Dokkyo Journal of Medical Sciences
33(1) : 35～41, 2006

Originals

Effects of Norepinephrine on Left Ventricular Hemodynamics and Myocardial Blood Flow in Rats with and without Calcium Overload

Takahide Nakajima[a], Ryuko Matsuda[b], Makoto Shinozaki[a], Shichiro Abe[a] and Hidehiko Suzuki[a]

Departments of [a]Cardiology and Pneumology and [b]Clinical Laboratory Medicine, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan

SUMMARY

Heart failure patients have been shown to have an increased blood norepinephrine concentration, and patients with a high norepinephrine concentration have a poor prognosis. Norepinephrine is a catecholamine with α_1 and β_1 effects, which lead to a vasoconstrictive action and enhancement of myocardial contraction. However, the consequences of norepinephrine-induced changes in myocardial blood flow in heart failure patients remain unknown. In this study, the influence of norepinephrine on hemodynamics and blood flow in the left ventricular myocardium was investigated using rats with and without a calcium load. Norepinephrine without a calcium load induced a 29.3% reduction of myocardial blood flow (MBF), but had no significant effect on ejection fraction (EF) and left ventricular end-diastolic pressure (LVEDP). With simultaneous calcium administration, norepinephrine induced a 33.2% reduction of MBF and increased LVEDP significantly, but caused no reduction in EF. These results suggest that norepinephrine decreases MBF but has no effects on systolic function, and increases LVEDP and decreases MBF more markedly in combination with calcium.

Key Words : norepinephrine, diastolic dysfunction, LVEDP, calcium, myocardial blood flow

INTRODUCTION

Heart failure patients with a high plasma norepinephrine concentration have been shown to have a poor prognosis[1], and increased norepinephrine concentration is an important prognostic factor in patients with a history of ischemic left ventricular dysfunction[2]. The role of a high level of plasma norepinephrine with regard to heart failure remains to be explained. Norepinephrine is a catecholamine with α_1 and β_1 effects that enhances contraction of vascular smooth muscle and cardiac muscle[3]. The β_1 effect of norepinephrine increases intracellular calcium ($[Ca^{2+}]i$) and leads to increased myocardial contraction, while the α_1 effect induces vasoconstriction, inhibits oxygenation in tissues, and may increase organ failure.

Calcium ions (Ca^{2+}) play a central role in excitation-contraction coupling, and variation in the amount of active calcium plays a major role in the regulation of myocardial contractility[4]. An increase of diastolic $[Ca^{2+}]i$ level has been demonstrated in postischemic reperfusion[5] and heart failure[6]. An intracoronary bolus of

Received October 31 2005 ; accepted December 15, 2005
Reprint requests to : Takahide Nakajima, M.D.
Department of Cardiology and Pneumology, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan

calcium, given after the onset of ischemic diastolic dysfunction, increased diastolic $[Ca^{2+}]i$, but not diastolic ventricular pressure[7]. It is not clear whether there is a cause-and-effect relationship between increase of diastolic $[Ca^{2+}]i$ and diastolic ventricular pressure. Recently, we reported that norepinephrine under a calcium load induces diastolic dysfunction in rats[8]. Diastolic heart failure is cardiac dysfunction without reduced left ventricular contraction, and is associated with delayed relaxation, impairment of blood filling and left ventricular stiffness[9~12]. At present, the underlying mechanism of diastolic heart failure, as well as the effects of increased norepinephrine on myocardial blood flow (MBF) and hemodynamics in heart failure patients, remains poorly understood.

In this study, to elucidate the relationship between norepinephrine and MBF, and the relationship between MBF and LVEDP, the effects of norepinephrine on left ventricular hemodynamics and MBF were examined in rats with and without a calcium load.

