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Carvedilol blockade of α_1- and β-adrenoceptor induced inotropic responses in rats with congestive heart failure

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Abstract

Carvedilol is a combined α_1- and β-adrenoceptor antagonist. We investigated the ability of carvedilol to antagonize functional effects mediated through myocardial α_1-adrenoceptors in failing vs. non-failing (sham-operated) control hearts and compared such antagonisms to those of myocardial β-adrenoceptors.

Congestive heart failure was induced in Wistar rats by coronary artery ligation. Papillary muscles experiments were performed. Carvedilol antagonized inotropic effects mediated through myocardial α_1-adrenoceptors with similar potencies in failing (pK_i=7.7 (95%, CI; 7.4–8.0)) and sham-operated hearts (pK_i=7.9 (95%, CI; 7.6–8.1)). The potency for the α_1-adrenoceptors was 10–30-fold lower than that for the β-adrenoceptors. In failing hearts, the α_1-adrenoceptor mediated response was similar in size to the attenuated β-adrenoceptor mediated inotropic response. The β-adrenoceptor mediated lusitropic effects were not, however, attenuated in failing compared to sham-operated hearts.

A low degree of α_1-adrenoceptor blockade in the myocardium may contribute to the beneficial effects of carvedilol in heart failure.

Keywords: Carvedilol; Congestive heart failure; α_1-Adrenoceptor; β-Adrenoceptor; Lusitropic; Inotropic

1. Introduction

Carvedilol is essentially a non-selective β-adrenoceptor antagonist devoid of intrinsic sympathomimetic activity. It also exhibits antioxidative and antiproliferative effects (Brixius et al., 2001; Ohlstein et al., 1993; Yue et al., 1992). The vasodilatory actions of carvedilol result primarily from its α_1-adrenoceptor blocking effects (Sponer et al., 1992). Accordingly, carvedilol provides a more comprehensive adrenergic blockade (α_1 and $\beta_{1/2}$) compared to conventional β-adrenoceptor antagonists.

β-adrenoceptor antagonists, such as carvedilol, are now considered standard treatment for patients with heart failure arising from left ventricular systolic dysfunction. Studies indicate that carvedilol has additional benefits over conventional β-adrenoceptor blockade (Metra et al., 2000; Packer et al., 2001) and recently The Carvedilol Or Metoprolol European Trial (COMET) demonstrated superiority for carvedilol on morbidity and mortality in patients with chronic heart failure (Poole-Wilson et al., 2003). However, it is not known whether this is partly attributable to the α_1-adrenoceptor antagonizing effect of carvedilol. Currently, it is still uncertain whether it is beneficial to antagonize myocardial α_1-adrenoceptors during chronic heart failure (Messerli, 2001; Ball, 2000; Osnes et al., 2000).

The α_1-adrenoceptor blocking properties of carvedilol have been investigated with regard to vasodilating effects (Monopoli et al., 1989). However, Pönicke et al. (2002) demonstrated that carvedilol inhibited α_1-adrenoceptor mediated hypertrophy in cardiomyocytes from normal hearts with a potency similar to that we observed for the

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α_1-adrenoceptor mediated inotropic effects in normal hearts (Qvigstad et al., 2003). The ability of carvedilol to antagonize functional α_1-adrenoceptor mediated effects in failing hearts has not, however, been explored. During congestive heart failure, β-adrenoceptor mediated effects are attenuated, and the sensitivity (EC_{50} value) to β-adrenoceptor stimulation is altered. In contrast, α_1-adrenoceptor mediated effects is reported to be unchanged in experimental heart failure (Sjaastad et al., 2003; Chess-Williams et al., 1994). Despite of lower receptor density of α_1-adrenoceptors in human than in rat hearts, the α_1-adrenoceptor mediated inotropic effects in failing human ventricle are of comparable size to that elicited by β-adrenoceptors (Skomedal et al., 1997). Accordingly, the relative contribution of the two respective receptor systems is altered during chronic heart failure. Thus, the principal aim of the present study was to compare the ability of carvedilol to antagonize the inotropic responses induced by myocardial α_1- and β-adrenoceptors in failing and sham-operated control hearts. This will elucidate the effect of carvedilol on the balance between the α_1- and the β-adrenoceptors in the failing heart.

Activation of β-adrenoceptors regulates cardiac function by mediating relaxant effects (lusitropic) in addition to the inotropic effects. Acceleration of the relaxation shortens the systole. This allows less reduction in diastolic filling time at higher heart rates. We have previously demonstrated that lusitropic effects to β-adrenoceptor stimulation seem to remain intact in chronic heart failure in contrast to the well-known attenuation of the inotropic effects (Sjaastad et al., 2003). In the present study, a further characterization of the lusitropic effects of β-adrenoceptor stimulation was performed in failing and sham-operated control hearts, and a comparison to the inotropic effect was made. The ability of carvedilol to antagonize the lusitropic effects of β-adrenoceptor stimulation was studied as well.

2. Material and methods

2.1. Animals

Animals were cared for according to the Norwegian Animal Welfare Act which conforms with the European Convention for the protection of Vertebrate animals used for Experimental and other Scientific Purposes (Council of Europe no. 123, Strasbourg 1985). Two rats were kept in each cage and housed in a temperature regulated room with a 12-h day/12-h night cycling and allowed free access to water and food.

