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RESEARCH PAPER

Effects of treatment with a 5-HT_4 receptor antagonist in heart failure

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Background and purpose: Positive inotropic responses (PIR) to 5-hydroxytryptamine (5-HT) are induced in the left ventricle (LV) in rats with congestive heart failure (CHF); this is associated with upregulation of the G_s-coupled 5-HT_4 receptor. We investigated whether chronic 5-HT_4 receptor blockade improved cardiac function in CHF rats.
Experimental approach: Rats were given either the 5-HT_4 antagonist SB207266 (0.5 mg kg^{-1} $24h^{-1}$; MI_{int}) or placebo (MI_{pl}) through mini-osmotic pumps for 6 weeks subsequent to induction of post-infarction CHF. *In vivo* cardiac function and *ex vivo* responses to isoprenaline or 5-HT were evaluated using echocardiography and isolated LV papillary muscles, respectively. mRNA levels were investigated using real-time quantitative RT-PCR.
Key results: LV diastolic function improved, with 4.6% lower LV diastolic diameter and 24.2% lower mitral flow deceleration in MI_{int} compared to MI_{pl}. SB207266 reduced LV systolic diameter by 6.1%, heart weight by 10.2% and lung weight by 13.1%. The changes in posterior wall thickening and shortening velocity, cardiac output, LV systolic pressure and $(dP/dt)_{max}$, parameters of LV systolic function, did not reach statistical significance. The PIR to isoprenaline (10 μM) increased by 36% and the response to 5-HT (10 μM) decreased by 57% in MI_{int} compared to MI_{pl}. mRNA levels for ANP, 5-$HT_{4(b)}$ and 5-HT_{2A} receptors, MHCβ, and the MHCβ/MHCα -ratio were not significantly changed in MI_{int} compared to MI_{pl}.
Conclusions and implications: Treatment with SB207266 to some extent improved *in vivo* cardiac function and *ex vivo* myocardial function, suggesting a possible beneficial effect of treatment with a 5-HT_4 receptor antagonist in CHF.
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Keywords: 5-HT; congestive heart failure; 5-HT_4 receptor blockade; SB207266; piboserod

Abbreviations: CHF, congestive heart failure; CO, cardiac output; CRC, contraction relaxation cycles; $(dP/dt)_{max}$, $(dP/dt)_{min}$ maximum and minimum derivative of the pressure curves; $(dF/dt)_{max}$, maximal development of force; FS, fractional shortening; LAD, left atrial diameter; LV, left ventricle; LVDd, LV diameter in diastole; LVDs, LV diameter in systole; LVEDP, LV end diastolic pressure; MI, myocardial infarction; MI_{int}, MI intervention; MI_{pl}, MI placebo; PIR, positive inotropic response; Polr2A, polymerase (RNA) II (DNA directed) polypeptide A; PW, posterior wall; PWSV, PW shortening velocity; RT, time from peak force to 80% relaxation; RT–PCR, real-time reverse transcriptase–polymerase chain reaction; SBP, systolic blood pressure; TBP, TATA box binding protein; TPF, time to peak force

Introduction

The treatment of congestive heart failure (CHF) has changed during the recent years from treating haemodynamic imbalance to correction of maladaptive neurohumoral changes (Bristow, 2000). Both the renin–angiotensin–aldosterone system (Poole-Wilson, 2003; Rajagopalan and Pitt, 2003) and the β-adrenoceptors (Lohse *et al*., 2003) have proven important in activating myocardial remodelling.

The rationale for β-blocker therapy in CHF is that chronic β-adrenoceptor-mediated signalling is a harmful compensatory mechanism in the failing heart (Bristow, 2000). In end-stage heart failure, the total signal transducing potential through β-adrenoceptors is reduced by 50–60% owing to desensitization of β-adrenoceptors. This process involves increased activity of the inhibitory G protein (Lohse *et al*., 2003) and the G-protein-coupled receptor kinases responsible for modulating receptor activity by phosphorylation, and decreased abundance and activity of the adenylyl

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cyclase enzyme (Ishikawa *et al*., 1994; Ungerer *et al*., 1994; Lohse *et al*., 2003). Remaining signalling capacity is believed to be the basis for the beneficial effects of β-adrenoceptor blockade in CHF (Bristow, 2000). With this in mind, other compensatory mechanisms might also be maladaptive and represent targets for pharmacotherapy. However, despite the impressive clinical evidence for the effectiveness of β-blockade in human CHF, several studies have failed to demonstrate a similarly beneficial effect in rats with CHF (Omerovic *et al*., 2003).

Recently, we demonstrated a positive inotropic response (PIR) to 5-hydroxytryptamine (5-HT; serotonin) in the left ventricle (LV) of rats with CHF (Qvigstad *et al*., 2005a). This response was not observed in control hearts, but became apparent after large myocardial infarctions (MI), also in the absence of CHF. The PIR was mediated through the 5-HT$_4$ receptor, which also mediates a PIR in human failing ventricle (Brattelid *et al*., 2004b). The 5-HT$_4$-mediated response was characterized by an increase in contractile force as well as by a more rapid relaxation, features similar to those of the β-adrenoceptor-mediated contractile response.

The 5-HT$_4$ receptor is known to activate adenylyl cyclase through the stimulating G protein G_s, and signals by increasing cAMP (cyclic AMP) levels (Hoyer *et al*., 1994). This was also found in failing myocardium of humans and rats (Brattelid *et al*., 2004b; Qvigstad *et al*., 2005a). This parallels the signalling mechanism for the β-adrenoceptor system, suggesting that the induction of the 5-HT$_4$ receptor could be maladaptive, for example, through induction of myocardial remodelling.

We hypothesized that the upregulation of the 5-HT$_4$ receptor in postinfarction CHF is maladaptive and that chronic blockade of the 5-HT$_4$ receptor would reduce myocardial remodelling and improve cardiac function. We found that treatment with a 5-HT$_4$ receptor antagonist to some extent improved *in vivo* cardiac function. Also, *ex vivo* β-adrenoceptor responsiveness increased, and 5-HT$_4$ receptor-mediated signalling decreased, consistent with some beneficial effects of the treatment.