MATERIALS AND METHODS

1. Study design

This study was approved by the Animal Study Committee of Dokkyo University School of Medicine, and carried out in accordance with the ethical code for animal studies of Dokkyo University School of Medicine. Thirty-three male Wistar rats aged 8 to 9 weeks, body weight : 270 to 310 g, were purchased and fed food and water *ad libitum* for several days. Animals were anesthetized with an intraperitoneal injection of urethane (1000 mg/kg ; Sigma) and α-chloralose (80 mg/kg ; Wako) under artificial respiration, using a respirator for small animals (model SN480-7, Shinano). An electrocardiograph was monitored and recorded using a bio-amplifier (P55 GRASS Inc.). A Mikro-Tip pressure transducer catheter was inserted from the right common carotid artery, and left ventricular pressure was determined. A polyethylene catheter for solution injection was inserted from the right femoral vein, and test solutions were injected using a continuous injector (CFV-3200, Nihon Koden). Anesthetized rats were monitored by ECG, and the respective test solutions were injected after confirming that the pulse and ventricular pressure of the animals were stable.

Three groups of rats were established : one receiving norepinephrine only, a second receiving simultaneous calcium chloride and norepinephrine, and a third (control) group receiving saline. Norepinephrine (Sigma) was dissolved in 5% glucose solution and administered at 30 μg/kg/min for 20 min in animals in the norepinephrine group. In rats of the calcium chloride and norepinephrine group, calcium chloride (dissolved in 5% glucose solution) was administered for 20 min at 12.0 mg/kg/min prior to norepinephrine administration. Norepinephrine was then administered at 30 μg/kg/min for 20 min with concomitant administration of calcium chloride at 12.0 mg/kg/min.

2. Determination of hemodynamics

A Mikro-Tip pressure transducer catheter (2F ; SPC-320, Millar Instruments Inc.) was inserted from the right common carotid artery and advanced to the left ventricle, and left ventricular pressure (LVP) was recorded. The ECG and LVP data were loaded onto a personal computer via an A/D converter (PowerLab/200). Left ventricular end-diastolic pressure (LVEDP) was defined as the pressure at the beginning of left ventricular isovolumetric contraction, if possible, or as the pressure in synchronization with the R wave of the ECG[13]. LVP, heart rate (HR) and LVEDP were measured every 5 min. LVEDP was determined in 10 consecutive cardiac cycles using the Mikro-Tip pressure transducer catheter, and mean values were calculated.

3. Echocardiography

After shaving the precordial hair of anesthetized rats, echocardiography was conducted at 7 MHz using an echograph (Toshiba Powervision SSA-380) and a high-speed sector probe (PSK-70LT ; Toshiba). As an index of systolic function, left ventricular ejection fraction (EF %) was estimated using the Teichholz method[14]. EF was measured in rats in which left ventricular pressure was determined. Echocardiography was carried out by two cardiologists, one of whom was in charge of imaging during the test period, while the other analyzed the waves.

