

β -adrenergic blockade attenuates cardiac dysfunction and myofibrillar remodelling in congestive heart failure

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Abstract

Although β -adrenoceptor (β -AR) blockade is an important mode of therapy for congestive heart failure (CHF), subcellular mechanisms associated with its beneficial effects are not clear. Three weeks after inducing myocardial infarction (MI), rats were treated daily with or without 20 and 75 mg/kg atenolol, a selective β_1 -AR antagonist, or propranolol, a non-selective β -AR antagonist, for 5 weeks. Sham operated rats served as controls. All animals were assessed haemodynamically and echocardiographically and the left ventricle (LV) was processed for the determination of myofibrillar ATPase activity, α - and β -myosin heavy chain (MHC) isoforms and gene expression as well as cardiac troponin I (cTnI) phosphorylation. Both atenolol and propranolol at 20 and 75 mg/kg doses attenuated cardiac hypertrophy and lung congestion in addition to increasing LV ejection fraction and LV systolic pressure as well as decreasing heart rate, LV end-diastolic pressure and LV diameters in the infarcted animals. Treatment of infarcted animals with these agents also attenuated the MI-induced depression in myofibrillar Ca^{2+} -stimulated ATPase activity and phosphorylated cTnI protein content. The MI-induced decrease in α -MHC and increase in β -MHC protein content were attenuated by both atenolol and propranolol at low and high doses; however, only high dose of propranolol was effective in mitigating changes in the gene expression for α -MHC and β -MHC. Our results suggest that improvement of cardiac function by β -AR blockade in CHF may be associated with attenuation of myofibrillar remodelling.

Keywords: atenolol • propranolol • myofibrillar ATPase • myosin heavy chain • troponin I

Introduction

Prolonged and excessive activation of sympathetic nervous system is considered to play a pivotal role in the initiation of left ventricular (LV) remodelling and progression of congestive heart failure (CHF) after myocardial infarction (MI) [1]. β -adrenoceptor (β -AR) blockers, once contraindicated in CHF, are now widely utilized in its therapy [2–5]. To date, there are several proposed mechanisms mediating the beneficial effects of β -AR blockers at cellular and molecular levels [2] but very little are known regarding their effects at the level of myofibrils in CHF. Although it is clear that intracellular Ca^{2+} homeostasis is critical for efficient myocardial cell function [6–8], the ability of the cardiac muscle to generate contractile force is primarily dependent upon the myofibrillar

Ca^{2+} -stimulated ATPase activity [9–12]. Furthermore, alterations in the responsiveness of myofibrils to Ca^{2+} are central to the depressed contractility and diminished contractile reserve in CHF [6–8]. It is also well established that the myofibrillar ATPase activity in the heart is determined by the relative amounts of myosin heavy chain (MHC) isoforms, namely α -MHC and β -MHC [10–12]. In various models of cardiac hypertrophy and heart failure, depression in myosin ATPase as well as myofibrillar Ca^{2+} -stimulated ATPase activity and contractile function are associated with a shift in the composition (an increase of β -MHC and a decrease of α -MHC expression in the LV) of myosin isoforms with respect to α -MHC and β -MHC content [10, 13–15]. Therefore, this study was undertaken to evaluate the effects of a selective β_1 -blocker (atenolol) and a non-selective β -blocker (propranolol) on myofibrillar Ca^{2+} -stimulated ATPase, and to examine if these actions are involved in attenuating cardiac contractile dysfunction in CHF due to MI. In order to investigate the molecular mechanisms of β -AR blockade, we measured changes in the mRNA levels and protein content for α -MHC and β -MHC upon treatment of heart failure rats with these β -AR blockers. Since myofibrillar Ca^{2+} -stimulated

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ATPase activity is regulated by cardiac troponin I (cTnI) [11], the effect of β -blockade on the unphosphorylated and phosphorylated protein content for cTnI was also studied in CHF rats. Thus in view of the paucity of information regarding the effects of selective or non-selective β -AR blocking agents, this study can be seen to provide further evidence that improvement of cardiac function in CHF due to MI by both atenolol and propranolol is associated with attenuation of myofibrillar remodelling.

Methods

Experimental model and study design

All experimental protocols were approved by the Animal Care Committee of the University of Manitoba following the guidelines established by the Canadian Institutes of Health Research. CHF due to MI was induced in male Sprague-Dawley rats (175–200 g) by occlusion of the left coronary artery as described earlier [16, 17]. Briefly, rats were anaesthetized and the heart was exposed and the left coronary artery was ligated at about 2 mm from its origin at the aorta. The heart was repositioned in the chest and the incision was closed with a purse string suture. Sham operated rats underwent similar procedure except coronary ligation. All rats received standard care, kept at 12 hrs day/night cycle and fed rat chow and water *ad libitum*. Since myocardial infarct is fully healed by about 3 weeks after coronary occlusion, this time-point was chosen for starting drug treatment. At the end of 3 weeks after surgery and after echocardiographic assessment of cardiac function, surviving coronary occluded rats were assigned to vehicle-treated (MI + V, $n = 14$) or low dose of atenolol (20 mg/kg; ATN₂₀-MI, $n = 12$), high dose of atenolol (75 mg/kg; ATN₇₅-MI, $n = 12$), low dose of propranolol (20 mg/kg; PRP₂₀-MI, $n = 11$) and high dose of propranolol (75 mg/kg; PRP₇₅-MI, $n = 11$) groups. The drugs were given daily for 5 weeks *via* a gastric tube; the dose of each drug was adjusted to body weight (wt.) twice a week. At the end of the treatment period, echocardiographic and haemodynamic measurements were performed and rats were killed. Heart was removed for cardiac morphometry; the LV (including septum) and the scar tissue were dissected, weighed, frozen in liquid nitrogen and stored at -70°C . The lung wet/dry wt. ratio, an index of pulmonary congestion, as well as the heart/body wt. ratio, an index of cardiac hypertrophy, was assessed in these animals. Since scar wt./total LV wt. (including septum and infarcted tissue) ratio was found to exhibit a linear relationship with infarct size (as measured morphometrically), the scar wt. was used as a marker to determine the extent of infarct size.

