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Effects of endocardial endothelium in myocardial mechanics of hypertrophied myocardium of rats[1]

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AIM: To investigate the effects of endocardial endothelium (EE) in myocardial mechanics of hypertrophied myocardium of rats. **METHODS**: Hypertrophied myocardium has been developed through long-term renovascular hypertension in rats. Selective stripping of EE was achieved chemically and the effects of EE on the activation and relaxation of hypertrophied myocardium were investigated and compared to those in the presence and absence of intact EE. **RESULTS**: Resting tension (RT) and peak developed tension (DT) of isometric contraction of the left ventricular papillary muscles of renovascular hypertensive rats (RHR) remained similar, but maximal rate of tension development and fall ($+dT/dt_{max}$ and $-dT/dt_{max}$) were lowered, time to $+dT/dt_{max}$ (TPP) and time to $-dT/dt_{max}$ (TPN) were increased, and time to peak developed tension (TPT) and half relaxation time ($RT_{\frac{1}{2}}$) were prolonged, *vs* sham-operated rats (Sham). After EE denudation, TPT, and $RT_{\frac{1}{2}}$ were significantly abbreviated in RHR. The $-dT/dt_{max}$ was significantly augmented while the $+dT/dt_{max}$ was unaltered, resulting in a prominent decrease in the ratio of $+dT/dt_{max}$ to $-dT/dt_{max}$. **CONCLUSION**: EE predominantly influences the relaxation in isolated myocardium and early diastolic filling events in hearts, and is involved in the cardiac compensatory mechanism in hypertrophied myocardium.

KEY WORDS endocardium; renovascular hypertension; heart hypertrophy; myocardial contraction; papillary muscles

Heart hypertrophy is a positive adaptive process allowing temporary compensation of raised demands on blood circulation[1]. As a consequence of long-standing cardiac overloading, various hormonal systems, eg, catecholamine, renin-angiotensin, vasopressin, atrial natriuretic factor (ANF), are also activated. Thus, these intrinsic and extrinsic cardiac compensatory mechanisms are elicited and act in concert to compensate for the overloading conditions. Recently, however, there has been growing experimental evidences that endocardial endothelium (EE) directly modulates the performance of subjacent myocardium[2-5] and that EE is capable of altering the inotropic responses to a variety of agents related to cardiac compensatory mechanisms. Stimulation of EE by ANF[4], vasopressin[7], and substance P[8] results in a similar abbreviation of contraction, and the effect is abolished by prior removal of EE. The EE has also been shown to modulate the inotropic responses of other agents, eg, serotonin, ATP, endothelin, angiotensin, phenylephrine, platelets, eosinophil, and other circulating substances. Thus, EE may participate either directly or indirectly in the cardiac compensatory mechanisms. Although several lines of investigation suggested us to postulate that hypothesis, there has been no direct supporting evidence. In an attempt to examine the functional role and pathophysiological sig-

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nificance of EE in the cardiac compensatory mechanism, the present study has been developed through long-term renovascular hypertension in rats. Selective stripping of EE was achieved chemically and the effects of EE on the activation and relaxation of hypertrophied myocardium were investigated and compared to those in the presence and absence of intact EE.

MATERIALS AND METHODS

Heart hypertrophy Wistar rats, ♂, $n = 20$, weighing 178±21 g, anesthetized with pentobarbital sodium (45 mg kg^{-1} ip). The renal hypertension was made by placing a silver clip with a circle (ID 0.3 mm) around the left renal artery, which was occluded by the silver clip to about 50 %–70 %[9]. The contralateral kidney remained untouched. Age-matched, sham-operated rats (Sham) underwent the same procedures but no clip was used. All rats were given a standard dry chow and water. Systemic blood pressure (SBP) was measured by the tail-cuff method (Buffington 101A, USA) before clipping and at 1-wk intervals thereafter. Rats were considered hypertensive only if SBP increased to 20.0 kPa (150 mm Hg) within 3 wk after clipping.