Methods

CHF model

Animal care was according to the Norwegian Animal Welfare Act, which conforms to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe no. 123, Strasbourg, 1985). Two animals per cage in a temperature-regulated room (21°C) with 12-h day/12-h night cycling were given access to food and water *ad libitum*. An extensive MI was induced in anaesthetized (68% N_2O/29% O_2/2–3% isoflurane) male Wistar rats (320 g) by proximal ligation of the left coronary artery as described previously (Sjaastad *et al*., 2000). After 3 days, echocardiography was performed under similar anaesthesia to verify the size of the infarction (see below). Sham-operated rats (Sham) were used as controls.

Study design

Only those rats with a large infarction defined, with echocardiography, as an infarction including more than 2/3 of the anterior wall and the apex of the LV in the long axis view, and more than 2/3 of the LV-free wall in the short axis view, were included in the study (MI rats). MI rats were randomly allocated to treatment with the 5-HT$_4$ blocker SB207266 (MI_{int} (MI intervention)) or vehicle (MI_{pl} (MI placebo)), administered with 2 ml Alzet mini-osmotic pumps (Alza, Palo Alto, CA, USA) placed subcutaneously on the neck. The pumps delivered drug at a rate of 0.5 mg kg^{-1} 24 h^{-1}, which gave a serum concentration of $\sim$15 nM as determined by high-performance liquid chromatography (HPLC). To control the effect of drug administration *per se*, Sham rats were similarly randomly allocated to receive drug ($Sham_{int}$) or vehicle ($Sham_{pl}$). Three weeks later, the mini-osmotic pumps were replaced as they only contained a volume sufficient for 4 weeks of drug administration. After 6 weeks of drug administration, *in vivo* cardiac performance was evaluated by echocardiography and haemodynamic measurements under anaesthesia. Subsequently, the heart was excised and LV myocardium was harvested for real-time reverse transcriptase–polymerase chain reaction (RT–PCR) or papillary muscle experiments. The individuals obtaining and analysing the various data were unaware of the treatment of the animals.

Reduced LV end diastolic and systolic diameters, measured by echocardiography, were used as primary indicators of less cardiac remodelling. Also, mitral flow deceleration and cardiac output (CO), parameters of diastolic and systolic function, were used as primary end points of *in vivo* function. *Ex vivo*, increased inotropic response to isoprenaline in papillary muscles was used as an indicator of improved cardiac function.

HPLC

SB207266 was extracted and concentrated from serum by solid-phase extraction on 1 ml Oasis HLB (Waters Corp., Milford, MA, USA). Analysis was performed by HPLC on a Nova-Pak C18 (Waters Corp., Milford, MA, USA) analytical column with electrochemical detection (ESA Coulochem III, Chelmsford, MA, USA) at 800 mV.

Echocardiography, haemodynamic and infarct size measurements

Doppler echocardiography and analysis were performed with a VIVID 7 echocardiograph (GE Vingmed Ultrasound, Horten, Norway (GE)) as described previously (Sjaastad *et al*., 2000). Infarct size was determined and left-ventricular end-diastolic pressure (LVEDP), systolic blood pressure (SBP) and the maximum and minimum derivative of the pressure curves ($(dP/dt)_{max}$ and $(dP/dt)_{min}$) were obtained as described previously (Sjaastad *et al*., 2000; Qvigstad *et al*., 2005a).

Isolated papillary muscles

Posterior left-ventricular papillary muscles were prepared and field-stimulated at 1 Hz, and the contraction–relaxation cycles (CRCs) were recorded and analysed as described

previously (Skomedal *et al*., 1997; Sjaastad *et al*., 2003) with respect to maximal developed force (F_{max}, mN), maximal development of force ($(dF/dt)_{max}$), time to peak force (TPF), and relaxation time (RT = time from peak force to 80% relaxation). The PIR was defined as an increase in $(dF/dt)_{max}$. Retrograde perfusion (15 min) of the heart before removal of the papillary muscle, repetitive buffer changes and an equilibration of 90 min also served as a washout procedure for the 5-HT$_4$ antagonist SB207266 given *in vivo*. The experiments were performed in the presence of blockers (added 90 min before agonist) of adrenoceptors (prazosin 0.1 μM, timolol 1 μM, except when the β-adrenoceptor agonist isoprenaline was used) and muscarinic cholinoceptors (atropine 1 μM). 5-HT was added to the organ bath as a bolus (10 μM), and maximal response was verified by subsequently increasing the 5-HT concentration to 100 μM (Qvigstad *et al*., 2005a). Concentration–response curves were constructed by estimating centiles ($-\log EC_{10}$ to $-\log EC_{100}$) (Sjaastad *et al*., 2003).

Real-time quantitative RT–PCR

LV tissue was prepared, and real-time quantitative RT–PCR was performed as described previously (Brattelid *et al*., 2004a). In addition to normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the results were normalized to Polr2A and TBP. Sets of primers and probes and quantitative RT–PCR conditions are listed in Qvigstad *et al*. (2005a). The names and sequences of additional upper (U) and lower (L) primers (Eurogentec, Seraing, Belgium) and probes (P; Eurogentec, Seraing, Belgium) used were (5′–3′): rat β_1-adrenoceptor (β_1-AR): ON309(U), GCAAGGACCC GAGTGGAAA; ON310(L), CAAACCAGAGCTGAACACTTA GGTT; rat β_2-AR: ON311(U), CTCATCCCTAAGGAAGTTTA CATCCT; ON312(L), CTGGAAGGCAATCCTGAAATCT; myosin heavy chain (MHC)α: ON369(U), CTCAAACTCATGGC CACACTCTT; ON370(L), GAGCCTTTCTTCTTGCCTCCTT; TM33(P), FAM-CCACCTATGCTTCTGCTGATACCGGTGA-DQ; MHCβ: ON371(U), CCTCCCTCAAGCTCCTAAGTAATCT; ON372(L), AAAGGATGAGCCTTTCTTTGCTT; TM34(P), FAM-TTTGCCCTTGTCTACAGGTGCATCAGCT-DQ. SYBRGreen (Eurogentec, Seraing, Belgium) was used to quantify the mRNA for the β_1- and β_2-adrenoceptors.

