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# Effects of propranolol treatment on left ventricular function and intracellular calcium regulation in rats with postinfarction heart failure

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**1** Chronic treatment with beta-adrenergic blocking agents can improve survival in patients with heart failure. The mechanisms underlying the beneficial effects and whether these effects are generalizable to ischaemic heart failure are unresolved.

**2** We performed echocardiographic-Doppler examinations in rats ($n=28$) 1 and 6 weeks after myocardial infarction (MI) or sham surgery. Rats were randomized to no treatment or propranolol (500 mg/l in drinking water) after the first echocardiogram. Isometric contractions and intracellular Ca transients were recorded simultaneously in noninfarcted left ventricular (LV) papillary muscles.

**3** Untreated MI rats had significant LV dilatation ($10.6\pm0.4$* vs $8.9\pm0.3$ mm, MI vs control), impaired systolic function (fractional shortening $=11\pm2$* vs $38\pm2$%), and a restrictive LV diastolic filling pattern. MI rats receiving propranolol had similar LV chamber sizes ($10.6\pm0.5$ mm) and systolic function ($13\pm2$%). The propranolol treated animals had higher LV end-diastolic pressures ($27\pm2$* vs $20\pm3$ mmHg) and a more restricted LV diastolic filling pattern (increased ratio of early to late filling velocities and more rapid E wave deceleration rate). Contractility of papillary muscles from untreated MI rats was depressed ($1.6\pm0.3$ vs $2.4\pm0.5$ g mm$^{-2}$). In addition, Ca transients were prolonged and the inotropic response to isoproterenol was blunted. Propranolol treatment did not improve force development ($1.6\pm0.3$ g mm$^{-2}$) or the duration of Ca transients during isoproterenol stimulation.

**4** Chronic propranolol treatment in rats with postinfarction heart failure did not improve LV remodeling or systolic function. LV diastolic pressures and filling patterns were worsened by propranolol. Treatment also did not produce appreciable improvement in contractility, intracellular Ca regulation or beta-adrenergic responsiveness in the noninfarcted myocardium.

**Keywords:** Myocardial infarction; echocardiography; blood flow velocity; diastole; calcium; heart failure; congestive; myocardial contractility; beta-adrenergic receptors

**Abbreviations:** LV, Left ventricular; MI, Myocardial infarction

## Introduction

There is growing enthusiasm for the use of beta adrenergic blocking agents as a treatment for patients with depressed left ventricular (LV) function (Heidenreich *et al*., 1997). However, there are several issues that continue to be controversial with regard to the use of beta blockers in this setting. First, some studies suggest that patients with ischemic disease as the etiology of heart failure derive less benefit from long term beta blockade than those patients with nonischaemic heart failure (Andersson *et al*., 1991; Fisher *et al*., 1994; The CIBIS investigators, 1994; Woodley *et al*., 1991). Other studies suggest that these agents are beneficial in patients with both ischaemic and nonischaemic etiologies for heart failure (Australia/New Zealand Heart Failure Research Collaborative Group, 1997). Several components of the beta adrenergic signal transduction pathway appear to be affected differently in ischemic and idiopathic dilated cardiomyopathy (Bohm *et al*., 1990; Bristow *et al*., 1991). These differences have been postulated to account for the discordant effects of beta blockade in patients with these distinct types of heart muscle disease (Woodley *et al*., 1991). Second, while many studies suggest that beta blockade is associated with improvement in LV ejection fraction (Bristow *et al*., 1996; The CIBIS investigators, 1994), it is less clear whether there is improvement in contractility at the cellular or tissue level (Wagner *et al*., 1997). Finally, there are conflicting data on whether beta blockade improves LV diastolic function (Eichhorn *et al*., 1994; Haber *et al*., 1993). In view of the uncertainty about efficacy and the possible mechanisms of beneficial effects from beta blockade in postinfarction LV failure, we designed this study to answer the following questions. In postinfarction heart failure, does chronic nonselective beta adrenergic blockade: (1) attenuate or reverse LV remodelling? (2) alter the development of LV systolic and diastolic dysfunction? and (3) prevent abnormalities of excitation-contraction coupling in the noninfarcted myocardium?

Animal studies can be advantageous in defining mechanisms of drug effects since animals typically provide a much more homogeneous group of subjects than those in clinical trials. In addition, animal studies provide the opportunity to perform serial studies and to obtain myocardial tissue. We have previously demonstrated the feasibility and reproduci-

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bility of quantifying LV structure and function using transthoracic echocardiography in rats following large anterior MI (Litwin *et al*., 1994). The noninvasive nature of this technique is ideal for these studies because it allows longitudinal assessment of LV geometry and function before and after institution of treatment. In the present study, we combined echocardiographic examinations with *ex vivo* assessment of myocardial contractility and excitation-contraction coupling in noninfarcted myocardium. Our data suggest that in postinfarction heart failure, 5 weeks of propranolol treatment: (1) has little effect on LV remodelling or the progression of systolic dysfunction, (2) worsens diastolic function, (3) does not significantly affect the prolongation of intracellular Ca transients, and (4) does not improve beta-adrenergic signalling in the surviving myocardium.

## Methods

### *Model of ischaemic heart failure*

All studies described in this manuscript were performed according to the guidelines of the American Physiological Society. Anterior myocardial infarction (MI) was produced in male Sprague-Dawley rats (age approximately 12 weeks, weight 250–300 g) as previously described ($n=14$) (Litwin *et al*., 1994; 1996). Sham-operated rats ($n=14$) underwent identical surgery but did not sustain a myocardial infarction. We studied rats with large infarctions (>40% LV circumference) since these animals develop heart failure characterized by progressive LV remodelling and contractile dysfunction. Rats with MI or sham surgery were randomized to chronic propranolol treatment or no treatment beginning 1 week after surgery. The dose of propranolol (500 mg/l in drinking water) has previously been shown to produce clinically significant beta blockade in this model of heart failure (Gay *et al*., 1990). Propranolol or no treatment was continued for a total of 5 weeks (6 weeks after surgery). Treatment was not initiated until 1 week after surgery because of concerns that earlier beta blockade might be detrimental in animals with markedly compromised LV function.

