and preload-dependent index. Because heart rate was higher in the nontreated group than in the donepezil group (354 ± 37 vs. 324 ± 23 bpm, difference of approx. 9%) and LVEDP was higher in the nontreated group than in the donepezil group, the difference in heart rate and preload would have underestimated the true difference in contractility. Moreover, decreased LVEDP with decreased RAP in the donepezil group suggested that body fluid retention was suppressed.

Neurohumoral factors

Figure 3 shows the blood concentrations of norepinephrine, epinephrine, and BNP measured 6 weeks after donepezil administration was started. Compared to the nontreated group, donepezil administration resulted in significant decreases in the concentrations of norepinephrine (316 ± 248 vs. 1,885 ± 1423 pg/ml, $P < 0.01$), epinephrine (347 ± 153 vs. 1,694 ± 1,355 pg/ml, $P < 0.05$), and BNP (362 ± 80 vs. 457 ± 68 ng/ml, $P < 0.05$) in the blood. These results indicated that donepezil effectively suppressed the overactive sympathetic nervous system, which is a hallmark pathophysiology of heart failure.

Infarct size and heart weight

Figure 1b shows representative ventricular sections in the nontreated and the donepezil groups. The myocardial infarction resulted from obliteration of the left coronary artery was 48 ± 6% of the left ventricular perimeter in the nontreated group and 53 ± 3% in the donepezil group, with no significant difference in infarct size between two groups. Therefore, donepezil administration started 2 weeks after myocardial infarction did not reduce the size of the infarct, suggesting that infarct size did not account for the differences in hemodynamics and neurohumoral factors described above.

Figure 1c compares the ventricular weight per body weight between the nontreated and the donepezil groups. The combined weight of the left and right ventricles was significantly lower in the donepezil group than in the nontreated group (3.02 ± 0.21 vs. 3.40 ± 0.13 g/kg body weight, $P < 0.05$). This result indicated that donepezil reduced cardiac remodeling after myocardial infarction was completed.

Power spectral analysis of heart rate variability

The left panel of Fig. 4a shows a representative change in RR intervals with respect to time in a rat from the donepezil group. The RR intervals connected with dotted lines were assessed to be extrasystoles or post-extrasystoles and were removed before spectral analysis. The right panel shows the result of spectral analysis from the same data. The solid area was calculated as the HF component. The HF components during the daytime (0600–1800 hours, Fig. 4b) and nighttime (1800–0600 hours, Fig. 4c) were calculated for the donepezil group ($n = 6$) and the nontreated group ($n = 5$). The log-transformed HF components [log(HF)] of the two groups were analyzed statistically.

During the night, log(HF) significantly increased in the donepezil group compared to the untreated group. On the other hand, there was no significant difference in log(HF) during the day between the two groups. These results indicated that heart rate variability at night was enhanced by donepezil administration in rats.

Discussion

Imbalances in the autonomic nervous system, particularly overactive sympathetic activity together with reduced

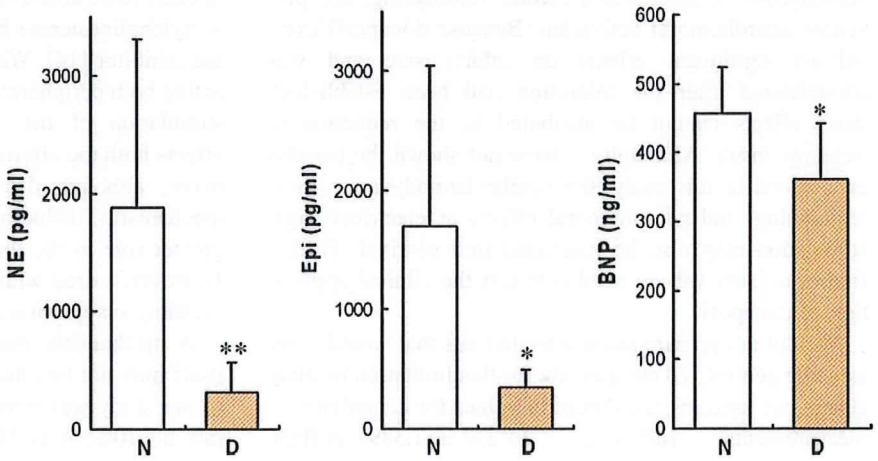

Fig. 3 Blood concentrations of norepinephrine (*NE*), epinephrine (*Epi*), and brain natriuretic peptide (*BNP*) at week 6 of treatment. Significant decreases (*$P < 0.05$, **$P < 0.01$) in blood NE, Epi, and BNP concentrations were observed in the donepezil group (*shaded bar*, *D*) compared to the nontreated group (*open bar*, *N*)

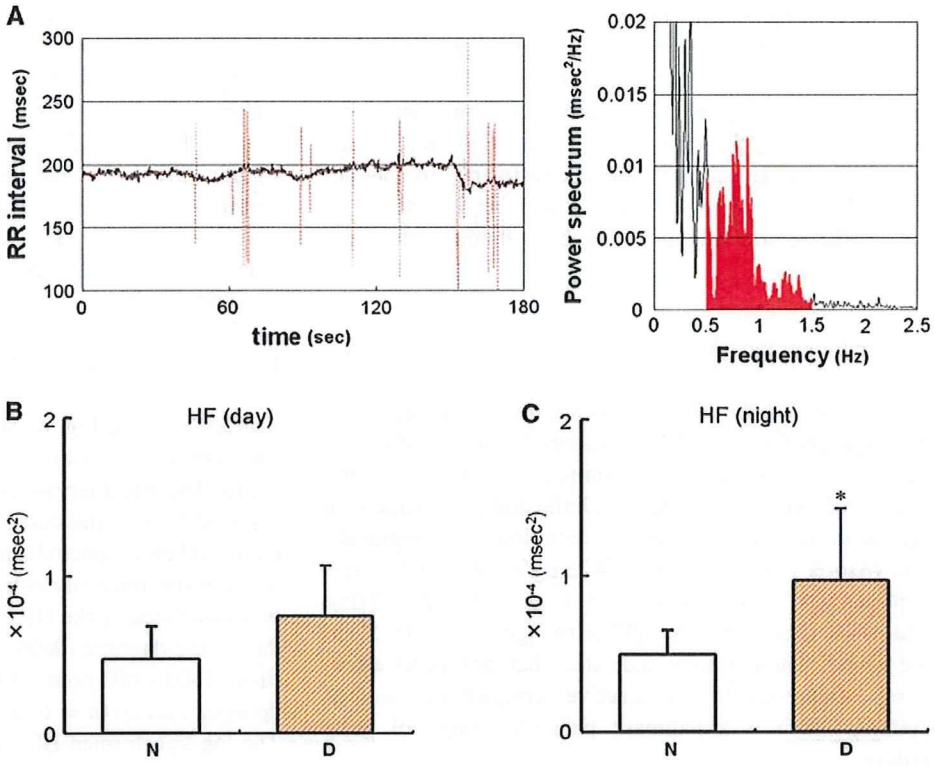

Fig. 4 **a** A representative example of time series of RR interval (*left*) and its power spectrum (*right*) in a donepezil-treated rat. RR intervals shown with *dotted lines* were assessed to be extrasystoles or post-extrasystoles and were removed before the power spectrum was calculated. *Solid area* indicates the high-frequency (HF) component. **b** HF of heart rate variability during the day. No significant difference in daytime HF value was observed between the donepezil group (*shaded bar*, *D*) and the nontreated group (*open bar*, *N*). **c** HF component of heart rate variability during the night. The nocturnal HF value of the donepezil group (*shaded bar*, *D*) was significantly higher than that of the nontreated group (*open bar*, *N*). $^*P < 0.05$ by *t* test using log(HF) values

vagal activity, have been considered to be major factors aggravating heart failure. In an earlier study, we demonstrated that upstream treatment using electrical stimulation of the vagal nerve improves the survival rate in rats with heart failure after a healed extensive myocardial infarction. Although pharmacological reproduction of the vagotonic treatment of heart failure would be of benefit clinically, no vagotonic drugs have successfully shown anti-remodeling, which is the most direct evidence of a lack of progression of heart failure.

The results presented here clearly demonstrate that, in our rat model system, donepezil treatment improved hemodynamics, ameliorated cardiac remodeling, and prevented neurohumoral activation. Because donepezil exerted no significant effects on infarct size and was administered after the infarction had been established, these effects cannot be attributed to the reduction in ischemic insult. Although we have not shown the benefits on survival in this study, the similar hemodynamic, anti-remodeling, and neurohumoral effects as electrical vagal stimulation may also be translated into survival. Further studies on survival are needed to test the clinical application of donepezil.

We did not prepare sham-operated rats that would serve as a true control. To compensate for this limitation in study design, we used historical control values for hemodynamic measurements (dP/dt_{max} 11,237 ± 1,389 mmHg/s, LVEDP 6.5 ± 2.3 mmHg; RAP 1.9 ± 1.3 mmHg), neurohumoral factor measurements (NE 392 ± 205 pg/ml, Epi 164 ± 46 pg/ml, BNP 62 ± 7 pg/ml), and biventricular weight (2.22 ± 0.11 g/kg) obtained from the same strain and similar age of rats. These control values indicate that hemodynamic deterioration, neurohumoral activation, and cardiac remodeling were only partially reversed, with the exception of NE. Notwithstanding, the results with the electrical stimulation of vagal nerves indicate that these small benefits may accompany a larger improvement in survival.

We selected donepezil, a novel cholinesterase inhibitor, in order to be able to maximize inhibitor action on neuronal acetylcholinesterase but not on hepatic butyrylcholinesterase inhibitor [14]. We intentionally used donepezil, a drug acting both peripherally and centrally, to simulate electrical stimulation of the vagus nerve. Electrical stimulation affects both the afferent and efferent pathways of the vagus nerve, although detailed knowledge of the therapeutic mechanisms, including which of the two pathways plays a greater role in the therapeutic effect, is not yet available. However, a drug with dual central and peripheral action is certainly inappropriate for deepening mechanistic insights.

A mechanistic study would be important as donepezil itself may not be clinically applicable. The dose we chose in our study was aimed at decreasing the heart rate in the rats by 10%; it is 50-fold larger than the dose used for

treating Alzheimer's disease. Although the objective of our study was not to elucidate how large the contribution of each effect of donepezil is on the peripheral vagus nerve, ganglion, and central nervous system, we would like to add discuss some mechanistic aspects in terms of designing future studies.

