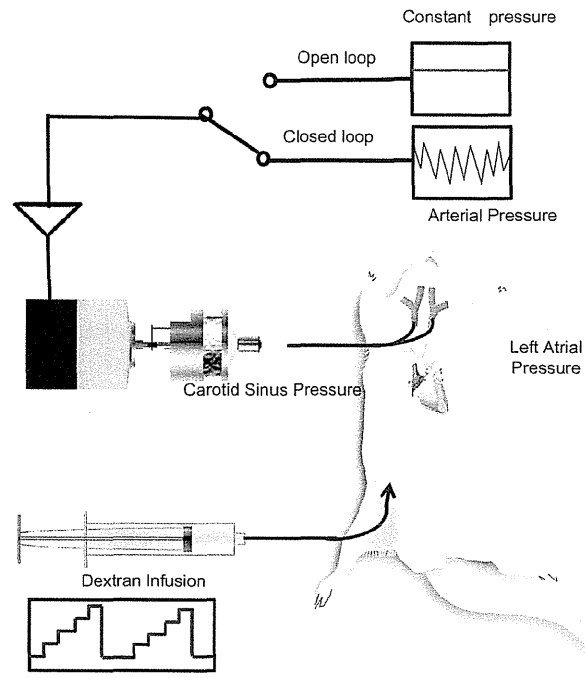

Fig. 1. Schematic representation of the preparation in the present study. Responses in arterial pressure and left atrial pressure after stepwise volume infusion were compared under native open-loop arterial baroreflex conditions (switching upward) and closed-loop arterial baroreflex conditions (switching downward).

Methods

Animals

All procedures and animal care were approved by the Committee on Ethics of Animal Experiment, Kyushu University Graduate School of Medical and Pharmaceutical Sciences, and were performed in accordance with the Guideline for Animal Experiment of Kyushu University. Five 14- to 16-week-old male Sprague-Dawley rats (SLC, Hamamatsu, Japan) weighing 450–650 g were used.

Surgical Preparations

Figure 1 illustrates the experimental preparation. Rats were anesthetized by intraperitoneal injection (2 mL/kg) of a mixture of urethane (250 mg/mL) and α-chloralose (40 mg/mL). Anesthesia was maintained by continuous intravenous infusion of the anesthetics at a rate of 0.1 mL/kg/h with the use of a syringe pump (CFV-3200; Nihon Kohden, Tokyo, Japan). Lactated Ringer solution also was infused continuously (6 mL/kg/h) to prevent dehydration. The rats were intubated and ventilated artificially with room air (SN-480-7; Shinano, Tokyo, Japan). We vascularly isolated bilateral carotid sinuses from the systemic circulation as reported previously.[17–19] Carotid sinuses were isolated and filled with lactated Ringer solution through catheters inserted via the common carotid arteries. We measured the carotid sinus pressure (CSP) with the use of a fluid-filled pressure transducer (AP-630G; Nihon Kohden) and controlled CSP with the use of a servo-controlled piston pump (ET-126A; Labworks, Costa Mesa, California). Bilateral vagal nerves were sectioned at the middle of the neck to eliminate reflexes from the cardiopulmonary region and to prevent efferent conduction. Bilateral aortic depressor nerves were sectioned to eliminate the reflexes from the aortic arch. Using this preparation, we mimicked the normal arterial baroreflex (NORM) by matching CSP to instantaneous AP (closed loop) and FAIL by maintaining CSP at a constant value regardless of AP perturbations (open loop).

Protocols

We infused dextran stepwise every minute in a volume of 1–4 mL/kg until LAP reached 14–16 mm Hg and measured LAP and AP in response to the infused volume (Vi). The measurements were repeated in alternating interleaved fashion in NORM and FAIL. Each volume challenge was followed by blood withdrawal until CSP, LAP, and systemic AP returned to the basal level. The sequence of the volume challenge was randomized to minimize order bias.