Assessment of cardiac function

To assess the cardiac function non-invasively, serial echocardiograms were obtained at baseline (4 days prior to surgery), 3 weeks after surgery (before drug treatment) and 8 weeks after surgery (after 5 weeks drug treatment). Transthoracic 2D-guided M-mode echocardiographic tracings were recorded as described elsewhere [18]. Briefly, rats were anaesthetized with isoflurane and echocardiograms were performed with SONOS 5500 ultrasound imaging system equipped with a S12 phased-array transducer (Agilent Technologies Inc., Andover, MA, USA). A 2D short-axis view of the LV cavity was obtained at the level of the papillary muscles and then 2D-guided

M-mode tracings were recorded. LV end diameters at the diastolic and systolic phases were measured. Global LV systolic function was assessed by calculating left ventricular ejection fraction (LVEF) using the formula $(\text{LV end-diastolic diameter}^2 - \text{LV end-systolic diameter}^2) \times 100 / \text{LV end-diastolic diameter}^2$. Since the infarcted rats showed 50% to 55% reduction in LVEF at 3 weeks after surgery, no animal was excluded from this study. On the other hand, haemodynamic measurements were carried out at 8 weeks after surgery as described previously [16, 17]. Briefly, animals were anaesthetized with an intraperitoneal injection of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The right carotid artery was exposed and cannulated with a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, TX, USA). The catheter was advanced carefully into the LV. Arterial blood pressure, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), heart rate (HR), rate of pressure development (+dP/dt) and rate of pressure decay (-dP/dt) were measured using AcqKnowledge software (3.0.3 MP100, BIOPAC System, Inc., Goleta, CA, USA).

Myofibrillar Ca^{2+} -stimulated and Mg^{2+} -ATPase activities

The myofibrillar fraction was isolated as described earlier [19, 20] and suspended in a final solution containing 100 mM KCl, 20 mM Tris-HCl (pH 7.0). Mg^{2+} -ATPase activity was measured at 30°C in a medium containing (in mM) 20 imidazole (pH 7.0), 2 MgCl_2 , 2 Na_2ATP , 10 NaN_3 , 1.6 ethylene glycol-bis(aminoethylether)- $\text{N,N,N}',\text{N}'$ -tetraacetic acid (EGTA) and 50 KCl [19, 20]. Total ATPase activity was determined in the same medium except that EGTA was replaced by 10 μM of free Ca^{2+} . Ca^{2+} -stimulated ATPase activity was taken as the difference between values obtained for total and Mg^{2+} -ATPase activities. All reactions were terminated at 5 min. by the addition of 12% trichloroacetic acid. The samples were centrifuged at $1000 \times g$ and the phosphate was determined in the supernatant by colorimetric method.

Cardiac myosin heavy chain isoform analysis

Cardiac MHC isoforms were determined under denaturing conditions; both α - and β -MHCs were separated on a 4% polyacrylamide gel upon loading 4 μg protein/well as described previously [19, 20]. The electrophoresis was carried out at a constant 220 V for 3.5 hrs with cooling between 9°C and 13°C . The gels were stained with Coomassie brilliant blue R250 for 2 hrs and were destained with acetic acid and methanol. The relative amount of isoforms was estimated by GS-800 Imaging Densitometer (Quantity One 4.4.0 Software, Bio-Rad Laboratories, Mississauga, ON, Canada).

RNA isolation and Northern blot analysis

Total RNA was isolated from the viable LV of sham controls and infarcted rats with or without drug treatment by the acid guanidinium thiocyanate-phenol-chloroform method [19, 20]. Briefly, frozen samples of viable LV were homogenized with a Polytron homogenizer (model PT3000) at 12,000 rpm in the presence of 1.5 ml of TRIzol Reagent (1 ml/100 mg tissue); (GIBCO-BRL Life Technologies, Grand Island, NY, USA). The mixture was centrifuged at $12,000 \times g$ for 10 min. at 4°C . The supernatant was incubated with chloroform (0.3 ml/sample) for 5 min. at room temperature and then centrifuged at $12,000 \times g$ for 15 min. at 4°C . The RNA containing upper aqueous phase was kept at -20°C for 4 hrs after the addition of 0.75 ml of isopropyl alcohol. Upon centrifugation at $17,500 \times g$ for 10 min. at 4°C ,

RNA pellets were washed two times in 75% ethanol and vacuum dried by Speed Vac (model SC110, Savant Instruments, Farmingdale, NY, USA). The final RNA pellet was resuspended in DEPC-treated water and stored at -70°C . The RNA concentration was calculated from the absorbance at 260 and 280 nm with SPECTRAMax PLUS (Molecular Devices, Sunnyvale, CA, USA). Total RNA (20 μg) was denatured at 65°C for 10 min. and size fractionated on a 1.2% agarose gel containing 1.2 M formaldehyde. The blotted samples were transferred onto positively charged nylon membranes (Schleicher & Schuell, Keene, NH, USA), UV cross-linked, and hybridized to randomly primed cDNA. Cardiac α - and β -MHC as well as 18S cDNA probes were labelled by random primer DNA labelling system using Klenow fragment [21]. The α -MHC probe was a 39-mer oligonucleotide derived from the 3'-untranslated region of the rat α -MHC gene as follows: 5'-GGG ATA GCA ACA GCG AGG CTC TTT CTG CTG GAC AGG TTA-3'. Similarly, the cardiac β -MHC probe was derived from the 3'-untranslated region of the gene (rat genome) and was 5'-CAG GCA TCC TTA GGG TTG GGT AGC ACA AGA -3'. Filters were exposed to X-ray film (Kodak X-OMAT) at -80°C with intensifying screens. Results of autoradiographs from Northern blot analysis were quantified by using imaging densitometer GS 670 (Bio-Rad Laboratories). The signal of α - and β -MHC isoform mRNA was normalized to that of 18S mRNA to account for differences in loading and/or transfer. The mRNA abundance of the MHC α -isoform and β -isoform was expressed as MHC α -isoform/18S and MHC β -isoform/18S ratio, respectively.

