Activated Matrix Metalloproteinase and Disrupted Myocardial Collagen Matrix in Increased Sympathetic Activity Following Stimulation of Dorsal Medulla in the Vagotomized Feline Model

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Abstract

Sympathetic hyperactivation in many kinds of neurocardiogenic injury can result in obvious heart failure. We generated a vagotomized feline model in which sympathetic hyperactivation was induced by electrical stimulation of dorsal medulla (ESDM) of brain stem to investigate the relationship between disruption of extracellular collagen matrix (ECM) and activation of matrix metalloproteinases (MMPs) in myocardium in the sympathetic hyperactivity. Mean blood pressure, heart rate and plasma norepinephrine were all significantly increased from baseline to a peak at 5 min after ESDM. Echocardiographic study showed significant left ventricular dilatation and hypokinesia (ejection fraction: from 87.7 ± 6.3% to 39.4 ± 7.8%) from baseline to 180 mm after ESDM. Histopathological finding revealed significant overstretching or spring-like disappearance and disruption of ECM. MMP-2 expression was significantly increased in left ventricular myocardium as compared to sham. These results suggest that ESDM-induced sympathetic hyperactivity causes the expression of MMP-2 that disrupts myocardial ECM, contributing to the development of cardiac dysfunction.

Key Words: extracellular collagen matrix, matrix metalloproteinases, sympathetic nervous activity, heart failure, animal model

Introduction

Sympathetic activation by neurocadiogenic injuries (NCI) including subarachnoid hemorrhage (SAH) (14, 15, 24, 32), rhombencephalitis (10) and stroke (3, 11), is a complicated process involving

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cardiac dysfunction and myocardial damage. The cardiac dysfunction is induced by an excessive secretion of norepinephrine (NE) following the remarkable activation of the sympathetic nervous system immediately after onset of NCI. The excessive NE secretion elevates the levels of myocardial matrix metalloproteinases (MMPs) which can degrade extracellular collagen matrix (ECM) in the myocardium (1, 2, 31). Merters *et al*., however, proposed that in animal models the direct release of cardiotoxic levels of NE into the myocardium by the cardiac sympathetic nerve terminals was a more likely cause of NCI than adrenal release of NE into the systemic circulation (16). Disruption of ECM therefore may compromise cardiac function and even induce heart failure.

The existence of neurons in the dorsal medulla essential for integration of basal vasomotor tone has been well documented (4, 26, 30). Electrical stimulation of dorsal medulla (ESDM) of brain stem produces large pressor responses and increases in sympathetic outflow, a state of sympathetic activation (4, 26). Therefore, we proposed that the ESDM-induced sympathetic activation might elicit excessive NE secretion, over expression of MMPs, and disruption of ECM, finally leading to cardiac dysfunction. We for the first time demonstrated that ESDM in a vagotomized feline model induced over expression of MMP-2 and disruption of myocardial ECM to cause left ventricle (LV) dysfunction.

Materials and Methods

Animal and Experimental Procedure

The investigation was conformed by the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committees of Taichung Veterans General Hospital.

ESDM was performed in five cats (3.23 ± 0.26 kg), as described previously (6). Five cats without ESDM were used as the sham group. Mean blood pressure (MBP) and heart rate (HR) were determined at 0, 5, 15, 30, 60, 120 and 180 min after ESDM. After completion of the experiment, each cat was immediately sacrificed by intravenous potassium chloride injection. Myocardial specimens were stored in 10% neutralized formalin, 2.5% glutaraldehyde, and liquid nitrogen for histological, ultrastructural, and zymographic examinations, respectively.

Evaluation of Sympathetic Activity

Plasma NE was measured by high performance liquid chromatography at 0, 5, 15, 30, 60, 120 and 180 min after ESDM (5).

Evaluation of Cardiac Size and Function

Transthoracic echocardiography with a 12 mHz probe (Hewlett-Packard 5500, Bothell, WA, USA) was performed at 0, 15, 30, 60, 120 and 180 min after ESDM (6).

