Alterations of responsiveness to adrenoceptor agonists and calcium of non-infarcted hypertrophied muscles from rats with chronic myocardial infarction

Takehiko Itaya, Hisakuni Hashimoto, Toshihiko Uematsu & Mitsuyoshi Nakashima

Department of Pharmacology, Hamamatsu University School of Medicine, 3600 Handa Hamamatsu, 431–31 Japan

- 1 The responsiveness of hypertrophied right ventricular muscle from rats with chronic myocardial infarction (MI) was examined. Myocardial infarction was produced by ligating the left coronary artery. The positive inotropic effects of α - and β -adrenoceptor agonists and calcium on the isolated non-ischaemic muscles were determined at one and four weeks after ligation, and were compared with those in sham-operated (SO) rats.
- 2 The contractile force of electrically-driven right ventricular papillary muscles was measured isometrically. We also determined the change of beating rate of the right atrium in response to a β -adrenoceptor agonist.
- 3 Basal contractile force increased at four weeks after ligation.
- 4 The pEC₅₀ values of the α_1 -adrenoceptor agonist, methoxamine, and calcium increased at four weeks after ligation, but the pEC₅₀ values of the β -adrenoceptor agonist, dobutamine did not change.
- 5 Maximal contractile force did not change with these agonists at either one and four weeks after ligation.
- 6 Tissues from rats four weeks after MI exhibited slight increases in the B_{max} of α - but not β -receptors, without any changes of affinity.
- 7 The increases in the sensitivity to an α_1 -adrenoceptor agonist and calcium may be compensatory mechanisms of non-infarcted ventricular tissue which has undergone hypertrophy in chronic MI.

Introduction

The gradual morphological change of the non-ischaemic cardiac muscle after myocardial infarction in rats has been described by many investigators (Anversa *et al.*, 1980a,b; 1984; 1986; Fishbein *et al.*, 1978; Rubin *et al.*, 1983). The necrotic myocardial tissue is completely replaced by connective tissue within 21 days and changes in the morphology of the right ventricle begin from three to twelve weeks (Fishbein *et al.*, 1978). In guinea-pigs with myocardial infarction, the responsiveness to β -adrenoceptor stimulation of non-ischaemic ventricular muscle has been shown to decrease three days after ligation of the coronary artery, and to return to normal seven days after ligation (Baumann *et al.*, 1981). However, the responsiveness of non-infarcted muscles over a longer period of time has not been investigated. In this study, we determined the responsiveness to drugs of hypertrophied ventricular muscles at one and four weeks after ligation of the coronary artery in rats.

Methods

Operative procedure

Myocardial infarction (MI) was induced in male Wistar rats (200–220 g) by a method similar to that described previously (Johns *et al.*, 1954; Selye *et al.*, 1960; Fishbein *et al.*, 1978). Briefly, the rats were anaesthetized with ether, followed by pentobarbitone (35 mg kg⁻¹, i.p.), intubated and ventilated by a positive pressure respirator. The heart was exposed through an incision between the fourth to fifth, or fifth to sixth ribs on the left side. The left coronary artery was then ligated 1–2 mm from its origin with 5-0 nylon. The chest was closed, and the rats were allowed to recover. There was a 20–30% mortality rate within the first 24 h following this procedure. The sham-operated (SO) rats were treated similarly without the pro-

cedure of coronary ligation. Surviving rats were maintained under the same conditions in a 12 h light/dark cycle and given standard chow and water *ad libitum*.