Analysis of troponin I content and phosphorylation

As cTnI is involved in the modulation of contractile kinetics of myocardium, cTnI content and phosphorylation were estimated in tissue homogenates of LV from all the groups according to the method used in our laboratory [20, 22]. The muscle was homogenized by Brinkmann homogenizer with Polytron PT 300 for 8–10 sec. (15,000 rpm) in the homogenizing buffer (50 μl buffer/mg tissue) containing 60 mM KCl, 1 mM cysteine, 20 mM imidazole (pH 6.9), 1 mM MgCl_2 , 1 mM ouabain, 10 mM NaN_3 , 1 mM CaCl_2 , 0.01% leupeptin, 250 μM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol. The homogenization procedure was carried out at 4°C and the samples were placed on a rotator and mixed at least 15 min. The concentration of protein in the homogenate was adjusted to 2 mg/ml with homogenizing buffer. In order to obtain maximum reaction of cTnI phosphorylation, all samples were incubated for 10 min. in the presence of 1 mM Mg^{2+} -ATP and 1 mM CaCl_2 . Then the samples were boiled in equal volume of SDS-PAGE loading buffer. Equal amount of sample (10 μg total protein/well) of all experimental groups was loaded onto 12% polyacrylamide gels and was separated by SDS-PAGE at 200 V for 40–45 min. The proteins were electroblotted to polyvinylidene fluoride (PVDF) membranes using a wet transfer method. Membranes were blocked in 5% fat-free powdered milk-containing tris buffered saline-tween 20 (TBS-T) at room temperature for 1 hr, then were incubated in monoclonal anti-cTnI (1:1000, Cell Signalling Technology, Inc., Beverly, MA, USA) or anti-phosphorylated cTnI antibodies specific for the phosphorylated form of Ser22/Ser23 (1:1000, Cell Signalling Technology, Inc.). The membranes were subsequently incubated with biotinylated anti-rabbit IgG (1:3000) for 40 min. and finally with streptavidin conjugated horseradish peroxidase (1:3000) for 40 min. cTnI bands were visualized by enhanced chemiluminescence (Amersham, Biosciences Inc., Baie d'Urfe, Quebec, Canada). Band intensities were analysed by GS-800 Calibrated Imaging Densitometer (Bio-Rad Laboratories) using Quantity One Software Version 4.4 (Bio-Rad Laboratories). The level of cTnI phosphorylation was expressed as percentage of total cTnI {cTnI phosphorylation = $(\text{OD}_{\text{phosphorylated}}/\text{OD}_{\text{total}}) \times 100$ }.

Statistical analysis

Data are reported as mean \pm S.E.M. Statistical differences between two mean values were evaluated by Student's t-test and one-way ANOVA. All statistical analyses were performed with the ORIGIN software (version 7.1, OriginLab Corporation, Northampton, MA, USA). A P -value of <0.05 was considered significant.

Results

Mortality and general characteristics

A total of 97 rats underwent coronary occlusion. Thirty-one rats died within the first 2 days after surgery, which corresponds to 32% mortality. Three rats died 1wk after surgery, one rat died 2 weeks after surgery and two rats died 4 days before therapy. We lost 5/14 rats during the treatment-equivalent period in the MI + V group, 4/12 died in the ATN₂₀-MI, 3/12 in the ATN₇₅-MI, 2/11 in the PRP₂₀-MI and 2/11 in the PRP₇₅-MI groups. None of the drug-treated rats died during the first week of treatment. There was no animal loss in the sham group of 12 rats.

The general characteristics of sham-operated, MI + V, atenolol-treated and propranolol-treated animals are shown in Table 1. The body wt. was similar in all the groups. Occlusion of the left coronary artery resulted in scar formation occupying 40–50% of LV free wall. The MI + V rats also had clinical signs of CHF such as pulmonary congestion (as reflected by an increase in lung wet/dry wt. ratio), cardiac hypertrophy, pleural effusion and ascites. Treatment with low and high doses of atenolol and propranolol led to significant decreases in total ventricular wt., RV wt., ventricular/body wt. ratio and pulmonary congestion. The decreases in RV wt. and lung wet/dry wt. ratio by 75 mg/kg doses were greater than those by 20 mg/kg doses of both atenolol and propranolol. There was no significant difference in liver wet/dry wt. ratio among different groups (Table 1). Also, there was no significant difference in scar wt. among the β -blocker treated and MI + V rats, indicating that the extent of myocardial infarct produced by coronary occlusion was comparable in all groups (Table 1).