### *Echocardiographic studies*

One week after MI or sham surgery, and just before initiation of propranolol or no treatment, rats were anaesthetized with ketamine HCl (Parke Davis, Morris Plains, NJ, U.S.A.) 50 mg $kg^{-1}$ and xylazine (Lloyd laboratories, Shenendoah, IA, U.S.A.) 10 mg $kg^{-1}$ i.p. the echocardiographic procedure was performed as previously described (Litwin *et al*., 1994; 1996). Anterior and posterior wall thickness (end-diastolic and end-systolic), and LV internal dimensions were measured from at least three consecutive cardiac cycles on the M mode strip chart recordings. Pulsed-wave Doppler spectra of mitral inflow were recorded from an apical four chamber view with the sample volume placed at the tips of the mitral leaflets and adjusted to the position where velocity was maximal and the flow pattern was laminar. Stroke volume was estimated by integrating the pulsed-wave Doppler spectra recorded in the LV outflow tract. The mean of at least three consecutive cardiac cycles were used for each parameter measured. Propranolol was discontinued 18–24 h prior to the final echocardiogram (6 weeks after MI or sham surgery). Following the echocardiographic study, a 2 Fr. micromanometer-tipped catheter (Millar Inst., Houston, TX, U.S.A.) which had been calibrated at 37°C was passed retrogradely into the left ventricle via the right carotid artery. The electronic signal was passed through a differentiating circuit to obtain the first derivative of pressure (dP/dt).

### *Isometric muscle performance*

Immediately following haemodynamic measurements, the heart was rapidly excised. The noninfarcted posterior papillary muscle was dissected free in an oxygenated dissecting chamber and the muscular end was grasped with a spring clip. None of the muscles had gross evidence of infarction. Previous work has shown that 6–9 weeks after MI, the posterior papillary muscles do not have appreciable scar tissue on histologic examination although variable interstitial fibrosis is present (Litwin *et al*., 1991; Warner *et al*., 1992). The tendinous end of the muscle was tied to a 6–0 silk suture and vertically suspended from an isometric force transducer (MBL 341 Sensotech Inst, Columbus, OH, U.S.A.). The muscle was placed in a 50 ml tissue bath containing modified Krebs–Henseleit solution with the following composition (mmol $l^{-1}$): NaCl 120, KCl 5.9, dextrose 11, $NaHCO_3$ 25, $NaH_2PO_4$ 1.2, $MgCl_2$ 1.2, and $CaCl_2$ 1.0. The bath was maintained at a constant temperature of 30°C and bubbled with 95% $O_2$ and 5% $CO_2$. The muscle was stimulated to contract isometrically at 0.33 Hz using a punctate platinum electrode at the base of the muscle. Five ms square wave pulses were delivered at a voltage approximately 10% above threshold. The muscle was stretched to the length at which maximum tension development occurred. At the conclusion of all studies, the muscle length was measured. The muscle was then gently blotted dry and weighed. Papillary muscle cross-sectional area was then calculated assuming a cylindrical geometry and specific gravity of muscle = 1.05:

$$\text{cross sectional area} = (\text{muscle weight}/1.05)/\text{muscle length} \quad (1)$$

### *Aequorin signal measurements*

Aequorin (purchased from J.R. Blinks, Friday Harbor, WA, U.S.A.) was loaded using a previously described technique (Litwin & Morgan, 1992). When the background light had decreased to a stable baseline, the aequorin signal was measured using a light collecting apparatus designed by Dr John R. Blinks. Light and isometric tension were simultaneously recorded from the force transducer and a photon counter (C10, Thorn EMI Gencom Inc, Fairfield, NJ, U.S.A.) attached to the photomultiplier tube. To improve the signal-to-noise ratio, the data from 20–40 steady state light signals and isometric twitches were averaged (#4562 Nicolet, Madison, WI, U.S.A.).

### *Inotropic responses to isoproterenol*

After baseline measurements were completed, aequorin signals and isometric tension were recorded during exposure to the beta-adrenergic agonist isoproterenol. Isoproterenol HCl (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in distilled water and added to the bath to produce cumulative concentrations of $10^{-9}$–$10^{-6}$ mol $l^{-1}$. Light signals and isometric contractions were measured when the response was maximal (5–10 min after each addition of isoproterenol).

### *Pathologic studies*

After removal of the papillary muscle from the heart, the atria were trimmed from the ventricles, and the right ventricle and left ventricle plus septum were separated and weighed. The tissues were then immersion-fixed in 10% buffered formalin. Each heart was cut in cross-section at four levels from apex to base and prepared for routine histology. Thin sections from each level were stained with Masson's trichrome. The section corresponding to the midventricular short axis echocardiographic image was projected and infarct size was estimated by measuring the percentage of the endocardial circumference replaced by scar tissue.

### *Statistics*

All data are shown as mean ± s.e.mean. Intergroup comparisons at week 1 and week 6 were done using a factorial ANOVA followed by a *post hoc* multiple comparisons procedure (Fishers least protected significant difference test) where appropriate (Statview 4.01, Abacus Concepts, Berkeley, CA, U.S.A.). A probability of $<0.05$ was considered to be significant. Based on the variability seen in our previous studies, we estimated that we would have an 80% chance of detecting differences of approximately 30% in LV diastolic dimension, E wave deceleration rate, or in myocardial contractility (peak rate of tension rise) with $n=7$ rats in each group. For purposes of clarity, only the *post hoc* comparisons of each group vs untreated sham, and propranolol MI vs untreated MI are shown in the tables and figures.