Regarding the mechanism downstream of the neuro-effector junction, the neurotransmitter acetylcholine per se may provide some protective effect for cardiomyocytes. Based on their results from acute studies, Sato et al. have obtained several lines of evidence supporting this hypothesis. First, acetylcholine promotes the phosphorylation of connexin 43, a gap junction molecule located between cardiomyocytes, which in turn normalizes the intercellular ion flow and prevents the occurrence of fatal arrhythmia [19]. Second, acetylcholine directly enhances the phosphorylation of Akt via PI3K in the cardiomyocytes and activates the PI3/Akt pathway to enhance the expression of hypoxia-inducible factor-1α (HIF-1α), which may protect the cardiomyocytes from the hypoxic state induced by ischemia [20]. As shown by these findings, the acetylcholine concentration increases in the neuro-effector junction by vagal efferent activation; this acetylcholine possesses various functions that support the survival of cardiomyocytes. Further studies are required to study the contribution of acetylcholine in cardiomyocytes at the molecular level. Vagal enhancement at the effector site may potentiate its anti-inflammation effects [21] and may ameliorate progression of heart failure through alpha 7-nicotinic receptors.

On the other hand, experiments using rat and canine models of heart failure suggest the presence of abnormalities in the ganglia of the vagus nerve. For example, a comparison of control rats to those with heart failure following myocardial infarction revealed that the bradycardiac response to pre-ganglionic vagus stimulation in the rats with infarction was attenuated, while the bradycardiac response to acetylcholine was unchanged [22]. In dogs with heart failure induced by tachypacing, pre-ganglionic vagus stimulation showed lower heart rate responses, while postganglionic stimulation at the fat pad showed no difference in heart rate response compared to control dogs [23]. Taken together the above observations, in our model system, donepezil may act on the ganglia of the vagus nerve.

As donepezil passes the blood–brain barrier, the drug can act on the central nervous system. To gain an insight into the central effect, we conducted an analysis of heart rate variability. Heart rate variability, especially its HF component (at respiratory frequency) reflects background vagal tone and has been shown to be a strong prognostic determinant [15, 16]. Our results revealed that donepezil increased the HF of heart rate variability during the night, indicating enhanced vagal activity. On the other hand, the HF of the heart rate variability tended to increase, although not significantly, during the day. These finding may suggest a central effect of donepezil, but again a secondary effect of improved hemodynamics cannot be ruled out. Regardless of the detailed mechanism, increased HF may be associated to a better outcome in these rats, as shown in the ATRAMI study [24, 25]. These issues require further investigations.

In summary, the results of the study reported here suggest that donepezil treatment, similar to electrical stimulation of the vagus nerve, confers beneficial effects in terms of the prevention of cardiac remodeling in rats with heart failure following myocardial infarction. Future studies should examine if survival would be improved by the administration of donepezil in rats with healed myocardial infarction.

Acknowledgments This study was supported by Health and Labor Sciences Research Grants (H19-nano-Ippan-009, H20-katsudo-Shitei-007) from the Ministry of Health, Labor and Welfare of Japan.

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Structural Heterogeneity in the Ventricular Wall Plays a Significant Role in the Initiation of Stretch-Induced Arrhythmias in Perfused Rabbit Right Ventricular Tissues and Whole Heart Preparations

Kinya Seo, Masashi Inagaki, Satoshi Nishimura, Ichiro Hidaka, Masaru Sugimachi, Toshiaki Hisada, Seiryo Sugiura

Rationale: Mechanical stress is known to alter the electrophysiological properties of the myocardium and may trigger fatal arrhythmias when an abnormal load is applied to the heart.

Objective: We tested the hypothesis that the structural heterogeneity of the ventricular wall modulates globally applied stretches to create heterogeneous strain distributions that lead to the initiation of arrhythmias.

Methods and Results: We applied global stretches to arterially perfused rabbit right ventricular tissue preparations. The distribution of strain (determined by marker tracking) and the transmembrane potential (measured by optical mapping) were simultaneously recorded while accounting for motion artifacts. The 3D structure of the preparations was also examined using a laser displacement meter. To examine whether such observations can be translated to the physiological condition, we performed similar measurements in whole heart preparations while applying volume pulses to the right ventricle. At the tissue level, larger stretches (≥20%) caused synchronous excitation of the entire preparation, whereas medium stretches (10% and 15%) induced focal excitation. We found a significant correlation between the local strain and the local thickness, and the probability for focal excitation was highest for medium stretches. In the whole heart preparations, we observed that such focal excitations developed into reentrant arrhythmias.

Conclusions: Global stretches of intermediate strength, rather than intense stretches, created heterogeneous strain (excitation) distributions in the ventricular wall, which can trigger fatal arrhythmias. (*Circ Res.* 2010;106:176-184.)

Key Words: stretch-induced arrhythmia ■ mechanoelectric feedback ■ optical mapping

Alterations to the mechanical state of the myocardium affect its electrophysiological properties, a phenomenon termed mechanoelectric feedback (MEF).[1,2] MEF is considered to play a significant role in the genesis of cardiac rhythm disturbances in various disease states, such as myocardial infarction and heart failure, in which myocardial tissues are subjected to abnormal loading conditions.[3–5] This speculation is supported by previous observations that in myocardial infarction, ventricular ectopic excitations are initiated by acute stretches of the border zone between the infarct and the normal myocardium.[6–8] A more definite causality is suspected in the etiology of commotio cordis, where sudden death occurs owing to a nonpenetrating chest wall impact in the absence of injury to the ribs, sternum, and heart.[9,10] Using anesthetized juvenile swine, Link et al[10] found that ventricular fibrillation can be produced by a baseball strike, and examined the effects of the phase, strength and speed of the strike for the induction of arrhythmias.

To elucidate the mechanisms underlying MEF and related arrhythmias, extensive studies have been carried out using various preparations from various species, including rabbits, lambs and dogs.[11–13] Stretch-activated channels (SACs) have been regarded as the most likely candidates for the primary transducers of mechanical stress.[14–16] Although such findings at the molecular level can account for changes in the action potential duration, amplitude, effective refractory period and resting potential induced by mechanical interventions at the cellular level, we still face a huge gap between these laboratory findings and clinical arrhythmias observed at the organ level. In this context, Franz et al[17] investigated the effects of increases in ventricular volume and pressure on epicardial monophasic action potentials in both isolated cross-circulated hearts and

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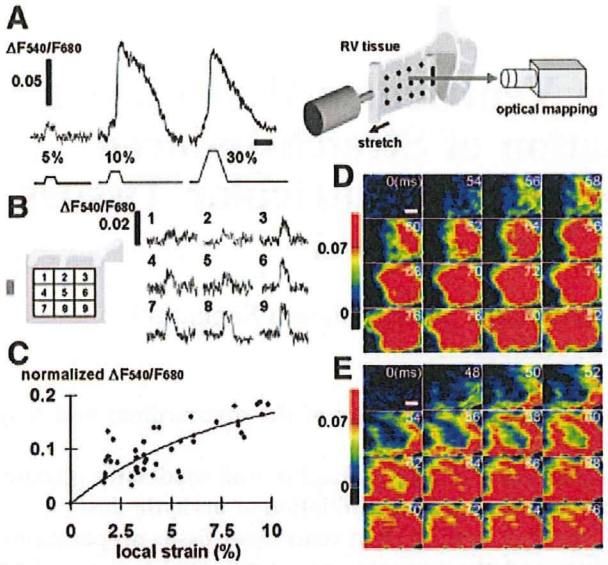

Figure 1. Alterations in the electric response in a cardiac tissue. A, Ratiometric optical signals ($\Delta F_{540}/F_{680}$) in response to 5%, 10%, and 30% stretches from left to right. Scale bar: 100 ms. B, Spatiotemporal pattern of the depolarizations (typical optical signals in each segment) in response to a 5% stretch. C, Relationship between the changes in the normalized optical signals and the local strain under the excitation threshold (n=5). The smooth curve through the data points was fitted with a nonlinear regression model. D and E, Representative action potentials and optical maps in response to 10% and 30% stretches, respectively. The stretch starts at 0 ms. Scale bar: 4 mm.

in situ canine hearts to clearly demonstrate the manifestation of MEF. However, these volume and/or pressure alterations do not allow detailed evaluation of the changes in myocardial stress or strain, which are believed to be the keys for establishing a link between the macroscopic and microscopic phenomena.

To elucidate how the cellular responses to stretches lead to arrhythmias in the heart, we focused on the morphology of tissue preparations and its role in the modulation of the electric responses. We developed an experimental set-up in which controlled uniaxial stretches were applied to crystalline perfused rabbit ventricular walls while monitoring the local strain. The use of optical transmembrane potential mapping combined with a tissue tracking technique enabled us to examine the relationship between local strain and excitation of the myocardium. By applying acute stretches of varying amplitudes, we demonstrate that global stretches applied to the ventricular wall tissue can create strain dispersion in the heterogeneous structure of the ventricular wall and that mechanical insults of intermediate, rather than intense, strength induce focal excitation, thus potentially triggering fatal arrhythmias. Finally, using whole heart preparations, we confirm that only medium stretches of the myocardium can evoke spiral wave formation.

Methods

Japanese white rabbits weighing 2.4 to 2.9 kg were used. The distribution of strain and the transmembrane potential were simultaneously recorded while applying an acute stretch to right ventricle (RV) tissue preparations. The 3D structure of the preparations was also examined. Similar measurements were conducted in whole heart preparations while applying acute volume pulses to the RV.

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Non-standard Abbreviations and Acronyms	
MEF	mechanoelectric feedback
SAC	stretch-activated channel
RV	right ventricle

Results

Effect of the Stretch Amplitude on Excitation of the Tissue

To elucidate the relationship between the electric response and the stretch level, we measured the optical transmembrane potential signals of stretched tissues. Figure 1 shows representative transmembrane potential signals in response to stretches of varying amplitudes. When a uniaxial stretch with a small amplitude (5%) was applied, the myocardial tissue was depolarized but an action potential did not develop (Figure 1A, left). The distribution of these depolarizations was heterogeneous and the amplitudes of these depolarizations had a positive dependence on the local strains (n=5) (Figure 1B and 1C). However, above a certain level of amplitude (≥10%), we observed focal excitation (development of an action potential in less than 4 segments of 9 blocks) (Figure 1A, middle; Figure 1D). A larger stretch (30%) only induced multiple occurrences of excitation in the tissue (Figure 1E). Figure 2A shows the relationship between the probability of tissue excitation (development of an action potential in at least one locus within the tissue) and the amplitude of the stretch applied (global strain). We found a fairly abrupt transition in the tissue responses to a uniaxial stretch (n=7). Specifically, excitation was rare when the amplitude was small (5%), but its rate increased with stretches in the medium range (10% and 15%) to reach 100% (sure observation) in response to large stretches (20%, 25% and 30%).