Sample size and statistics

We would, with a power of 0.80 and an α of <0.05, detect a difference in the echocardiographic parameters LV diameter in diastole (LVDd), LV diameter in systole (LVDs), CO and mitral flow deceleration of 5–10% (depending on parameter) between the MI groups with 30 rats included in each group (Sjaastad *et al*., 2000). Thus, 40 MI animals were scheduled to be included in both the MI$_{int}$ and MI$_{pl}$ groups. Owing to a higher than expected mortality rate during the primary operations, only 64 MI rats were included in the study. The random allocation procedure assigned 29 rats to treatment (MI$_{int}$) and 35 to placebo (MI$_{pl}$), but two rats in the MI$_{int}$ group lost the pump and were thus excluded. Mortality rate was not significantly different between the MI$_{int}$ group (four out of 27) and the MI$_{pl}$ group (five out of 35). In both Sham groups, 13 rats were included, and none died. In both Sham$_{pl}$ and Sham$_{int}$, one of the rats managed to remove the pump. All results are expressed as mean ± s.e.m., where *n* denotes number of animals. Statistical analysis was performed with a two-way analysis of variance with a *post hoc* Student–Newman–Keuls test or a nonparametric Mann–Whitney analysis. $P<0.05$ was considered statistically significant.

Drugs

SB207266 (*N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2*H*[1,3]-oxazino[3,2_a]indole-10-carboxamide) hydrochloride (Gaster *et al*., 1995) was synthesized by Drug Discovery Laboratory AS (Oslo, Norway). Prazosin hydrochloride, timolol maleate and atropine sulphate were from Sigma (Steinheim, Germany).

Results

Effects of treatment on animal and cardiac characteristics

Infarction size was not significantly different between the MI groups (Table 1). However, the heart weight was 10.2% lower in MI$_{int}$ than in MI$_{pl}$. Heart rate was not significantly different between the two MI groups, but overall heart rate was lower in the intervention groups than in the placebo groups. Also, lung weight (LW) was 13.1% lower in MI$_{int}$ than in MI$_{pl}$. The other characteristics of the animals were not significantly different between the groups. However, some trends in the direction of improvement were observed (right ventricular weight, SBP, LVEDP and $(dP/dt)_{min}$), whereas none of the characteristics indicated aggravated heart failure. Body weights and tibia lengths were not significantly different among the four groups. In the Sham group, administration of SB207266 did not have a significant effect on heart-, lung- or right-ventricle weights, or on LV pressures (Table 1). The Sham$_{int}$ rats did not exhibit any signs of adverse drug effects as judged by visual observation.

Effects of treatment on in vivo *cardiac function evaluated by echocardiography*

The treatment improved LV diastolic function, as assessed by 4.6% lower LVDd and 24.2% lower mitral flow deceleration (Table 2 and Figure 1). Consistent with this, left atrial diameter (LAD) was reduced, although nonsignificantly. LVDs was reduced by 6.1% in MI$_{int}$ compared to MI$_{pl}$, which suggests improved systolic function, although the change was minor. Also, the 15% higher fractional shortening (FS) in MI$_{int}$ than in MI$_{pl}$ was in accordance with this finding, but this difference did not reach significance ($P=0.25$). Posterior wall (PW) thickening, PW shortening velocity and CO were not significantly different in MI$_{int}$ compared to MI$_{pl}$ (Table 2). Neither M-mode nor Doppler parameters revealed any significant difference between the Sham groups (Table 2), suggesting that SB207266 does not have major effects on normal *in vivo* cardiac function.

Table 1 Animal characteristics

	MI_{pl}	MI_{int}	$Sham_{pl}$	$Sham_{int}$
n	30	23	12	12
BW (g)	373±6	368±6	382±6	382±5
HW (g)	2.26±0.05	2.03±0.06$^{\#}$	1.14±0.03*	1.13±0.02*
HW/TL (g cm^{-1})	0.58±0.01	0.52±0.02$^{\#}$	0.29±0.01*	0.29±0.01*
RVW (g)	0.44±0.02	0.40±0.02	0.17±0.01*	0.17±0.01*
RVW/TL (g cm^{-1})	0.112±0.001	0.101±0.006	0.042±0.002*	0.043±0.002*
LW (g)	3.97±0.27	3.45±0.21$^{\#}$	1.53±0.03*	1.49±0.05*
LW/TL (g cm^{-1})	1.01±0.07	0.87±0.05$^{\#}$	0.39±0.01*	0.38±0.01*
TL (mm)	39.3±0.2	39.6±0.2	39.3±0.2	39.1±0.2
SBP (mm Hg)	91±3	94±3	111±5*	108±5*
LVEDP (mm Hg)	24.5±1.9	23.5±1.9	2.5±0.5*	4.4±0.6*
$(dP/dt)_{max}$ (mm Hg s^{-1})	5.19±0.24	5.23±0.22	13.16±1.04*	12.64±0.78*
$-(dP/dt)_{min}$ (mm Hg s^{-1})	5.05±0.28	5.52±0.28	9.42±0.83*	10.71±0.72*
Infarct size (%)	48.4±1.3	46.2±1.4		

Abbreviations: BW, body weight; HW, heart weight; LVEDP, left ventricular end diastolic pressure; LW, lung weight; RVW, right ventricular weight; SBP, systolic blood pressure; TL, tibia length.
*$P<0.05$, Sham vs corresponding MI; $^{\#}P<0.05$, MI_{int} vs MI_{pl}.