## Results

### *Effects of MI and propranolol on cardiac chamber weights and haemodynamics*

Surgical mortality was ~40% in the first 24 h. No rats died during the treatment period or during echocardiographic and haemodynamic measurements. Untreated MI rats had evidence of chronic LV dysfunction such as the development of significant right ventricular hypertrophy (Table 1). Haemodynamic abnormalities were characteristic of those previously reported in rats with postinfarction heart failure (Table 1) (Litwin & Morgan, 1992; Pfeffer *et al*., 1979). Specifically, rats with large MI had decreased LV systolic pressure, depressed maximal and minimal LV dP/dt, and elevated LV end-diastolic pressure. Infarct size determined histologically was not different in untreated and propranolol treated MI rats (Table 1). This was expected since propranolol was not given until after the infarctions were completed. Propranolol treated MI rats weighed slightly less than the untreated MI rats, and significantly less than the sham operated rats (Table 1). Propranolol did not alter the development of right ventricular hypertrophy and also did not change LV weight/body weight as compared to the untreated MI rats. Heart rate was not different in the four groups of rats. This was anticipated since measurements were performed 18–24 h after discontinuation of treatment. Propranolol slightly reduced both + and $-dP/dt$ in the sham and MI groups compared to their respective untreated groups ($P$ = NS, Table 1). LV end-diastolic pressure was significantly higher in the propranolol treated MI rats compared to the untreated MI rats.

### *Effects of propranolol on postinfarction LV remodelling and systolic function*

As we have previously shown, echocardiographic measurements demonstrated marked LV remodelling following transmural anterior MI (Table 2, Figure 1) (Litwin *et al*., 1994; 1996). Postinfarction LV remodelling was not significantly affected by propranolol treatment. MI also was associated with marked LV systolic dysfunction (Table 2, Figure 1). LV fractional shortening was depressed to a similar extent in the two groups of infarcted rats prior to initiation of propranolol treatment (Figure 1). Five weeks later both treated and untreated rats showed further declines in both of these parameters. At the termination of the study, fractional shortening was not different in the propranolol treated vs the untreated MI rats. Systolic thickening of the noninfarcted posterior wall tended to be improved in the propranolol treated MI rats compared to the untreated group ($P$ = NS).

### *Effects of propranolol on LV diastolic filling*

Left ventricular diastolic filling was markedly abnormal in rats with MI (Table 3, Figure 2). Rats with MI had a restrictive LV filling pattern defined as an increased ratio of early (E) to late (A) filling velocities, rapid deceleration of the early filling wave, and shortening of the isovolumic relaxation time (Appleton *et al*., 1988). This was evident 1 week after MI. By 6 weeks, rats

**Table 1** Cardiac chamber weights and haemodynamics in rats 6 weeks after myocardial infarction (MI) or sham surgery

| | *Sham* (n = 8) | *Sham propranolol* (n = 6) | *MI* (n = 7) | *MI propranolol* (n = 7) |
|---|---|---|---|---|
| BW (g) | 431 ± 28 | 489 ± 16 | 392 ± 22 | 355 ± 14* |
| RV/BW (mg/g) | 0.59 ± 0.02 | 0.52 ± 0.02 | 1.32 ± 0.12* | 1.30 ± 0.04* |
| LV/BW (mg/g) | 1.98 ± 0.7 | 2.05 ± 0.06 | 2.07 ± 0.07 | 2.16 ± 0.06 |
| Histologic Infarct size (% LV) | – | – | 49 ± 3 | 46 ± 4 |
| Heart rate (bpm) | 244 ± 7 | 240 ± 12 | 243 ± 5 | 255 ± 12 |
| LVSP (mmHg) | 133 ± 6 | 165 ± 7* | 104 ± 7* | 105 ± 6* |
| LVEDP (mmHg) | 4.8 ± 0.6 | 9.6 ± 1.5 | 20.5 ± 3.4* | 27.0 ± 2.0*† |
| +dP/dt (mmHg/s) | 7632 ± 370 | 6059 ± 317* | 3929 ± 361* | 3110 ± 226* |
| −dP/dt (mmHg/s) | 5934 ± 495 | 4502 ± 147* | 2586 ± 233* | 2401 ± 138* |

Rats were randomly assigned to no treatment or 5 weeks of propranolol, beginning 1 week after sham surgery or MI. Propranolol was discontinued 18–24 h prior to studies. All values are mean ± s.e.mean. BW = body weight; $dP/dt$ = peak rate of pressure rise or fall; LV = left ventricular weight; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; RV = right ventricular weight. *$P<0.05$ vs sham. †$P<0.05$ MI propranolol MI vs untreated MI.

receiving propranolol showed a further increase in the E to A ratio.

### *Effects of propranolol on isometric contractility and intracellular Ca regulation*

Isometric function and intracellular calcium transients were recorded in papillary muscles isolated from a noninfarcted portion of the heart. Papillary muscle cross-sectional areas were not significantly different in the four groups of rats (Table 4). Papillary muscles from rats with untreated heart failure had significant impairment of contractility and relaxation and prolongation of the isometric twitch (Table 4). The time course of the aequorin light signal (both time to peak light and time to 50% decline in light) was also prolonged in muscles from rats with MI compared to the sham rats (Table 4). The isometric twitch and the intracellular Ca transient were also prolonged in these muscles. Propranolol treatment caused some depression of myocardial contractility in sham operated rats. Treatment had little effect on contractility of muscles from infarcted hearts, although the time to peak tension and the time to the peak of the Ca transient were partially normalized (Table 4).