Contractions of right ventricular papillary muscles

At one and four weeks after the left coronary artery ligation or sham-operation, rats were killed by a blow on the head. The hearts were rapidly removed and papillary muscles of the right ventricle excised. The isolated preparations were suspended in 10 ml organ baths containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, MgSO₄ 1.2, CaCl₂ 2.0, KH₂PO₄ 1.2, NaHCO₃ 25.0 and glucose 1.4. To determine the sensitivity to exogenous Ca²⁺, Ca²⁺-free perfusate was prepared and Ca²⁺ 0.2 to 5 mM was added in a cumulative manner. The solution was maintained at a temperature of 32.0 ± 0.2°C and bubbled with a gas mixture of 95% O₂–5% CO₂. Preparations were stimulated at 100 min⁻¹ through an electrode by a square wave pulse of 3 ms duration and a voltage of approximately twice threshold, delivered by a stimulator (Nihon Kodens SE-1101). Papillary muscles were stretched by a resting tension of about 1.0 g, and tension development was recorded via force-displacement transducers (Nihon Kodens, TB-612T, TB-652T) on a thermal array recorder (Nihon Kodens, WS-641G). After a stabilization period of one hour, cumulative concentration-response curves were determined (Hashimoto *et al.*, 1978). In preliminary experiments, resting tension-active tension development curves for muscles from one and four week old MI and SO rats were determined (Figure 1). The basal contractile forces were maximal with a tension of 1.0 g in one and four week old MI and SO rats. We measured the diameter of the centre of the papillary muscles under tension after all experimental procedures had been completed. Cross-sectional area of individual papillary muscles was calculated as a circle. The tension development was represented as mg mm⁻².

Beating rate of right atrium

At one week and four weeks after ligation or sham-operation, rats were killed and the hearts were rapidly removed. Right

¹ Author for correspondence.

Figure 1 The active tension development of the isolated right ventricular papillary muscles with various resting tensions in one and four week old myocardial infarcted (MI) or sham-operated (SO) rats. (○—○) One week SO rats ($n = 6$); (●—●) one week MI rats ($n = 6$); (○---○) four week SO rats ($n = 6$); (●---●) four week MI rats ($n = 6$). Each point represents the mean and vertical lines show s.e.mean in this and subsequent figures.

atria were dissected and suspended in 10 ml organ baths containing Krebs-Henseleit solution. The solution was maintained at $32.0 \pm 0.2^\circ\text{C}$ and bubbled with 95% O_2 -5% CO_2 . The spontaneously beating atria were connected to force-displacement transducers and contractions were recorded. The beating rate was counted with a tachometer (Nihon Koden, AT-601G). The resting tension on the preparations was set to 0.5 g. Concentration-response curves were determined cumulatively.

α - and β -adrenoceptor binding assays

Four weeks after the left coronary artery ligation or sham-operation, rats were killed by a sharp blow on the head. The hearts were rapidly removed from the rats, placed in ice-cold buffer and the right ventricular free walls were dissected. Due to the large amount of protein required for receptor binding assays and Scatchard analysis, segments from two to five hearts were combined for the preparation of the membrane fraction. The right ventricular free walls were minced with a pair of scissors and homogenized in 10 to 20 times volume (w/v) of ice-cold buffer (0.25 M sucrose, 5 mM Tris/HCl, 1 mM MgCl_2 , pH 7.5: buffer A) by a Brickman Polytron PT-10 at setting 6 for 20, followed by 10 strokes of motor-driven teflon-glass homogenizer.

These homogenates were centrifuged at 1000 g for 10 min at 4°C . The pellet was removed, centrifuged at 30,000 g for

20 min at 4°C . The membrane pellet was washed in 20 times volume of the original wet weight of ice-cold buffer (50 mM Tris/HCl, 10 mM MgCl_2 , pH 7.5: buffer B) and recentrifuged at 30,000 g for 20 min at 4°C . The final pellet was resuspended in 20 times volume (for the [^3H]-prazosin and [^3H]-dihydroalprenolol ([^3H]-DHA) binding assay) of original wet weight of ice-cold buffer B.

α_1 - and β -adrenoceptor binding was measured by a method similar to that described previously (Asano *et al.*, 1983).