Bionic Arterial Baroreflex System

To implement arterial baroreflex transduction as a native arterial baroreceptor (bionic arterial baroreflex system), we stimulated afferent nerves (the aortic depressor nerve) in a previously reported fashion.[27] In brief, the bionic arterial baroreflex system consists of an arterial pressure sensor (DX-360, MEG-5200; Nihon Kohden), a neurostimulator (SEN-3401, Nihon Kohden; AD202JN, Analog Devices, Norwood, Massachusetts), and a regulator (Studio 1410; Dell, Round Rock, Texas). The bionic pressure sensor senses AP, and the regulator translates AP into neurostimulation (stimulation of the aortic depressor nerve), thus mimicking the native arterial baroreceptor. Therefore, the bionic baroreflex system is expected to substitute impaired baroreceptors.

The neurostimulator generates an electrical rectangular pulse train (5 V intensity, 0.2 ms pulse width) according to the command signal from the regulator. The regulator updates the frequency of the pulse train every 10 ms. The encoding rule for translating intracarotid sinus pressure into stimulation of the aortic depressor nerve was identified with the use of a white noise technique and applied to the regulator.

Data Acquisition and Analysis

We recorded the pressures and digitalized the recordings at a sampling rate of 200 Hz with the use of a 12-bit analog-to-digital converter. The 10-s data of LAP and AP starting from 50 s after dextran infusion were used in the analysis.

Statistical Analysis

The data are presented as least-squares mean ± standard error. Multiple data sets of NORM (repeated twice) and FAIL (repeated twice) were compared using a linear mixed-effects model. Rat identity was specified as a random effect. Assumptions of normality and homoscedasticity were fulfilled. The linear mixed-effects models were carried out using JMP version 9.0.2 (SAS Institute, Cary, CA, USA). The estimated values from the mixed-effect model are presented as least squares mean ± standard error. Differences were considered to be significant when the P value was $<.05$. Curve fitting in nonlinear regression was performed with the use of Graphpad Prism version 5.04 (Graphpad, San Diego, California).

Results

Baseline Characteristics

The heart rate, mean AP (mAP), and LAP before volume load sequences are presented in Table 1. Baseline hemodynamic parameters did not differ significantly between NORM and FAIL.

Representative Recording

Figure 2 shows representative recordings of AP, CSP, and LAP in response to stepwise dextran infusion. In NORM, matching AP and CSP indicated normal activation of the arterial baroreflex. In contrast, in FAIL, CSP was unaltered despite AP perturbation, indicating no arterial baroreflex activation. In NORM, volume loading by dextran infusion reproducibly increased both LAP and AP, but the changes were relatively small. In FAIL, however, a much smaller volume load increased LAP and AP markedly, indicating striking volume intolerance. Before switching from FAIL to NORM or NORM to FAIL, we terminated volume infusion and determined the recovery of CSP, AP, and LAP to each basal level. Duration of the entire experiment from anesthesia induction to killing the animal under artificial ventilation was ~4 hours.

Table 1. Baseline Hemodynamic Parameters

	NORM	FAIL	P
HR (bpm)	433.4 ± 10.8	433.7 ± 10.9	NS
mAP (mm Hg)	90.5 ± 5.4	85.2 ± 5.3	NS
LAP (mm Hg)	6.6 ± 0.5	6.4 ± 0.5	NS

bpm, beats per minute; FAIL, arterial baroreflex failure; HR, heart rate; LAP, left atrial pressure; mAP, mean arterial pressure; NORM, normal arterial baroreflex; NS, not significant. Values are least-squares means ± standard error.

LAP-Vi Relationship

The volume load intolerance in FAIL is clearly demonstrated in the plot of the LAP-Vi relationship (Fig. 3A). The LAP-Vi relationship was markedly steeper in FAIL than in NORM, indicating that FAIL induces significant volume intolerance.