The renovascular hypertension was maintained for 8 consecutive weeks after the onset of hypertension. Then the renovascular hypertensive rats (RHR) and Sham were decapitated and their hearts were excised. After a left ventricular papillary muscle was prepared for the mechanical study, the ventricles were trimmed of atria and visible blood vessels. The left and right ventricles were thoroughly washed with ice-cold buffer solution. The left ventricular weight (LVW), right ventricular weight (RVW), the ratio of LVW and RVW (LV/RV), and the ratio of ventricular weight (VW) and body weight (BW) were utilized to assess the degree of left ventricular hypertrophy.

Papillary muscle preparation The rats were stunned. A left ventricular papillary muscle was mounted in a bath with Krebs-Ringer solution, as previously described[10]. The solution was kept at 30 C and bubbled with 95 % O_2+5 % CO_2 at pH 7.4. The muscle was stimulated at 50 % above threshold at 12 stimuli/min with a stimulator (SEN 3201 Nihon Kohden) through platinum field electrodes. The preload was adjusted so that the muscle was at a length at which maximal developed tension occurred (L_{max}). The muscle was stabilized for 2 h, and then the endothelial layer of endocardium was denuded by immersing the papillary muscle in 0.5 % Triton X-100 (Sigma Co) dissolved in Krebs-Ringer solution at 30 C for 1 s and then washed thoroughly. This technique has been shown to remove the EE without damaging the myocardial cells[11]. The preparations were restabilized for 2 h, and isometric contraction was resumed. The first derivatives were recorded at $CaCl_2$ 1.25 mmol L^{-1} by polygraph (RM-6000, Nihon Kohden). An isometric transducer (UC2, Gould Statham, USA) was used.

After equilibration of isometric contraction at a resting tension (RT) of 1 g, the tension of each muscle was measured by the increase of muscle length with a micrometer in 0.1 mm steps from a length at which minimal developed tension occurred (L_0) to L_{max}. The passive and active length-tension relationships were obtained. At L_{max}, the baseline values of the following parameters were measured: RT, peak developed tension (DT), maximal rate of tension rise and fall ($+dT/dt_{max}$, $-dT/dt_{max}$), time to $+dT/dt_{max}$ (TPP), time to $-dT/dt_{max}$ (TPN), time to peak developed tension (TPT), and time to half relaxation ($RT_{\frac{1}{2}}$). After the mechanical data in basal state were collected, the frequency of stimulation was increased from 0.2 to 1.5 Hz and the relation between stimulation and DT or relaxation time were observed. At the end of the experiment, the muscle was blotted dry and weighed. The cross-sectional area (CSA) of the muscle was scrutinized and the force and velocity were normalized by CSA.

Statistical analysis Results were analyzed by t test for unpaired observations. For multiple comparisons, an ANOVA followed by a Newman-Keuls test was used to compare the individual groups.

RESULTS

Heart hypertrophy There was no significant decrease in the BW. SBP in RHR was much higher than that in Sham. Persistent renovascular hypertension for 8 consecutive weeks led to the heart hypertrophy evidenced

by LVW and VW/BW. Compared to the Sham, the left ventricular mass increased by approximately 20 %, while the ratio of VW/BW increased by 21 %. SBP and VW/BW were closely correlated. The papillary muscle weight (PMW) and CSA were slightly increased in RHR (Tab 1).

Tab 1. Characteristics of Sham and RHR and their papillary muscles with/without EE. $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* sham; $^dP>0.05$ *vs* HT+EE.

Parameters	Sham	HT+EE	HT−EE
BW/g	292±28	284±36[d]	274±22[ad]
SBP/kPa	15.3±0.7	27.1±1.7[c]	28±3[c]
DBP/kPa	8.7±0.7	20.5±2.9[c]	20±4[c]
LV/mg	657±98	772±97[b]	747±106[b]
RV/mg	160±31	154±16[d]	154±31[ad]
LV/RV	3.8±0.5	5.0±0.5[c]	4.9±0.6[c]
VW/BW	2.8±0.3	3.4±0.2[c]	3.4±0.3[c]
PMW/mg	3.93±1.33	5.35±2.38[a]	4.92±1.42[ad]
L_{max}/mm	4.65±1.00	5.13±0.99[a]	5.42±0.66[ad]
CSA/mm^2	0.76±0.17	0.98±0.34[a]	0.91±0.23[ad]

Myocardial mechanics in intact hypertrophied heart RT and DT of isometric contraction of the left ventricular papillary muscles remained similar in both Sham and RHR. The $+dT/dt_{max}$ and $-dT/dt_{max}$ were declined, but the ratio of them was unaltered compared with sham control. Simultaneously, TPT and $RT_{\frac{1}{2}}$ were significantly prolonged, TPP and TPN showed a tendency to increase ($P>0.05$) compared with sham control (Tab 2).