For binding to α_1 -adrenoceptors, membrane preparations (250 μl) were incubated, with shaking, for 20 min at 30°C with [^3H]-prazosin (0.1–2.0 nM) in a total volume of 550 μl of buffer A containing 0.1 mg ml^{-1} bovine serum albumin. The incubations, which were performed in duplicate, were terminated by the addition of 4 ml ice-cold buffer B, followed by rapid filtration through Whatman GF/C glass fibre filter disks. The filter disks were washed three times with 4 ml of ice-cold buffer B, and the radioactivity trapped on the filter disks was measured in an Aloka scintillation spectrometer (LSC-671) with a counting efficiency of 34%. Non-specific binding was defined as non-displaceable binding in the presence of 10 μM phentolamine and the specific binding was defined as the difference between total and non-specific binding.

The binding assay with [^3H]-DHA was carried out in the same way as that for [^3H]-prazosin, using [^3H]-DHA (1.0–20 nM) instead of [^3H]-prazosin, and non-specific binding of [^3H]-DHA was defined in the presence of 10 μM propranolol.

The protein concentration was measured by the method of Lowry *et al.* (1951). We analysed the data with the aid of a weighted non-linear square curve fitting programme, 'LIGAND' (Munson & Rodbard, 1980).

Drugs

The drugs used were as follows: dobutamine hydrochloride (Shionogi Chem.), methoxamine hydrochloride (Tanabe Chem.), [^3H]-prazosin (80.9 Ci mm^{-1}), [^3H]-DHA (54.8 Ci mm^{-1}) were purchased from New England Nuclear Corporation (Boston, MA, U.S.A.), bovine serum albumin (Sigma), phentolamine hydrochloride (Chiba-Geigy), propranolol (Sigma).

Statistical analysis

The results are expressed as mean $\pm$ s.e.mean. Statistical analysis of the data was performed by Student's t test and differences were considered significant only if $P < 0.05$.

Results

Heart weights and right ventricular weights

Table 1 shows the body weight and heart weight of MI and SO rats. The body weights of four week old MI and SO rats

Table 1 Body, heart and ventricular weight ratios in rats with myocardial infarction (MI) and sham-operated (SO) rats

	1 week		4 weeks	
	SO	MI	SO	MI
n	6	7	9	8
BW (g)	236 ± 4	$204 \pm 6^{**}$	278 ± 4	253 ± 10
HW (mg)	742 ± 10	$797 \pm 18^*$	827 ± 20	$1139 \pm 65^{**}$
RV (mg)	117 ± 2	$134 \pm 4^{**}$	137 ± 3	$247 \pm 17^{**}$
RA (mg)	34.1 ± 2.1	$41.2 \pm 3.1^*$	38.3 ± 2.6	$69.5 \pm 4.9^*$
SC (mg)	—	90 ± 8	—	86 ± 18
HW/BW (mg g^{-1})	3.12 ± 0.04	$3.91 \pm 0.10^{**}$	2.98 ± 0.06	$4.40 \pm 0.20^{**}$
RV/BW (mg g^{-1})	0.50 ± 0.02	$0.65 \pm 0.03^{**}$	0.50 ± 0.01	$0.98 \pm 0.06^{**}$
SC/HW (%)	—	11.3 ± 0.5	—	$7.6 \pm 0.4^{\dagger\dagger}$

Results are expressed as mean $\pm$ s.e.mean. BW = body weight, HW = heart weight, RV = right ventricular weight, RA = right atrial weight, SC = scar weight.

* $P < 0.05$, ** $P < 0.01$ (MI vs SO).

$\dagger\dagger P < 0.01$ (1 week vs 4 weeks).