Haemodynamic and echocardiographic effects

Haemodynamic studies revealed impaired cardiac performance in MI + V rats. It can be seen from Table 2 that HR and LVEDP were significantly increased whereas LVSP, $+dP/dt$ and $-dP/dt$ were significantly decreased in MI + V rats. Both low and high doses of atenolol and propranolol significantly decreased HR when compared to MI + V rats. Although elevated LVEDP and decreased LVSP were attenuated by both low and high doses of atenolol and propranolol, 75 mg/kg dose of propranolol was more effective in attenuating these alterations induced by MI. The changes in $+dP/dt$ after treatment with atenolol or propranolol showed dose

Table 1 General characteristics of sham and infarcted rats with or without 20 mg/kg and 75 mg/kg atenolol or propranolol treatments at 8 weeks after surgery

	SHAM	MI + V	ATN ₂₀ -MI	ATN ₇₅ -MI	PRP ₂₀ -MI	PRP ₇₅ -MI
Body wt. (g)	480 ± 9.1	473 ± 11.3	478 ± 8.5	475 ± 13.0	472 ± 8.2	460 ± 11.8
Ventricular wt. (mg)	1327 ± 13	1543 ± 55*	1398 ± 26 [#]	1370 ± 45 [#]	1412 ± 24 [#]	1369 ± 35 [#]
Ventricular/body wt. ratio	2.76 ± 0.05	3.26 ± 0.10*	2.92 ± 0.09 [#]	2.88 ± 0.07 [#]	2.99 ± 0.08 [#]	2.98 ± 0.04 [#]
RV wt. (mg)	267 ± 25	535 ± 40*	380 ± 25 [#]	300 ± 20 [#]	371 ± 30 [#]	280 ± 50 [#]
Scar wt. (mg)	ND	260 ± 22	255 ± 17	262 ± 22	250 ± 20	247 ± 18
Lung wet/dry wt. ratio	3.90 ± 0.11	5.48 ± 0.07*	5.00 ± 0.20 [#]	4.60 ± 0.10 [#]	4.85 ± 0.09 [#]	4.50 ± 0.05 [#]
Liver wet/dry wt. ratio	2.92 ± 0.06	3.00 ± 0.06	2.94 ± 0.04	2.95 ± 0.03	3.00 ± 0.04	2.93 ± 0.05

Values are means ± S.E.M. LV: left ventricle; ND: not detectable; RV: right ventricle; wt.: weight; MI + V: vehicle-treated infarcted; ATN₂₀-MI: 20 mg/kg atenolol-treated infarcted; ATN₇₅-MI: 75 mg/kg atenolol-treated infarcted; PRP₂₀-MI: 20 mg/kg propranolol-treated infarcted; PRP₇₅-MI: 75 mg/kg propranolol-treated infarcted. **P* < 0.05, significantly different from sham control group, [#]*P* < 0.05 versus MI + V rats.

Table 2 Haemodynamic parameters of sham and infarcted rats with or without 20 mg/kg or 75 mg/kg atenolol and propranolol treatments at 8 weeks after surgery

	SHAM	MI + V	ATN ₂₀ -MI	ATN ₇₅ -MI	PRP ₂₀ -MI	PRP ₇₅ -MI
HR (beats/min.)	250 ± 5.0	270 ± 8.8*	242 ± 9.2 [#]	232 ± 5.0 [#]	235 ± 7.0 [#]	224 ± 6.3 [#]
LVSP (mm Hg)	130 ± 5.2	99 ± 5.0*	115 ± 4.8 [#]	119 ± 6.1 [#]	119 ± 7.0 [#]	125 ± 6.0 [#]
LVEDP (mm Hg)	8.8 ± 0.90	16.8 ± 1.00*	14.0 ± 0.85 [#]	11.7 ± 1.20 [#]	13.2 ± 0.92 [#]	10.1 ± 1.25 [#]
+dP/dt (mm Hg/s)	8102 ± 280	4657 ± 292*	4681 ± 340	5533 ± 270 [#]	4750 ± 301	7170 ± 310 [#]
-dP/dt (mm Hg/s)	8244 ± 350	4270 ± 297*	4415 ± 258	4660 ± 308	4390 ± 304	6705 ± 220 [#]

Values are means ± S.E.M. HR: heart rate; LVEDP: left ventricular end-diastolic pressure; LVSP: left ventricular systolic pressure; MI: myocardial infarction; +dP/dt: rate of pressure development; -dP/dt: rate of pressure decay; MI + V: vehicle-treated infarcted; ATN₂₀-MI: 20 mg/kg atenolol-treated infarcted; ATN₇₅-MI: 75 mg/kg atenolol-treated infarcted; PRP₂₀-MI: 20 mg/kg propranolol-treated infarcted; PRP₇₅-MI: 75 mg/kg propranolol-treated infarcted. **P* < 0.05, significantly different from sham control group, [#]*P* < 0.05 versus MI + V rats.

dependency because it was not improved by low doses but at high doses both drugs induced a significant increase; propranolol being most effective. Interestingly, -dP/dt was increased only by high doses (75 mg/kg) of propranolol. Table 3 shows the data on LVEF, LV end-diastolic and LV end-systolic diameters as measured by echocardiography at 8 weeks after surgery. Both LV end-systolic and end-diastolic diameters were increased whereas LVEF was markedly decreased in the vehicle-treated infarcted animals. Unlike LV end-diastolic diameter, LV end-systolic diameter was significantly reduced by low and high doses of atenolol and propranolol when compared to vehicle-treated MI rats (Table 3). Treatment of infarcted animals with low and high doses of atenolol or propranolol also improved the LVEF (Table 3).

Myofibrillar ATPase activity

Myofibrils isolated from the viable LV of infarcted hearts exhibited a lower Ca²⁺-stimulated ATPase activity compared to sham-

operated rats (Table 3). β-adrenergic blockade with 20 and 75 mg/kg atenolol or 20 mg/kg propranolol partially normalized the myofibrillar Ca²⁺-stimulated ATPase activity (Table 3). Treatment of infarcted animals with 75 mg/kg propranolol completely restored the MI-induced depression in Ca²⁺-stimulated ATPase activity. There was no change in myofibrillar Mg²⁺-ATPase activity among different groups (Table 3).