Myocardial responses in EE-denuded hypertrophied heart The muscle length at the apex of the active length-tension curves was defined as L_{max}, and changes of muscle length were expressed as % of L_{max}. In the RHR, RT and DT at L_{max} were similar before and after EE removal (Tab 2) and no alteration in active and passive length-tension relationships was seen, even compared with Sham. Analysis of the RT showed no obvious differences in

Tab 2. Twitch configuration of electrically-paced (0.2 Hz) left ventricular papillary muscles with intact EE from Sham, and of papillary muscles with/without EE from RHR. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* HT+EE; $^dP>0.05$, $^eP<0.05$ *vs* Sham.

Parameters	Sham	HT+EE	HT−EE
RT, mN/mm^2	5.9±0.7	6.0±1.3[d]	5.5±0.9[a]
DT, mN/mm^2	9.2±2.4	9.4±2.6[d]	9.1±2.0[a]
TPT, ms	106±20	131±16[c]	103±11[c]
$RT_{\frac{1}{2}}$, ms	87±8	110±20[c]	78±11[c]
$+dT/dt_{max}$, mN mm^{-2} s^{-1}	140±18	116±17[a]	118±20[a]
$-dT/dt_{max}$, mN mm^{-2} s^{-1}	74±15	61±16[d]	84±18[b]
Ratio	1.89±0.22	1.90±0.13[d]	1.40±0.13[c]
TPP, ms	44±8	50±10[a]	40±8[a]
TPN, ms	85±12	101±14[d]	76±11[c]

the hypertrophied myocardium with intact EE (HT+EE) and denuded ones (HT−EE). EE stripping displayed a slight tendency to decrease the DT. With the rise in stimulation frequency to 1.5 Hz, the DT decreased dramatically in RHR, being more marked in EE-denuded muscle preparations, even though the difference between the groups was insignificant for statistical analysis (Fig 1).

After EE denudation, $+dT/dt_{max}$ was unaltered in RHR. In contrast, $-dT/dt_{max}$ was significantly augmented, resulting in a prominent decline in the ratio of $+dT/dt_{max}$ to $-dT/dt_{max}$ (Tab 2). Meanwhile, TPN was decreased and similar results were founded in TPT and $RT_{\frac{1}{2}}$. In addition, the shortening of relaxation of isometric contraction remained stable at different muscle lengths (Fig 2) or stimulation frequencies (Fig 3) in EE-denuded muscle preparations.

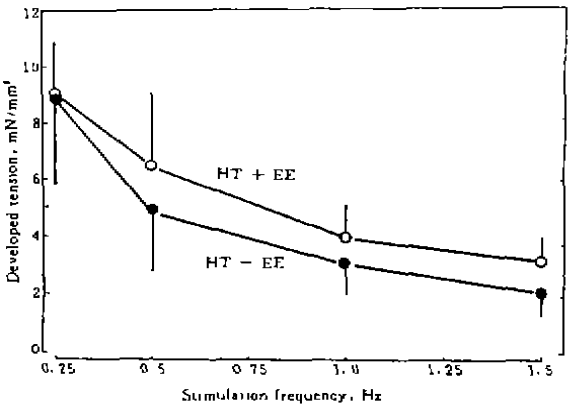

Fig 1. Relation between stimulation frequency and DT of rat hypertrophied papillary muscle with/without EE. $n=6$, $\bar{x}\pm s$.

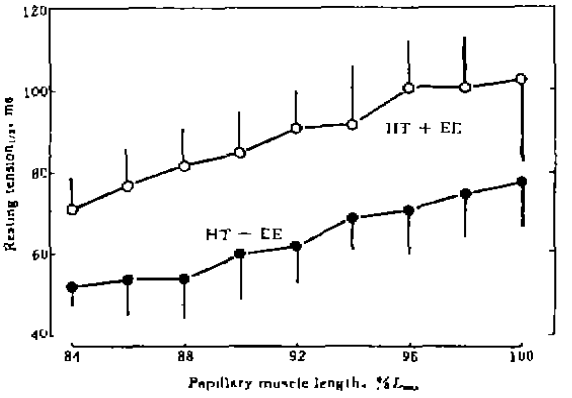

Fig 2. Papillary muscle length *vs* $RT_{\frac{1}{2}}$ in hypertrophied papillary muscle with/without EE. $n=6$, $\bar{x}\pm s$.