2.2. Heart failure model

Male Wistar rats (Møllegaard Breeding and Research Centre, Skensved, Denmark), weighing about 320 g were intubated and ventilated with 68% N_2O, 29% O_2 and 2–3% isoflurane (Abbot Laboratories, USA). An extensive myocardial infarction was induced by a proximal ligation of the left coronary artery. Six weeks later, the rats were anaesthetized and ventilated on the respirator with 2.2% isoflurane. Left ventricular pressures were measured (Sjaastad et al., 2000) and congestive heart failure rats were included if left ventricular end diastolic pressure was >15 mm Hg and the rats had clinical symptoms of congestive heart failure (such as tachypnoe, pleural effusion, ascites, pulmonary congestion and dilated left atrium). In this model, echocardiography has previously demonstrated severely depressed myocardial function (Sjaastad et al., 2000). The sham-operated control animals were subjected to the same surgical procedure except the coronary artery ligation.

2.3. Isolated papillary muscles

Hearts were isolated from the anaesthetized animals (see above) and carefully dissected free of connective tissue and transferred to ice cold 0.9% NaCl and weighed. The aorta was cannulated, and the coronaries were perfused at 31 °C with a salt solution described below. Posterior left ventricular papillary muscle was ligated and dissected free and mounted in organ baths. In order to prevent contracture of the papillary muscles during dissection and mounting, we used a relaxing solution with a Ca^{2+}/Mg^{2+} concentration ratio of 1:20. The relaxing solution contained (mmol/l): NaCl 118.3; KCl 3.0; $CaCl_2$ 0.2; $MgSO_4$ 4.0; KH_2PO_4 2.4; $NaHCO_3$ 24.9; glucose 10.0; mannitol 2.2 and equilibrated with 95% O_2/5% CO_2 at 31 °C (pH 7.4).

The papillary muscles were mounted in organ baths containing the relaxing solution and allowed to adapt at 31 °C for about 20 min before the solution was changed to one containing the following (in mmol/l): NaCl 119.2; KCl 3.0; $CaCl_2$ 1.8; $MgSO_4$ 1.2; KH_2PO_4 2.4; $NaHCO_3$ 24.9; glucose 10.0; mannitol 2.2 and equilibrated with 95% O_2/5% CO_2 at 31 °C (pH 7.4).

The muscles were field stimulated with alternating polarity at 1 Hz with impulses of 5 ms duration and current about 20% above individual threshold (10–15 mA, determined in each experiment). The isometrically contracting muscles were stretched to the maximum of their length-tension curve. The force was recorded and analysed as previously described (Skomedal et al., 1997). The muscles were allowed to equilibrate for 90 min. When used, prazosin (α_1-adrenoceptor antagonist), timolol (β-adrenoceptor-antagonist) and carvedilol were allowed to act for 90 min before addition of agonist. To avoid possible interference from muscarinic acetylcholine receptor stimulation atropine (0.1 μmol/l) was present throughout all experiments.

Signal averaged contraction–relaxation cycles were calculated for different experimental periods. Representative descriptive parameters like maximal development of force $(dF/dt)_{max}$, time to peak force and time to relaxation to 20% level were obtained from the averaged cycles. $(dF/dt)_{max}$ was used as an index of contractility and inotropic responses to adrenoceptor agonists were expressed by increases in $(dF/dt)_{max}$. Relaxation time (index of relaxation) was calculated as time to relaxation to 20% level minus time to peak force. Lusitropic responses were expressed as reduction in relaxation time. The descriptive parameters at the end of the equilibrium period were used as the control values.

2.4. Experimental design

Pure α_1- and β-adrenoceptor mediated inotropic responses were obtained in the presence of appropriate receptor antagonists.

Table 1
Animal characteristics

	SHAM[a]	CHF[b]
Animals, *n*	90	60
Body weight (g)	420±4	379±5[c]
Heart weight (g)	1.50±0.02	2.58±0.04[c]
RV weight (g)	0.23±0.01	0.44±0.01[c]
Heart wt/body wt (g/kg)	3.6±0.1	6.9±0.1[c]
Lung weight (g)	1.55±0.02	3.84±0.09[c]
LVEDP (mm Hg)[d]	6±0.2	25±0.8[c]
LVSP (mm Hg)[e]	134±2	94±2[c]

[a] Sham-operated rats.
[b] Congestive heart failure rats.
[c] $P<0.001$ compared to SHAM.
[d] Left ventricular end diastolic pressure.
[e] Left ventricular systolic pressure.

Accordingly, either the β-adrenoceptor antagonist timolol (10 μmol/l) or the α_1-adrenoceptor antagonist prazosin (0.1 μmol/l) was used in all experiments. At these concentrations, the receptor antagonists prevented the response to different agonists through the respective receptor system.The concentration–response curves for α_1-adrenoceptor stimulation by phenylephrine and noradrenaline in papillary muscles from failing and sham-operated hearts were performed in the absence and presence of 20 and 80 nmol/l of carvedilol, respectively.

The concentration–response curves for β-adrenoceptor stimulation by isoprenaline were performed in the absence and presence of 4 and 20 nmol/l carvedilol or 2.5 and 20 nmol/l in failing and sham-operated hearts, respectively. The presence of antagonists did not influence the basal function of the muscles with regard to mechanical performance or electrical stimulation threshold.The different agonists were added directly to the organ baths in increasing concentrations until supramaximal concentrations of agonist were obtained with respect to responses. The time to maximal inotropic response after adding agonist to the baths was 5–10 min during α_1-adrenoceptor stimulation and 3–5 min during β-adrenoceptor stimulation.