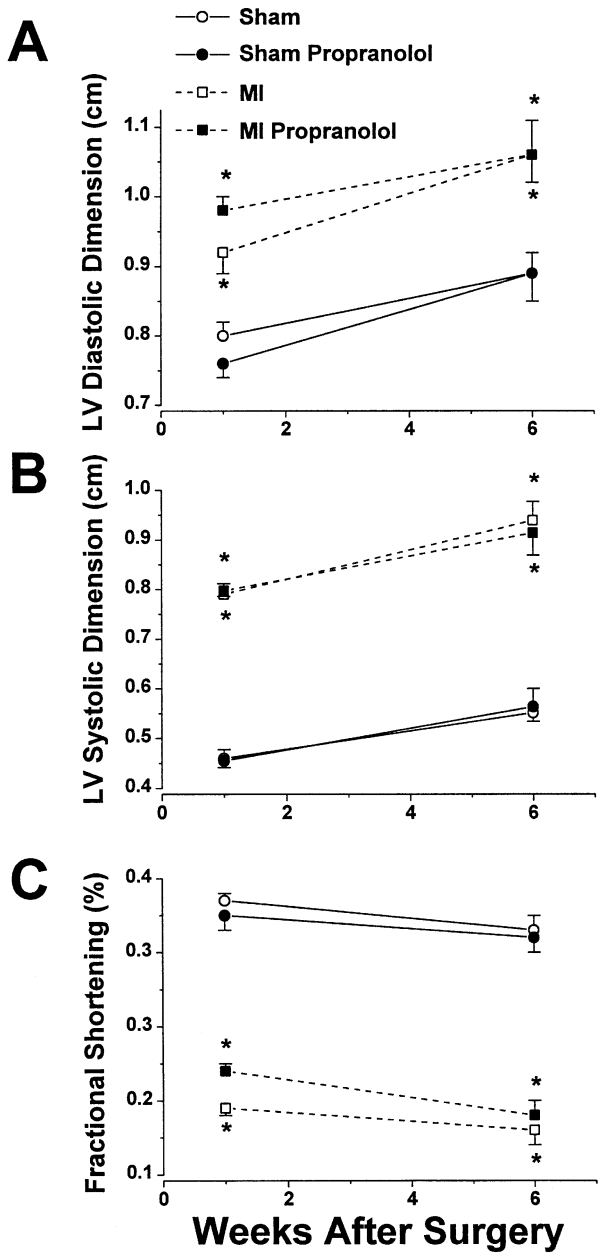


**Figure 1** Time course of echocardiographically assessed changes in left ventricular (LV) geometry and systolic function in rats after transmural anterior wall myocardial infarction (MI; $n=14$) or sham surgery ($n=14$). Immediately following the 1 week echocardiogram, rats from each group were randomized to propranodol (500 mg $l^{-1}$ in the drinking water) or no treatment. Treatment was continued for 5 weeks. (A) Rats with MI showed prominent pathological LV remodelling manifest as increased LV internal diastolic dimension. The extent of LV dilatation was not significantly altered by chronic propranodol treatment. MI caused marked and progressive LV systolic dysfunction as shown by increased LV end-systolic dimension (B) and decreased fractional shortening (C). These changes were not prevented by the administration of propranodol. *$P<0.005$ vs sham. Propranodol MI vs untreated MI = NS.

### *Effects of isoproterenol on excitation-contraction coupling in papillary muscles*

Isoproterenol produced dose-related increases in $+dT/dt$ in sham operated rats. This inotropic effect was slightly diminished in the propranolol treated sham rats. Isoproterenol also caused prominent reductions in the time to peak tension in the muscles from both sham and infarcted rats (Figure 4). Propranolol treatment did not alter this effect. Isoproterenol had less effect on the time course of the intracellular Ca transient than on the isometric twitch in muscles from all four groups of rats (Figure 4C and D). Interestingly, isoproterenol had a stronger effect on the time course of the fall in Ca concentration in the infarcted than in the sham rats (Figure 4D). Thus, at maximal stimulation, the time to 50% decline in light was comparable in the sham and MI muscles. Propranolol treatment did not significantly alter these effects.

## Discussion

We found that ischaemic heart failure in the rat was characterized by marked LV dilatation, global and regional systolic dysfunction, diastolic filling abnormalities and contractile dysfunction in isolated muscle preparations. Long term

**Table 2** Indexes of left ventricular geometry and systolic function measured from M-mode and Doppler echocardiography 6 weeks after MI or sham surgery

| | *Sham* | *Sham propranol* | *MI* | *MI propranolol* |
|---|---|---|---|---|
| LVIDd (mm) | 8.9±0.3 | 8.9±0.4 | 10.6±0.4* | 10.6±0.5* |
| LVIDs (mm) | 5.5±0.2 | 5.6±0.4 | 9.4±0.4* | 9.1±0.4* |
| PWd (mm) | 1.6±0.05 | 1.6±0.06 | 1.6±0.07 | 1.6±0.1 |
| PWs (mm) | 2.7±0.1 | 2.7±0.1 | 2.2±0.2 | 2.4±0.3 |
| PWT (%) | 74±3 | 67±5 | 39±8* | 55±11* |
| RWT | 0.36±0.02 | 0.37±0.01 | 0.31±0.02 | 0.30±0.03 |
| FS (%) | 38±2 | 37±2 | 11±2* | 13±2* |
| LVOT VTI (mm) | 49.8±2.3 | 42.2±2.6 | 47.5±2.2 | 43.0±3.3 |

Propranolol was administered weeks 1–6 postoperatively. LVIDd = left ventricular internal diastolic dimension; LVIDs = left ventricular internal systolic dimension; PWd = posterior wall thickness in diastole; PWs = posterior wall thickness in systole; PWT = posterior wall thickening; RWT = relative wall thickness ((2 × PWD)/LVIDd); FS = fractional shortening; LVOT VTI = velocity time integral of the pulsed wave Doppler spectrum recorded in the left ventricular outflow tract. All values are mean±s.e.m. *$P<0.05$ vs sham-operated rats. MI propranolol vs untreated MI = NS.