4. Measurement of myocardial blood flow (MBF)

Myocardial blood flow was measured using the col-

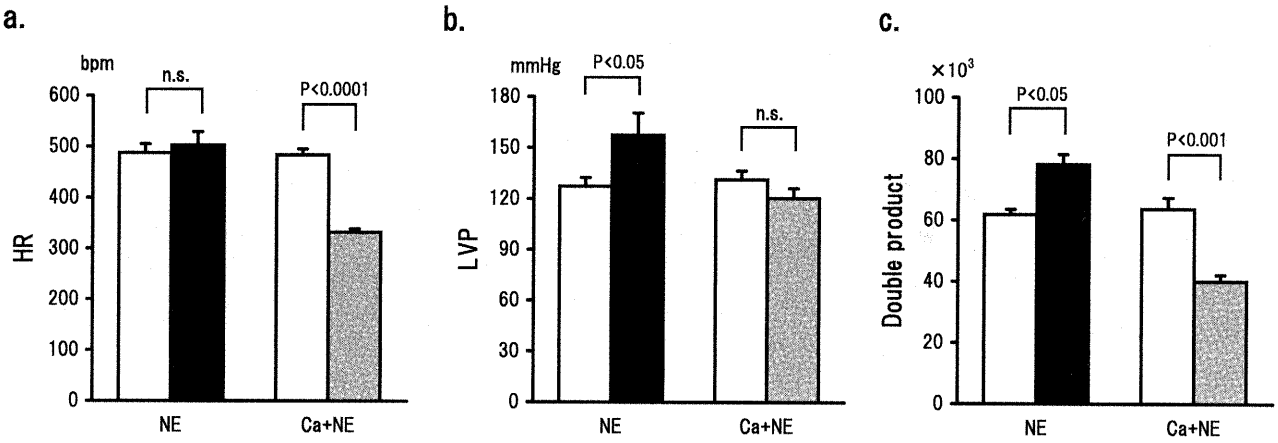

Fig. 1 Changes in heart rate (HR ; a), left ventricular pressure (LVP ; b) and double product (c) after administration of norepinephrine, or coadministration of calcium chloride and norepinephrine.
White bars show value (mean ± SE) before administration. Black bars show value after administration of norepinephrine (NE ; n = 6). Gray bars show value after coadministration of calcium chloride and norepinephrine (Ca + NE ; n = 6).

ored microsphere method, which has been described by Hakkinen et al[15]. Microspheres (Dye-Trank VII + ; Triton Technology) containing yellow or persimmon-colored dyes were dissolved in saline and injected into the left ventricle using an indwelling polyethylene catheter at two time points : 5 min after saline administration and 20 min after administration of saline (control ; n = 6), norepinephrine (n = 6), or calcium chloride and norepinephrine (n = 6). Approximately 200,000 microspheres were injected into each rat using an infusion pump at 0.6 ml/min for 50 sec. For 75 sec from 10 sec before microsphere injection, blood was collected using a withdrawal pump at 0.84 ml/min via the indwelling catheter in the femoral artery to measure the microspheres in the blood. After this measurement, the left ventricle was removed and weighed. The left ventricle and blood were dissolved to extract the dyes as previously described[15], and each absorbance of the tissue or of blood (considered to be the microsphere volume) was determined using a dual wavelength spectrometer (150-20, Hitachi, Japan). Myocardial blood flow (MBF) (Qm) was calculated according to the method of Hakkinen et al.[15], as follows :

$$Qm = (Am \times Qr)/Ar,$$

where Qm is the blood volume of the sample (ml/min/g) ; Qr is the recovery rate of blood (ml/min) ; Am is the absorbance per 1 g of tissue (microsphere volume) ; and Ar is the absorbance of blood (microsphere volume).

5. Statistical analysis

Data are shown as mean ± S.E.M. Intergroup comparison was performed using unpaired t-test, repeated measures ANOVA, and post hoc test (Tukey-Kramer method). The significance level was considered to be less than 0.05.

RESULTS

Hemodynamic data

Heart rate (HR), LVP and the double product (HR × LVP) before administration of norepinephrine were 487 ± 18 beats per minute (bpm), 127 ± 5.0 mmHg and 62,000 ± 1500, respectively. There was no change in HR after administration of norepinephrine, but a significant decrease was recognized after coadministration of calcium chloride and norepinephrine ($P < 0.0001$; Fig. 1a). There was a significant increase in LVP after administration of norepinephrine ($P < 0.05$; Fig. 1b), but no significant difference between before and after coadministration of calcium chloride and norepinephrine was recognized.

The double product after administration of norepinephrine was 73,000 ± 8900, showing a significant difference ($P < 0.05$). The double product before and after coadministration of calcium chloride and