2.5. Calculation and statistics

The inotropic responses to either agonist are presented as percentage of initial maximal development of force $(dF/dt)_{max}$. When appropriate, the values after responses to agonist were also expressed as percent of maximal response (100%). The concentration–response curves were constructed according to Ariëns et al. (1964), by estimating centiles (EC_{10} to EC_{100}) for each single experiment and calculating the corresponding means. This calculation provides mean curves that express the response as fractional response or percent of maximum and display horizontal positioning and the correct mean slope of the curves. The horizontal positions of the concentration–response curves were expressed as pD_2-values ($-\log EC_{50}$).

Data are expressed as mean or median as appropriate with a 95% confidence interval (CI) and the number of animals expressed as *n*. In all the graphs, the data are presented as the mean±S.E.M. The significance levels of differences were calculated according to Students *t*-test. $P<0.05$ is considered to indicate a statistically significant difference. Inhibition constants (K_i) for carvedilol were calculated from the Schild equation, based on the relative shift, ΔpD_2-values, of the concentration–response curves for receptor stimulation and expressed as median pK_i.

2.6. Drugs

Carvedilol was kindly supplied by Roche Diagnostics GMBH (Mannheim, Germany); Prazosin hydrochloride, Timolol bitartrate, (−)-Phenylephrine hydrochloride and (−)-Isoprenaline hemisulphate were purchased from Sigma. (−)-Noradrenaline bitartrate was purchased through Norwegian Medical Depot. Stock solutions were prepared in double distilled water and kept at −20 C to avoid oxidation. Stock solution of carvedilol was dissolved in DMSO (Sigma). Further solutions of the drugs were made fresh daily and kept cool (0–4 °C).

3. Results

3.1. Cardiac function in rats with post-infarction heart failure

The rats displayed clinical signs of heart failure with tachypnoe, pleural effusion and pulmonary congestion. The failing hearts had large anterior-lateral infarctions. Haemodynamic data suggested diastolic and systolic heart failure (Table 1).

3.2. α_1-Adrenoceptor blockade by carvedilol in failing and sham-operated hearts

The functional α_1-adrenoceptor blocking property of carvedilol was compared in papillary muscles from failing and sham-operated hearts and all experiments reported in this section were performed in the presence of 10 μmol/l timolol. Concentration–response experiments for pure α_1-adrenoceptor mediated inotropic effects were performed with two agonists, the physiologically relevant endogenous agonist noradrenaline and for comparison the more selective synthetic agonist phenylephrine. The concentration–response curves were shifted to a higher concentration of both agonists in the presence of carvedilol.

3.2.1. α_1-Adrenoceptor stimulation by noradrenaline

The mean maximal inotropic responses to noradrenaline in failing and sham-operated hearts were 48% (CI; 44–55, $n=19$) and 54% (CI; 44–64, $n=25$), respectively. Thus, the maximal inotropic response to noradrenaline was not significantly changed in failing compared to sham-operated hearts. The presence of 20

Table 2
α_1-Adrenoceptor mediated inotropic responses

	Control	20 nM carvedilol	80 nM carvedilol
CHF[a] noradrenaline	51% (37–65)	46% (30–63)	46% (29–62)
SHAM[b] noradrenaline	48% (20–77)	56% (38–75)	57% (41–72)
CHF[a] phenylephrine	44% (38–49)	57% (40–73)	40% (33–46)
SHAM[b] phenylephrine	40% (28–52)	39% (31–47)	40% (24–57)

α_1-Adrenoceptor mediated inotropic response above control induced by noradrenaline and phenylephrine in congestive heart failure[a] and sham-operated rats[b], respectively. No significant differences were observed between the inotropic response in the absence and presence of carvedilol.