DISCUSSION

In the present study, left ventricular hypertrophy was definitely achieved, as evidenced by weight variables of left ventricle and the heart. Normal contractility of hypertrophied myocardium was observed even though the twitch configuration of isometric contraction was altered. This was supported by the findings of Ding & Li[9]. The resting length-tension curves, reflecting the relaxant

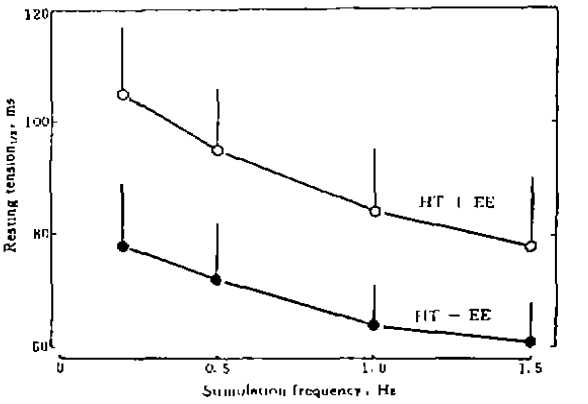

Fig 3. Stimulation frequency *vs* $RT_{\frac{1}{2}}$ of hypertrophied papillary muscle with/without EE. $n=6$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* HT+EE.

stiffness, showed no significant alteration. But, hypertension for 8 consecutive weeks caused prolongation of isometric time to DT and $RT_{\frac{1}{2}}$. $+dT/dt_{max}$ was depressed. The results thus further supported the idea that the hypertrophied myocardium was characterized by impaired intrinsic contractility in basal state although muscle force development was well maintained through a compensatory mechanism.

There is growing evidence that relaxation is intrinsically a much slower process than activation[12] and impaired relaxation is one of the earliest manifestations of hypertrophy and usually determines the overall function of the heart[13]. In this study, effects of EE on inotropic and relaxant responses were investigated for the first time in the hypertrophied myocardium. EE removal did not affect DT and the velocity of tension development. But the duration of twitch contraction, in particular relaxation time was shortened and relaxation velocity was slowed. It was therefore interesting that EE was likely to influence predominantly relaxation and early diastolic filling events. This could have pathophysi-

ological relevance, especially in hypertrophy where filling was impaired. Thus, damage to the EE might also reduced the stroke volume. Furthermore, in various animal models of experimental congestive heart failure, Rouleau *et al* observed extensive morphological damage of EE in both ventricles[10,11]. So from these studies, it was implied that EE could play a role in the development of cardiac hypertrophy.

In summary, the results of our study showed that myocardial mechanics of hypertrophied myocardium was intrinsically modulated by EE, even though muscle force development was well maintained. The data provided direct evidence for the pathophysiological significance of EE in hypertrophied myocardium.

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心内膜对大鼠肥厚心肌力学的影响[1]

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目的：观察心内膜对大鼠肥厚心肌力学的影响。**方法**：采用两肾一夹肾性高血压大鼠离体乳头肌标本，化学去内膜法。

结果：肾血管性高血压大鼠肥厚乳头状肌等长收缩静息张力、发展张力与假手术组相似，$\pm dT/dt_{max}$降低，$+dT/dt_{max}$及$-dT/dt_{max}$时程、收缩峰值时间及半数舒张时间延长。去除心内膜心肌标本收缩峰值时间及半数舒张时间显著缩短，$-dT/dt_{max}$显著升高而$+dT/dt_{max}$无改变，因而两者比值显著下降。

结论：心内膜主要影响心肌舒张反应及舒张早期充盈，在心肌肥厚代偿机制中起到一定作用。

关键词 心内膜；肾血管性高血压；心脏肥厚；心肌收缩；乳头状肌