The use of a trapezoidal command with constant rates of rise and fall necessarily made the entire duration of the stretch longer for larger stretches, which may thus have led to modulation of the responses of the myocardium through different mechanisms. To exclude these possibilities, we applied stretches of varying amplitudes while keeping the entire duration constant at 50 ms. We found similar responses, thereby indicating that the amplitude rather than the duration is the major determinant of stretch-induced activation of the myocardium (Online Figure V, A). We also confirmed that stretches applied during the action potentials could modulate their shapes, and sometimes found stretch-activated depolarizations followed by premature ventricular contractions (Online Figure V, B).

Relationship Between Stretch-Induced Excitation and Epicardial Local Strain

We also evaluated the relevance between stretch-activated excitation and epicardial local strain (n=7). To compare the

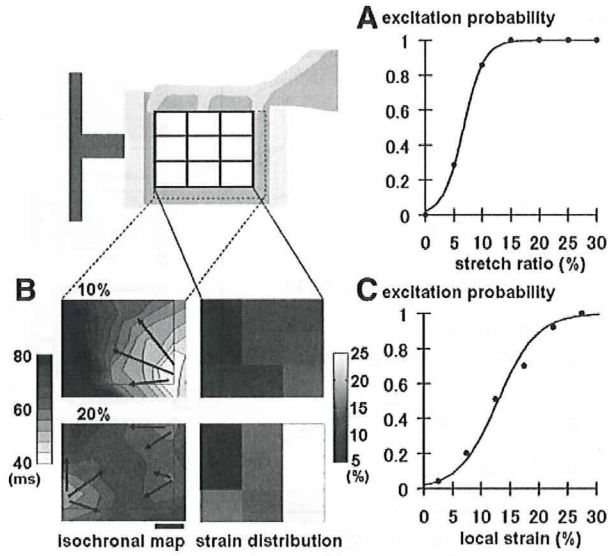

Figure 2. Electric responses and strain distributions. A, Probability that an action potential develops in at least 1 region of the whole tissue as a function of global stretch (n=7). The smooth curve through the data points was fit with a logistic regression model. B, Representative isochronal maps of a transmembrane potential showing the point of initial depolarization (left) and distributions of local strain (right). Top and bottom show 10% and 20% stretch, respectively. Scale bar: 4 mm. C, Relationship between the probability of stretch-induced excitation in the local area and the strain in the corresponding area (n=7). The smooth curve through the data points was fit with a logistic regression model.

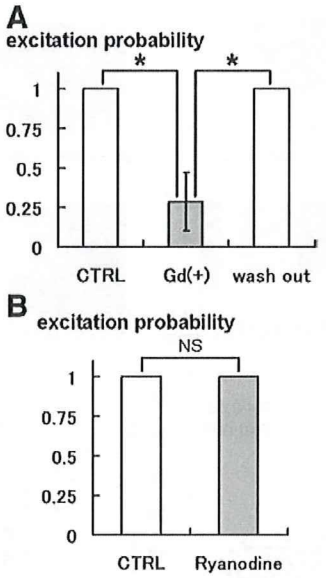

Figure 3. Modulation of stretch-induced excitation by drugs. A, Effect of Gd^{3+} on the probability of stretch-induced excitation after a 15% stretch (n=7). *$P<0.05$. CTRL indicates control condition. B, Effect of ryanodine on the probability of stretch-induced excitation after a 15% stretch (n=3).

strain distribution with the isochronal electric responses, the whole tissue area was divided into 9 blocks and the average strain value in each block was shown in grayscale. The local strain maps at each level of stretch with the corresponding isochronal maps are shown in Figure 2B (right). Initial excitation tended to take place at the locus of high strain (top: right lower block with 14% strain; bottom: left lower block with 14% strain; right upper 2 blocks with 23% and 24% strains). The excitation probability was clearly found to be more prominent for higher strains (Figure 2C), when the probability of local excitation was plotted as a function of the corresponding local strain (n=7).

Involvement of SACs in Stretch-Induced Excitations

To examine the involvement of SACs in the genesis of stretch-induced excitation, we repeated the experiments with a 15% stretch in the presence of 10 μmol/L Gd^{3+}, a blocker of nonspecific SACs. Gd^{3+} inhibited the stretch-induced excitation by 71.4±18.4% compared with the control condition and its effect was reversed by washout of Gd^{3+} (Figure 3A; n=7; $P<0.01$, Gd(+) versus control condition and washout). We also administered ryanodine to examine whether stretch-induced Ca^{2+} release from the sarcoplasmic reticulum and the triggered activity are involved in the activation process. When we applied 15% stretches, action potentials developed similarly in both ryanodine-treated and untreated (control condition) tissues (Figure 3B; n=3).

Strain Distribution and Tissue Structure

Because we applied uniaxial stretches to the ventricular tissue, the strain distribution on the epicardial surface was most probably created by heterogeneity within the tissue structure. To clarify the relationships between the strain distribution and the tissue structure, we measured the thickness distribution in each preparation using a laser displacement meter (Figure 4A; n=7). We divided the tissue into 9 blocks and calculated the average thickness in each block to facilitate comparisons with the strain data. Figure 4B shows a comparison between the thickness and local strain distributions after a 10% stretch from a single experiment. We found that the strain was high in regions where the tissue thickness was thin. For further comparisons between the tissue structure and the strain, we calculated the normalized thickness value of each block (mean thickness value of each block relative to the mean thickness value of all the blocks). Figure 4C summarizes the relationships between the local strain and the local thickness under different levels of stretch. Local strain was negatively correlated with the local thickness, which supported our hypothesis (10% stretch: n=7, $r=-0.52$, $P<0.0001$; 20% stretch: n=7, $r=-0.53$, $P<0.0001$).

Heterogeneous Excitation in Accordance With the Tissue Thickness and Stretch Level

We then plotted the relationship between the local wall thickness and the probability of stretch-induced local excitation for various levels of stretches (Figure 5A; n=7; closed circles, 5% stretch; closed triangles, 15% stretch; open circles, 30% stretch). When the applied stretch was small (5%), there was hardly any excitation (low probabilities over the entire range of thickness) because the local strain was below the threshold. As the amplitude of the stretch increased, the probability of excitation started to rise from the

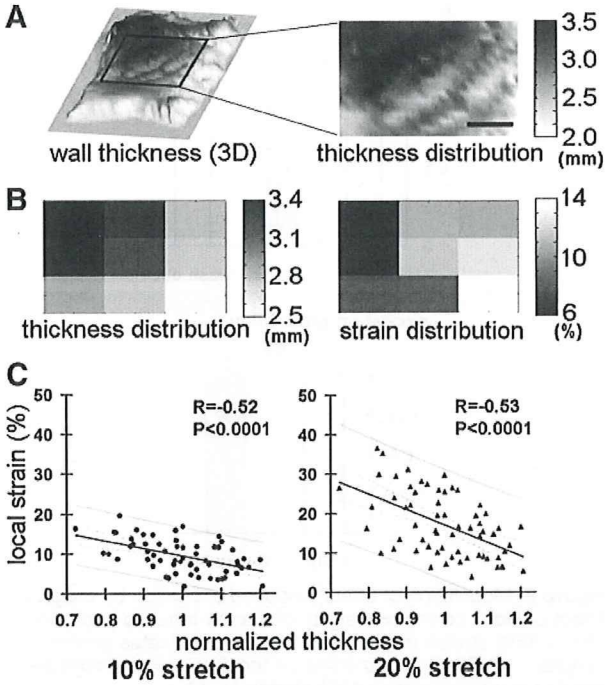

Figure 4. Thickness and local strain distributions of cardiac tissue. A, Representative case of the wall thickness distribution (laser-scanned data). Scale bar: 4 mm. B, Thickness distribution (left) and strain distribution (right) in response to a global 10% stretch in a representative experiment. C, Relationship between the normalized wall thickness and local strain in response to 10% (left) and 20% (right) global stretches. Lines are linear regression lines (10% stretch: n=7, $r=-0.52$, $P<0.0001$; 20% stretch: n=7, $r=-0.53$, $P<0.0001$).

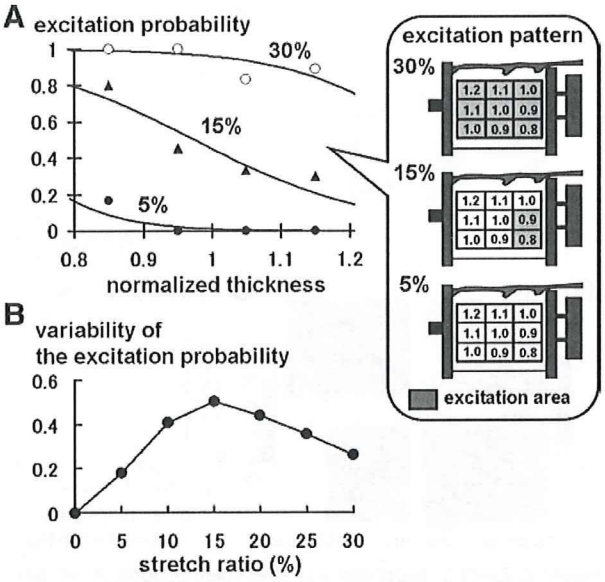

Figure 5. Relationship between the probability of focal excitation and the stretch amplitude. A, Probabilities of stretch-induced local excitation as a function of the relative wall thickness for 5% (closed circles), 15% (closed triangles), and 30% (open circles) stretches (n=7). The smooth curves through the data points were fit with logistic regression models. Right, Distributions of the wall thicknesses, in which the segments where the action potentials developed are depicted in gray for 30% (top), 15% (middle), and 5% (bottom) stretches. B, From the data shown in A, the variability of the probability for the development of local excitation over the entire range of wall thickness was plotted as a function of the global stretch (n=7). Heterogeneous excitation is induced by a stretch of intermediate amplitude rather than a large stretch.

thin area (15%) and all areas were finally excited in response to a large stretch (30%). We calculated the variability (standard deviation) of the excitation probability over the entire thickness range for each stretch amplitude, and these data are plotted in Figure 5B (n=7). In regions of small (5%) or large (30%) stretches, the variability was low (0.18 or 0.26) because the whole tissue was either unresponsive or responsive to the stretch, respectively, whereas heterogeneous excitation was achieved in response to a stretch of intermediate amplitude (0.50 on 15% stretch).