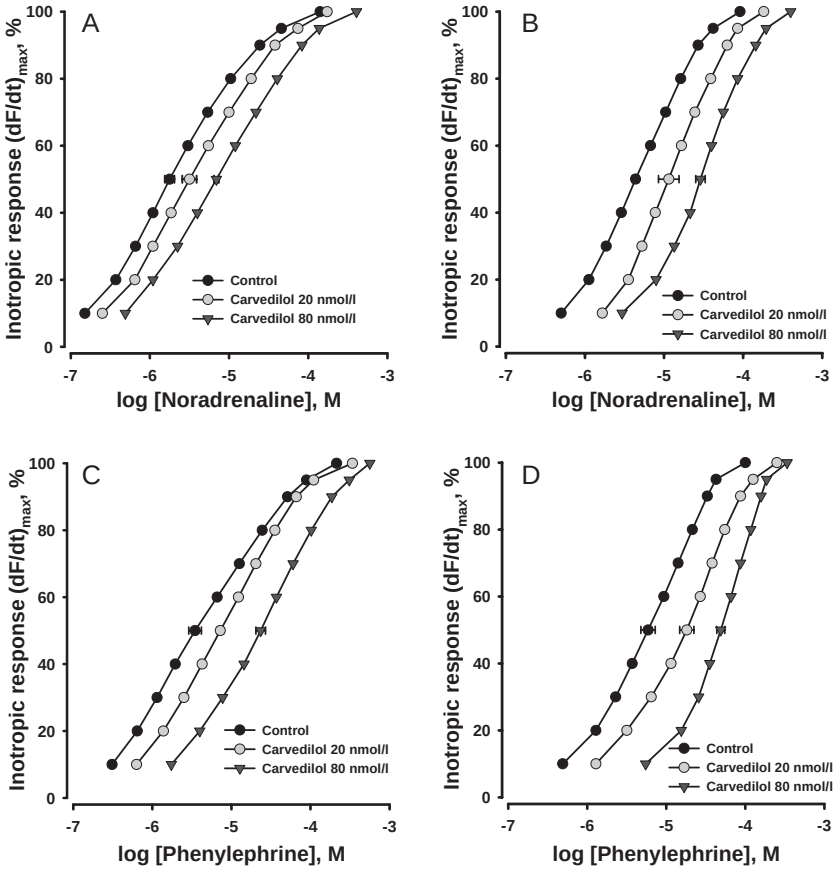

Fig. 1. The concentration–response curves for α_1-adrenoceptor stimulation by noradrenaline (A,B) or phenylephrine (C,D) in congestive heart failure rats (A,C) and sham-operated rats (B,D) in the absence and presence of 20 nmol/l or 80 nmol/l carvedilol, respectively. Ordinate; Inotropic response (increase in $dF/dt)_{max}$ expressed in percent of maximum. Abscissa; logarithmic concentration of noradrenaline (A,B) or phenylephrine (C,D). Horizontal error bars represent S.E.M. of pEC_{50}.

nmol/l and 80 nmol/l carvedilol did not alter the maximal response in either of the groups (Table 2).

The pD_2-values for noradrenaline were 5.75 (CI; 5.93–5.57, $n=7$) in failing and 5.36 (CI; 5.48–5.25, $n=10$, $P<0.05$) in sham-operated control hearts.

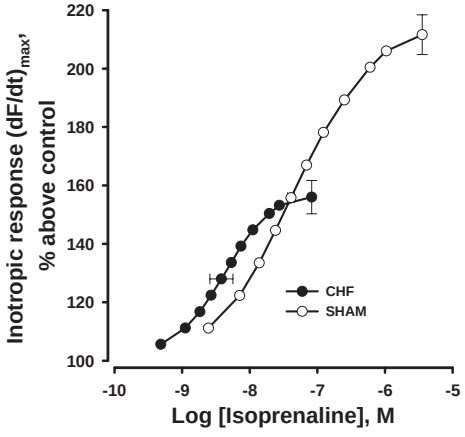

Fig. 2. Concentration–response curves for the inotropic response to β-adrenoceptor stimulation by isoprenaline in congestive heart failure (CHF) and sham-operated (SHAM) rats, respectively. Ordinate; Inotropic response (increase in $dF/dt)_{max}$ expressed in percent of control. Abscissa; logarithmic concentration of isoprenaline. Error bars represent S.E.M.

In failing hearts, 20 nmol/l and 80 nmol/l of carvedilol shifted the pD_2-values for noradrenaline to 5.50 (CI; 5.75–5.25, $n=9$) and 5.16 (CI; 5.24–5.09, $n=7$, $P<0.05$), respectively (Fig. 1A). The calculated median pK_i value for carvedilol in failing hearts was 7.52 (7.85–7.26).

By comparison, 20 nmol/l and 80 nmol/l of carvedilol in sham-operated hearts shifted the curve and the corresponding pD_2-values to 4.94 (CI; 5.25–4.63, $n=8$, $P<0.05$) and 4.53 (CI; 4.68–4.40, $n=7$, $P<0.05$), respectively (Fig. 1B). Accordingly, the median pK_i value of 7.86 (7.98–7.54) calculated revealed an insignificant

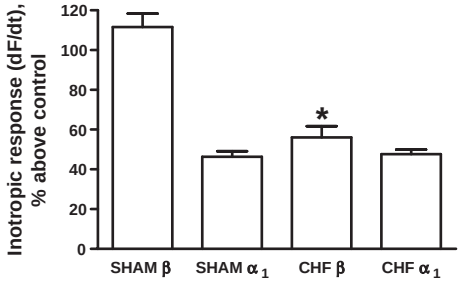

Fig. 3. α_1- and β-adrenoceptor mediated inotropic response above control in congestive heart failure (CHF) and sham-operated (SHAM) rats, respectively. Ordinate; Inotropic response (increase in $dF/dt)_{max}$ expressed in percent of control. *$P<0.05$ compared to SHAM β.

Table 3
β-Adrenoceptor mediated inotropic and lusitropic responses

	Control	4 nM carvedilol	20 nM carvedilol
CHF[a] inotropic	61% (26–87)[c]	54% (38–70)[c]	51% (32–70)[c]
CHF[a] lusitropic	30 ms (27–34)	–	30 ms (25–35)
	Control	2.5 nM Carvedilol	20 nM Carvedilol
SHAM[b] inotropic	123% (80–176)	108% (91–125)	109% (83–136)
SHAM[b] lusitropic	30 ms (26–34)	–	30 ms (24–36)

β-Adrenoceptor mediated inotropic and lusitropic response above control induced by isoprenaline in congestive heart failure[a] and sham-operated[b] rats, respectively. [c]$P<0.05$ compared to SHAM.

difference in potency for carvedilol on myocardial α_1-adrenoceptors in failing compared to sham-operated hearts.