Pathological Examination

Myocardial tissues of LV free wall and ventricular septum were fixed in 10% neutralized formalin and then embedded in paraffin. The 6 μm sections were stained by sirius red and fast green, and the 25 to 50 μm sections were stained by silver impregnation method to assess myocardial collagen matrix (7, 8, 13, 29). Scanning electron microscopy was used to investigate the changes of inter-cardiomyocytic matrix (struts) (7).

Detection of MMPs Activity by Substrate Zymography

Activities of MMPs in myocardial extracts were measured by gelatin zymography as described previously with some modifications (9, 11, 25, 29). Frozen LV myocardium from each cat was homogenized with lysis buffer. The 20 μg of proteins in each extract was electrophorized in 8% SDS-PAGE impregnated with 1 mg/ml gelatin. After electrophoresis, gels were renatured, incubated, stained and discolored (29). The images of gels were digitalized and gelatinolytic zones were quantified by Gel-pro Analyzer.

Statistical Analysis

All data are expressed as mean ± SD. Analysis of variance (ANOVA) was used with multiple comparison procedure. The difference between two groups were considered significant with $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$.

Results

Hemodynamic Recordings of MBP and HR

The MBP values were significantly increased at 5 (peak), 15, and 30 min and returned to baseline at 60 min after ESDM (Fig. 1A). HR was significantly increased from 199 ± 22 to 240 ± 15 beats/min at 5 min and then maintained throughout the stimulation period (Fig. 1B). In the sham control, MBP and HR values remained steady at each time point (data not shown).

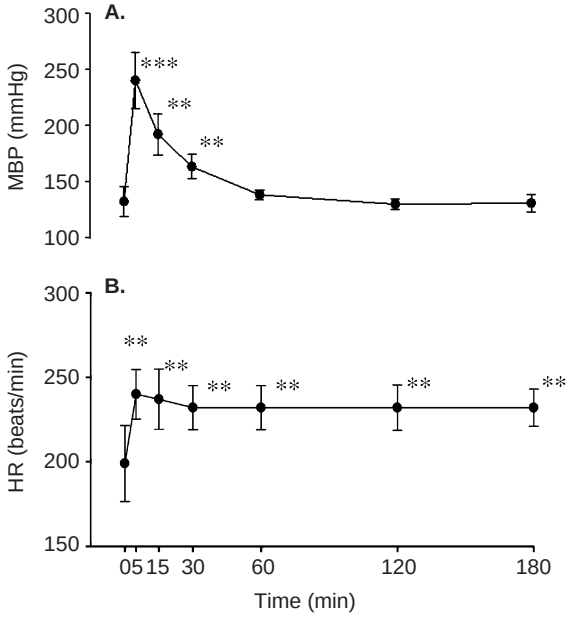

Fig. 1. Time course of hemodynamic recordings after ESDM. Mean blood pressure (MBP) and heart rate (HR) increased immediately and thereafter ESDM. **: $P < 0.01$, ***: $P < 0.001$ *vs.* baseline values.

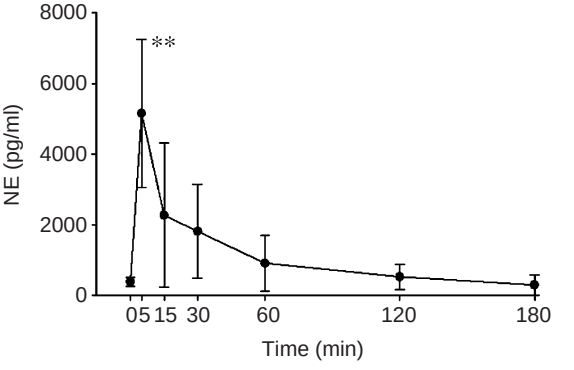

Fig. 2. Sympathetic effects after ESDM. Plasma concentration of norepinephrine (NE) showed significant immediate rise at 5 min peak, compared with basal line ($P = 0.008$), and deescalated to basal line as the similar downside to MBP.

Sympathetic Activity

Plasma concentrations of NE showed a peak rise at 5 min (5.16 ± 2.11 *vs.* 0.38 ± 0.12 ng/ml, $P = 0.008$) after ESDM. After the peak, NE concentrations deescalated to baseline similar to MBP (Fig. 2).