To quantify the LAP sensitivity to volume load, we fitted a monoexponential curve [LAP = Exp (AVi) + B, where A denotes the coefficient of the curve and B is a constant] to the LAP-Vi relationship (Fig. 3B). The coefficient of the monoexponential curve (A), an index of LAP sensitivity to Vi, was significantly higher in FAIL than in NORM (0.27 ± 0.03 vs 0.16 ± 0.03; n = 10; $P < .01$).

Critical Volume to Provoke Pulmonary Edema

To assess the extent of volume load intolerance that provokes pulmonary edema, we estimated critical Vi as the Vi at which LAP reaches 20 mm Hg.[28] As shown in Figure 4, critical Vi was significantly smaller in FAIL than in NORM (15.0 ± 1.6 mL/kg vs 19.4 ± 1.6 mL/kg; n = 10; $P < .01$), indicating that FAIL induces volume load intolerance The least-squares mean of differences between NORM and FAIL was 4.5 ± 1.2 mL/kg, which represents one-fifth of the stressed volume.

LAP in response to the same volume load was compared between NORM and FAIL. At the critical Vi for FAIL (19.4 mL/kg), LAP was by definition 20 mm Hg in FAIL but was significantly and markedly lower in NORM (8.6 ± 2.3 mm Hg; $P < .01$).

AP-Vi Relationship

Figure 5A shows the mAP-Vi relationship. For a given Vi, mAP was consistently higher in FAIL than in NORM. We fitted a logarithmic curve [mAP = A × ln (Vi − B), where A denotes the coefficient of the curve and B is a constant] to the mAP-Vi relationship . The coefficient of the logarithmic curve (A) was significantly higher in FAIL than in NORM (69.0 ± 3.3 vs 49.1 ± 3.3; n = 10; $P < .01$), indicating that AP is supersensitive to volume overload under FAIL conditions.

We examined the systolic AP at which LAP reached 20 mm Hg (ie, at the critical Vi). Systolic AP at the critical Vi was significantly higher in FAIL than in NORM (194 ± 6 mm Hg vs 163 ± 6 mm Hg; n = 10; $P < .01$; Fig. 5B). The least-squares mean of differences in systolic AP between NORM and FAIL was 30 ± 6 mm Hg.