Stretch-Induced Focal Excitations Develop Into Reentrant Arrhythmias in the Ventricle

To examine whether the findings at the tissue level are applicable to more physiological situations, we applied volume pulses to the RV in whole heart preparations and recorded the transmembrane potential responses. Figure 6A and 6B shows representative optical signals in response to 2 different amplitudes of volume pulses. When we applied a small volume pulse (0.5 mL), virtually no response was observed. However, local excitation (excitation from less than one-third of all the blocks) was induced by a 1.0-mL volume pulse (Figure 6A), and a large volume pulse (2.0 mL) elicited excitation from a larger area simultaneously (global excitation) (Figure 6B). The corresponding thickness distribution in the optically mapped region revealed that the focal excitation originated from a thin region (Figure 6C). As summarized in Figure 6D (n=6), focal excitation was only induced with pulses of intermediate volumes (1.0 and 1.5 mL). Structural measurements revealed that such focal excitations tended to take place in regions where the wall thickness was thinner (Figure 6E; n=6, $P<0.05$), similar to the case for the tissue preparations. All of these findings were in accordance with the tissue experiments, thus confirming that only global stretches of medium intensity can induce focal excitation in the ventricular wall.

Focal excitation is a prerequisite for the initiation of reentrant arrhythmias, but may not fulfill the conditions. Therefore, we hypothesized that when the propagation of the focal excitations induced by medium mechanical stimuli interacts with the preceding electric activations, it can develop to fatal reentrant arrhythmias. To assess this hypothesis, we applied the volume pulses to the RV for 50 ms at various coupling intervals (90 to 130 ms) with a preceding electric stimulus. Similar to the electric "pinwheel experiment" protocol,[18] this protocol involves the simultaneous establishment of a spatial gradient of momentary stretch-induced excitability together with a spatial gradient of refractoriness induced by the prior passage of an activation. As shown in Figure 7A, a 1.5-mL volume pulse after a 110-ms coupling interval initiated vortex-like reentrant waves pivoting around phase singularities. As clearly shown in Figure 7B, a large volume pulse (2.0 mL) never elicited arrhythmias, whereas an intermediate volume pulse (1.5 mL) applied after a proper coupling interval (110 ms) triggered reentrant arrhythmias (n=3, 66.7% probability).

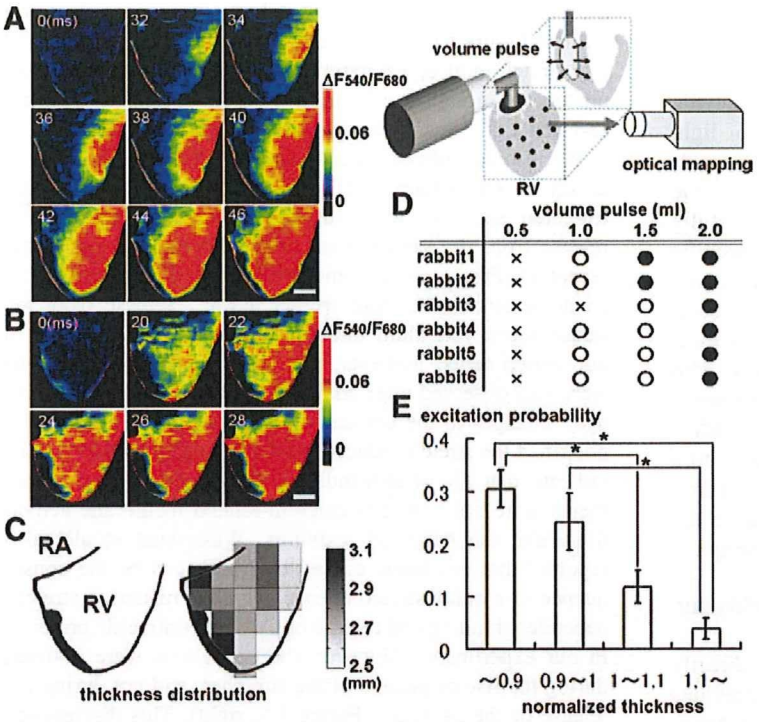

Figure 6. Alterations in the electric responses in a whole heart preparation. A and B, Representative optical maps of the responses of the RV to volume pulses of 1.0 mL (A) and 2.0 mL (B). The stretches start at 0 ms. Scale bar: 4 mm. C, Corresponding thickness distribution in the ventricle. RA indicates right atrium; RV, right ventricle. D, Response patterns to stretches in 6 rabbit hearts. Crosses indicate no excitation; focal excitation, open circles; global excitation, closed circles. E, Excitation probability for each normalized thickness range in the initiation of focal excitation (n=6). *$P<0.05$.

Discussion

In the present study, we simultaneously measured the transmembrane potentials and local strains while applying uniaxial stretches of varying amplitudes to rabbit RV wall tissue to clarify the linkage of electric activity between cells and organs. The use of optical transmembrane potential mapping coupled with local strain measurements based on bead markers enabled us to record the strain–electric response relationship of myocardial tissue. In addition, structural measurements of the preparations suggested that the complex architecture of the ventricular wall could cause heterogeneous strain responses to mechanical stimuli, thereby leading to the initiation of focal excitation. We confirmed this hypothesis under more physiological conditions by successfully inducing reentrant arrhythmias using a volume pulse of medium amplitude.

Optical Mapping of the Transmembrane Potential

Owing to its high temporal and spatial resolutions, optical recording of transmembrane potentials has been widely used, but most studies have only dealt with immobile preparations where the motion was inhibited mechanically and/or pharmacologically.[19–21] These stabilizations of the preparations were conducted to prevent motion artifacts caused by changes in the fluorescence intensity along the light path, and also

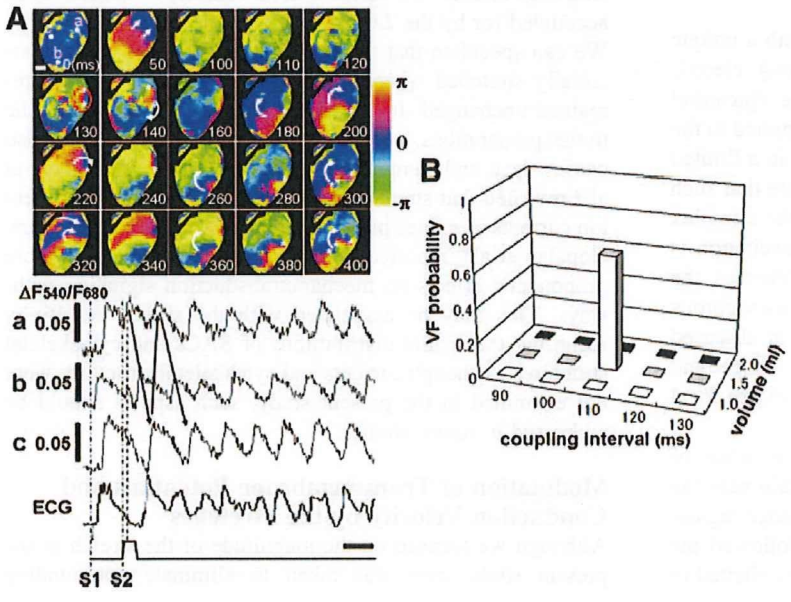

Figure 7. Initiation of spiral waves by volume pulses. A, Representative phase maps of spiral wave formation. The volume pulse was applied at 110 ms after the electric stimulus. Scale bar: 4 mm. a through c represent the ratiometric optical signals ($\Delta F_{540}/F_{680}$) for the corresponding positions shown in the 0-ms optical map (top left). The electric stimulus starts at 0 ms. The ECG is shown at the bottom. Scale bar: 100 ms. *Phase singularity points. B, Excitation probabilities in relation to the coupling intervals and the intensities of the volume pulses (n=3).

changes in the *x-y* position. In the present study, we tried to account for the motion-induced contamination of optical signals by using 2 methods to accurately evaluate the transmembrane potentials of the local myocardium while applying a stretch to the whole tissue. First, the fluctuation of light intensity was cancelled by ratiometry of the 2 emission bands of the fluorescent indicators. Second, by using the affine transformation based on motion tracking, we successfully traced the tissue points, presumably a cluster of specific myocytes, during a stretch and induced contraction, and showed the sequential changes in the transmembrane potential in the reference position. As shown in Figure 1A, the local action potential triggered by the stretch reconstructed with these techniques is similar to that recorded by an electrode with its clear zero phase characteristics. Compared with a previous study of the local response of electric activity to a linear acute stretch using a pair of electrodes in isolated frog ventricular tissue at only 2 points,[22] detailed maps of the action potentials were obtained with the present technique.

Heterogeneity in the Tissue Structure for Bridging Cellular Responses to Arrhythmias

Although the activity of ion channels was not directly measured, the present results were consistent with previous studies demonstrating the involvement of SACs in MEF. Zeng et al[23] recorded the stretch-dependent inward current, which was blocked by Gd^{3+} in rat cardiac myocytes. They also observed that a 10% stretch induced an immediate contraction of the myocytes. Although the threshold for excitation varies among studies, similar observations were made for rat (>20%),[24] guinea pig (20% to 25%),[25] and frog (15%)[26] myocytes. In our probability curves of stretch-induced excitation for both whole tissues and segments (Figure 2A and 2C), the transition from nonresponse to excitation took place within a similar range of stretch amplitudes. Furthermore, the response was inhibited by Gd^{3+} and recovered by washout of the agent. Taken together, these results suggest that a uniaxial stretch applied to the tissue induces strain in the myocytes, which in turn triggers the activation of molecular mechanotransducers, most probably SACs.

The use of a tissue preparation provided us with a unique opportunity to elucidate the relationships among electric excitation, global strain and local strain on the epicardial surface. Although a uniaxial global stretch was applied to the preparation, excitation was usually only induced in a limited area where the local strain was high. We speculate that such heterogeneity in the strain distribution reflects the complex structure of the ventricular wall, such that the excitation is initiated in regions where the wall is thin. Whereas the complex structure of the ventricles normally allows vigorous contraction, different hemodynamic overloads in diseased states lead to abnormalities in the ventricular shape and regional wall motion,[27,28] which may sometimes evoke focal excitations.