3.2.2. α_1-Adrenoceptor stimulation by phenylephrine

The mean maximal inotropic responses above basal to phenylephrine in failing and sham-operated hearts were 48% (CI; 41–54, $n=24$) and 40% (CI; 34–45, $n=21$), respectively. Accordingly, the maximal α_1-adrenoceptor mediated response to phenylephrine was not significantly changed in failing compared to sham-operated hearts. Nor did the presence of carvedilol significantly alter the maximal response in the individual groups (Table 2). The pD_2-values for phenylephrine were 5.46 (CI; 5.66–5.27, $n=8$) in failing and 5.23 (CI; 5.43–5.02, $n=8$) in sham-operated hearts ($P=0.1$).

In failing papillary muscles, 20 nmol/l and 80 nmol/l of carvedilol shifted the concentration–response curve and lowered the corresponding pD_2-values for phenylephrine significantly to 5.14 (CI; 5.23–5.05, $n=9$, $P<0.05$) and 4.63 (CI; 4.76–4.49, $n=7$, $P<0.05$), respectively (Fig. 1C). The calculated median pK_i value for carvedilol in failing hearts was 7.82 (CI; 8.06–7.52). By comparison, the shifts of the concentration–response curve by 20 nmol/l and 80 nmol/l carvedilol in sham-operated hearts gave pD_2-values of 4.74 (CI; 4.96–4.53, $n=7$, $P<0.05$) and 4.31 (CI; 4.42–4.20, $n=6$, $P<0.05$), respectively (Fig. 1D). Accordingly, the median pK_i value of 7.94 (CI; 8.25–7.69) calculated in sham-operated hearts was not significantly different from that of failing hearts.

3.3. β-Adrenoceptor blockade by carvedilol in failing and sham-operated hearts

The functional β-adrenoceptor blocking property of carvedilol was studied in papillary muscles from both failing and sham-operated hearts in order to compare the potency of carvedilol as an antagonist on both α_1- and β-adrenoceptors in the same preparation.

Concentration–response experiments for functional β-adrenoceptor responses were performed by the synthetic agonist isoprenaline in the presence of 0.1 μmol/l prazosin. The concentration–response curves were shifted to higher concentrations of isoprenaline by 4 nmol/l and 20 nmol/l in failing and 2.5 nmol/l and 20 nmol/l in sham-operated hearts.

3.3.1. β-Adrenoceptor stimulation by isoprenaline

In contrast to the unchanged functional α_1-adrenoceptor mediated response, we observed the expected attenuation of the β-adrenoceptor mediated inotropic response above basal in failing (56%, CI; 44–68, $n=21$) compared to sham-operated hearts (112%, CI; 98–126, $n=21$) (Figs. 2 and 3, $P<0.05$). There were no statistically significant differences in the maximal responses to isoprenaline in the absence and presence of carvedilol in both groups (Table 3).

The potency, expressed as pD_2-values, for isoprenaline at the β-adrenoceptors was significantly higher in failing (8.42, CI; 8.81–8.04, $n=7$) compared to sham-operated hearts (7.39, CI; 7.50–7.28, $n=7$, $P<0.05$) demonstrating a marked increase in sensitivity for isoprenaline in failing hearts despite lower efficacy (Fig. 2).

The presence of 4 nmol/l and 20 nmol/l carvedilol shifted the concentration–response curve for failing hearts significantly to higher concentrations of isoprenaline giving pD_2-values of 7.75 (CI; 7.95–7.55, $n=7$, $P<0.05$) and 7.34 (CI; 7.73–6.96, $n=7$, $P<0.05$), respectively (Fig. 4A). Consequently, the calculated median pK_i value for carvedilol in failing hearts was 8.85 (CI; 9.36–8.33).

In sham-operated hearts, carvedilol at 2.5 nmol/l and 20 nmol/l significantly shifted the concentration–response curve and lowered the corresponding pD_2-values to 6.53 (CI; 6.61–6.44, $n=7$, $P<0.05$) and 5.66 (CI; 5.79–5.52, $n=7$, $P<0.05$), respectively (Fig. 4B). Consequently, the median pK_i value of 9.44 (CI; 9.57–

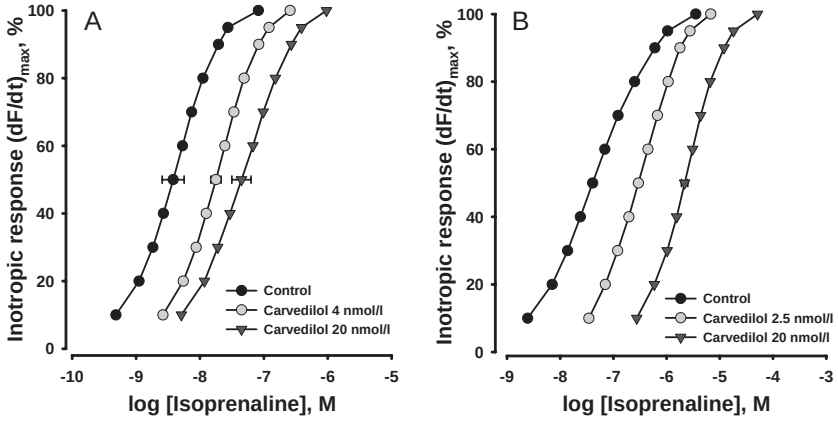

Fig. 4. The concentration–response curves for β-adrenoceptor stimulation by isoprenaline in congestive heart failure rats (4A; 4 nmol/l and 20 nmol/l carvedilol) and sham-operated rats (4B; 2.5 nmol/l and 20 nmol/l carvedilol) in the absence and presence of carvedilol. Ordinate; Inotropic response (increase in $dF/dt)_{max}$ expressed in percent of maximum. Abscissa; logarithmic concentration of isoprenaline. Horizontal error bars represent S.E.M. of pEC_{50}.