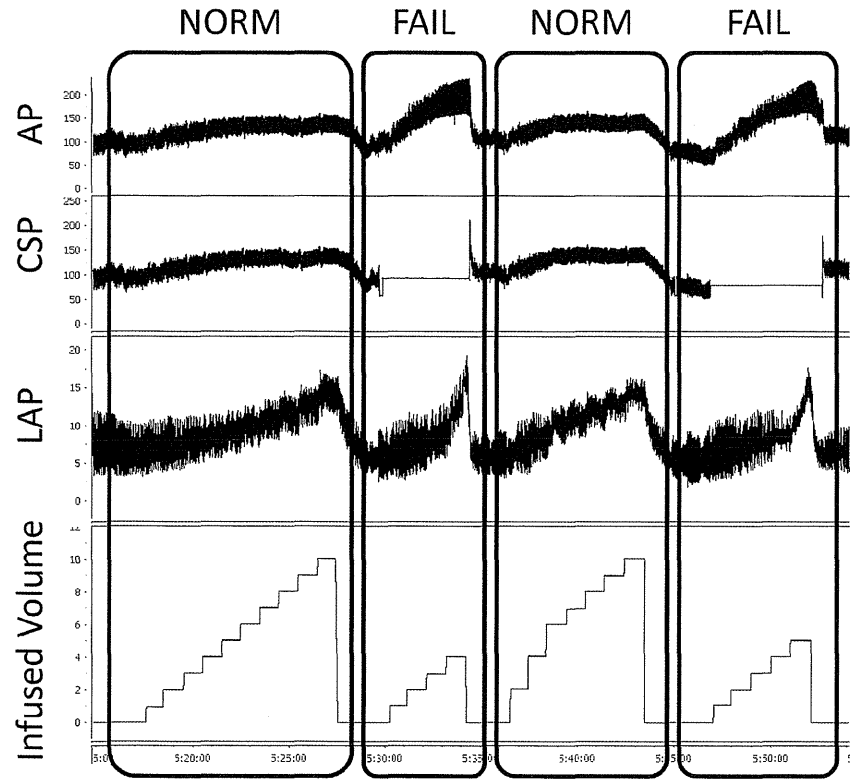

Fig. 2. Typical example of recordings obtained during volume loading by dextran infusion. Arterial blood pressure (AP), carotid sinus pressure (CSP), left atrial pressure (LAP), and volume of dextran infused (Infused Volume) are shown. FAIL, arterial baroreflex failure; NORM, normal arterial baroreflex.

Benefit of Bionic Arterial Baroreflex System on Volume Intolerance

Typical recordings are shown in Figure 6A. Our bionic arterial baroreflex system was able to fully reverse the physiologic volume intolerance in the FAIL animals (Fig. 6B).

Discussion

We demonstrated that the estimated critical volume load at which LAP reaches 20 mm Hg was significantly smaller and systemic AP at the critical volume load was significantly higher under FAIL conditions mimicked by constant CSP than under NORM conditions. These findings indicate that FAIL induces volume intolerance with an increase in systemic AP with normal LV function. FAIL might be involved in the pathogenesis of acute decompensated heart failure with a striking increase in LAP regardless of LVEF.

The novel finding is that volume intolerance was prominent in FAIL with normal LV function. Because all animals in the present study had normal LV function, we can consider that LV dysfunction was not a prerequisite for

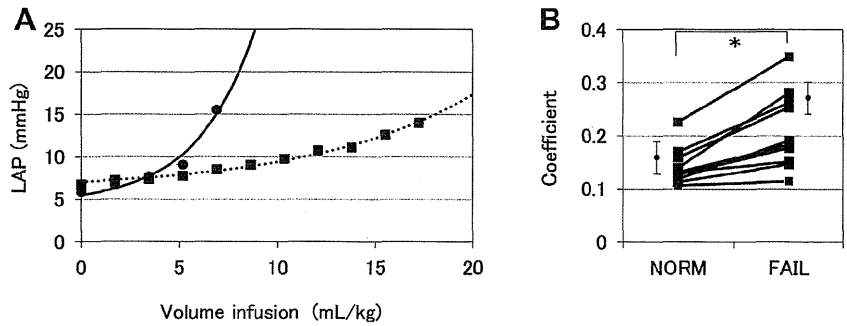

Fig. 3. (A) Typical example of the response of left atrial pressure (LAP) to infused volume (Vi) of dextran. We fitted a monoexponential curve [LAP = Exp (AVi) + B] to the LAP-Vi relationship. The curves were drawn from the least-squares means of the fitting parameters under normal arterial baroreflex (NORM; dotted line) and arterial baroreflex failure (FAIL; solid line) conditions. (B) Summarized data of the LAP-Vi relationship. Coefficient of the monoexponential curve was significantly higher in FAIL than in NORM (0.27 ± 0.03 vs 0.16 ± 0.03; n = 10; $P < .01$). *Significantly different means (Tukey post hoc comparison).

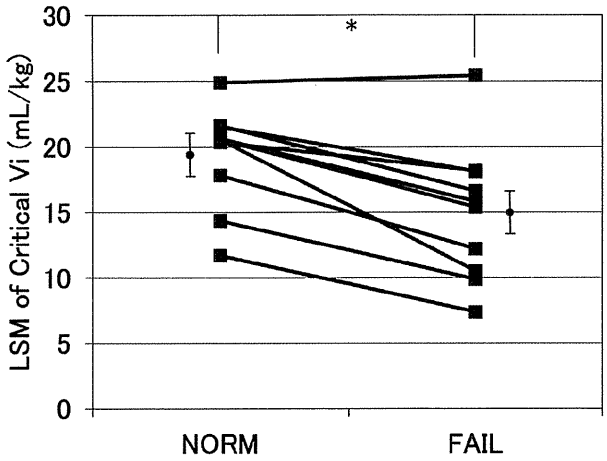

Fig. 4. Effect of baroreflex failure on critical infused volume (Vi) at which left atrial pressure reaches 20 mm Hg. The points of the least-squares means (LSMs) of each subject are connected. *Significantly different means (Tukey post hoc comparison; $P < .05$). The number of repeated measures was 10 for both normal arterial baroreflex (NORM) and arterial baroreflex failure (FAIL).