Table 2 Echocardiographic characteristics

	MI_{pl}	MI_{int}	$Sham_{pl}$	$Sham_{int}$
n	30	23	12	12
M-mode				
IVSd (mm)	0.48±0.03	0.55±0.04	1.77±0.05*	1.68±0.05*
IVSs (mm)	0.51±0.05	0.57±0.06	3.00±0.06*	2.80±0.13*
LVDd (mm)	10.8±0.16	10.2±0.14$^{\#}$	7.36±0.13*	7.10±0.57*
LVDs (mm)	9.8±0.16	9.2±0.18$^{\#}$	4.44±0.16*	4.82±0.22*
FS (%)	9.1±0.5	10.5±1.0	39.9±1.7*	40.2±4.0*
PWd (mm)	2.07±0.08	2.12±0.06	1.75±0.05*	1.84±0.08*
PWs (mm)	2.63±0.10	2.72±0.10	2.72±0.09	2.75±0.13
PW thickening (%)	29.3±1.8	28.3±2.6	56.0±6.3*	51.3±6.4*
PWSV (cm s^{-1})	1.94±0.13	1.84±0.24	2.92±0.23*	2.70±0.27*
LAD (mm)	7.64±0.28	7.13±0.22	4.67±0.07*	4.69±0.13*
Doppler				
Peak mitral flow (m s^{-1})	1.00±0.04	0.94±0.03	0.90±0.04	0.89±0.05
Mitral flow deceleration (m s^{-2})	49.5±4.2	37.5±2.6$^{\#}$	17.7±0.8*	20.8±2.3*
Heart rate (beats min^{-1})	341±9	322±7	400±9*	375±10*
Peak LVOT flow (m s^{-1})	1.18±0.08	1.17±0.05	1.19±0.03	1.21±0.06
CO in LVOT (ml min^{-1})	102±8	100±6	154±8*	145±10*

Abbreviations: CO, cardiac output; FS, fractional shortening in LVD; IVSd/s, inter-ventricular septum thickness in diastole and systole, respectively; LAD, left atrial diameter; LVDd/s, left ventricular diameter in diastole and systole, respectively; LVOT, left ventricular outlet tract; PWd/s, posterior wall thickness in diastole and systole, respectively; PWSV, posterior wall shortening velocity.
*$P<0.05$, Sham vs corresponding MI; $^{\#}P<0.05$, MI_{int} vs MI_{pl}.

Effects of treatment on β*-adrenoceptor-mediated inotropic and lusitropic responses*

The PIR to isoprenaline (10 μM) was 36% higher in MI_{int} than in MI_{pl} (Figure 2d and f, $P<0.05$), representing a partial normalization. SB207266 treatment did not change EC_{50} significantly (Figure 2e). Basal TPF was 35.0 and 38.6 ms longer and RT 12.2 and 20.4 ms longer in MI_{int} and MI_{pl} than in the respective Sham groups ($P<0.05$), but these variables were not influenced by treatment with SB207266 (Table 3). Isoprenaline shortened TPF by 19 and 20% and RT by 33 and 32% in MI_{int} and MI_{pl}, respectively, with no significant difference between the groups (Table 3). There was no significant difference in basal or β-adrenoceptor-mediated PIR or CRC characteristics between the two Sham groups (Table 3 and Figure 2). SB207266 treatment did not change the EC_{50} for isoprenaline stimulation in Sham (Figure 2b), demonstrating that it did not change the sensitivity of the β-adrenoceptor system.

Effects of treatment on 5-HT-mediated inotropic and lusitropic responses

Because a 5-HT-mediated PIR appears after MI and is related to development of CHF (Qvigstad *et al*., 2005a), we studied the PIR induced by 5-HT in the MI groups. After washout of SB207266 given *in vivo*, the PIR elicited by a supramaximal concentration of 5-HT (10 μM) was 57% smaller in MI_{int} than in MI_{pl} (Figure 3a and b). Further increasing the 5-HT concentration 10-fold to 100 μM did not increase the response, confirming proper washout of SB207266. The

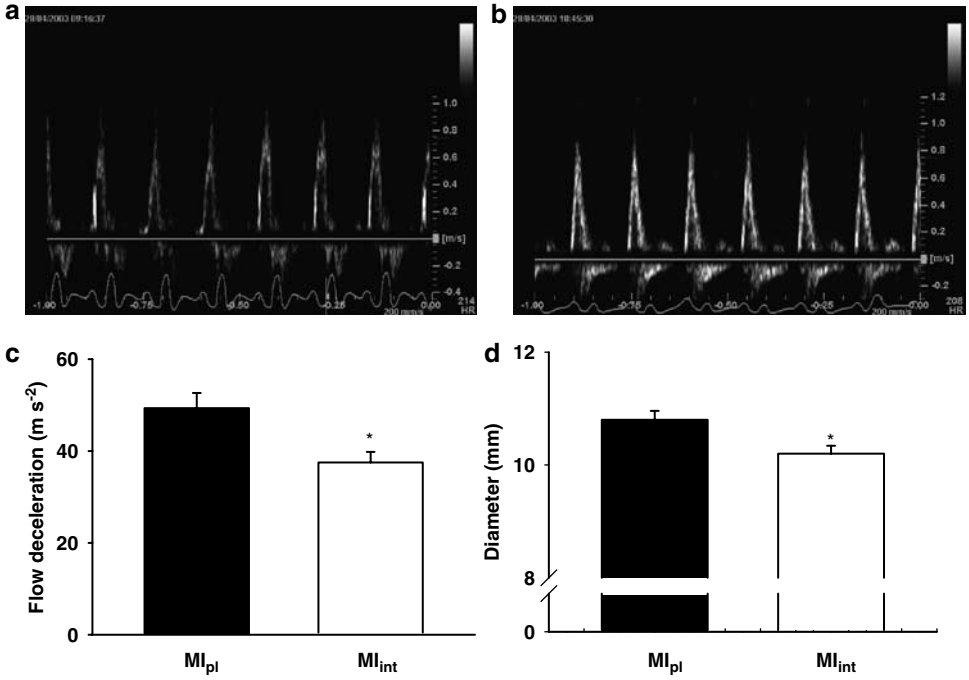

Figure 1 Echocardiography of MI. The figure shows original mitral Doppler recordings from representative MI$_{pl}$ (**a**) and MI$_{int}$ (**b**) rats, average mitral flow deceleration in the MI-groups (**c**) and left ventricular end diastolic diameter, LVDd, in the MI-groups (**d**). *$P<0.05$ MI$_{int}$ vs MI$_{pl}$.