Cardiac Size Function and Morphologic Changes

After ESDM was started, the LV chamber remarkably dilated in long, short-axis view and M-mode of echocardiography (Fig. 3). LV end-diastolic diameter significantly increased at 60 min, compared with baseline (1.95 ± 0.12 *vs.* 1.36 ± 0.14 cm, $P = 0.003$), and remained elevated thereafter (Fig. 4A). LV end-systolic diameter increased from 0.67 ± 0.22 to 1.60 ± 0.21 cm (0 to 180 min, $P = 0.006$) and was significantly dilated after 30min ($P < 0.05$) (Fig. 4A). These changes were accompanied with hypokinesia and decreased ejection fraction from 87.7 ± 6.3 % to 39.4 ± 7.8 % (0 to 180 min, $P < 0.001$) after ESDM and significantly different 15 min later ($P = 0.007$) (Fig. 4B). The morphological changes of experimental heart revealed remarkable dilation of ventricle, congestion and round apex, as compared to sham heart (Fig. 5, A and B).

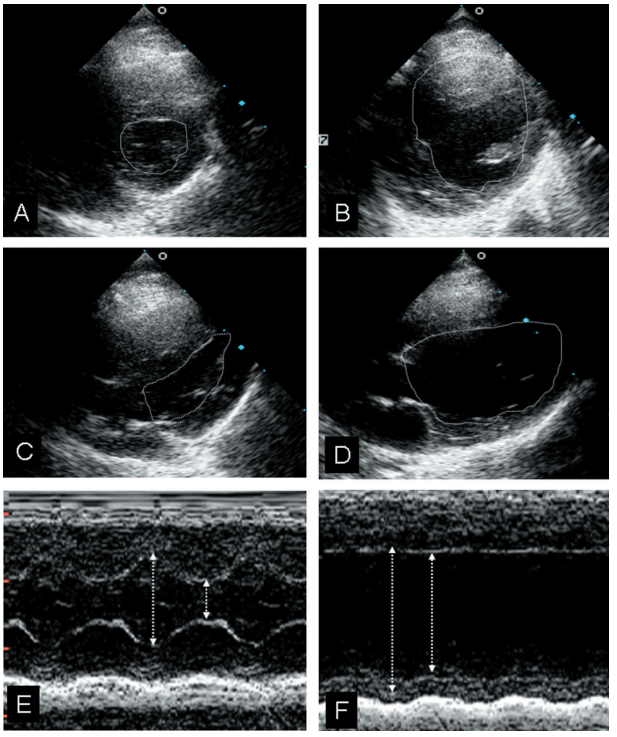

Fig. 3. Two-dimensional echocardiography of basal line and ESDM 60 min later. The short- (Panel A, B), long- (Panel C, D) axis and M-mode (Panel E, F) views show significant dilatation and hypokinesia of the LV after ESDM (right panels), as compared to basal line (left panels).

Cardiac Pathology and ECM Remodeling

In histopathological findings, cardiomyocytes in experimental myocardium exhibited significant elongation, weaving, and fragmentation (Fig. 5, D and F). Myocytolysis and coagulation were also noticed in the myocardium (Fig. 5, E and F). ECM (epimysial weaves, perimysial coils) showed overstretching, spring-like disappearance, fragmentation and disruption