volume intolerance. This model could explain the unique feature of acute decompensated heart failure. Although the pathophysiology of HFpEF was initially thought to be due principally to LV diastolic dysfunction, recent studies have suggested more complex involvement of multiple abnormalities.[29,30] Risk factors are closely similar between in HFpEF and FAIL.[5,7,25,26] Considering these earlier findings, our present results may suggest that FAIL could be one of the cardinal causes of volume intolerance in acute decompensated heart failure regardless of LV function.

The cardiopulmonary baroreflex is also quite important in the pathogenesis of heart failure via autonomic regulation.[20] However, our present results indicate the effect of the arterial baroreflex on volume tolerance, because we did vagal sectioning that would lead to the exclusion of the cardiopulmonary baroreflex. Moreover, the effect of the arterial baroreflex on volume tolerance should be quite important clinically, because sympathetic nerve activity and gain of the arterial baroreflex is significantly smaller in the anesthetized condition than in the conscious state.[31] In addition, our results could support the concept that redistribution rather than net accumulation of fluid might contribute importantly to acute decompensated heart failure.

Risk factors of HFpEF are quite similar to those of FAIL.[5,25,26] Atherosclerosis stiffens the arterial wall in which the arterial baroreflex receptors are located.[22–24] Chronic hypertension and cardiovascular changes associated with aging are the most common causes of HFpEF.[7] Pulse wave velocity is increased in HFpEF to a greater extent than in HFrEF.[32] These clinical findings also support the notion that FAIL is one of the main factors in HFpEF pathogenesis regardless of LV dysfunction. However, it has not been clarified whether FAIL induces the transient and repeated increase in LAP in acute decompensated heart failure. The present study provides a novel concept that FAIL induces, in part, striking volume intolerance in the absence of LV dysfunction. Furthermore, to prevent flash pulmonary edema in acute decompensated heart failure, we should focus on not only LV dysfunction but also FAIL, in addition to attenuation of atherosclerotic risk factors.

Approximately one-half of the patients with heart failure have HFpEF, and the morbidity and mortality are similar to HFrEF. Therapies with unequivocal benefit for HFrEF, such as angiotensin-converting enzyme inhibitors, β-blockers, diuretics, and digitalis, have not shown consistent benefits for HFpEF.[16] Because the major risk factors of HFpEF are known to promote atherosclerosis,[5,25,26] statins given for the treatment of atherosclerosis could be effective for HFpEF. In support of this observation, a clinical study with a mean follow-up of 21 months indicated that statin therapy was associated with significantly lower mortality in patients with diastolic heart failure.[33] However, the GISSI-HF trial that evaluated the effectiveness of rosuvastatin on death or cardiovascular hospitalization in patients with chronic heart failure found no benefit in the subgroup of patients with HFpEF.[34] Direct carotid sinus stimulation has been proposed as a nonpharmacologic therapy, because this therapy might improve symptoms in patients with heart failure through reducing heart rate, improving baroreflex