We must consider the possibility that the presence of damaged ends may have caused an abnormal strain near the tissue supports to initiate the excitation from the edge region. However, the locus of focal excitation always followed the thickness distribution, such that the excitation was elicited in the center of a preparation that had a thin central region (Online Figure VI, A). Furthermore, ter Keurs et al[29] reported that stretch-induced excitations from the damaged myocardium occur through a calcium-related triggering mechanism, and that Gd^{3+} does not suppress these phenomena.

We also considered the relevance of a Ca^{2+}-related mechanism to our experiments. Fujiwara et al[30] showed that triggered activities were subsequently evoked by a Ca^{2+} release from the sarcoplasmic reticulum through ryanodine receptors. Furthermore, some previous studies reported that an acute stretch can also trigger a Ca^{2+} release from the sarcoplasmic reticulum through ryanodine receptors.[31,32] In our experiments, however, the stretch-induced excitations were still observed after administration of ryanodine. Moreover, changes in the extracellular calcium concentration did not affect the stretch-induced excitability. These observations indicate that the stretch-induced excitations in our experiments were not linked to calcium-related membrane activations like the triggered activities. Wakayama et al[33] also reported that excitation caused by MEFs can be the consequence of a quick stretch release, which is related to stretch-dependent binding and release of Ca^{2+} to contractile proteins. In our experiments, however, the excitations were initiated during the rise or plateau of the stretches, and not during the release of the stretches (Figure 1A, right). This discrepancy may be caused by the fact that the excitation as a consequence of a quick release in the previous report was only observed at a high Ca^{2+} concentration (5.2 ± 0.73 mmol/L), whereas our experiments were carried out with a lower Ca^{2+} concentration (1.8 mmol/L). These observations indicate that the stretch-induced excitations observed in our study are not related to the release of Ca^{2+} to contractile proteins following the stretch release.

Translation of data obtained with tissue preparations to the intact heart requires consideration in terms of both the magnitude and the nature of the deformation. A volume pulse of 2.0 mL induced global excitation, the effect caused by a 20% stretch of the tissue. However, if we simply assume a spherical ventricle, a 20% increase in its circumference would lead to an almost 70% increase in its volume, which cannot be accounted for by the 2.0-mL volume pulse in the rabbit RV. We can speculate that the thinner RV free wall was preferentially stretched whereas the thick ventricular septum remained unchanged. In addition to the stretch applied to the tissue preparations, volume expansion of the ventricle also causes shear and compression of the wall. In fact, Isenberg et al[34] revealed that stretch and compression activated different ion currents in guinea pig ventricular myocytes. Furthermore, Gopalan et al[35] reported that transverse stretches have more pronounced effects on mechanotransduction signaling pathways. This may be associated with the stretch sensitivity regarding the spatial distributions of SACs and cytoskeletal structures. Although currents and cytoskeletal structures were not examined in the present study, such aspects should be addressed in future studies.

Modulation of Transmembrane Potentials and Conduction Velocity by the Stretches

Although we focused on the magnitude of the stretch in the present study, care was taken to eliminate confounding

factors. Fasciano and Tung[22] revealed that the stretch speed significantly affects the stretch-induced excitability. In this context, we made the speed of the stretch constant in all the experiments (Online Figure IV). We checked the influence of the stretch duration in another set of experiments in which the stretch duration was made constant at 50 ms. We confirmed that these 2 types of protocols did not cause any significant differences in the excitability induced by the stretch.

We also examined the effect of the stretch timing relative to the action potentials. Similar to previous reports,[17,36] stretches applied in each phase (2, 3 and 4) of the action potentials modulated the transmembrane potentials differently (Online Figure V, B).

We calculated the conduction velocity of the focal excitations elicited by 10% stretches and compared it with that elicited by an electric stimulus (Online Figure VI, B). In these experiments, the spread of conduction between 2 recording positions (crosses) was completed during the stretch plateau. Although the number of observations was limited owing to the technical difficulty, we confirmed that the conduction velocities of the stretch-induced excitations in both the horizontal and vertical directions tended to be slower (31.7% and 38.7% decrease in vertical and horizontal direction, respectively). In addition, we also examined the relationship between the normalized dV/dt_{max} (evaluated by the time derivative of the ratiometric optical signal, dF/dt_{max}) of action potential upstrokes and local strains. Normalized dV/dt_{max} of the action potential upstroke was decreased in regions where local strain was high (Online Figure VII). Although the effects of stretches on the conduction velocity are still controversial,[37] conduction slowing has been reported in previous studies.[21,38] Eijsbouts et al[38] reported that the anisotropic nature in the heterogeneous wall thickening may play an important role in conduction disturbances attributable to dilation. Geometric and structural changes during an acute stretch should be some of the causes of this effect, and SACs and the intracellular calcium dynamics may also be involved in this phenomenon. In either case, such changes in the propagation characteristics could also contribute to the development of reentrant arrhythmias.

Clinical Implications

When a mechanical stimulus of moderate amplitude was applied to the ventricular wall, local excitation was induced in regions where the wall thickness was thin and, if other facilitatory conditions were met, it was propagated to the adjacent area to develop into fatal arrhythmias. We expect that further increases in the intensity of the stimulus would induce multiple excitations to exaggerate the electric heterogeneity, thereby increasing the possibility of arrhythmias. However, if a very intense stimulus is applied, the whole tissue can be synchronously excited, which considerably decreases the possibility of arrhythmias (Figure 5B). Interestingly, we can see a similar tendency in the relationship between the ventricular fibrillation probability and the rise in ventricular pressure produced by a baseball impact in an experimental study on commotio cordis by Link et al,[10] who did not provide any mechanistic comments.

In this study, a volume pulse of 1.5 mL at a 110-ms coupling interval after the last electric stimulus initiated a reentrant arrhythmia. No reentrant arrhythmia, however, was induced by 1.0-mL volume pulses that triggered focal excitations when applied at 500-ms coupling intervals. These findings probably arise from a dependence of the strength of the mechanical stimuli required to generate focal activity on the phase of the action potential at which it is applied. In contrast to the protocol (a), in which the pulses were applied to the fully relaxed ventricle after a long coupling interval (500 ms), we confirmed that the myocardium in activated states has higher thresholds for activation (Online Figure VIII). Based on these observations, a 1.0-mL volume pulse cannot initiate the excitation with coupling intervals from 90 to 130 ms, whereas a 1.5-mL volume pulse can initiate focal excitations with coupling intervals of >100 ms. Although the focal excitations were frequently initiated with coupling intervals of >120 ms, the excitations did not develop into reentrant arrhythmias because a unidirectional conduction block cannot be formed at these timings. Owing to the trapezoidal volume change and viscoelastic nature of the tissue, the effect of the volume pulse was realized with some delay. In fact, although we applied a volume pulse after a 110-ms coupling interval, excitation was initiated at around 130 ms corresponding to the late phase 2 of the action potential. We speculate that these findings correspond to the observation that ventricular fibrillations were triggered when the chest wall impacts were applied during the vulnerable portion of the T wave.[9]

Our present results suggest that the complex structure of the ventricular wall functions to modulate a mechanical impact and create a heterogeneous excitation distribution in response to a stimulus of intermediate intensity, rather than an intense stimulus, to initiate ventricular fibrillation in otherwise healthy young subjects.

The structural complexity of the ventricular wall may also contribute to the genesis of arrhythmias in old myocardial infarctions. Regarding myocardial infarction, it is considered that the conduction abnormality in the infarct area acts as the substrate for arrhythmias,[39] but its trigger still remains unclear. Bogen et al[40] reported that a large mechanical load is added to the border zone in regions where the wall thickness is thin in systole. Moreover, Josephson[41] revealed that arrhythmias are often initiated from these borders. Calkins et al[8] observed that ventricular dilation shortens the refractoriness of the surviving myocardium in the infarct area rather than the healthy myocardium. Taken together, the following scenario is conceivable. In an old myocardial infarction, a systolic rise in ventricular pressure can induce a large stretch in the functional border zone, where the wall thickness is thin to provoke an ectopic excitation, which may develop into fatal reentrant arrhythmias promoted by the conduction abnormality in the infarct area.

In either case, the structural and/or functional heterogeneity of the myocardial tissue serves to create a heterogeneous strain distribution, and establishes a MEF-mediated electrophysiological dispersion in the tissue, which is known to be a potent substrate for arrhythmias.

Study Limitations

Although the use of flattened tissue preparations made it easy to evaluate local strain, the results cannot be translated directly to the clinical setting where volume/pressure loading or external compression distorts the ventricular tissue in a complex manner. Furthermore, although uniaxial stretches may cause 3D strain within the tissue with reductions in the width and thickness, these effects were not taken into consideration. On the other hand, the intact heart preparations pose a problem for potential mapping and the measurement of strain. In either case, because the action potentials and strains were recorded at the epicardial surface, we did not evaluate the heterogeneity in the transmural structure from the epicardium to the endocardium. Furthermore, as stated above, we did not measure the ion currents in response to the stretches, although they seemed to greatly promote our understanding of stretch-induced arrhythmias in the intact heart. Finally, we only used the RV in our experiments based on our assumption that the RV is more vulnerable to mechanical stimuli because of its weak elasticity, and stretch-induced arrhythmias could also be evoked in the left ventricle.

In summary, a global stretch applied to the ventricular wall tissue can create a heterogeneous strain distribution in the heterogeneous structure of the ventricular wall. Such heterogeneity in the strain distribution can lead to local excitation, which in turn leads to fatal reentrant arrhythmias.

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Disclosures

None.

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ORIGINAL PAPER

Right ventricular stiffness constant as a predictor of postoperative hemodynamics in patients with hypoplastic right ventricle: a theoretical analysis

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Abstract One and a half ventricle repair (1.5VR) is a surgical option for hypoplastic right ventricle (RV). The benefits of this procedure compared to biventricular repair (2VR) or Fontan operation remain unsettled. To compare postoperative hemodynamics, we performed a theoretical analysis using a computational model based on lumped-parameter state-variable equations. We varied the RV stiffness constant (B_{RV}) to simulate the various RV hypoplasia, and estimated hemodynamics for a given B_{RV}. With $B_{RV} < 150\%$ of normal, cardiac output was the largest in 2VR. With $B_{RV} > 150\%$, cardiac output became larger in 1.5VR than in 2VR. With $B_{RV} > 250\%$, RV end-diastolic volume was almost the same between 1.5VR and 2VR, and a rapid increase in atrial pressure precluded the use of 1.5VR. These results indicate that the beneficial effect of 1.5VR depends on the RV stiffness constant. Determination of management strategy should not only be based on the morphologic parameters but also on the physiological properties of RV.