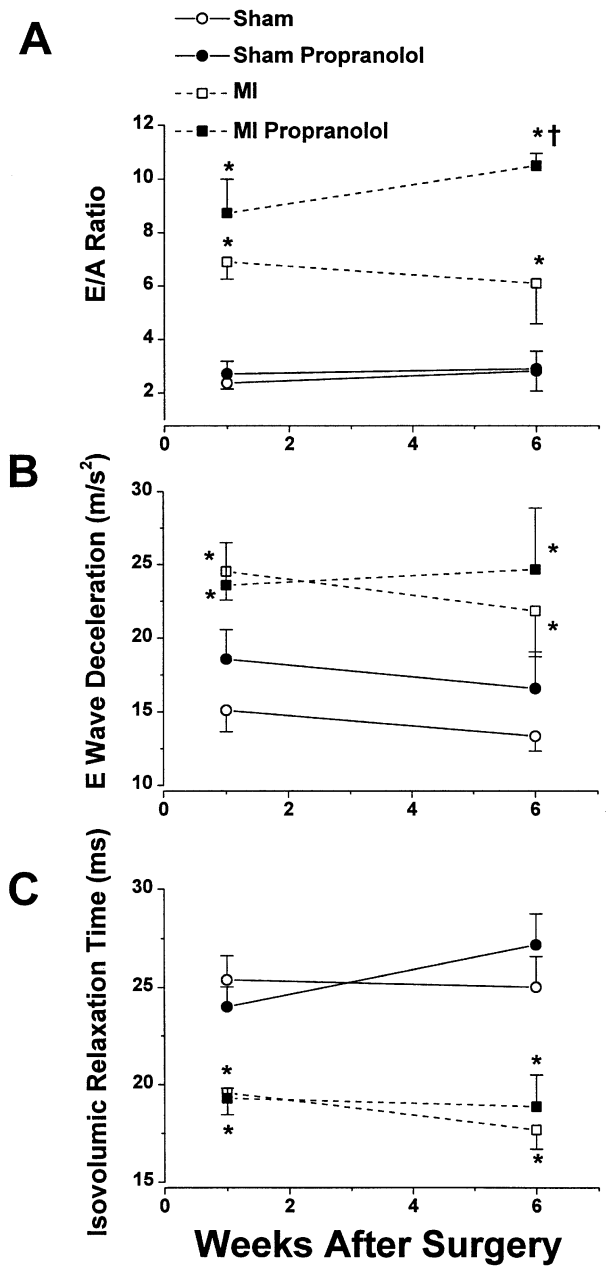


**Figure 2** Changes in left ventricular (LV) diastolic filling in sham operated rats and rats with myocardial infarction (MI) randomized to propranodol or no treatment. Untreated MI rats developed restrictive LV filling patterns as assessed by (A) the ratio of early to late filling velocities (E/A), (B) the E wave deceleration slope, and (C) the isovolumic relaxation time. Propranodol treatment significantly worsened the E to A ratio in MI rats, tended to cause further increases in the E wave deceleration rate, and did not change the isovolumic relaxation time. $*P<0.05$ vs sham operated rats. $\dagger P<0.05$ propranodol MI vs untreated MI.

beta-adrenergic blockade in this model of heart failure did not alter the development of LV enlargement or the progression of systolic dysfunction. Instead, active treatment further increased LV end-diastolic pressures and modestly worsened the LV diastolic filling abnormalities. Treatment did not produce significant improvement in isometric contractility of papillary muscles although the time to peak tension and the time to the peak of the Ca transient were slightly decreased ($P=$NS). Propranolol treatment also did not restore inotropic responsiveness to beta adrenergic stimulation. Thus, chronic nonselective beta-adrenergic receptor blockade in this model of postinfarction heart failure appears to produce very few beneficial effects and may worsen diastolic abnormalities.

Acute and long-term treatment with beta adrenergic blocking agents has been associated with improved survival in patients with recent MI (beta blocker heart attack trial research group, 1982; Norwegian Study Group, 1981). Several mechanisms may contribute to these effects. Given acutely, beta blockers may limit infarct size. Another component of the improvement with beta blockade seems to be related to reductions in sudden cardiac death (beta blocker heart attack trial research group, 1982). Finally, prevention of recurrent MI or other ischemic events also may be important (Roberts *et al*., 1991). Our study was not intended to evaluate effects on infarct size or long-term survival. Rather, we were primarily interested in whether beta blockade was effective in improving structural or functional abnormalities in established heart failure. In this model, most of the arrhythmic deaths occur in the first 24 h post MI, and late deaths generally begin to occur more than 30 days out (Opitz *et al*., 1995; Pfeffer *et al*., 1985a). Since treatment was administered during a time period in which mortality was expected to be very low in the untreated MI rats, we did not expect to see a treatment-associated reduction in mortality. Recurrent or ongoing ischaemia was not an issue in our model since the coronary circulation is normal except in the area of ligation. Although the anti-ischaemic and antiarrhythmic effects of beta blockade would not be evident because of our study design, we hypothesized that chronic propranolol treatment might be associated with improvements in LV remodelling and haemodynamics. Further, we posited that improvement of myocardial function and excitation-contraction coupling might explain such beneficial effects.