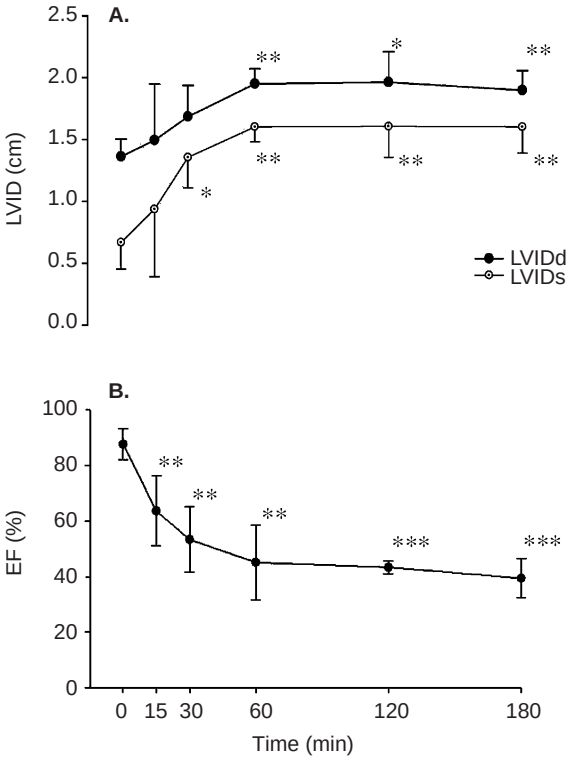

Fig. 4. ESDM induced changes in cardiac size and function. LV end-diastolic ($LVID_d$) and end-systolic diameters ($LVID_s$) increased with time and showed significant increase after ESDM 60 min and 30 min (Panel A). These changes were accompanied with significant hypokinesia with deescalated ejection fraction (EF) after ESDM 15 min (Panel B).

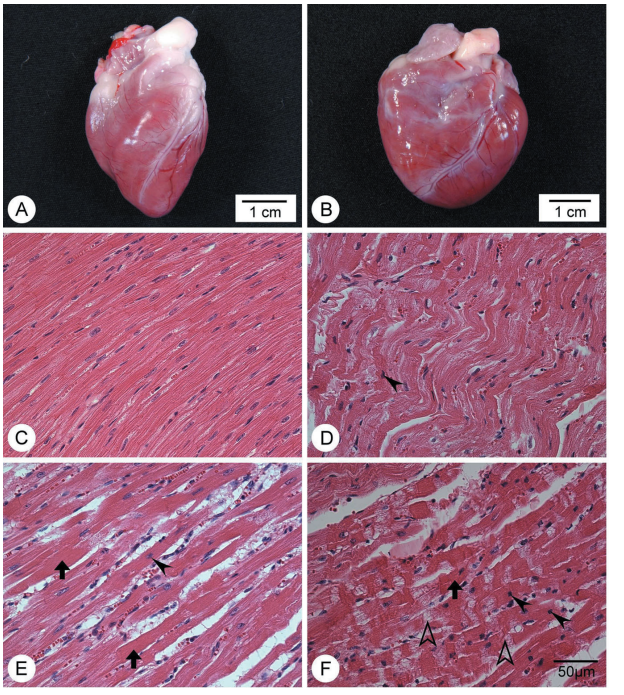

Fig. 5. Gross and histopathologic findings in myocardium. The heart of experimental cat (Panel B) reveals more dilation of both ventricles and round apex than sham (Panel A). Cardiomyocytes in experimental myocardium (Panel D-F) were characterized by significant necrosis (solid arrowhead), coagulation (arrow), weaving (Panel D), elongation (Panel E), and fragmentation (Panel F, hallow arrowhead), which were not found in sham normal myocardium (Panel C).

in Sirius red & Fast green (Fig. 6, A and B) and silver impregnation staining (Fig. 6, C and D). Ultrastructural changes of endomysial struts revealed disruption and decreased number in the intercellular space (Fig. 6, E and F).

MMPs Activity of Myocardium

The gelatinase activity was assessed by zymography analysis, as shown in Fig. 7A, and quantified in Fig. 7B. Only MMP-2 and MMP-9 bands were detectable in both experimental and sham groups. The MMP-2 activity was significantly elevated in the experimental group than sham (P = 0.009).

Discussion

In the present study, we for the first time demonstrated that ESDM induced excessive release of NE, over expression of MMP-2 and disruption of myocardial ECM to cause LV dysfunction.

The ECM-linked cardiomyocytes plays an important role in maintaining normal geometric structure and contractile function of heart. Importantly, alteration or remodeling of these ECM structures leads to morphologic abnormality and contractile dysfunction of the heart (20, 27). A positive correlation between MMPs and myocardial ECM remodeling has been demonstrated in various animal models of heart failure (12, 17, 19, 29). Moreover, increased MMPs levels in myocardium were accompanied by the progression of LV dilation and dysfunction in an LV-failure animal model (21, 22, 29). These changes in myocardial MMPs causes remarkable alteration of ECM structure in microscopic histopathology (29) and ultrastructural pathology (19). Increase in MMP activities is attributed to excessive plasma cardiac NE in patients with CHF and animal models with infusion of NE (18, 23, 28). Similarly, in this study, we also found LV dilation or dysfunction and same pathological changes of ECM structure with increased MMP-2 in myocardium after ESMD in vagotomized cats (Figs 5, 6, and 7).