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Keywords One and a half ventricle repair · Right ventricular stiffness · Hypoplastic right ventricle · Computational model

Introduction

One and a half ventricle repair (1.5VR) is a surgical option for hypoplastic right ventricle (RV) caused by various congenital heart diseases including pulmonary atresia with intact ventricular septum (PA/IVS), Ebstein's anomaly or their relatives. In this procedure, the superior vena cava (SVC) is directly connected to the pulmonary artery (PA). Therefore, the blood from SVC directly enters PA, whereas the blood from the inferior vena cava (IVC) is pumped by RV to PA. This procedure is clinically acceptable because of its low surgical risk [1, 2]. However, the benefits of this procedure on postoperative hemodynamics in patients with a wide spectrum of RV hypoplasia compared to other procedures such as biventricular repair (2VR) and Fontan operation remain unsettled [3]. Furthermore, conversion to Fontan circulation was required late after 1.5VR in a possibly inappropriate candidate [4].

Although various authors reported an arbitrary selection scheme for the procedures based on RV morphology such as RV end-diastolic volume (RVEDV) [1, 2, 5], the long-term outcomes of 1.5VR have remained insufficiently known [5]. The previous criteria do not likely predict postoperative hemodynamics of these complex circulations accurately because morphological values measured preoperatively largely depend on the RV preload and afterload conditions, which change remarkably between subjects and between before and after the operation.

Hypoplastic RV is physiologically characterized by increased RV stiffness, caused by hypertrophy and

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fibroelastosis of RV muscles [6]. However, how RV stiffness influences the postoperative hemodynamics has not been reported. Given the small number of patients with each of the wide variety of preoperative RV conditions [7, 8], the influence of RV stiffness on 1.5VR, 2VR, and Fontan operation cannot be examined by clinical study. It is also difficult to experimentally reproduce hemodynamics before and after 1.5VR for hypoplastic RV with various stiffness. In view of the above, we attempted to clarify postoperative hemodynamics by a theoretical analysis using a computational model based on lumped-parameter state-variable equations. The present results indicate that the RV stiffness constant may provide selection criteria for 1.5VR.

Materials and methods

The electrical analogs of the model used to simulate the cardiovascular system are shown in Fig. 1. We modeled the postoperative cardiovascular system mathematically by a combination of the time-varying elastance cardiac chamber model and the three-element Windkessel vascular model. We set the normal values of parameters to be appropriate for a 75-kg man. These values were obtained from the literature [9–13] and are listed in Table 1. Since the data of the pressure–volume relationship of the atrium were scarcely available, parameters of the atrium were surmised from the literature [10–12].

Heart

The right and left ventricular chambers as well as the atrial chambers are represented by the time-varying elastance model [9, 10, 13]. The end-systolic pressure–volume relationship is described by a linear formula:

$$P_{es,cc} = E_{es,cc}\left[V_{es,cc} - V_{0,cc}\right] \quad (1)$$

where $P_{es,cc}$ and $V_{es,cc}$ are end-systolic pressure and volume, respectively; $E_{es,cc}$ is the maximal volume elastance; $V_{0,cc}$ is the volume at which $P_{es,cc}$ is equal to 0 mmHg. cc denotes each chamber, i.e., RA for the right atrium, LA for the left atrium, RV for the right ventricle, or LV for the left ventricle. The end-diastolic pressure–volume relationship is represented by a non-linear formula:

$$P_{ed,cc} = A_{cc}\left[e^{B_{cc}(V_{ed,cc}-V_{0,cc})} - 1\right] \quad (2)$$

where $P_{ed,cc}$ and $V_{ed,cc}$ are end-diastolic pressure and volume, respectively; A_{cc} and B_{cc} are constants [9, 10, 13]. We assumed the time course of the time-varying elastance by defining normalized elastance curve $e_{cc}(t)$ as:

Table 1 Parameters used in modeling

Heart rate (HR), beats/min	75			
Duration of cardiac cycle (T_c), ms	800			
Time advance of atrial systole (DT), ms	16			
Total stressed blood volume (V_s), ml	750	(control only)		
	LV	RV	LA	RA
Time to end systole (T_{es}), ms	200	200	120	120
End-systolic elastance (E_{es}), mmHg/ml	3.0	0.7	0.5	0.5
Scaling factor of EDPVR (A), mmHg	0.35	0.35	0.06	0.06
Exponent scaling factor for EDPVR (B), ml^{-1}	0.033	0.023	0.264	0.264
Unstressed volume (V_0), ml	0	0	5	5
	Aortic	Pulmonary	Mitral	Tricuspid
Valvular resistance (forward), (mmHg s)/ml	0.001	0.001	0.001	0.001
	Systemic		Pulmonary (p)	
	Superior (ss)	Inferior (si)		
Arterial resistance (R_a), (mmHg s)/ml	2.25	1.5	0.03	
Characteristic impedance (R_c), (mmHg s)/ml	0.075	0.05	0.02	
Venous resistance (R_v), (mmHg s)/ml	0.0375	0.025	0.015	
Arterial capacitance (C_a), ml/mmHg	0.528	0.792	13	
Venous capacitance (C_v), ml/mmHg	28	42	8	

LV Left ventricle, *RV* right ventricle, *LA* left atrium, *RA* right atrium, *EDPVR* end-diastolic pressure–volume relationship

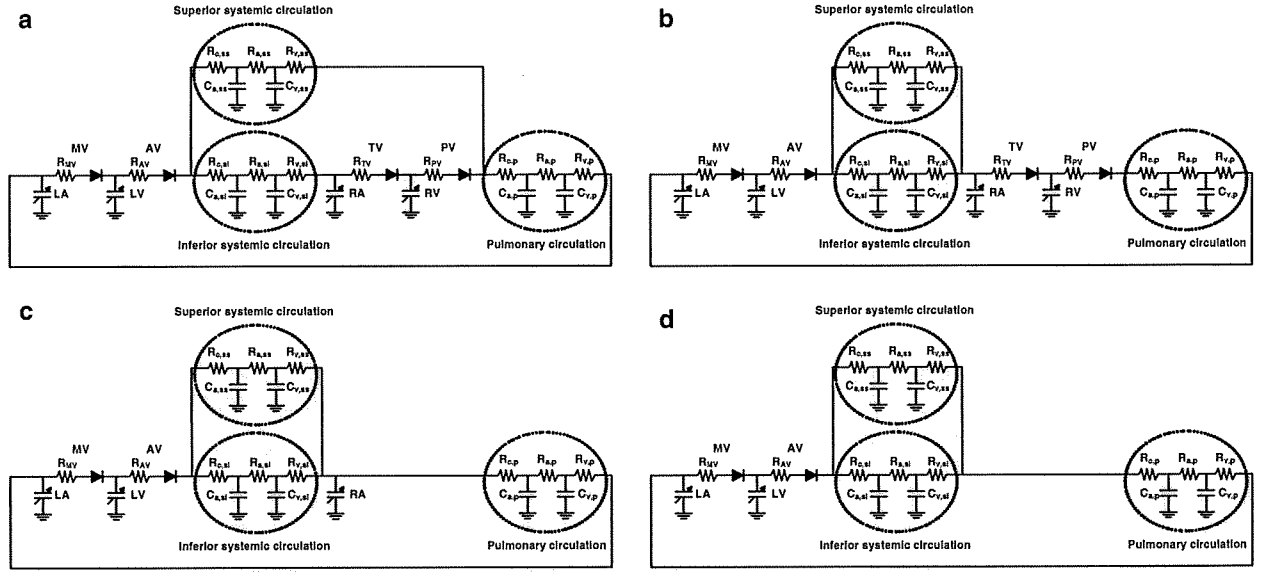

Fig. 1 **a** The electric equivalent circuit of one and a half ventricle repair. **b** Biventricular repair (normal circulation). **c,d** Variations of Fontan operation [**c** atriopulmonary connection (APC); **d** total cavopulmonary connection (TCPC)]. *LV* and *RV* left and right ventricles, *LA* and *RA* left and right atria, *AV* and *MV* aortic and mitral valves, *PV* and *TV* pulmonary and tricuspid valves, C_a and C_v lumped arterial and venous capacitances, R_c characteristic impedances, R_a lumped arterial resistances, R_v venous resistances, *ss* superior systemic circulation, *si* inferior systemic circulation, *p* pulmonary circulation

$$\begin{aligned} e_{cc}(t) &= 0.5[1-\cos(\pi t/T_{es,cc})]\ (0 \le t < 2T_{es,cc}) \\ e_{cc}(t) &= 0\ (2T_{es,cc} \le t < T_c) \end{aligned} \quad (3)$$

where t is the time from the start of systole, $T_{cs,cc}$ is the duration of systole, and T_c is the duration of cardiac cycle. Using $e_{cc}(t)$, the instantaneous pressure, $P_{cc}(t)$, is described by:

$$P_{cc}(t) = [P_{es,cc}(V_{cc}) - P_{ed,cc}(V_{cc})]e_{cc}(t) + P_{ed,cc}(V_{cc}) \quad (4)$$

Ventricular systole is preceded by atrial systole. The time advance of atrial systole (DT) is calculated as the fixed fraction of T_c (DT = $0.02T_c$). Function of each chamber is characterized by the parameters $E_{es,cc}$, $T_{es,cc}$, $V_{0,cc}$, A_{cc}, B_{cc}, and $e_{cc}(t)$. The same $e_{cc}(t)$ was used for all chambers, but the other parameters were different between chambers, as shown in Table 1.

Vascular system

Basically, the pulmonary and systemic circulations are modeled as modified Windkessel impedances. Each vascular system is modeled by lumped venous (C_v) and arterial (C_a) capacitances, a characteristic impedance (R_c) that is related to the stiffness of the proximal aorta or pulmonary artery, a lumped arterial resistance (R_a), and a resistance proximal to C_v (R_v). This framework is similar to that used in deriving Guyton's resistance to venous return [14].