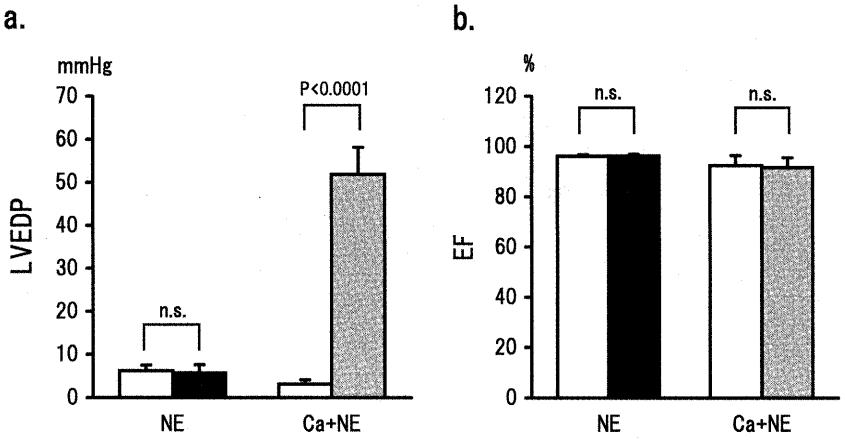

Fig. 2 Changes in left ventricular end-diastolic pressure (LVEDP ; a, n = 6 in each group) and ejection fraction (EF ; b, n = 4 in each group) after administration of norepinephrine, or coadministration of calcium and norepinephrine.
White bars show value (mean ± SE) before administration. Black bars show value after administration norepinephrine (NE) and gray bars shows value after coadministration of calcium chloride and norepinephrine (Ca + NE).

norepinephrine was 64,000 ± 3700 and 40,000 ± 2100, respectively, showing a significant decrease ($P < 0.001$; Fig. 1c).

There was no significant difference in LVEDP between before and after administration of norepinephrine. LVEDP before and after norepinephrine administration was 6.2 ± 1.3 and 5.7 ± 1.9 mmHg, respectively. However, LVEDP was significantly increased by coadministration of calcium chloride and norepinephrine. LVEDP before and after coadministration of calcium chloride and norepinephrine was 3.1 ± 1.0 mmHg and 52.0 ± 6.2 mmHg, respectively ($P < 0.0001$; Fig. 2a). There was no significant difference in EF between before and after norepinephrine administration ; 96.1 ± 0.6 % and 96.7 ± 0.9 %, respectively. There was no significant change in EF between before and after coadministration of calcium chloride and norepinephrine : 92.5 ± 3.9 % and 91.7 ± 3.9 %, respectively (Fig. 2b).

Myocardial blood flow (MBF)

There was no significant difference in myocardial blood flow (MBF) between before and 20 min after administration in the control group (before administration : 4.1 ± 0.6 ml/min/g ; 20 min after administration : 4.1 ± 0.6 ml/min/g). MBF before and after norepinephrine administration was 4.1 ± 0.3 and 2.8 ± 0.2 ml/min/g, respectively, showing a 29.3 % reduction. MBF in the calcium-treated group before and after norepinephrine administration was 4.3 ± 0.5 and 2.7 ± 0.2 ml/min/g, respectively, showing a 33.2 % reduction (Fig. 3a, b).

There was no correlation between percent reduction of MBF and LVEDP after norepinephrine administration with calcium (Fig. 4).

ECG data

During administration of norepinephrine, sinus rhythm was maintained, while administration of calcium chloride induced sinus bradycardia and premature atrial beats. Similar ECG changes were also recognized after administration of norepinephrine with calcium chloride (Fig. 5).

DISCUSSION

Heart failure patients generally have high blood norepinephrine concentrations, and the blood norepinephrine concentration reflects the severity of heart failure. NYHA class III and IV heart failure patients with plasma norpinephrine levels of 800 pg/ml or higher have an extremely poor prognosis[1, 2], and increased norepi-