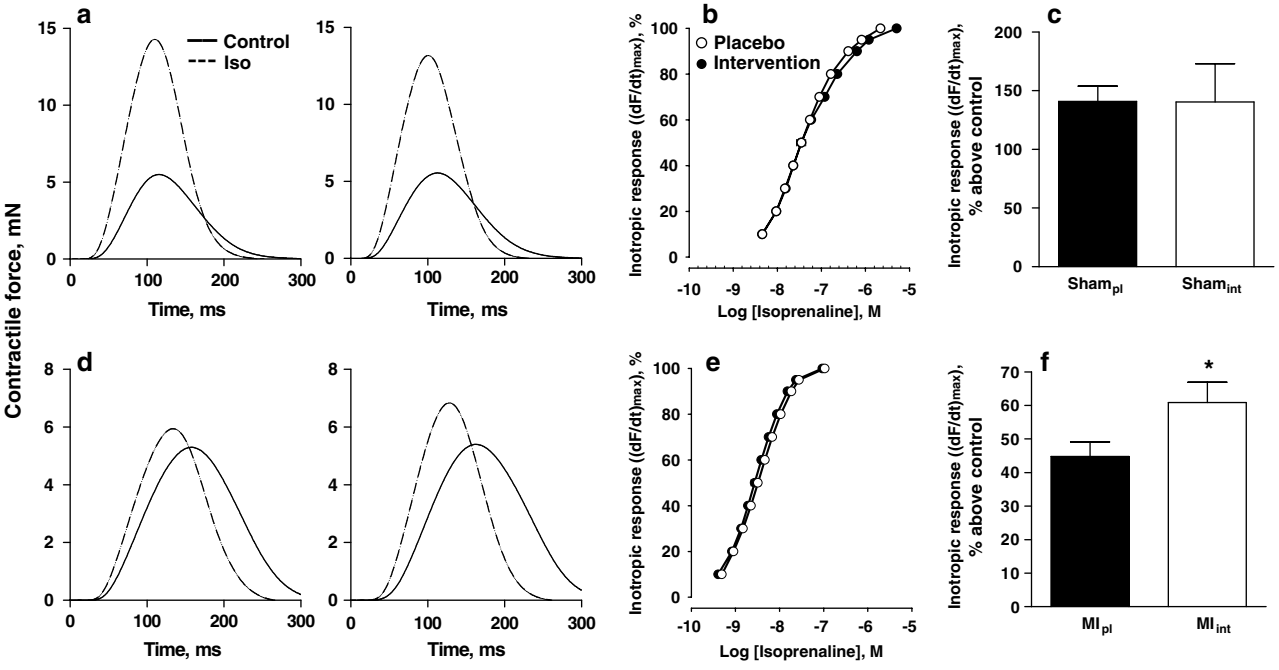

Figure 2 Inotropic responses to isoprenaline in Sham (**a**–**c**) and MI (**d**–**f**) papillary muscles. The figure shows averaged (20–40 cycles) CRCs from Sham$_{pl}$ (**a**, left), Sham$_{int}$ (**a**, right), MI$_{pl}$ (**d**, left) and MI$_{int}$ (**d**, right) before and at maximal steady state after addition of 100 μM isoprenaline (Iso), concentration–response curves for isoprenaline (**b**, **e**) and maximal inotropic response to isoprenaline (**c**, **f**). *$P<0.05$ MI$_{int}$ vs MI$_{pl}$.

effect of 5-HT on the CRC characteristics was less pronounced in MI$_{int}$ than in MI$_{pl}$ (ΔTPF was 61% lower and ΔRT 52% lower in MI$_{int}$ than in MI$_{pl}$; Table 3), in line with the finding of reduced 5-HT-induced responses in MI$_{int}$ compared to MI$_{pl}$.

mRNA profile following treatment with 5-HT$_4$ receptor blocker

There were no significant changes in the mRNA profile after treatment with SB207266, regardless of whether the real-time RT–PCR results were normalized to GAPDH (Figure 4), Polr2A or TBP. However, several parameters, which changed

significantly between Sham and MI, showed an expression pattern that could encourage future studies. These nonsignificant effects of treatment were all in a direction consistent with improvement of CHF. Thus, mRNA for ANP, 5-HT$_4$ and 5-HT$_{2A}$ receptors, induced in CHF (Qvigstad *et al*., 2005a, b), tended to be reduced (Figure 4a–c). The level of MHCα is known to be reduced in CHF (Wang *et al*., 2004), whereas the level of MHCβ is usually found to increase (Yue *et al*., 1998; Wang *et al*., 2004). Nonsignificant trends consistent with an opposite effect of treatment were observed for the levels of MHCβ mRNA (Figure 4d), MHCα mRNA (Figure 4e) and the MHCβ mRNA/MHCα mRNA ratio (Figure 4f). Whereas the individual mRNAs were normalized to GAPDH mRNA (Figure 4) as well as Polr2A and TBP (not shown), the calculated MHCβ/MHCα ratio is independent of the normalization standard. Expression of β_1- and β_2-adrenoceptor mRNA did not differ between the MI and Sham groups (not shown), and was not influenced by treatment (Figure 4g and h).

Table 3 Effect of 5-HT and isoprenaline on CRCs characteristics

	MI_{pl}	MI_{int}	$Sham_{pl}$	$Sham_{int}$
Iso				
N	11	13	4	4
TPF basal	148.1±2.6	148.0±3.0	109.5±3.1	113.0±5.1
TPF stim	118.4±2.2*	119.8±2.0*	101.0±3.2*	101.3±4.0*
ΔTPF	−29.7±1.5	−28.2±1.0	−8.5±1.6	−11.8±1.4
RT basal	110.2±2.4	109.0±1.9	89.8±2.3	96.8±1.8
RT stim	74.8±1.4*	72.9±0.5*	61.0±1.2*	66.3±2.5*
ΔRT	−35.3±2.3	−36.1±1.8	−28.8±2.5	−30.5±1.2
5-HT				
N	4	6		
TPF basal	141.3±4.5	147.7±4.1		
TPF stim	132.3±4.4	144.2±2.9		
ΔTPF	−9.0±2.0	−3.5±1.5†		
RT basal	100.8±2.8	103.3±3.7		
RT stim	89.8±3.6	98.0±4.1		
ΔRT	−11.0±2.4	−5.3±0.8†		

Abbreviations: CRC, contraction relaxation cycle; Iso, isoprenaline; RT, time from peak force to 80% relaxation; ΔRT, RT stim – RT basal; *n*, number of rats (one papillary muscle per rat); TPF, time to peak force; ΔTPF, TPF stim – TPF basal.