To simulate the postoperative hemodynamics of 1.5VR, the systemic circulation is divided into two parts, the superior and the inferior circulation. Therefore, the parameters of the systemic circulation are also divided into the superior and inferior ones, as shown in Fig. 1. Blood flow in the descending aorta is reported to be 63.8% of the left ventricular output [15]. The compliance of the IVC is considered to be 66.6% of the total venous compliance [16]. Thus, in our model, arterial and venous compliances of the inferior systemic circulation are adjusted to 0.6 times those of the compliance of the total circulation, and the blood flow of the inferior systemic circulation is controlled to be 60% of the left ventricular output by adjusting the resistances of R_c, R_a, and R_v.

The capacitance of the superior systemic circulation is also divided into arterial ($C_{a,ss}$) and venous ($C_{v,ss}$). Similarly, arterial and venous capacitances are defined for the inferior systemic circulation ($C_{a,si}$ and $C_{v,si}$) and for the pulmonary circulation ($C_{a,p}$ and $C_{v,p}$). The ratio of C_a to C_v was obtained from the literature [9, 10, 13]. The relationship between pressure (P_c) and volume (V_c) in each capacitance is described by the following linear formula.

$$P_c = \frac{V_c}{C} \quad (5)$$

The changes in volume in each capacitance ($dV(t)/dt$) are described by the differential equations below

$$\frac{dV(t)}{dt} = \sum Q_{\text{in-flow}}(t) - \sum Q_{\text{out-flow}}(t) \quad (6)$$

where $\Sigma Q_{\text{in-flow}}(t)$ and $\Sigma Q_{\text{out-flow}}(t)$ indicate the sum of instantaneous volumetric flow rates at the inlet and outlet of each compartment, respectively. Each of the aortic, mitral, pulmonary, and tricuspid valves is described as an ideal diode with a serially connected small resistor.

In the 1.5VR model, the superior circulation flows from SVC to PA, while the inferior blood flow returns to RA through IVC as shown in Fig. 1a. The models of 2VR (Fig. 1b) and variations of Fontan operation [Fig. 1c, atriopulmonary connection (APC); Fig. 1d, total cavopulmonary connection (TCPC)] are constructed for comparisons. Although the superior and inferior systemic circulations return to RA in both 2VR and APC models, RA is directly connected to PA in the APC model. In the TCPC model, SVC and IVC are directly connected to PA. All parameter values were the same for all of these models except total stressed blood volume (see below) (Table 1).

Hypoplastic RV

Hypoplastic RV is physiologically characterized by an increase in RV stiffness caused by hypertrophy and fibroelastosis of RV muscles [6]. Recalling Eq. 2 for RV, we have:

$$P_{\text{ed,RV}} = A_{\text{RV}}\left[e^{B_{\text{RV}}\left(V_{\text{ed,RV}} - V_{0,\text{RV}}\right)} - 1\right] \quad (7)$$

where B_{RV} is stiffness constant of RV. The value of B_{RV} was changed stepwise from 0.023/ml (normal RV) to 0.143/ml (extremely stiff RV) in increments of 0.01/ml to simulate the various degrees of RV stiffness associated with hypoplasia (Fig. 2).

Protocols

First, the control state was simulated by the 2VR model with normal RV stiffness constant ($B_{\text{RV}} = 0.023$). The total stressed blood volume (V_s), equal to the sum of the stressed volumes in each capacitance and the volume of each chamber, was set as 750 ml to reproduce normal hemodynamics.

$$V_s = V_{\text{LV}} + V_{\text{RV}} + V_{\text{LA}} + V_{\text{RA}} + V_{\text{Ca,ss}} + V_{\text{Cv,ss}} + V_{\text{Ca,si}} + V_{\text{Cv,si}} + V_{\text{Ca,p}} + V_{\text{Cv,p}} \quad (8)$$

We solved these simultaneous equations (Eqs. 1–8) using the component ODE45 of MATLAB, based on the Runge–Kutta method (MathWorks). The hemodynamic parameters of 2VR with normal RV stiffness constant are listed in Table 2.

Next, systemic cardiac output, pulmonary arterial pressure (PAP), right atrial pressure (RAP), and RVEDV after each procedure were calculated for each RV stiffness constant. Heart rate was kept constant and mean systemic arterial pressure (MAP) was controlled at the same value as that of the control state, by adjusting the total stressed blood volume.

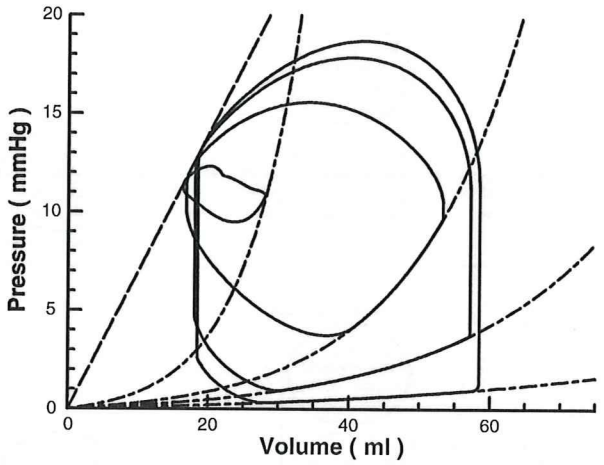

Fig. 2 Right ventricular pressure–volume loops (PV loop) after one and a half ventricle repair. With the increase in the right ventricular stiffness constant, the PV loop became smaller. The *horizontal axis* is the instantaneous right ventricular volume (ml) and the *longitudinal axis* is the instantaneous right ventricular pressure (mmHg)

Table 2 Control hemodynamic parameters (2VR with normal RV stiffness constant)

Parameter	Value
Heart rate (HR), beats/min	75
Mean systemic arterial pressure (MAP), mmHg	80.3
Mean pulmonary arterial pressure (PAP), mmHg	13.6
Mean right atrial pressure (RAP), mmHg	2.34
Mean left atrial pressure (LAP), mmHg	8.26
Left ventricular cardiac output (CO), l/min	4.95

Results

Figure 3a shows the impact of the RV stiffness constant on systemic cardiac output after each procedure. In the Fontan circulation (APC and TCPC), systemic cardiac output was independent of the RV stiffness constant and remained at 4.40 l/min. Under the condition of normal RV stiffness constant, systemic cardiac output was 4.95 l/min in 2VR and 4.73 l/min in 1.5VR, being 13 and 8% greater than that of Fontan circulation, respectively. As the RV stiffness constant was increased from the control value to mimic increased severity of RV

hypoplasia, systemic cardiac output decreased in both 2VR and 1.5VR circulations. Within the range between 100 and 150% of the control RV stiffness constant, systemic cardiac output of 2VR circulation was obviously greater than those of other two circulations. With the RV stiffness constant >150%, systemic cardiac output became greater in 1.5VR than in 2VR. In this situation, 2VR needed larger stressed blood volume than 1.5VR to maintain MAP (Fig. 3d).

The results for PAP and RAP are shown in Fig. 3b. As the RV stiffness constant increased, PAP decreased and RAP increased in both 2VR and 1.5VR circulations. In 2VR circulation, RAP increased steeply as the RV stiffness constant increased up to 150% of normal, and exceeded the atrial pressure of TCPC when the RV stiffness constant increased above 150% of normal. In 1.5VR circulation, RAP also increased but more slowly and exceeded the atrial pressure of TCPC only when the RV stiffness constant increased above 250% of normal. PAP in 1.5VR circulation, which was equal to SVC pressure, became higher than PAP in 2VR circulation in the range of RV stiffness constant >150% of normal.

In the control state, RVEDV in 2VR was 87.7 ml, which was treated as the value of 100% of RVEDV. The influence of the RV stiffness constant on RVEDV is shown in Fig. 3c. In 2VR circulation, RVEDV decreased as the RV stiffness constant increased. In 1.5VR circulation, RVEDV reduced only slightly with an increase in the RV stiffness constant until 250% of normal. In the range of RV stiffness constant >250% of normal, RVEDV showed a relatively linear decay in both 2VR and 1.5VR circulations, and there was no difference in RVEDV between 2VR and 1.5VR. In this situation, both 1.5VR and 2VR needed larger stressed blood volume than Fontan circulation (Fig. 3d).