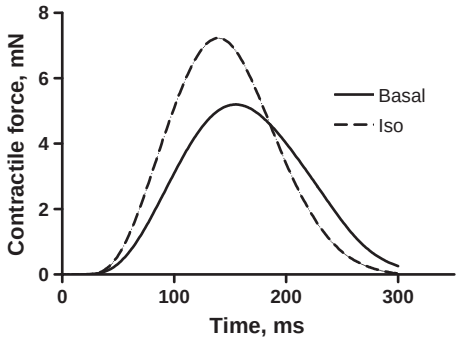

Fig. 5. Example of signal averaged contraction–relaxation cycle in a papillary muscle from a congestive heart failure rat before and after β-adrenoceptor stimulation by isoprenaline. Abcissa: time in ms after initiation of contraction. Ordinate: contractile force in mN.

9.29) calculated for sham-operated hearts was slightly but not significantly higher than the value obtained for failing hearts.

3.4. Lusitropic effects of β-adrenoceptor stimulation in failing and sham-operated hearts

The concentration-dependent lusitropic effect of β-adrenoceptor stimulation was studied in both failing and sham-operated hearts and related to the inotropic effect. The ability of carvedilol to antagonize the lusitropic effect was explored with 20 nmol/l carvedilol.

The mean maximum lusitropic (expressed as reduction of relaxation time) effects elicited by isoprenaline were 30.3 ms (27.8–32.7, $n=14$) and 29.9 ms (27.1–32.8, $n=14$) in failing and sham-operated hearts, respectively. This reflected a marked shortening of the relaxation phase (Fig. 5). In contrast to the inotropic response, the lusitropic effect of β-adrenoceptor stimulation was not attenuated in the failing hearts (Fig. 6A,B).

The concentration–response curves for isoprenaline exhibited pD_2-values of 7.85 (CI; 8.24–7.46, $n=7$) and 7.18 (CI; 7.41–6.95, $n=7$, $P<0.05$) in failing and sham-operated hearts, respectively, demonstrating a significant increase in sensitivity to β-adrenoceptor induced lusitropic effect in failing hearts.

In the presence of 20 nmol/l carvedilol, the concentration–response curves for the lusitropic effect were shifted to higher concentrations of agonist giving pD_2-values of 7.07 (CI; 7.22–6.92, $n=7$) and 5.53 (CI; 5.72–5.36,$n=7$) for failing and sham-operated hearts, respectively (Fig. 7A,B, $P<0.05$). The corresponding median pK_i values calculated for carvedilol were 8.51 (CI; 8.82–7.76) and 9.33 (CI; 9.59–9.09) for failing and sham-operated hearts, respectively. These pK_i values were similar to those obtained for the inotropic responses in failing and sham-operated hearts, respectively.

4. Discussion

The present study demonstrates for the first time that carvedilol is able to antagonize functional effects exerted through myocardial α_1-adrenoceptors in failing hearts. In addition, the study also for the first time fully characterizes and compares the lusitropic effect of β-adrenoceptor stimulation in papillary muscles from failing and sham-operated control hearts and the capability of carvedilol to antagonize this effect.

4.1. Characteristics of carvedilol

The ability of carvedilol to antagonize the functional mechanical effects mediated through myocardial adrenoceptors was not significantly changed in failing compared to sham-operated hearts irrespective of agonist used. In addition, the maximal inotropic responses obtained in the absence and presence of carvedilol were similar (Tables 2 and 3). Accordingly, carvedilol acted as a competitive reversible antagonist with similar potencies for myocardial α_1- and β-adrenoceptors, respectively, in failing and control hearts. Thus, in failing hearts, carvedilol antagonized myocardial α_1-adrenoceptors with a 10–30-fold lower potency than the β-adrenoceptors similar to previously observed data in non-operated non-failing hearts (Qvigstad et al., 2003).