Isoprenaline (100 μM) in the presence of atropine (1 μM) and prazosin (0.1 μM), or 5-HT (10 μM) in the presence of atropine (1 μM), prazosin (0.1 μM) and timolol (1 μM). Values are mean±s.e.m. of average results from 20–40 CRCs in each group of papillary muscles.

*$P<0.05$ stim vs basal, $^{\dagger}P<0.05$ MI_{int} vs MI_{pl}.

Discussion

The present study was conducted to test the hypothesis that sustained treatment with a 5-HT$_4$ antagonist could improve cardiac function in rats with postinfarction CHF. Although some of the results were consistent with this idea, the effects were generally small. The most pronounced effects were observed on *in vivo* diastolic function, as assessed by reduced LVDd and mitral flow deceleration. The treatment was also associated with several other changes representing a shift towards normality, such as reduced heart and lung weights, increased myocardial β-adrenoceptor-mediated PIR and reduced 5-HT$_4$ receptor-mediated PIR. Taken together, treatment with a 5-HT$_4$ receptor blocker appeared to be beneficial in postinfarction CHF, but further studies are required to verify this.

Changes in cardiac remodelling

Some of the echocardiographic data are consistent with reduced cardiac remodelling in MI_{int} compared to MI_{pl}. Both LVDd and LVDs were reduced, and taken together with the observed reduction in heart weight, this is consistent with improved function and attenuated postinfarction remodelling in response to treatment. This finding may be important as the postinfarction remodelling process is believed to be maladaptive and lead to myocardial dysfunction (Vaughan and Pfeffer, 1994). Clinical trials have also shown that indices of remodelling are correlated with poor prognosis after MI (White *et al*., 1987). During the last decade, several studies have established β-blockers and ACE-inhibitors as the standard treatment for postinfarction CHF. These studies have shown that the ability to attenuate the remodelling process is one of the most important properties of these drugs (Sharpe *et al*., 1988; Nicolosi *et al*., 1996; Omerovic *et al*., 2003; Doughty *et al*., 2004; Frantz *et al*., 2005; Karram *et al*., 2005). Thus, the observed reduction of remodelling in response to treatment in the present study could improve prognosis in postinfarction CHF.

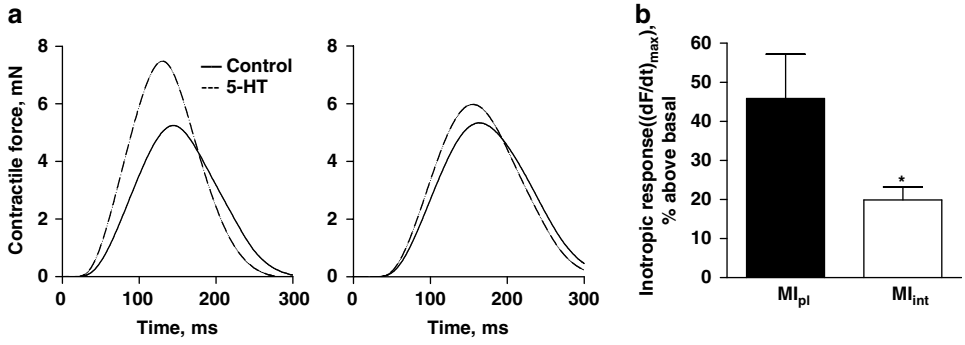

Figure 3 Inotropic response to 5-HT in MI papillary muscles. The figure shows averaged (20–40 cycles) CRCs before and at maximal steady state after addition of 100 μM 5-HT (**a**) from rats receiving vehicle (left) and SB207266 (right) as well as maximal inotropic response to 100 μM 5-HT in MI papillary muscles (**b**) *$P<0.05$ MI_{int} vs MI_{pl}.