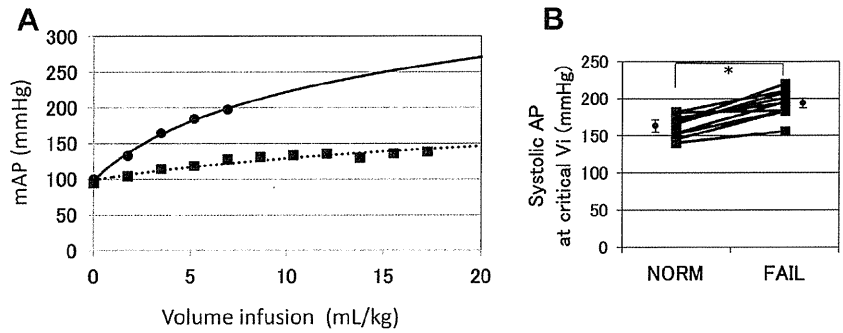

Fig. 5. (A) Response of mean arterial pressure (mAP) to infused volume (Vi) of dextran. We fitted a logarithmic curve [mAP = A × ln (Vi − B)] to the mAP-Vi relationship. The curves were drawn from the least-squares means of the fitting parameters under normal arterial baroreflex (NORM; dotted line) and arterial baroreflex failure (FAIL; solid line) conditions. (B) Systolic arterial pressure (AP) at critical Vi for FAIL under NORM and FAIL conditions. *Significantly different means (Turkey post hoc comparison).

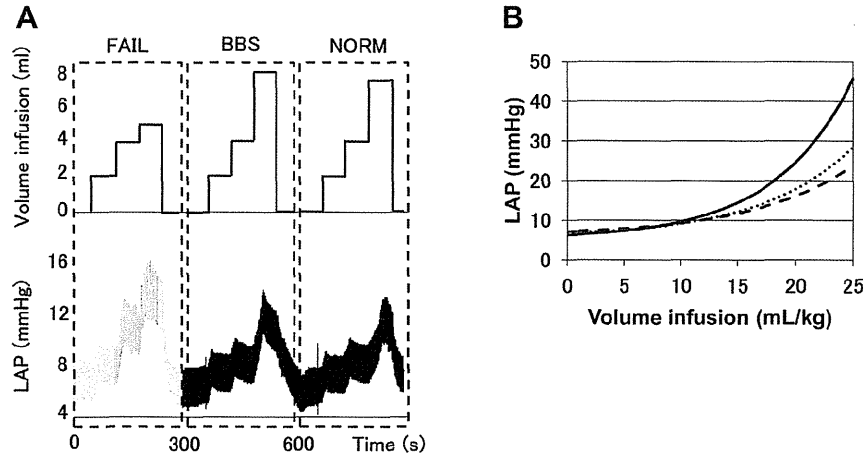

Fig. 6. (A) Typical recordings of changes in left atrial pressure (LAP) in response to infused volume (Vi) of dextran under arterial baroreflex failure (FAIL), bionic arterial baroreflex system (BBS), and normal arterial baroreflex (NORM) conditions. (B) Summarized data of LAP-Vi relationship in FAIL (solid line), BBS (dashed line), and NORM (dotted line).

sensitivity, altering nitric oxide bioavailability, antiarrhythmic effects, or central modulation.[35] However, the effects of parasympathetic activation on HFpEF have not been fully clarified. Given these results, we should recognize that the proven therapies for HFpEF are not consistent and that the treatment for acute decompensating heart failure is largely empirical, focusing on blood pressure control and treatment or avoidance of intravascular volume overload.[16,36] The present study provides a possible concept for the precise mechanisms of flash pulmonary edema in acute decompensated heart failure, in both HFpEF and HFrEF.

Interestingly, our bionic arterial baroreflex system was able to fully reverse the physiologic volume intolerance in FAIL. These results could suggest that the bionic arterial baroreflex system would be an attractive therapeutic tool in preventing volume intolerance in acute decompensated heart failure. A recent study also indicated that arterial baroreflex activation could be a novel therapeutic strategy for diastolic heart failure.[37] To develop a clinically useful system, further investigations to develop durable pressure sensors and electrodes are essential.