Figure 2 The positive inotropic effect of methoxamine on the isolated right ventricular papillary muscles of one and four week old myocardial infarcted (MI) or sham-operated (SO) rats. (a) Concentration-response curves, (○—○) one week SO rats ($n = 6$), (●—●) one week MI rats ($n = 7$), (○—○) four week SO rats ($n = 7$), (●—●) four week MI rats ($n = 6$). (b) The contractile force per cross-sectional area with methoxamine. Hatched columns, basal contractile force. Hatched + open columns, maximal contractile force with methoxamine. 1 w, one week old rats; 4 w, four week old rats. * $P < 0.05$ vs SO rats.

were not significantly different. However, the body weights of one week old MI rats were significantly less than those of the SO rats. The heart weights and right ventricular weights in both one and four week old MI rats were significantly greater than those of SO rats. Thus, the ratios of whole heart weight/body weight and of right ventricular free wall weight/body weight in one and four week old MI rats significantly increased in comparison with the SO rats. The right ventricular weight to body weight ratios increased 32% in one week old MI rats and 100% in four week old MI rats. The ratios of the scar weight/heart weight were $11.3 \pm 0.5\%$ in one week old and $7.6 \pm 0.4\%$ in four week old rats.

Positive inotropic effect of adrenoceptor agonists on right ventricular papillary muscles

Figure 2a shows the concentration-response curves of contractile force in response to an α_1 -agonist, methoxamine. In one week old SO rats, low concentrations of methoxamine produced a slight negative inotropic effect, but in other SO or MI rats these responses were not observed. The pEC_{50} values for methoxamine of tissues from one week old MI and SO rats were identical, but those from four week old MI and SO rats were significantly different (Table 2). Figure 2(b) shows the values of the basal and the maximal contractile force per cross-sectional area of right ventricular papillary muscles with methoxamine. In four week old rats, the basal contractile

Figure 3 The positive inotropic effect of dobutamine on the isolated right ventricular papillary muscles of one and four week old myocardial infarcted (MI) or sham-operated (SO) rats. (a) Concentration-response curves, (○—○) one week SO rats ($n = 6$), (●—●) one week MI rats ($n = 6$), (○—○) four week SO rats ($n = 6$), (●—●) four week MI rats ($n = 7$). (b) The contractile force with dobutamine. Hatched columns, basal contractile force. Hatched + open columns, maximal contractile force with dobutamine. 1 w, one week old rats; 4 w, four week old rats. * $P < 0.05$ vs SO rats.

force per area was greater in MI rats. The maximal contractile force per area with methoxamine was not different in MI rats and SO rats.

In preliminary experiments, the pEC_{50} of dobutamine in the papillary muscle was 6.30 ± 0.12 ($n = 6$). The pEC_{50} of dobutamine with 10^{-6} M propranolol was 5.36 ± 0.15 ($n = 6$, $P < 0.01$). The pEC_{50} of dobutamine with 3×10^{-6} M phenolamine was 6.22 ± 0.17 ($n = 6$). Thus it can be regarded as a β -adrenoceptor agonist in papillary muscle of rats. Figure 3a shows the concentration-response curves of contractile responses to dobutamine. The values of pEC_{50} of one or four week old MI rats were not significantly different from those of SO rats (Table 2). Figure 3b shows the values of the basal and the maximal contractile force per cross-sectional area with dobutamine. In four week old rats, the basal contractile force was larger in MI than SO rats. The maximal contractile force with dobutamine was not different in MI and SO rats.

Positive inotropic effects to calcium on right ventricular papillary muscles

Figure 4a shows the concentration-response curves for contractile force in response to exogenous calcium. The sensitivity of right ventricular muscle to calcium significantly increased in one and four week old MI rats (Table 2). Figure 4b shows the absolute increases in the contractile force of right ventricular papillary muscles with calcium. The maximal contractile

Table 2 pEC_{50} (negative logarithm of EC_{50}) of methoxamine, dobutamine and calcium in the papillary muscles of rats with myocardial infarction (MI) and sham-operated (SO) rats

	1 week		4 weeks	
	SO	MI	SO	MI
Methoxamine	5.09 ± 0.11 ($n = 6$)	5.34 ± 0.08 ($n = 7$)	5.09 ± 0.04 ($n = 7$)	$5.62 \pm 0.04^*$ ($n = 6$)
Dobutamine	6.26 ± 0.11 ($n = 6$)	6.60 ± 0.16 ($n = 6$)	6.19 ± 0.10 ($n = 6$)	6.54 ± 0.17 ($n = 7$)
Calcium (mM)	3.10 ± 0.06 ($n = 6$)	$3.38 \pm 0.10^*$ ($n = 7$)	2.98 ± 0.05 ($n = 6$)	$3.33 \pm 0.09^{**}$ ($n = 6$)

Results are expressed as mean $\pm$ s.e.mean.