In conclusion, ESDM can induce excessive secretion of NE that stimulates increase of MMPs to degrade ECM in myocardium, resulting in heart

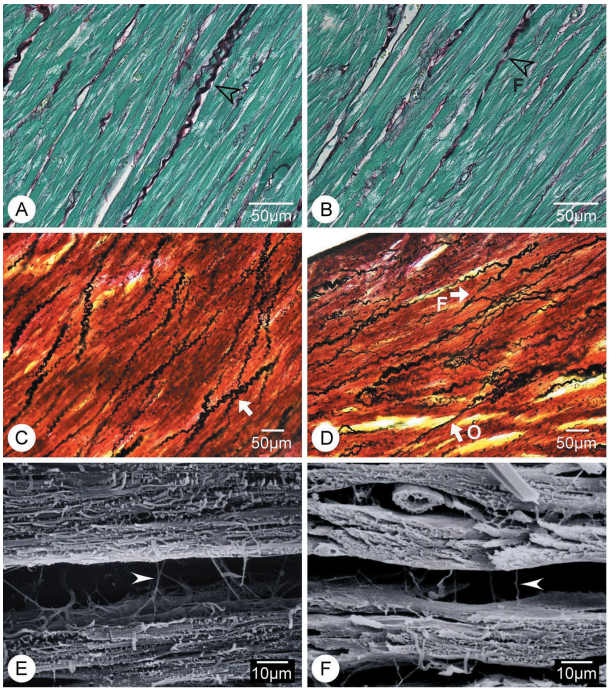

Fig. 6. Comparison of ECM remodeling in special stains and ultrastructure between experimental (right panels) and sham myocardium (left panels). The epimysial weaves (hallow arrowhead), perimysial coils (arrow) showed overstretching or spring-like disappearance (O), fragmentation (F) and disruption in sirius red and fast green (Panel A, B) and silver impregnation staining (Panel C, D). Ultrastructural changes of endomysial struts (arrowhead) revealed disruption and decrease in number in the intercellular space (Panel F), compared to sham (Panel E).

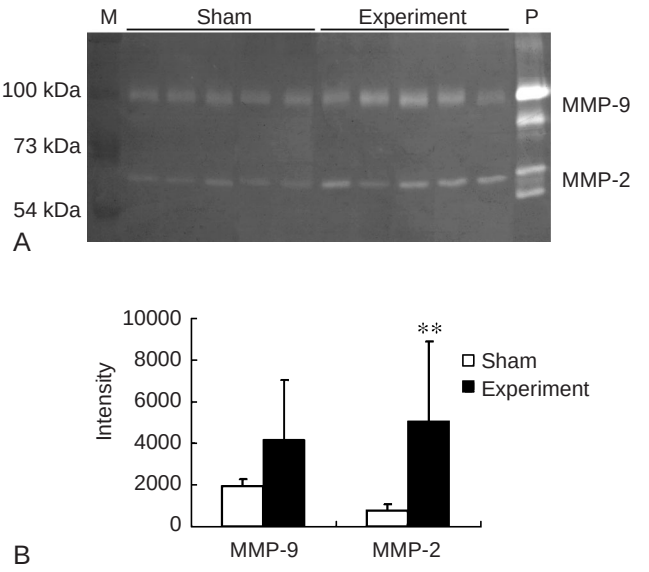

Fig. 7. MMPs activity of left ventricular wall. The gelatin-degrading activity was assessed by zymogram analysis (Panel A) and quantified intensity presented as bar (Panel B). The MMP-2 activity was significantly elevated in the experimental group as compared to sham (P = 0.009).

dysfunction. This model may be applicable for studying therapeutic strategies on heart failure caused by sympathetic hyperexcitation occurring in neurocardiogenic injury patients.

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