Study Limitations

There are several limitations to the present study. First, our model creates a condition of absolute FAIL mimicked by constant CSP, and the arterial baroreceptor of the carotid sinus is unable to perceive any change in blood pressure. In the clinical situation, patients with atherosclerosis of the carotid sinus may have impaired arterial baroreflex transduction, but they maintain at least marginal baroreflex function. Therefore, the difference in the clinical situation could be smaller than our estimation. On the other hand, because the surgical procedure and injury to the carotid sinus may damage NORM function, the difference in patients could be larger than our estimation. However, the importance of this study is that the arterial baroreflex system is capable of preventing striking volume intolerance regardless of LVEF. Second, we defined the critical volume as the infused volume resulting in LAP at 20 mm Hg. However, we continued the volume infusion until LAP was 14–16 mm Hg, and the critical volume was only estimated because it was necessary for us to repeat NORM and FAIL in the same rat. Volume infusion resulting in LAP at 20 mm Hg caused the real flash pulmonary edema, and we were not able to continue the experiments. Third, we fitted only a monoexponential curve to the obtained data of LAP and Vi, and we could not exclude a third curve that is not quite vertical (Fig. 3A). However, even if there is a third curve, we also consider that the LAP-Vi relationship was still markedly steeper in FAIL than in NORM, and we can conclude that FAIL induces significant volume intolerance.

Conclusion

FAIL induces, in part, striking volume intolerance with an increase in systemic AP in a rat model with normal LV function. Extrapolating this finding to patients with acute decompensated heart failure, FAIL might greatly increase the sensitivity to volume overload irrespective of LV function.

Disclosures

None.

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Multidisciplinary Approach to Genome-Wide Association Study for Heart Failure Based on the Different Ethnicity

-An overview of the bioinformatics and a new concept of BWAS-

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Preface

Heart failure (HF), a serious syndrome with diverse ethnicity and complicated etiology, is one of the leading causes of death. A part is heritable, though its underlying factors still remain elusive. GWAS (genome-wide association study) is promising for identifying the causative genes, directly or indirectly related genes as well as modifier genes that aggravate or ameliorate the clinical process to the advanced phase (1). This overview mainly focused our on-going HF-GWAS trial for the pathogenesis and/or progression of HF at the worldwide and whole genome levels, irrespective of the variable pathogenic factors common-in or specific-to different ethnicity, without missing biologically significant genes. We propose here a new concept of Bigenome (nuclear and mitochondrial)-Wide Association Study (BWAS) necessary for clarifying complexities of HF and providing therapeutic options.

Validity and Accuracy of a Case-Control Study

The case-control study has been defined as an observational epidemiological study of persons with the disease (or another outcome variable) of interest and "a suitable control group of persons without the disease (comparison group, reference group)". At a glance, it looks simple and persuasive but endogenously contains serious uncertainties. When in life-span? Under which circumstances and at where should be designated as the control group? What is "disease" or "normal"? Is it discernible to select candidate genes with majority rule? This paper aims not to discuss the medical determinism in case-control study but to realize an inevitable ambiguity to the principle of GWAS itself. The goal of GWAS targets identification of the potential, but often fuzzy gene(s), suggestive of a close or distant relationship to HF.

The current GWAS started as a post-genome project in Japan (Fig. 1). Genomic DNAs donated from the normal control and HF-cases and Affymetrix 6.0 for single nucleotide polymorphism (SNP)/single nucleotide variation (SNV)/ copy number variation (CNV) revealed ~1,500,000 haploid alterations and the

subsequent coding DNA sequence (CDS) analysis identified 7,287 SNPs with the χ^2 or Fisher's test. After restricting to the exons, or ORF, exon-intron border and stop codons, these SNPs reduced to ~1/3, consisting of 107 non-synonymous mutations, 54 stop codons, 23 insertion/deletions (in/del) to cause frame shift and 2,688 synonymous mutations (Fig. 2). These mutants did not overlap with Caucasian patients with dilated cardiomyopathy (1). Various cases with HF were diagnosed with clinical symptoms, physiological, morphological, biochemical and/or serological features. Candidate genes included adhesion molecules, channel proteins, cell signaling, transcription factors, proteases and anonymous proteins and would be opened elsewhere.