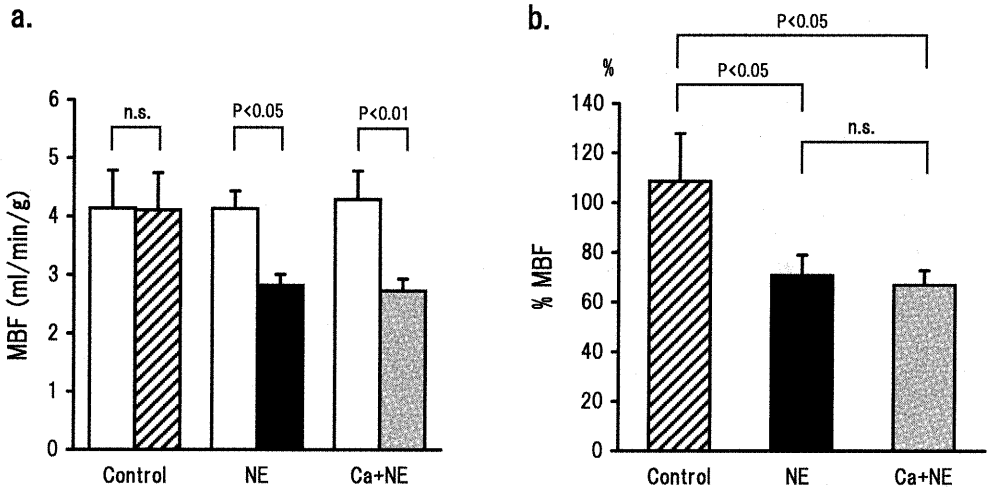

Fig. 3 Changes in myocardial blood flow (MBF ; a) and reduction (%) of MBF (b) after administration of norepinephrine, or coadministration of calcium chloride and norepinephrine.
White bars show value (mean ± SE) before administration. Stripe bas, black bars and gray bars show value after administration saline (control ; n = 5), norepinephrine (NE ; n = 6), and coadministration of calcium chloride and norepinephrine (Ca + NE ; n = 7), respectively.

nephrine in heart failure patients indicates a hyperactive sympathetic nervous system. However, the role of norepinephrine in heart failure has yet to be elucidated clearly.

Norepinephrine is a catecholamine with α_1 and β_1 effects. The β_1 effect of norepinephrine increases intracellular calcium and myocardial contraction, while the α_1 effect of norepinephrine induces vasoconstriction of vascular smooth muscle[3].

In this study, the effects of norepinephrine on MBF and hemodynamics were investigated in rats in the presence and absence of calcium. Norepinephrine administration alone induced a 29.3 % reduction of MBF, but had no effect on EF. The double product was increased, and LVEDP showed no change under these conditions. These results suggest that the α_1 effect of norepinephrine induced coronary vasoconstriction and reduced myocardial blood flow, but had no effect on cardiac contraction and relaxation. With simultaneous administration of calcium, norepinephrine had significant effects on hemodynamics, with a 33.2 % reduction of MBF and a significant increase in LVEDP, but did not reduce EF. There was no significant difference in MBF following norepinephrine administration with and without calcium, but MBF showed a tendency to decrease with increased diastolic dysfunction.

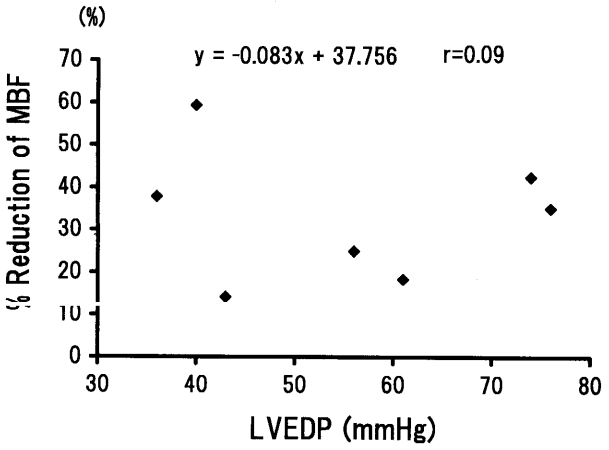

Fig. 4 Correlation between reduction of MBF (%) and LVEDP (n = 7).

In the present study, a significant decrease of HR was observed after administration of calcium chloride. It has been reported that hypercalcemia induced bradycardia[16] and cardiac arrest[17], although the underlying mechanism remains poorly understood.