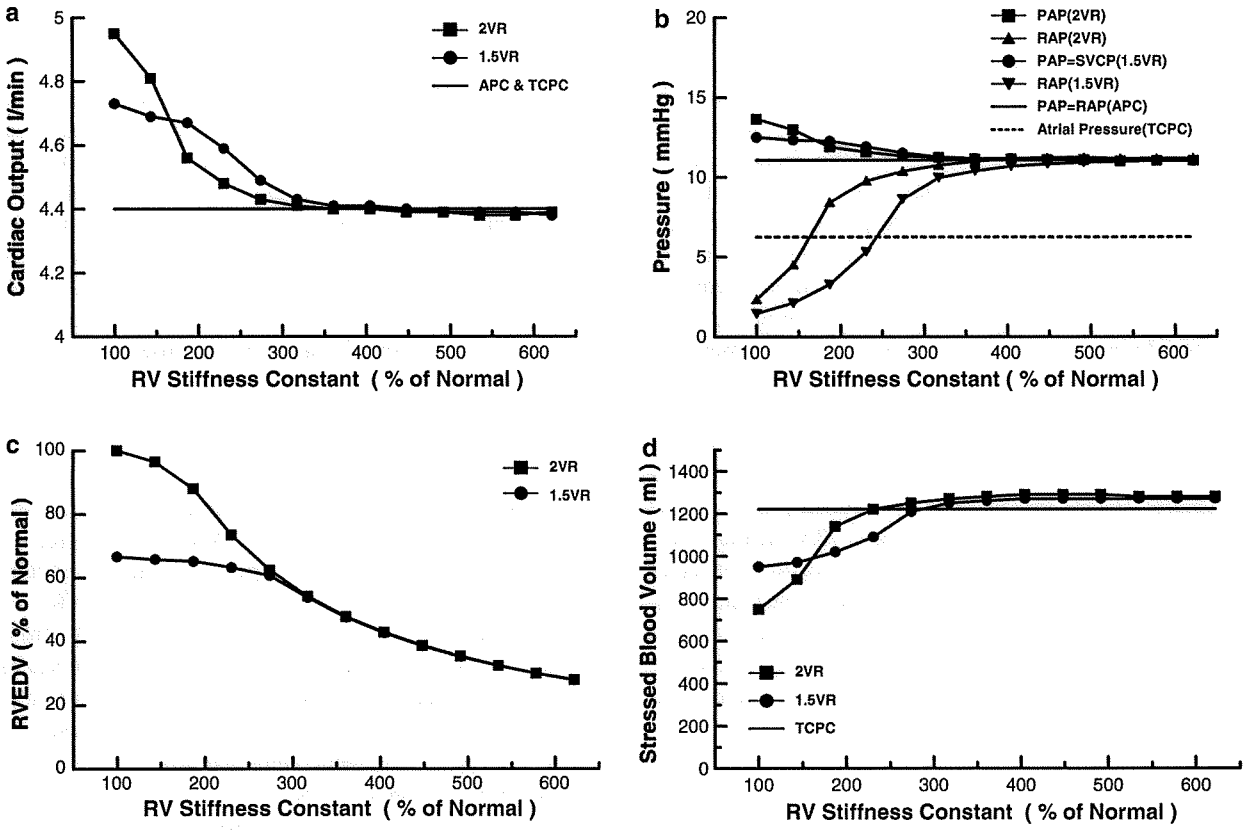

Fig. 3 **a** The relationship between systemic cardiac output (l/min) and % stiffness constant of hypoplastic right ventricle. The *horizontal axis* is the ratio of RV stiffness constant (% stiffness constant) to the normal value. **b** The relationship between pulmonary arterial pressure or right atrial pressure (mmHg) and % stiffness constant of hypoplastic RV. Pulmonary arterial pressure is the same as right atrial pressure in APC. **c** The relationship between % RVEDV and % stiffness constant of hypoplastic RV. **d** The relationship between stressed blood volume (ml) and % stiffness constant. *2VR* biventricular repair, *1.5VR* one and a half ventricle repair, *APC* and *TCPC* variations of Fontan operation (*APC* atriopulmonary connection, *TCPC* total cavopulmonary connection); *PAP* pulmonary arterial pressure, *RAP* right atrial pressure, *SVCP* superior vena caval pressure, *RVEDV* right ventricular end-diastolic volume

Discussion

The results of this theoretical analysis suggest that, in patients with hypoplastic RV, postoperative hemodynamics depends largely on the RV stiffness constant. PA/IVS, Ebstein's anomaly or their relatives are characterized by varying degrees of underdevelopment of RV. For a severely hypoplastic RV, the definitive treatment is single ventricular circulation. For a mildly hypoplastic RV, biventricular circulation is expected to have merit. Recently, 1.5VR has been proposed to reduce the surgical risk of 2VR. The use of 1.5VR has lowered the early or midterm mortality, and adequate growth of RV and the tricuspid valve has been documented in some patients [2]. However, the postoperative RV dysfunction or arrhythmic event has also been reported, in particular, when the patients are on the borderline of criteria between 1.5VR and Fontan operation [4, 5].

For the choice of surgical options among Fontan operation, 1.5VR, and 2VR, the previously used criteria were based on morphologic characteristics of the hypoplastic RV, such as RVEDV. However, simple anatomic indices may be inaccurate, since these values are dependent on the afterload and preload conditions. For that reason, the treatment strategy for hypoplastic RV based on the anatomic indices remains controversial. We focused on the intrinsic property of hypoplastic RV, i.e., RV stiffness constant. The fact that the RV stiffness constant, an index of chamber property, is relatively independent of the loading condition is important for the accurate prediction of postoperative hemodynamics. Based on the results of the present study, we propose that patients with hypoplastic RV can be classified into three groups according to the RV stiffness constant. The first group consists of patients with mild RV hypoplasia (RV stiffness constant <150% of normal), in whom enlargement of RV is expected after the operation. At the other extreme, the second group consists of patients with severe RV hypoplasia (RV stiffness constant >250%), in whom no RV reconstruction is expected to have merit. In addition, we have shown that there certainly exists a third group consisting of patients with intermediate RV hypoplasia (RV stiffness constant between 150 and 250%), who would benefit more from 1.5VR than from 2VR or Fontan operation.

Mild RV hypoplasia

When RV hypoplasia is mild (RV stiffness constant <150% of normal), systemic cardiac output is greater in 2VR than in 1.5VR or Fontan operation (APC or TAPC). Therefore, we recommend that 2VR should be chosen in the mild RV hypoplasia group. Although systemic cardiac output in 1.5VR is also greater than that in Fontan operation, SVC pressure (which is equal to PAP) is higher than that of APC. Accordingly, the upper part of the body is exposed to higher SVC pressure in 1.5VR, which may cause postoperative pleural effusion [2]. A large pressure gradient between SVC and IVC also results in abnormal venous collaterals from SVC to IVC [17–20], and they could effectively increase the venous return to RA in 1.5VR.

Intermediate RV hypoplasia

When RV hypoplasia is intermediate (RV stiffness constant between 150 and 250% of normal), systemic cardiac output in 1.5VR exceeds that in 2VR. Although SVC pressure is still higher in 1.5VR than in APC, RAP is lower in 1.5VR than in the other procedures. This condition is favorable to reduce supraventricular arrhythmias related to high RAP during the perioperative periods. This beneficial effect is not expected for 2VR since RAP in 2VR is higher than the atrial pressure of TCPC. Furthermore, 1.5VR is advantageous from the viewpoint of stressed blood volume because 1.5VR needs smaller stressed blood volume than does 2VR to maintain MAP (Fig. 3d).

In these patients, RVEDV in 1.5VR is relatively independent of the RV stiffness constant. However, abnormal systemic venous collateral channels might open after 1.5VR. These collateral channels would increase RV preload wastefully and decrease systemic cardiac output in the late postoperative phase. In such conditions, conversion to the Fontan circulation may be required in the late phase [4, 5]. Nevertheless, 1.5VR should be recommended for the intermediate RV hypoplasia group because high cardiac output and low RAP are anticipated.

Severe RV hypoplasia

When RV hypoplasia is severe (RV stiffness constant >250% of normal), neither 1.5VR and 2VR are expected to improve systemic cardiac output. In this condition, RVEDV is almost the same between 1.5VR and 2VR, and linearly decreases with an increase in the RV stiffness constant in spite of a rapid elevation in RAP. This indicates that RVEDV might be independent of the venous return to RA. Since RAP becomes higher than the atrial pressure of TCPC even in 1.5VR, supraventricular arrhythmias caused by high RAP are liable to occur [2, 5]. In this condition, 1.5VR is considered to have hemodynamics equivalent to APC and needs larger stressed blood volume than does TCPC to maintain systemic arterial pressure (Fig. 3d).

Therefore, TCPC should be chosen for patients with severe RV hypoplasia. In these patients, the arrhythmic events after TCPC are less than that after APC [21, 22]. Although a small pressure gradient between SVC and IVC

remains in 1.5VR, this may not be of clinical significance. Systemic venous collateral channels are expected to be rare, and an increase of RV volume after the operation is unlikely.

Clinical implication

The management strategy for patients with hypoplastic RV has been based on the morphological characteristics, which are dependent on the loading conditions. In contrast, we used a relatively load-independent index, RV stiffness constant, and simulated the postoperative hemodynamics. As a result, we identified the characteristics of hemodynamics after each of the surgical options, and clearly defined the indications of these operations.

Moreover, our results may be useful to theoretically speculate the reason for contrasting clinical findings. Chowdhury and colleagues [2] reported that the event rate of supraventricular arrhythmia was about 15% in the late postoperative phase of 1.5VR. On the other hand, Numata et al. [5] reported higher arrhythmic event rate. In the former report, the patients had a relatively high postoperative RV volume (45–75% of predicted normal RV; Fig. 3c) and a large pressure gradient between SVC and IVC (mean 7.6 mmHg; Fig. 3b) after 1.5VR. Indeed, there was significant pleural effusion in 22.7% of patients. Our results suggest that good systemic cardiac output, low IVC pressure, and high SVC pressure after 1.5VR can be expected under a condition of a relatively small RV stiffness constant. A great difference between SVC and IVC pressures may cause pleural effusion. Therefore, patients in the former report are likely to have low RV stiffness. In the latter report, the average RVEDV at 1 year after 1.5VR was about 50% of normal and there was no obvious collateral after the surgery in the patients examined. These data suggest a high RV stiffness (Fig. 3c), and a small difference between SVC and IVC pressures (Fig. 3b). Since higher arrhythmic event rate is likely to be associated with high RAP in patients with high RV stiffness, we can interpret the marked difference in arrhythmic event rate in these studies based on postoperative hemodynamics. Operations with 1.5 VR in potentially inappropriate patients (i.e., patients with stiffer RV) might impair long-term outcomes by continued high RAP-induced arrhythmia.

If we can assess the RV stiffness constant and other hemodynamic data in a catheter laboratory before operation, we will be able to select the most suitable operation for patients with hypoplastic RV. Recently, noninvasive methods for predicting LV chamber stiffness using echocardiography have been reported [23–25]. For example, LV chamber stiffness has been estimated from the deceleration time of LV early filling, effective mitral area and length. Such a method may be applied to estimate RV chamber stiffness using the deceleration time of RV early filling, effective tricuspid area and length. Moreover, it may be possible to choose an appropriate procedure for individual patients by performing simulation of postoperative hemodynamics from individual data using our model. Further clinical studies are needed to precisely assess the RV stiffness constant, including the above methods.

Limitations

A major limitation of this study is related to the parameters we used for the model. In our model, all parameters other than the RV stiffness constant are fixed. It is reported that RV end-systolic elastance as well as the RV stiffness constant depend upon RV histological changes such as RV hypertrophy [26]. The increase in RV end-systolic elastance moves the beneficial range of 1.5VR toward the stiffer range of the RV stiffness constant. The increase of heart rate also moves the range toward the stiffer range (Table 3). Moreover, ischemia caused by long-standing hypoxemia and hypertension of RV may influence other variables [6]. The existence of pulsatility of the pulmonary circulation may also affect the pulmonary vascular resistance [27]. Tricuspid regurgitation may also impair the postoperative hemodynamics. These limitations may be solved by using the preoperative data of individual patients. Santamore and Burkhoff have already reported the importance of ventricular interdependence using a computer model [13]. However, ventricular interdependence between small hypoplastic RV and relatively large left ventricle may be negligible.

Table 3 The influence of right ventricular end-systolic elastance and heart rate on the beneficial range of the one and a half ventricle repair

	Lower limit of RV stiffness constant (% of normal)	Upper limit of RV stiffness constant (% of normal)
$E_{es,RV} = 0.7$, HR = 75	150	250
$E_{es,RV} = 1.4$, HR = 75	200	300
$E_{es,RV} = 0.7$, HR = 100	175	275

RV Right ventricle, $E_{es,RV}$ right ventricular end-systolic elastance (mmHg/ml), *HR* heart rate (beats/min)