Evaluation of Candidate SNPs Related to Advanced HF

The SNP prevalence was analyzed with statistic test and the p value of each SNP was calculated with appropriate statistics. It should be notified that p values after Peason's χ^2 test or Fisher's exact test does not mean statistical potential of pathogenicity but denote the specificity. The Manhattan plot, –log (p) along the physical position on each chromosome, is critically dependent on case number. For the contribution potential to disease, effect size, β value, would be preferential. Both sex-chromosomes and mt-genes were excluded from this report, because they include many candidates to be commented and heteroplasmy, respectively, requiring other algorithms to discuss the genetic background (2-5).

Multiple Mutations in Each Nuclear Chromosome and Mitochondrion

The mutation locus was not disseminated but scattered in each autosome with some concentrated bias on 1, 2, 5, 11, 13, 15, 19 and 22 chromosomes (Fig. 3). These results may reflect natural selection under the *de novo* mutation and suggests the biological ontology, *i.e.* drastic modification of 2D/3D structure of the transcript or the transgene (4) may determine the viability or influence adoptability of mutants to environmental changes. These genetic modifications highlight the long-term mitochondrial (mt) symbiosis (5), because mt-genes often

show heteroplasmy or ethnicity-dependent haplogroup. More than 90% of nuclear SNPs are occupied by introns, pseudogenes or synonymous mutations (Fig. 3). In contrast, mt-gene has own exons, rRNAs and much less non-coding region in d-loop. Combination of nuclear and mt-SNPs are useful for identifying the haplogroup (3-5). Mt-mutations in heart require careful evaluation, since ORF-coding exons or tRNAs play a pivotal role in the ATP synthesis, reactive oxygen species (ROS) production, apoptosis induction and/or HF (3, 4).

A Novel Concept, "Bigenome-Wide Association Study (BWAS)", and Conclusion

In addition to nuclear genes, mt-gene also contributes to the incomparable pathophysiology at the cell/organ/body levels, particularly in muscular, cardiac and neural tissues (3) that require massive ATP. In addition to the nuclear gene, we intensify that mt-gene mutations should be considered along the time-course of HF and propose here a novel concept of BWAS, a combined research of nuclear and mt-genomes. This cross-talk between nuclear and mt-genes is different from the classic components located among mt-complexes coded by nuclear genes (NUMT, 3) and needed to maintain mt-function.

Our concept meant that combined mutations in nuclear and mt-gene may age-dependently aggravate HF and formed ROS synergistically peroxidizes adjacent constituents (DNAs, proteins or lipids), induce mutagenesis, reduce ATP production, enhance intracellular Ca^{2+} levels, decrease cardiac muscle cell contractility, activate endogenous proteases (1) and result in apoptosis and/or necrosis (1, 3, 6). Interruption of these sequential cascades would produce the beneficial outcome of the patients with HF. Compared with HF-related GWAS in European and African American (7), Japanese cases with HF indicated common SNPs to both ethnic groups. In addition, exome sequencing has recently revealed 25 % of causative loci in familial cases with exome-sequencing (8). Thus, we conclude that the gene analyses should be developed in both nuclear and mt-genomes and expanded to other candidate loci like iSNP, non-coding region (NCR) like miR to cover the whole aspects of genetic disorders.

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Figure Legends

Fig. 1. Road-map to identify pathogenic SNPs related to HF. All methods are informative, but still not definitive, because of gene interaction or cross-talk between candidate loci at each nt (epigenetic), intragenic (LD etc), intergenic (miR), and/or intergenomic (MUMT) complexities.

Fig. 2. Gene distribution of SNPs in exome or open-reading frame (ORF) of HF. Note that total SNP number is determined by the threshold of p value.

Fig. 3. Transgenomic distribution of SNP related with AdHF.multiple mutations in Accumulated Manhattan plots of >100 GWAS of HF-related SNP (B). Note that X-, Y-sex chromosomes and mt-gene contain the haplogroup linked to each algorithm (2, 5).

Fig. 4. Mutation numbers in each autosome indicating heterogeneous distribution of SNP.