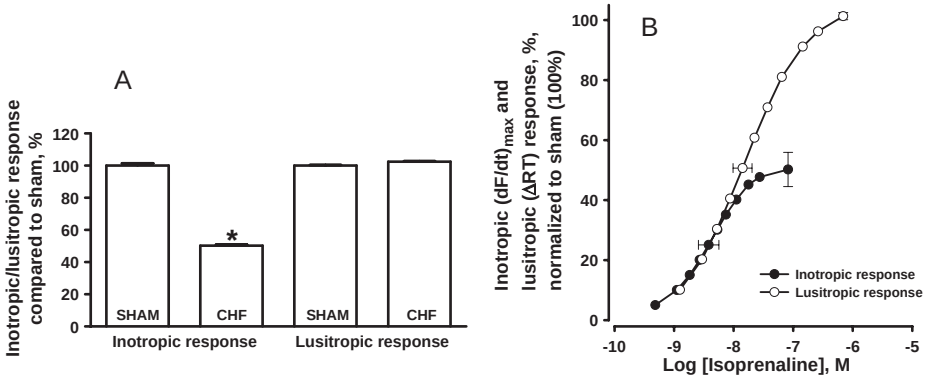

Fig. 6. (A) Inotropic and lusitropic responses to β-adrenoceptor stimulation in congestive heart failure rats (CHF) compared to sham-operated rats (SHAM). (B) Inotropic and lusitropic response to β-adrenoceptor stimulation in congestive heart failure rats normalized to sham-operated rats (100%). Inotropic response (increase in $dF/dt)_{max}$ and lusitropic (ΔRT) expressed in percent of sham-operated rats (100%). Ordinate (A,B); Inotropic response (increase in $dF/dt)_{max}$ and lusitropic (ΔRT) expressed in percent of sham-operated rats (100%). Abscissa (B); logarithmic concentration of isoprenaline. Error bars represent S.E.M. *$P<0.05$ compared to sham-operated rats.

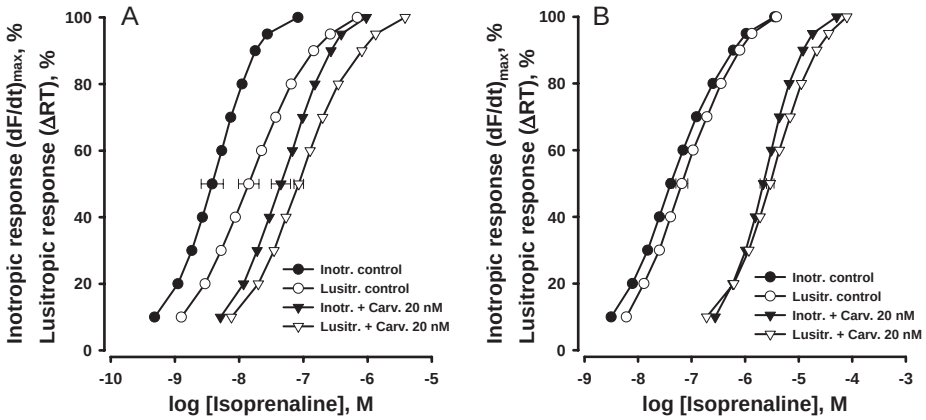

Fig. 7. Comparison of concentration–response curves for the inotropic (black symbols) and lusitropic (white symbols) response to β-adrenoceptor stimulation by isoprenaline in congestive heart failure (A) and sham-operated rats (B), respectively. The curves represent experiments in the absence and presence of 20 nM carvedilol. Ordinate; Inotropic response (increase in $dF/dt)_{max}$ and lusitropic response (ΔRT) expressed in percent of maximum (100%). Abscissa; logarithmic concentration of isoprenaline. Error bars represent S.E.M.

4.2. Inotropic responses in papillary muscles from failing and sham-operated hearts

The maximal α_1-adrenoceptor mediated inotropic responses were not significantly different in failing and sham-operated hearts (Fig 3). This is in agreement with previous reports demonstrating no attenuation of the inotropic response mediated through myocardial α_1-adrenoceptors during congestive heart failure (Sjaastad et al., 2003). The rat heart has a higher cardiac α_1-adrenoceptor density than the human heart (Steinfath et al., 1992). Despite the low receptor density, in failing human myocardium noradrenaline evoked an α_1-adrenoceptor mediated inotropic response of comparable size to that elicited by β-adrenoceptors (Skomedal et al., 1997). The inotropic response in human heart is also quantitatively comparable to the response observed in rat heart thus demonstrating a lack of coherence between receptor density and the size of a response mediated by the α_1-adrenoceptors.

In contrast, the failing rats demonstrated the expected attenuation of the β-adrenoceptor mediated inotropic response compared to sham-operated hearts (Fig. 2 and 3). Consequently, the size of functional α_1- and β-adrenoceptor mediated inotropic effects was similar in failing hearts. Thus, the increased contribution of myocardial α_1-adrenoceptor in the total mechanical response might provide inotropic support when β-adrenoceptor mediated effects are attenuated during congestive heart failure.

The concentration–response curves obtained with isoprenaline revealed a log unit leftward shift of pD_2-value in failing compared to control hearts. This demonstrates a 10-fold increase in sensitivity to isoprenaline in failing hearts. This finding was in agreement with Sjaastad et al. (2003). Thus, there is an increased sensitivity but a lower efficacy of β-adrenoceptor stimulation of inotropic effects in congestive heart failure. In contrast, Kompa et al. (1999) described both a decreased sensitivity and efficacy of β-adrenoceptor stimulation as a consequence of increased G_i in this model of heart failure.

4.3. Lusitropic effects

Activation of cardiac β-adrenoceptors leads to PKA dependent phosphorylation of e.g. phospholamban and troponin I and this facilitates augmentation of Ca^{2+}cycling in the heart and mediates lusitropic effect. Lusitropic effects in the rat heart are primarily mediated through β_1-adrenoceptors whereas the β_2-adrenoceptors elicit negligible lusitropic effects as a consequence of dual coupling to G_s/G_i (Brodde and Michel, 1999).