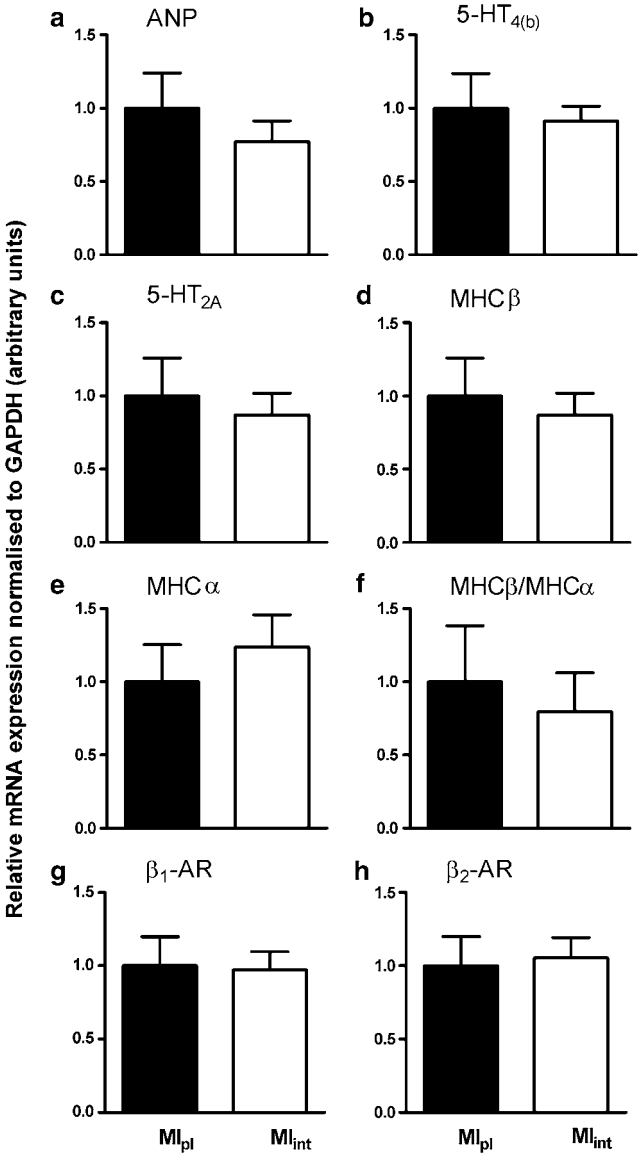

Figure 4 Expression of ANP, 5-HT$_{4(b)}$, 5-HT$_{2A}$, MHCβ, MHCα, β_1-AR and β_2-AR mRNA in LV of MI rats. mRNA for ANP (**a**), 5-HT$_{4(b)}$ receptor (**b**), 5-HT$_{2A}$ receptor (**c**), MHCβ (**d**), MHCα (**e**), β_1-AR (**g**) and β_2-AR (**h**) in the LV of MI$_{int}$ ($n=17$) and MI$_{pl}$ ($n=12$) rats was quantified by real-time quantitative RT–PCR. The data (**a–e**, **g** and **h**) were normalized to GAPDH mRNA, and are expressed relative to the mean values for MI$_{pl}$, assigned a value of 1. In addition to normalization to GAPDH, the results were normalized to Polr2A and TBP, with essentially the same results (not shown). The ratio of MHCβ to MHCα mRNA level (**f**) is independent of normalization standard.

Except for reduced LVDs, we did not detect any effect of treatment on cardiac systolic performance assessed by SBP, $(dP/dt)_{max}$ or echocardiographic parameters. This finding was not surprising, as experimental studies on β-blocker treatment also show conflicting results with regard to systolic function. Omerovic *et al*. (2003) did not find any effect on *in vivo* systolic function, whereas Sun *et al*. (2005) found improvement in FS. This could be explained by a different methodological sensitivity. Another possibility is that the cellular process leading to increased contractility does not occur simultaneously with the structural reorganization of the myocardium, since Omerovic *et al*. (2003) showed that β-blocker therapy in CHF improved ventricular dimension parameters without concomitant attenuation of functional impairment.

We also examined the effect of SB207266 on LV diastolic dysfunction. In MI$_{int}$, we found a significant reduction of the mitral flow deceleration, which is a reliable parameter of diastolic function (Sjaastad *et al*., 2000; Finsen *et al*., 2005). In line with this finding, LW was significantly reduced, and there was a trend towards reduced LAD, increased-$(dP/dt)_{min}$ (i.e. faster relaxation) and reduced LVEDP in MI$_{int}$ compared to MI$_{pl}$. This was, however, not reflected in the *ex vivo* parameters TPF and RT, both mainly reflecting lusitropic effects. An explanation may be that the papillary muscles are subjected to normalized stretching, which does not necessarily reflect the more complex *in vivo* situation. Taken together, these findings suggest enhanced LV diastolic function. Hence, treatment with a 5-HT$_4$ blocker seems to attenuate LV remodelling and improve diastolic function, but has only a minor impact on systolic function.

Changes in isoprenaline and 5-HT responsiveness

In postinfarction CHF, myocardial β-adrenoceptor responsiveness is reduced because of β_1- and β_2-adrenoceptor desensitization and β_1-adrenoceptor downregulation (Bristow *et al*., 1982; Lohse *et al*., 2003). In humans, β_1-adrenoceptor mRNA level was found to correspond to disease severity (Engelhardt *et al*., 1996), suggesting that β-adrenoceptor responsiveness is an indirect parameter of CHF severity. However, in rat postinfarction CHF, we previously found that the attenuation of the β-adrenoceptor-mediated inotropic response was not due to a reduced number of receptors (Sjaastad *et al*., 2003). Consistent with our previous results, there was no change in mRNA level for β_1- or β_2-adrenoceptors in CHF in the present study (not shown). In line with this, the treatment had no effect on β_1- or β_2-adrenoceptor mRNA expression (Figure 4g and h) or on total β-adrenoceptor level as measured by [^{125}I]iodocyanopindolol binding (not shown). In the present study, the maximum PIR to isoprenaline was 36% higher in MI$_{int}$ than in MI$_{pl}$ (Figure 2f). This may represent a partial restitution of the β-adrenoceptor-mediated response, in accordance with other studies that have shown increased responsiveness of myocardial β-adrenoceptors after treatment with ACE inhibitors (Sanbe and Takeo, 1995). An alternative hypothesis is that the increased β-adrenoceptor response in the treatment group represents a new example of 'cross-sensitization' of G$_s$-coupled receptors following chronic receptor blockade, as described for atrial β_2-adrenoceptors (Hall *et al*., 1990; Kaumann and Sanders, 1993), 5-HT$_4$ receptors (Kaumann and Sanders, 1994; Sanders *et al*., 1995; Pau *et al*., 2003) and H$_2$ histamine receptors (Sanders *et al*., 1996), following chronic β-adrenoceptor blockade in patients. However, the finding that 5-HT$_4$-mediated signalling was reduced, rather than increased in treated MI rats (Figure 3b), does not support this hypothesis.

We have previously demonstrated that the 5-HT$_4$-mediated PIR and 5-HT$_4$ receptor mRNA expression increase

with infarct size and are highest in hearts with large infarctions and CHF (Qvigstad *et al*., 2005a). In the present study, the 5-HT-induced PIR was attenuated in MI_{int} compared to MI_{pl}. The 5-$HT_{4(b)}$ mRNA level also tended to be reduced, although nonsignificantly and not in proportion with the change in 5-HT_4-mediated inotropic response. The reduced 5-HT_4-mediated PIR in MI_{int} is not likely to be explained by incomplete washout of SB207266 in the papillary muscles. Given the $\sim$50% reduction in PIR in MI_{int} compared to MI_{pl} and assuming readily reversible binding of SB207266 to the receptor, this would have implied that the remaining drug in the muscle preparation had shifted the concentration–response curve to 5-HT in such a way that the 10 μM 5-HT added was close to the EC_{50} value for 5-HT in the presence of remaining antagonist. Accordingly, a further 10-fold (10^{-5}–10^{-4} M) increase in 5-HT concentration would be expected to increase the PIR further by displacing SB207266 from the receptors, inconsistent with the data obtained. On the other hand, β_1-adrenoceptor expression increases in CHF patients treated with a selective β_1-adrenoceptor blocker (Sigmund *et al*., 1996). Thus, β_1-adrenoceptors and 5-HT_4 receptors are differently affected by treatment with receptor-selective antagonists in CHF, suggesting that the reduced 5-HT_4-mediated response in MI_{int} is indirectly caused by a reduced severity of CHF. The trend towards a reduction of 5-HT_{2A} receptor mRNA in MI_{int} compared with MI_{pl} can be interpreted similarly.