Protein content and gene expression for α-MHC and β-MHC isoforms

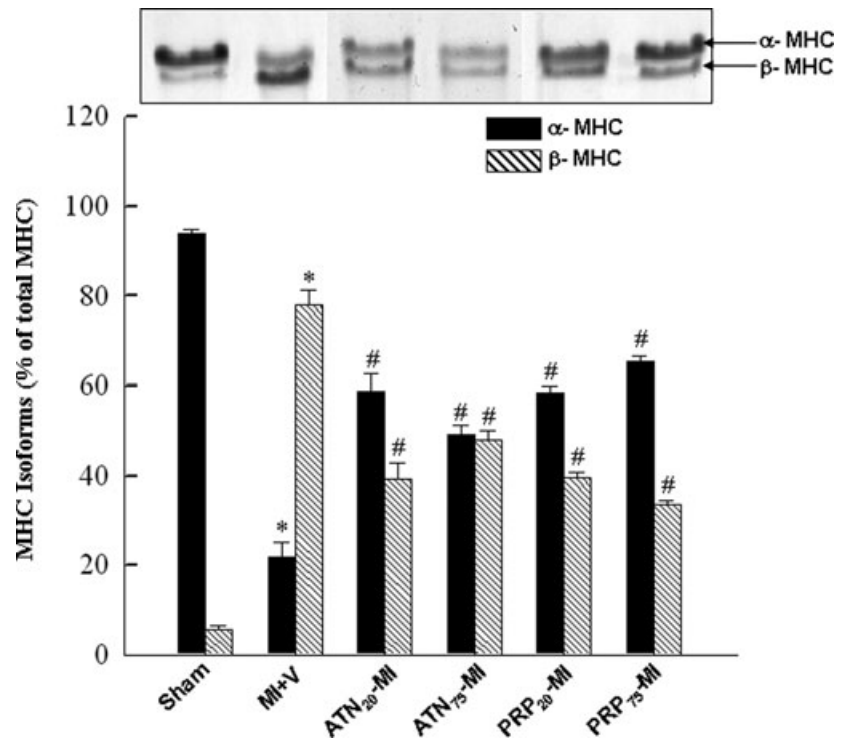
β-MHC was increased from 6.3% to 77.7% of total MHC whereas α-MHC was decreased from 93.7% to 22.3% of total MHC in the infarcted hearts (Fig. 1). β-blockade by atenolol or propranolol, partially prevented the increase in β-MHC as well as the decrease in α-MHC due to MI regardless of the dose used for treatment. Northern blot analysis of α- and β-MHC mRNA levels is shown in Fig. 2. The α-MHC mRNA level

Table 3 Echocardiographic parameters and myofibrillar ATPase activities in sham and infarcted rats with or without 20 mg/kg and 75 mg/kg atenolol or propranolol treatments at 8 weeks after surgery

	SHAM	MI + V	ATN ₂₀ -MI	ATN ₇₅ -MI	PRP ₂₀ -MI	PRP ₇₅ -MI
A. Echocardiographic parameters:						
LVEDD (mm)	7.39 ± 0.46	11.64 ± 1.01*	10.54 ± 1.12	10.23 ± 0.99	10.35 ± 1.02	10.14 ± 1.01
LVESD (mm)	3.5 ± 0.32	9.8 ± 0.92*	7.9 ± 0.64 [#]	7.8 ± 0.36 [#]	7.7 ± 0.75 [#]	7.2 ± 0.99 [#]
LVEF (%)	77.2 ± 1.36	28.74 ± 1.78*	43.26 ± 2.19 [#]	42.12 ± 2.4 [#]	45.68 ± 2.19 [#]	52.24 ± 3.00 [#]
B. Myofibrillar ATPase activities:						
Ca ²⁺ -stimulated ATPase (μmol Pi /mg protein / hr)	13.5 ± 0.4	9.9 ± 0.45*	11.75 ± 0.3 [#]	11.54 ± 0.32 [#]	11.65 ± 0.37 [#]	13.25 ± 0.45 [#]
Mg ²⁺ -ATPase (μmol Pi /mg protein / hr)	3.0 ± 0.09	3.26 ± 0.30	2.86 ± 0.10	3.27 ± 0.18	3.26 ± 0.09	3.12 ± 0.09

Values are means ± S.E.M. LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; MI + V: vehicle-treated infarcted; ATN₂₀-MI: 20 mg/kg atenolol-treated infarcted; ATN₇₅-MI: 75 mg/kg atenolol-treated infarcted; PRP₂₀-MI: 20 mg/kg propranolol-treated infarcted; PRP₇₅-MI: 75 mg/kg propranolol-treated infarcted. **P* < 0.05, significantly different from sham control group, [#]*P* < 0.05 versus MI + V rats.