We found little evidence that propranolol produced beneficial effects on LV, myocardial or cellular function in this model of heart failure. Given the growing body of literature regarding favourable effects of beta blockade, it is important to carefully consider the significance of our findings. First, it is possible that our study was simply underpowered to detect small, but significant improvements in LV geometry or function. This seems unlikely since the trend, if any, was towards worsening function in the treated animals. Furthermore, we have previously shown significant benefits of chronic angiotensin converting enzyme inhibition with captopril in this

**Table 3** Indexes of left ventricular filling measured from transmitral Doppler spectra 6 weeks after MI or sham surgery

| | *Sham* | *Sham propranolol* | *MI* | *MI propranolol* |
|---|---|---|---|---|
| E velocity (cm s) | 80±4 | 84±3 | 96±4* | 95±8* |
| A velocity (cm s) | 35±4 | 34±4 | 24±6 | 9±9*† |
| E/A | 2.8±0.7 | 2.9±0.7 | 6.1±1.5* | 10.5±0.5*† |
| E deceleration (cm $s^2$) | 13.3±1.0 | 16.5±2.4 | 21.8±3.1* | 24.6±4.2* |
| IVRT (ms) | 25.0±1.6 | 27.1±1.6 | 17.7±1.0* | 18.9±1.6* |

Propranolol was administered weeks 1–6 postoperatively. IVRT = isovolumic relaxation time. All values are mean±s.e.mean. $*P<0.05$ vs sham-operated rats. †MI propranolol vs untreated MI.

same model of heart failure, and with similar numbers of subjects (Litwin *et al*., 1996; Litwin & Morgan, 1992). In those studies we found that 5 weeks of captopril treatment produced significant improvement in myocardial function, intracellular calcium regulation, beta-adrenergic responsiveness and LV filling patterns (Litwin *et al*., 1996; Litwin & Morgan, 1992).

Second, it is possible that beta blockers are effective in some forms of heart failure, but not others (Steeds & Channer, 1997; The CIBIS investigators, 1994). In some studies, improvement in LV systolic function during treatment seems to be more pronounced in patients with idiopathic dilated cardiomyopathy than in those with ischaemic heart disease (Andersson *et*

**Table 4** Indexes of isometric function and the time course of the intracellular Ca transient (as determined from the aequorin light signal) in noninfarcted papillary muscles from sham-operated rats and rats with healed myocardial infarction (MI). Propranolol was administered weeks 1–6 after MI or sham surgery

| | *Sham* | *Sham propranolol* | *MI* | *MI propranolol* |
|---|---|---|---|---|
| Papillary muscle area ($mm^2$) | 0.87±0.06 | 1.17±0.08 | 1.09±0.18 | 1.03±0.11 |
| Developed tension (g $mm^2$) | 2.4±0.5 | 2.0±0.1 | 1.6±0.3* | 1.6±0.3* |
| Time to peak tension (ms) | 139±4 | 150±12 | 183±5* | 163±9* |
| Peak rate of tension rise (g $mm^2$ sec) | 32.4±6.9 | 22.5±2.0 | 15.4±2.7* | 16.7±3.3* |
| Peak rate of tension fall (g $mm^2$ sec) | 26.7±16.2 | 13.9±0.7 | 11.9±2.2* | 10.9±1.8* |
| Relaxation half time (ms) | 93±7 | 105±9 | 102±7 | 98±6 |
| Time to peak light (ms) | 41.7±4.4 | 46.4±1.3 | 62.0±3.3* | 53.3±3.6* |
| Time to 50% decline in light (ms) | 30.1±1.7 | 32.6±2.0 | 42.9±1.7* | 49.3±2.3* |

All values are mean±s.e.mean. *$P<0.05$ vs sham-operated rats. MI propranolol vs untreated MI = NS.