In the present study, the β-adrenoceptor mediated lusitropic effect was not attenuated in CHF in contrast to the current dogma that the β-adrenoceptor mediated responses are attenuated in heart failure (Brodde et al., 1995) (Fig. 6A,B).

In papillary muscles from sham-operated rats, the pD_2-values for β-adrenoceptor induced inotropic and lusitropic effects were similar (Fig. 7A). Thus, the maximal inotropic and lusitropic effects were achieved in the same concentration range describing a similar concentration–response relationship between the two effects mediated through the same receptor system (Fig. 7B).

In contrast, failing hearts displayed a significantly higher sensitivity for the inotropic effect compared to the lusitropic effect. The lusitropic effect increased monophasically to its maximum as opposed to the inotropic effect which has a bell-shaped concentration–response relationship as described by Sjaastad et al. (2003). Consequently, maximal lusitropic effects are achieved at concentrations of isoprenaline beyond those giving maximal inotropic effects (Fig. 6B). This results in increased relaxation in response to β-adrenoceptor stimulation even when the inotropic response is fully developed. Accordingly, the pronounced lusitropic effect in the failing heart can lead to an increased futile expense of

energy and increased oxygen demand (Hasenfuss et al., 1987; Shimizu et al., 1998) at concentrations of agonist that does not provide additional inotropic support. Thus, the ability of carvedilol to antagonize β-adrenoceptor mediated lusitropic effects in the failing heart may optimize the balance between lusitropic and inotropic effects and be beneficial in congestive heart failure. An interesting aspect is whether other potentially deleterious β-adrenoceptor mediated effects are preserved in chronic heart failure similar to the lusitropic effect. If so, this may partly explain the beneficial effects of β-adrenoceptor blockade in chronic heart failure in face of additional reduction of the inotropic response.

4.4. Functional antagonism of carvedilol

The present study shows for the first time that carvedilol is able to antagonize functional effects exerted through myocardial α_1-adrenoceptors in failing hearts. Although carvedilol exhibits lower affinity for the α_1-adrenoceptors than for the β-adrenoceptors, studies confer that concentrations used in a clinical setting cause an α_1-adrenoceptor mediated vasodilation in vivo (Sponer et al., 1992). The reported potency of carvedilol on vascular (Monopoli et al., 1989) α_1-adrenoceptors is similar to that we observe for myocardial α_1-adrenoceptors in the present study. Assuming that the inhibition constant of carvedilol on myocardial α_1-adrenoceptors observed after short time exposure (present study) is representative for chronic exposure, antagonism of myocardial α_1-adrenoceptors is present in parallel to the vascular antagonism in a clinical setting.

Chronic stimulation, as well as overexpression of myocardial α_1-adrenoceptors induces a hypertrophic phenotype (Ponicke et al., 2001; Simpson, 1983; Milano et al., 1994). Epidemiologic studies have shown an association between ventricular hypertrophy and increased cardiac morbidity and mortality (Levy et al., 1990). Consequently, it seems like a rational approach to attenuate cardiac α_1-adrenoceptor stimulation with e.g. carvedilol to reduce a main growth signal in chronic heart failure. Recently, it was demonstrated that carvedilol inhibits noradrenaline-induced increase in rate of protein synthesis, a marker of development of a hypertrophic phenotype (Pönicke et al., 2002). Myocardial α_1-adrenoceptors might provide beneficial effects and possible play an important role in mediating ischemic preconditioning (Salvi, 2001) and inotropic support during heart failure (Skomedal et al., 1997). Recently, the Data Safety Monitoring Board for the ALLHAT study decided to discontinue the doxazosin-treatment (α_1-adrenoceptor blocker) arm of the study due to increased development of congestive heart failure in the doxazosin group (ALLHAT Collaborative Research Group, 2000). Thus, monotherapy with an α_1-adrenoceptor antagonist may be of disadvantage because the benefit from reduction of myocardial hypertrophy is counteracted by an attenuation of α_1-adrenoceptor mediated beneficial effects and unopposed β-adrenoceptor effects (Osnes et al., 2000). Furthermore, a mutual inhibition between α_1- and β-adrenoceptors has been observed (Skomedal et al., 1988). Accordingly, monotherapy with an α_1-adrenoceptor antagonist might amplify the deleterious β-adrenoceptor mediated effects which can be prevented by β-blockers.

Thus, it is unclear whether blockade of myocardial α_1-adrenoceptors is beneficial or not in heart failure. Although speculative, a low but surmountable blockade of the α_1-adrenoceptors may be important attenuating chronic noradrenaline-mediated growth signals but allowing intermittent beneficial mechanical support when needed. Concomitant blockade of β-adrenoceptors may be a prerequisite for an optimal effect of a partial α_1-adrenoceptor blockade.

4.5. Conclusion

Carvedilol antagonized myocardial α_1-adrenoceptors in the failing heart with a potency similar to that observed in normal hearts and for vascular receptors. The β-adrenoceptor mediated lusitropic effects were not attenuated in failing hearts in contrast to the reduced inotropic response. The β-blocking properties of carvedilol were preserved in the failing heart. A low degree of antagonism of myocardial α_1-adrenoceptors by carvedilol in the failing heart might be of importance in the treatment of human heart failure.

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