Fig. 1 Coomassie blue-stained gels showing α- and β-MHC protein bands and their corresponding densitometric analysis in sham controls and infarcted rats with or without 20 mg/kg and 75 mg/kg atenolol or propranolol treatment at 8 weeks after surgery. The protein content of the isoforms was expressed as percentage of the total MHC. α-MHC: α-myosin heavy chain; β-MHC: β-myosin heavy chain; MI: myocardial infarction; MI + V: vehicle-treated MI; ATN₂₀: 20 mg/kg atenolol-treated MI; PRP₂₀: 20 mg/kg propranolol-treated MI; ATN₇₅: 75 mg/kg atenolol-treated MI; PRP₇₅: 75 mg/kg propranolol-treated MI. Values are means ± S.E.M. **P* < 0.05 versus sham control; [#]*P* < 0.05 versus MI + V.



decreased by 50% and that of β-MHC mRNA was increased by 282% in the infarcted animals. These changes in α- and β-MHC mRNA levels were significantly reversed by treatment with 75 mg/kg propranolol when compared to infarcted animals. However, the MI-induced depression in α-MHC mRNA was not

attenuated by 20 and 75 mg/kg atenolol or 20 mg/kg propranolol. Although the MI-induced increase in β-MHC mRNA was decreased significantly by 75 mg/kg atenolol, 20 mg/kg atenolol or propranolol did not depress the mRNA level of β-MHC (Fig. 2).

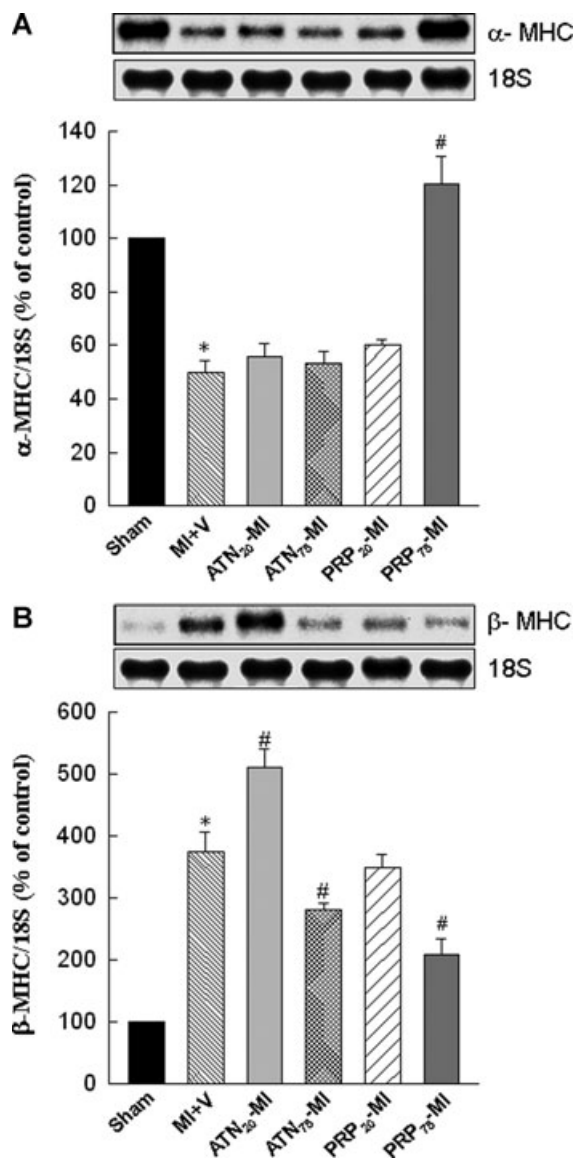


Fig. 2 Representative Northern blots and densitometric quantification of mRNA abundance for α -MHC and β -MHC in sham controls and infarcted rats with or without 20 mg/kg and 75 mg/kg atenolol or propranolol treatment at 8 weeks after surgery. α -MHC: α -myosin heavy chain; β -MHC: β -myosin heavy chain; MI: myocardial infarction; MI + V: vehicle-treated MI; ATN₂₀: 20 mg/kg atenolol-treated MI; PRP₂₀: 20 mg/kg propranolol-treated MI; ATN₇₅: 75 mg/kg atenolol-treated MI; PRP₇₅: 75 mg/kg propranolol-treated MI. Values are means \pm S.E.M. **P* < 0.05 versus sham control; #*P* < 0.05 versus MI + V.

Troponin I content and phosphorylation

By using a primary antibody, which reacts with the cTnI molecules regardless of their phosphorylation status, we found that the total

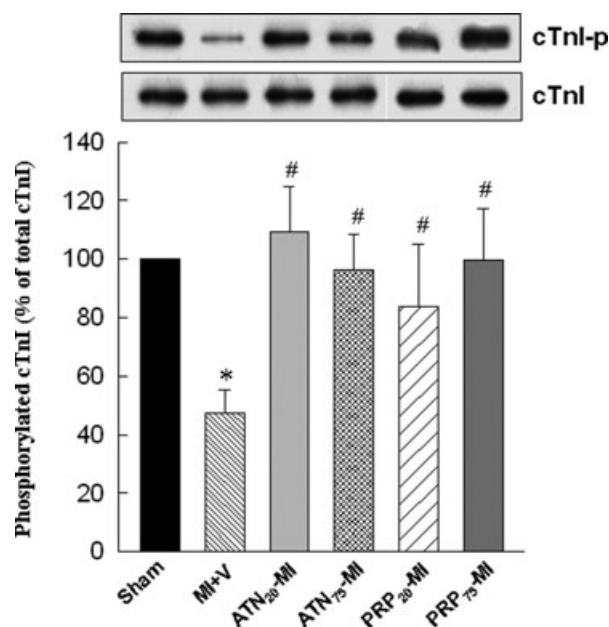


Fig. 3 Phosphorylation level of cTnI in sham controls and infarcted rats with or without 20 mg/kg and 75 mg/kg atenolol or propranolol treatment at 8 weeks after surgery. The phosphorylation level was expressed as percentage of the total cTnI based on the Western immunoblot band intensities obtained for the total cTnI (upper panel) and phosphorylated cTnI (lower panel). cTnI: cardiac troponin I; MI: myocardial infarction; MI + V: vehicle-treated MI; ATN₂₀: 20 mg/kg atenolol-treated MI; PRP₂₀: 20 mg/kg propranolol-treated MI; ATN₇₅: 75 mg/kg atenolol-treated MI; PRP₇₅: 75 mg/kg propranolol-treated MI. Values are means \pm S.E.M. **P* < 0.05 versus sham control; #*P* < 0.05 versus MI + V.

cTnI content was similar in sham controls, untreated infarcted and drug-treated infarcted groups (Fig. 3). However, a primary antibody specific for phosphorylated cTnI (Ser22/Ser23) showed significantly lower cTnI phosphorylation level in MI + V compared to sham controls. Furthermore, treatment of infarcted animals with low and high doses of atenolol or propranolol restored cTnI phosphorylation level to near control values (Fig. 3).