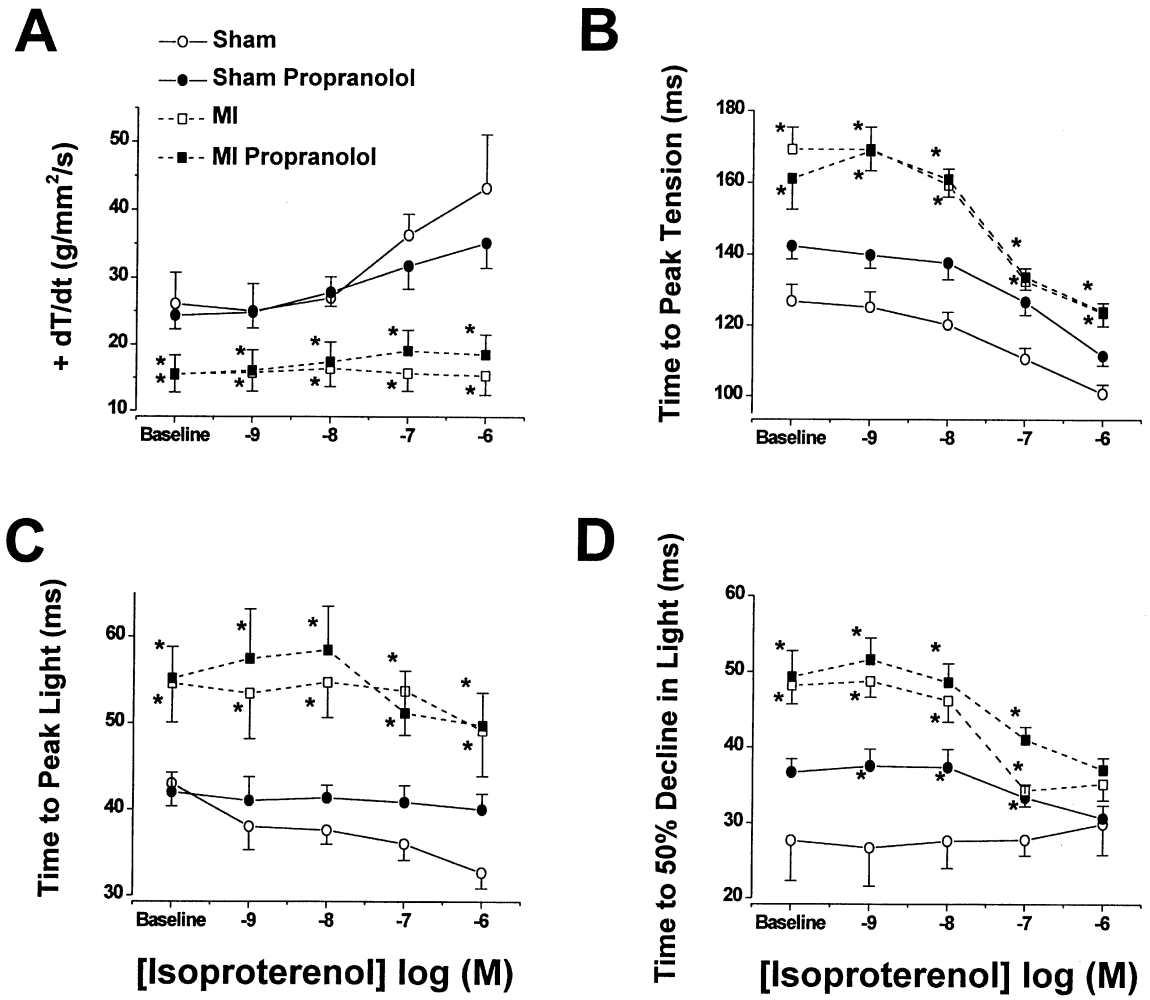


**Figure 3** Response to increasing isoproterenol concentration in papillary muscles from sham operated rats and rats with postinfection heart failure randomized to no treatment or propranodol. Papillary muscles from myocardial infarction (MI) rats had lower peak rate of tension rise (A) at all isoproterenol concentrations. Propanolol treatment did not improve the blunted inotropic response to isoproterenol in the papillary muscle from infarcted hearts. (B) Although the MI papillary muscles had prolongation of the isometric twitch at baseline, isoproterenol significantly shortened the time to peak tension. This effect was similar in muscles from untreated and propranodol treated rats. The time course of the intracellular Ca transient was also prolonged in papillary muscles from infarcted hearts (C and D). Again, the muscles from propranodol treated MI rats responded similarly to those from the untreated MI rats. Data are mean±s.e.mean. *$P<0.05$ vs sham. Propranodol MI vs untreated MI = NS.

al., 1991; Bristow et al., 1996; Doherty et al., 1992; Woodley et al., 1991). One possible explanation for this finding is that MI results in permanent myocyte loss in the infarcted territory. The amount of surviving viable myocardium may be insufficient to show significant improvement in global LV function, even during beneficial treatment. In contrast, the absolute number of viable myocytes may be greater in the hearts of patients with idiopathic dilated cardiomyopathy. The irreversible nature of ischaemic damage could help to explain why the prognosis is much worse in patients with ischaemic than in those with idiopathic cardiomyopathy (Bart et al., 1997).

Third, the beneficial effects of beta blockers may be specific to certain agents. Beta blockers vary widely in their receptor selectivity (beta 1 vs beta 2 receptor subtypes), lipid solubility, duration of action, intrinsic sympathomimetic activity, and vasodilatory effects. The various clinical trials have used different agents, enrolled different populations of patients, had different end points, and different durations of treatment. Thus, it is very difficult to directly compare the findings across these studies. There are few published data available directly comparing the effects of different agents in the treatment of heart failure. It has been suggested that carvedilol has a greater ability than propranolol to reduce infarct size in a rat model of ischaemia and reperfusion injury; however, this comparison was indirect (Feuerstein et al., 1993). Gilbert et al found that the vasodilating beta blocker, carvedilol, produced more improvement in functional classification and tended to produce greater improvement in LV ejection fraction than metoprolol (Gilbert et al., 1996). Recent studies showing beneficial effects of carvedilol in patients with ischaemic heart failure (Australia/New Zealand Heart Failure Research Collaborative Group, 1997; Doughty et al., 1997) suggests that this drug may be uniquely effective amongst the large class of beta blockers. Therefore, it is possible that some of the effects of carvedilol may be related to its vasodilating properties, antioxidant effects, or antineutrophil effects rather than its ability to block beta adrenergic receptors (Feuerstein et al., 1993; 1998). Some investigators believe that blockade of the beta-2 receptor subtype is important and may explain the relative lack of efficacy of highly beta-1 selective agents in some studies (Gilbert et al., 1996). Interestingly, at least two reports have shown that carvedilol does not increase beta adrenergic receptor density (Bohm et al., 1998; Gilbert et al., 1996). Thus, the beneficial effects of this drug are probably not related to alterations in membrane receptors. Propranolol is a widely used nonselective beta blocker that has been shown to improve survival in patients with recent MI (Beta blocker heart attack trial research group, 1982). In addition, propranolol has been shown to increase beta adrenergic receptor density in this animal model of heart failure (Warner et al., 1992). Thus it seemed to be an appropriate agent to study the relatively pure effects of beta adrenergic receptor blockade. Similar to our results, Cherng et al found that metoprolol produced no significant improvement when given to rats with previous myocardial infarction (Cherng et al., 1994). One additional consideration is that all of the recent clinical trials have added beta blockers to regimens of angiotensin converting enzyme inhibitors, diuretics, and dioxin (Packer et al., 1996). It is possible that the combination of these drugs is beneficial, while beta blockers without vasodilating effects are not effective when given alone.