* $P < 0.05$, ** $P < 0.1$ (MI vs SO).

Figure 4 The positive inotropic effect of calcium on the isolated right ventricular papillary muscles of one and four week old myocardial infarcted (MI) or sham-operated (SO) rats. (a) Concentration-response curves, (○—○) one week SO rats ($n = 6$), (●—●) one week MI rats ($n = 7$), (○---○) four week SO rats ($n = 6$), (●---●) four week MI rats ($n = 6$). (b) The maximal contractile force with calcium. 1 w, one week old rats; 4 w, four week old rats.

forces per area with calcium were not different in one and four week old MI rats.

Positive chronotropic effect of a β -agonist in right atrium

Figure 5a shows the concentration-response curves for the beating rate of right atria to the β -agonist, dobutamine. The values of pEC_{50} in one week old MI and SO rats were identical (6.70 ± 0.02 ($n = 6$), 6.93 ± 0.11 ($n = 7$)), and those of four week old MI and SO rats were also identical (6.55 ± 0.17 ($n = 6$), 6.38 ± 0.10 ($n = 6$)). Figure 5b shows the absolute beating rate of right atrium with dobutamine. The basal rate in four week old MI rats (162 ± 29) was significantly decreased compared with SO rats (232 ± 22). The maximal atrial rate with dobutamine in four week old MI rats (294 ± 25 beats min^{-1}) was also significantly decreased as compared with SO rats (365 ± 31 beats min^{-1}). The maximal increases in the atrial rate with dobutamine were not different between MI and SO rats.

Ligand binding at α - and β -adrenoceptors

The B_{max} of [^3H]-prazosin binding in four week old MI rats was slightly but significantly increased, but the K_D did not change (Table 3). The B_{max} and K_D of [^3H]-DHA binding in four week old MI rats were not significantly different from those of SO rats.

Table 3 Binding of [^3H]-prazosin and [^3H]-dihydroalprenolol ([^3H]-DHA) to right ventricle from sham-operated (SO) rats and rats with myocardial infarction (MI) at four weeks

	B_{max} (fmol mg^{-1} protein)	K_D (nM)
[^3H]-prazosin		
SO	67.4 ± 2.4 ($n = 6$)	0.25 ± 0.02 ($n = 6$)
MI	$77.6 \pm 1.8^{**}$ ($n = 6$)	0.26 ± 0.01 ($n = 6$)
[^3H]-DHA		
SO	42.9 ± 3.6 ($n = 6$)	1.49 ± 0.28 ($n = 6$)
MI	51.4 ± 3.8 ($n = 6$)	1.26 ± 0.21 ($n = 6$)

Results are expressed as mean $\pm$ s.e.mean.

$^{**}P < 0.01$ (MI vs SO).

Figure 5 The positive chronotropic effect of dobutamine on the isolated right atrial muscles of one and four week old myocardial infarcted (MI) or sham-operated (SO) rats. (a) Concentration-response curves for the effect of dobutamine on atrial rate; (○—○) one week SO rats ($n = 7$), (●—●) one week MI rats ($n = 6$), (○---○) four week SO rats ($n = 6$), (●---●) four week MI rats ($n = 6$). (b) The absolute beating rate of the atria with dobutamine. Hatched columns, basal rate; hatched + open columns, maximal atrial rate with dobutamine. 1 w, one week old rats; 4 w, four week old rats. $^{*}P < 0.05$ vs SO rats.