Discussion

The key findings of this study are that treatment of the 3 weeks infarcted rats for a period of 5 weeks with atenolol and propranolol improved cardiac dysfunction and ameliorated depression in myofibrillar Ca²⁺-stimulated ATPase activity, alterations in α - and β -MHC isoforms and gene expression, as well as decreased cTnI phosphorylation. It is pointed out that, to the best of our knowledge, this study is the first to compare the different effects of high and low doses of selective β_1 -AR blocker, atenolol

and non-selective β -AR blocker, propranolol on cardiac function and myofibrillar remodelling. These observations indicate that both selective and non-selective β -AR antagonists exert beneficial effects in CHF. In this regard, it may be noted that various selective and non-selective β -AR antagonists including atenolol and propranolol have been reported to attenuate LV remodelling, improve cardiac function and reduce sympathetic activity in CHF [23–28]. Moreover, treatment of CHF patients with metoprolol and carvedilol was found to improve myosin ATPase activity and restore cTn1 phosphorylation in the myocardium [29]. Likewise, improvement in cardiac function by treatment with a β -AR blocker, carteolol, attenuated myofibrillar ATPase activity in furazolidine-induced dilated cardiomyopathy in turkey [30]. Furthermore, as decreased sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) content is associated with reduced sarcoplasmic reticulum Ca^{2+} loading and elevated cytoplasmic Ca^{2+} levels [31, 32], the restoration of SERCA2a levels is likely to be a critical factor in the normalization of Ca^{2+} uptake. Therefore, owing to the importance of SERCA2a in restoring the sarcoplasmic reticulum Ca^{2+} load per excitation–contraction cycle, a study by Sun *et al.* [33], showed that 6 weeks of treatment of MI rats with metoprolol or carvedilol improved SERCA2a messenger RNA and protein levels. Such effects of β -AR blockade can lead to markedly improved cardiac function as assessed by echocardiography and haemodynamics. This particular investigation extended the results of another study that reported improved SERCA2a levels after propranolol treatment in dilated cardiomyopathy [34]. Thus these support the view that both selective and non-selective β -AR antagonists exert beneficial effects by attenuating alterations in contractile and regulating proteins in CHF.

Attenuation of ventricular remodelling and improvement in cardiac function

β -AR blockade with atenolol and propranolol in infarcted animals reduced cardiac preload, as illustrated by reduction in LVEDP. Interestingly, changes in $+dP/dt$ and $-dP/dt$ in CHF were improved only when propranolol was used in high doses. Atenolol in high doses was also observed to attenuate the depressed $+dP/dt$ without any changes in $-dP/dt$ in infarcted hearts. These effects of propranolol was similar to another study in which carvedilol (another non-selective β -blocker) was shown to have more improvement in haemodynamic parameters compared to metoprolol (a selective β_1 -blocker) [33]. The mechanisms for this significant haemodynamic improvement by non-selective β -blockade may be due to the reduction of wall stress and oxygen uptake, and an increase in coronary blood flow associated with blockade of excessive sympathetic activation [35, 36], which can protect and enhance the function of remaining surviving myocytes in the LV during CHF. Moreover, the decrease in LVEDP by both atenolol and propranolol suggest a trend towards a reduction in LV preload and afterload, which may further improve LV function.

LVEF was also significantly improved by treatments with these β -AR antagonists. This, together with the haemodynamic effects, as well as the observed reduction in LV end-diastolic and end-systolic diameters and LV dilation, may induce an improvement of LV filling and an increase in cardiac contractility. The increase in lung wet/dry wt. ratio due to CHF was attenuated by treatment with atenolol and propranolol indicating that the drugs were effective in improving pulmonary oedema in animals with CHF. Moreover, treatments with atenolol and propranolol depressed the increase in HR in rats with CHF, which may be due to the depression in the increased sympathetic activity and elevated levels of plasma catecholamines [27, 37].

Modulation of myofibrillar remodelling in CHF

The present study and previous reports from our laboratory have shown a depression in cardiac function and myofibrillar ATPase activity as well as a switch in α -MHC and β -MHC protein content and gene expression in post-MI CHF [19, 38]. The observed decrease in myofibrillar Ca^{2+} -stimulated ATPase activity in failing hearts would result in depression in cardiac function since the magnitude of cardiac contractile force is linearly related to myofibrillar Ca^{2+} -stimulated ATPase activity [39]. On the other hand, myofibrillar ATPase activity is mostly determined by the ratio of the expressed MHC isoforms as α -MHC has a low ATPase activity but produces high cross-bridge force with more economy of energy consumption [40]. The consequence of CHF has been studied in the failing human hearts and was shown that mRNA expression of α -MHC was significantly reduced and β -MHC was significantly increased compared to controls [41]. In rodent models of cardiac hypertrophy and failure, decrease in α -MHC mRNA and increase in β -MHC mRNA as well as corresponding changes in protein content were found to be associated with a reduction in velocity of shortening and other measures of systolic function [42–44]. However, it is pointed out that protein levels do not always correlate with the mRNA levels [45] as we have observed that 3.8-fold increase in β -MHC mRNA was associated with 15-fold increase in β -MHC protein whereas 0.5-fold decrease in α -MHC mRNA was associated with 4-fold decrease in α -MHC protein in the CHF due to MI in rats.