Calcium-treated rats are considered to have increased intramyocardial and sarcoplasmic calcium concentrations[18]. The intracellular calcium concentration is also increased in ischemic myocardium[7]. Norepi-

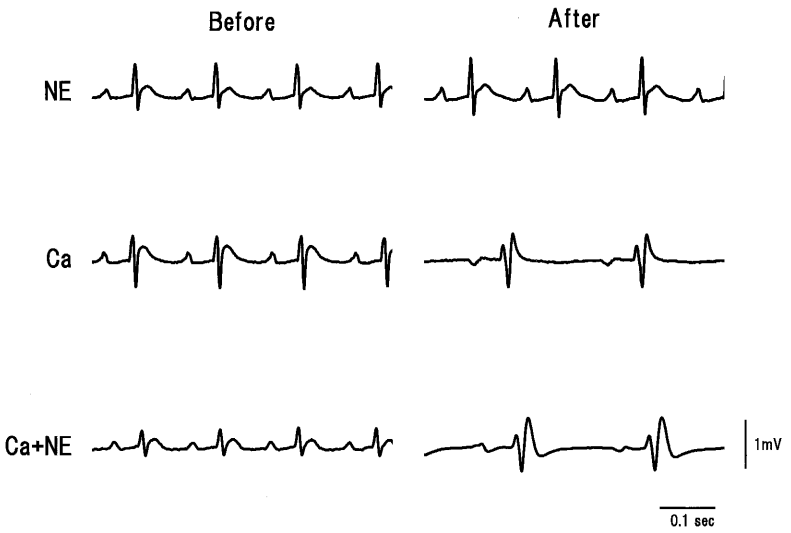

Fig. 5 ECG changes after administration of norepinephrine, calcium chloride and coadministration of both.

nephrine administration also increases the intracellular calcium concentration, which in turn is suggested to be associated with increased LVEDP ; although, another study has indicated that increased intracellular calcium concentration does not lead to increased LVEDP, and no consensus has been reached. The results of the present study show that significant diastolic dysfunction is induced by norepinephrine with calcium, suggesting that calcium may be an important factor in the development of diastolic dysfunction.

Our previous studies using Fura2/AM[18] and X-ray analysis[19] have shown that calcium treatment increases both intracellular and mitochondrial calcium concentrations, and it has been reported that the β_1 effect of norepinephrine administration with continuous calcium chloride treatment causes myocardial necrosis[18, 20]. In addition, echocardiography and the tissue Doppler method have been used to show that neither norepinephrine nor calcium alone induces diastolic dysfunction, but that coadministration of calcium chloride and norepinephrine decreases Ea and DCT and induces diastolic dysfunction[8].

Catecholamines such as norepinephrine stimulate protein kinase A and change intracellular Ca^{2+} handling by the myocardial sarcoplasmic reticulum (SR). In heart failure, abnormal intracellular Ca^{2+} handling is also induced[21, 22], suggesting that norepinephrine administration to the calcium-treated heart further increases the intracellular calcium concentration in the diastolic phase. Recent studies have indicated that binding of calstabin to the SR ryanodine receptor is reduced in heart failure patients[23, 24], leading to unstable calstabin binding and calcium efflux in the diastolic phase. Nevertheless, it has also been reported that intracellular calcium is not directly associated with diastolic dysfunction, and further studies need to be conducted to investigate the function of regulatory proteins in myocardial cells involved in relaxation[7].

In conclusion, our data show that norepinephrine administration reduces MBF and increases LVEDP, especially with concomitant calcium administration. These results suggest that norepinephrine may lead to exacerbation of heart failure due to development of diastolic dysfunction and a reduction of blood flow under a calcium load.

Acknowledgments. We thank Professor Noboru Kaneko of the Cardiology and Pneumology, Dokkyo University School of Medicine, for his advice and appreciate Rie Asaishi, Dokkyo University School of Medicine, for her technical assistance.

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