Discussion

In a previous study with guinea-pigs with myocardial infarction, the responsiveness to β -receptor stimulation of residual non-ischaemic ventricular papillary muscles decreased three days after ligation of the coronary artery and returned to normal by seven days after ligation (Baumann *et al.*, 1981). These alterations of responsiveness were due to a down-regulation of adrenoceptors caused by the increased level of plasma catecholamines. However, in chronic MI the alterations of the responsiveness of non-ischaemic muscle have not been examined. In the present study, we determined the responsiveness of hypertrophied muscles at one and four weeks after MI.

The hypertrophy of non-ischaemic areas in MI rats occurs during the early days after ligation and the necrotic myocardial tissue is completely replaced by connective tissue within 21 days (Fishbein *et al.*, 1978). According to previous studies, the increases in weight due to right ventricular hypertrophy in rats were 5–65% at 21 days after occlusion, and about 30–79% at 30 to 40 days after occlusion (Anversa *et al.*, 1984; 1986). It varied according to the infarct size (Pfeffer *et al.*, 1979). The right ventricular hypertrophy (as assessed by increase in tissue weight) in our studies was 32% and 100% at one and four weeks, respectively, which was larger than those found in previous studies, probably because of differences in infarct size. The marked increase in the basal contractile force in MI rats may be partially caused by hypertrophy, and the increased contractile force may contribute to the compensatory mechanisms in MI.

The sensitivity to the α -adrenoceptor agonist increased at four weeks after ligation. The α -adrenoceptor density slightly but significantly increased in MI rats at this time, and this may partially contribute to the increased sensitivity to the α -adrenoceptor agonist.

The sensitivity to the β -adrenoceptor agonist did not change in right papillary muscles or atrial preparations of MI rats. Maximal contractile force with methoxamine or dobutamine in one and four week old MI rats was not different from SO rats. However, basal contractile force in four week old MI rats was larger than that in four week old SO rats. Calcium (2 mM) produced a larger contractile force in MI rats than in SO rats, and this resulted in the increased basal contractile force in four week old MI rats. The basal atrial rate (beats min^{-1}) was significantly decreased in right atria from 4 week old MI rats; this may be due to some deterioration of pacemaker cells during hypertrophy of the right atrium, but this needs further clarification.

Clozel *et al.* (1987) found that at three weeks following MI, a slight increase in β -adrenoceptor density occurred, but no change in the EC_{50} of isoprenaline, in right papillary muscle from rats. In our study, the pEC_{50} of dobutamine did not significantly change, which is consistent with the result of Clozel *et al.* (1987). The B_{max} of β -adrenoceptors in MI rats did not significantly change in our study.

An alteration of cardiac α_1 -receptors has been observed in other models of cardiac hypertrophy, such as in spontaneously hypertensive rats (Hanna *et al.*, 1986) and in rats with aortic constriction (Karliner *et al.*, 1980). In hereditary cardiomyopathy of the Syrian hamster, responsiveness to stimulation of α - but not β -adrenoceptors was increased (Bohm *et al.*, 1986). It seems that an increase in cardiac α_1 -adrenoceptors in cardiac hypertrophy is a common phenomenon.

The sensitivity to calcium significantly increased in four week old MI rats, which led to the increased level of basal contractile force in this group. Thus, the increased sensitivity to calcium is considered to be one of the compensatory mechanisms in MI.

Anversa *et al.* (1985) found that the sensitivity to calcium of the left ventricle was significantly decreased after MI in working hearts. The difference between the results of Anversa *et al.* and ours may lie in the different muscles studied, i.e., the residual muscles included the infarcted area of the left ventricle in their study, but in our study only the intact papillary muscles of the right ventricle were used.

In conclusion, increases in the sensitivity to an α_1 -adrenoceptor agonist and calcium occur in four week old MI rats; these may be compensatory mechanisms of the non-infarcted hypertrophied ventricle in chronic MI.

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