A study in spontaneously hypertensive rats has shown that atenolol treatment reduced cardiac mass and affected the shift in MHC isozymes in the myocardium [46]. Likewise, we found that atenolol reduced ventricular hypertrophy, increased α -MHC and decreased β -MHC isoform in post-MI rats. However, the observed changes in MHC protein levels did not correspond to similar changes in mRNA levels. This finding suggests that MHC protein levels might be regulated by an altered mRNA turnover or translational activity, which may change the net mRNA level in the myocardium. Thus some caution should be exercised while interpreting the observed changes in α -MHC and β -MHC in terms of quantitative alterations in myofibrillar protein content because we did not determine the absolute values for the MHC isozymes in the failing heart by

employing mass spectroscopy [47], which is more accurate than the semi-quantitative Coomassie staining. Although treatment of hypertensive animals with propranolol induced partial regression in cardiac hypertrophy, it did not improve stroke volume significantly [46]. On the other hand, treatment of infarcted animals with propranolol decreased RV hypertrophy and improved both echocardiographic and haemodynamic parameters. This finding is consistent with a study [45] in which propranolol was observed to increase the LVEF, increase α -MHC mRNA level and decrease β -MHC mRNA level in patients with dilated cardiomyopathy. Furthermore, in another study by Boluyt *et al.* [48], β -adrenergic stimulation was shown to induce 'foetal' pattern of expression of MHC, which was reversed by non-selective β -blocker. Therefore, in view of the high levels of cardiac adrenergic activity present during CHF, we speculate that propranolol by blocking both β_1 - and β_2 -mediated adrenergic stimulation can contribute to reversal of induction of elements of the pathological 'foetal' gene program more efficiently than the selective β_1 -blocker atenolol. Thus it is likely that the effectiveness of both selective and non-selective β -AR antagonists on cardiac hypertrophy and myofibrillar remodelling during the development of cardiac dysfunction may depend on the type of CHF.

In CHF, the down-regulation of β -receptors and β -adrenergic signal transduction is mirrored at the molecular level as a decrease in cTnI phosphorylation, which may be responsible for the enhanced myofibrillar Ca^{2+} sensitivity and lower maximal ATPase activity observed in CHF [49, 50]. The phosphorylation of cTnI by protein kinase A (PKA), which is activated *via* β -receptors, results in a decrease in Ca^{2+} sensitivity of the contractile machinery [51]. Therefore, although we did not determine the protein levels of PKA, we used the phosphorylation-specific cTnI antibody (specific for the phosphoform of Ser22/Ser23, which is the main phosphorylation site for PKA [52]) in the present study to determine the phosphorylation of cTnI by PKA. Since treatment with both atenolol and propranolol restored the MI-induced decrease in cTnI phosphorylation without affecting total cTnI content, it appears that PKA-mediated cTnI phosphorylation correlates well with the contractile state of the heart. Moreover, when phosphorylated, cTnI induces conformational changes in the troponin molecules that reduce the Ca^{2+} affinity of cardiac troponin C and lead to enhanced relaxation [53]. Therefore, it is plausible that the beneficial effects of β -AR blockade on contractile function in CHF may partly rely on their ability to restore cTnI phosphorylation. Besides PKA-mediated cTnI phosphorylation, changes in PKC-mediated phosphorylation of cTnI can also contribute to altered contractile function. However, the importance of PKC-mediated phosphoryla-

tion is obscure because different PKC isozymes are not activated uniformly and may subserve distinct biological functions due to their different substrate specificities. Also, PKC-mediated phosphorylation of cTnI has been shown to have negative effects on myofibrillar function, such as inhibition of maximal Mg-ATPase activity, and to decrease myofilament Ca^{2+} sensitivity, maximal tension development and cross bridge cycling kinetics [54, 55]. Therefore, in this particular study, we studied only the cTnI phosphorylation by PKA, which is considered as more important than phosphorylation by PKC because functional consequences of cTnI phosphorylation by PKC are most probably affected by the phosphorylation status of the PKA phosphorylation sites and also PKC may cross-phosphorylate the PKA phosphorylation sites on cTnI [56]. Nonetheless, PKC has been reported to be up-regulated in secondary cardiac hypertrophy [57] and CHF [58]. Whether β -blockade affects changes in the PKC activity in the failing heart still needs to be elucidated.

In summary, β -receptor blockade is effective in preventing LV remodelling and cardiac contractile dysfunction in CHF after MI. The molecular mechanisms may be related with normalization of myofibrillar Ca^{2+} -stimulated ATPase activity and MHC protein content. However, only the non-selective β -AR blocker, propranolol at high dose was more effective in preventing the changes in $\pm\text{dP}/\text{dt}$ and gene expression for α -MHC and β -MHC in CHF than the selective β_1 -AR blocker, atenolol. It should be noted that propranolol being a non-selective β -blocker can inhibit the β -receptor stimulating action of epinephrine and increase the serum concentration of potassium. Potassium is first released from the intracellular space to the extracellular space through the α -adrenergic action of epinephrine and this subsequently stimulates the β -receptor action of epinephrine causing hyperkalemia [59]. Therefore, serum potassium concentration should be closely monitored when patients are on non-selective β -blocker therapy. Nonetheless, from this study, the beneficial effects of atenolol and propranolol on the activity and content of contractile and regulatory proteins may provide further insights regarding the relationship between myofibrillar remodelling and β -AR signalling in the heart.

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