MHCα and MHCβ mRNA and heart failure

It has been shown that MHCβ mRNA and the MHCβ/MHCα-ratio are decreased and MHCα increased with improvement of CHF, after treatment with β-blockers (Lowes *et al*., 2002; Yasumura *et al*., 2003) or with ACE inhibitor and AT_1 antagonist (Wang *et al*., 2004). The nonsignificant changes in MHC mRNA expression observed in MI_{int} compared with MI_{pl} were consistent with a slight improvement of CHF and might reflect beneficial effects of 5-HT_4 blockade.

Possible mechanisms

The mechanisms behind the possible beneficial effects of 5-HT_4 receptor blockade are not obvious as the intracellular effects of stimulating ventricular 5-HT_4 receptors have not been fully elucidated. However, several mechanisms are possible. First, 5-HT_4 receptors are known to be G_s-coupled in other tissues (Kaumann *et al*., 1990). 5-HT_4 stimulation increases cardiomyocyte cAMP content in the rat failing ventricle (Qvigstad *et al*., 2005a), and increased protein kinase A activity in porcine ventricle (Brattelid *et al*., 2004b). cAMP is known to increase cellular energy consumption, as indicated by the effects of β-adrenoceptor stimulation on myocardial energetics (Otorii *et al*., 1977; Hasenfuss *et al*., 1989). This is critical because the energy production in the failing heart is probably limited (Spindler *et al*., 2003). Thus, one of the possible mechanisms for the beneficial effects of β-blockade (Omerovic *et al*., 2001) and possibly 5-HT_4 receptor blockade could be reduced cAMP production and thereby reduced energy consumption.

Second, the beneficial effect of SB207266 could be mediated through alterations in Ca^{2+} homeostasis. Human atrial 5-HT_4 receptors are known to alter cellular Ca^{2+} handling, presumably by increasing cAMP (Kaumann *et al*., 1990; Ouadid *et al*., 1992; Jahnel *et al*., 1993), and we have unpublished data showing that the 5-HT_4 receptor in failing rat ventricle also exerts its PIR through changes in the activity of Ca^{2+} handling proteins. In CHF, the function of the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase SERCA and the sarcolemmal Na^+/Ca^{2+}-exchanger are altered (Houser *et al*., 2000). Also, the SR Ca^{2+} release channel (RyR) is suggested to be hyperphosphorylated, and this has been proposed to be important in the pathogenesis of CHF (Marx *et al*., 2000). In recent studies on two different CHF models, β-blockers partially attenuated these effects on myocardial Ca^{2+} handling (Plank *et al*., 2003; Sun *et al*., 2005). Thus, in analogy, the possible beneficial effects of SB207266 could also be exerted in part through a restorative effect on Ca^{2+} handling. However, this theory is not consistent with the lack of effect of SB207266 treatment on CRC characteristics, presumably reflecting Ca^{2+} handling. Thus, other mechanisms need to be invoked to explain the effects of SB207266 treatment on cardiac function.

Third, 5-HT_4 stimulation could induce myocardial remodelling by operating at the transcriptional or translational level, for example, through G_s-coupled pathways leading to altered transcription. Ponimaskin *et al*. (2002) have recently shown that 5-HT_4 receptors also couple to G_{13} in neuronal tissue. G_{13} is a G protein known to activate members of the Rho family of small GTPases, which in the heart play a role in the signalling cascades leading to hypertrophy (Hoshijima *et al*., 1998). We can speculate that a similar coupling between the 5-HT_4 receptor and G_{13} exists in the heart and plays a role in remodelling. Treatment with a 5-HT_4 blocker could thereby attenuate pathological remodelling.

So far, functional atrial 5-HT_4 receptors have not been demonstrated in the rat (Läer *et al*., 1998), and thus, a direct effect of 5-HT_4 blockade on heart rate was not expected. However, a significant reduction of heart rate was observed, but only when pooling Sham and MI groups and analyzing the effect of treatment. A larger study could determine whether this effect occurs in both groups, possibly reflecting functional atrial 5-HT_4 receptors with effects on heart rate.

Possibilities for improved study design?

In the present study, a possibly beneficial effect of 5-HT_4 receptor blockade was observed on cardiac remodelling and diastolic function. However, except for significantly reduced LVDs, parameters of systolic function yielded inconsistent results. In a β-blocker study in mice, cellular Ca^{2+} handling returned to normal after 2 weeks of propranolol treatment (Plank *et al*., 2003). However, 10 weeks of treatment was necessary to improve *in vivo* function assessed by echocardiography, suggesting a time lag between molecular restitution towards normality and detectable functional improvement. Extending the intervention for more than 6 weeks in the present study could thus have resulted in a more apparent improvement of *in vivo* function. Another possibly important factor for the effect of the treatment, is

time from start of MI to initiation of drug administration. Laser *et al*. (1996) demonstrated a beneficial effect of starting β-blocker administration to rats 30 min rather than 2 weeks after MI. In the present study, treatment was started 3 days after MI. At this stage only a small 5-HT$_4$-mediated PIR had appeared (Qvigstad *et al*., 2005b), but nevertheless it could have been beneficial to start treatment before this response evolved. However, an even earlier start time for the treatment was not possible because verification of a large infarction was required in the MI groups, and echocardiographic imaging quality is limited at the postoperative days 1–2.

In conclusion, treatment with a 5-HT$_4$ receptor blocker reduces LV remodelling and diastolic dysfunction and partially restores neurohormonal signalling to normal in a postinfarction rat model, whereas other parameters remained unchanged. The possible beneficial effects observed could imply a role for 5-HT$_4$ receptor blockers in the management of CHF.

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Conflict of interest

FOL is the inventor of a published patent application (WO03097065) covering the potential use of 5-HT$_4$ antagonists for treatment of heart failure. The patent is owned by the Norwegian biotech company Bio-Medisinsk Innovasjon AS and the contribution of several of the authors (IS, TB, EQ, KAK, TS, JBO, FOL) is acknowledged through contractual agreements.

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