Fourth, there may be species differences in effectiveness of beta blockade. It is possible that rats do not respond to beta blockade, while humans do. There is no literature available to directly support or refute this theory. However, propranolol produces clinically relevant inhibition of beta receptor mediated effects (i.e., blunting of chronotropic effects of isoproterenol) in the rat (Gay et al., 1990). The rat model of postinfarction heart failure, in many ways, closely mimics the same condition in humans. Angiotensin converting enzyme inhibitors improve survival and haemodynamics in this model, as in humans (Litwin & Morgan, 1992; Pfeffer et al., 1985b; Raya et al., 1989). Calcium channel blockers do not seem to have beneficial effects in rats with MI – also similar to findings in humans (Hagar et al., 1992). Finally, there is abundant data showing neurohumoral and sympathetic activation in this model of heart failure (Hodsman et al., 1988; Schunkert et al., 1993; Teerlink et al., 1994). If this overactivation of compensatory systems has a direct role in the deterioration of function that occurs after ischemic LV damage, then beta blockade should be effective in rats, like other species.

Fifth, the timing and duration of therapy may be important. We felt that by 1 week after surgery, animals would be stable to undergo echocardiography and to tolerate initiation of propranolol treatment. We were concerned that earlier initiation of treatment might be detrimental in animals with relatively acute heart failure. However, it is also possible that earlier initiation of treatment would have been more helpful. It is notable that treatment with captopril, initiated at the same time point and continued for the same duration, is associated with significant improvement in LV filling, myocardial function, and intracellular Ca regulation (Litwin et al., 1996; Litwin & Morgan, 1992). Interestingly, Hu et al suggested that late treatment (14 days after MI) with bisoprolol was more beneficial than treatment initiated early (30 min) after MI in the rat (Hu et al., 1998). The duration of treatment in our study was intended to allow any potential beneficial effects to become evident, but before significant mortality occurred in the untreated rats. It is possible that with longer treatment we would have seen more benefit. In this regard, some investigators have proposed that worsening of symptoms and LV function may occur initially, improvement in LV function may take months, and normalization of LV geometry may not be evident until patients have been treated with beta blockers for periods of more than one year (Hall et al., 1995). A given treatment duration in the rat is not equivalent to the same duration of treatment in a human. With a typical life span of approximately 3 years, most biological processes are accelerated in the rat compared with larger species. Thus, 5 weeks of therapy corresponds to a significantly longer period of treatment in humans.

Finally, the dose of propranolol used may not have been optimal. If the dose were either too high or too low, potentially beneficial effects of treatment may have been obscured. The dose of 500 mg $l^{-1}$ drinking water was chosen based on prior studies showing that it produces slowing of the heart rate and reduced chronotropic responsiveness to isoproterenol in intact animals. (Gay et al., 1990). Furthermore, this dose has been shown to increase beta adrenergic receptor density in this model of heart failure (Warner et al., 1992). Since the treated animals had evidence of worsened LV filling pressures and a more restrictive diastolic filling pattern, it seems unlikely that the dose was too low. In some clinical trials, higher doses of beta blockers were reported to have a more significant beneficial effect (Bristow et al., 1996). Thus, we doubt the dose that we used was too high.

### *Study limitations*

Several potential pitfalls of this study have been reviewed above. The limitations of the echocardiographic technique in

rats have been discussed elsewhere (Litwin *et al*., 1994). Although widely employed, the use of papillary muscle preparations for assessing myocardial function is open to criticism. Issues such as damaged muscle ends, core hypoxia, and inhomogeneous loading of the Ca indicator are all valid concerns. These issues have been extensively dealt with previously (Litwin *et al*., 1991; Litwin & Morgan, 1992). Despite the problems associated with papillary muscle studies, a wealth of knowledge about myocardial function has been derived from such work.

Although the results of the current study are largely 'negative' in the sense that a treatment benefit was not seen, this does not make the findings more or less important than a 'positive' study. Because there may be a tendency to preferentially report positive findings in clinical trials, the true effects of a certain treatment in unselected patients may be difficult to ascertain. In the case of beta blockers, most trial designs have excluded patients who did not tolerate the initial doses of the medication (Australia/New Zealand Heart Failure Research Collaborative Group, 1997; Packer *et al*., 1996). Thus, the published literature may contain a bias towards demonstrating efficacy of treatment. In addition, almost all patients enrolled in the clinical trials were first stabilized on angiotensin converting enzyme inhibitors prior to the institution of beta blockers. This may be important since there seems to be a synergistic effect between these classes of drugs (Exner *et al*., 1999). Our data do not rule out beneficial effects of beta blockade in patients with ischaemic heart failure, however, we found little evidence of a myocardial protective effect that could be directly attributable to beta adrenergic blockade.

## *Conclusions*

In a model of postinfarction heart failure, chronic propranolol treatment did not significantly alter postinfarction LV remodelling, and was associated with higher LV filling pressures, lower LV d$P$/d$t$, and a more restricted LV filling pattern. In the noninfarcted myocardium basal contractility and inotropic responsiveness to isoproterenol were not improved by treatment. Our study did not examine survival or possible antiarrhythmic effects of propranolol treatment. However, in this model, there seems to be little in the way of significant haemodynamic or contractile improvement. These data suggest that critical evaluation of beta blockers should be carried out to determine: (1) which subgroups of patients with LV dysfunction may gain the greatest benefit, (2) which agents are most efficacious, (3) are the ancillary effects of beta blocking agents (e.g., alpha blockade or antioxidant activity) important, and (4) is the simultaneous administration of vasodilating agents important in producing beneficial effects during long term beta adrenergic blockade?

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