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Carvedilol blocks β_2- more than β_1-adrenoceptors in human heart

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Abstract

Objective: To understand the basis of the effectiveness of carvedilol in heart failure by determining its specific properties at human heart β_1- and β_2-adrenoceptors.
Methods: The positive inotropic effects of noradrenaline (in the presence of the β_2-selective antagonist ICI118551) and adrenaline (in the presence of the β_1-selective antagonist CGP20712), mediated through β_1- and β_2-adrenoceptors, respectively, were investigated in atrial and ventricular trabeculae. The patch-clamp technique was used to investigate effects of noradrenaline and adrenaline on L-type Ca^{2+} current in human atrial myocytes.
Results: Carvedilol was a 13-fold more potent competitive antagonist of the effects of adrenaline at β_2-adrenoceptors ($-\log K_B = 10.13 \pm 0.08$) than of noradrenaline at β_1-adrenoceptors ($-\log K_B = 9.02 \pm 0.07$) in human right atrium. Chronic carvedilol treatment of patients with non-terminal heart failure reduced the inotropic sensitivity of atrial trabeculae to noradrenaline and adrenaline 5.6-fold and 91.2-fold, respectively, compared to β_1-blocker-treated patients, consistent with persistent preferential blockade of β_2-adrenoceptors. In terminal heart failure carvedilol treatment reduced 1.8-fold and 25.1-fold the sensitivity of right ventricular trabeculae to noradrenaline and adrenaline, respectively, but metoprolol treatment did not reduce the sensitivity to the catecholamines. Increases of current ($I_{Ca,L}$) produced by noradrenaline and adrenaline were not different in atrial myocytes obtained from non-terminal heart failure patients treated with metoprolol or carvedilol, consistent with dissociation of both β-blockers from the receptors.
Conclusions: Carvedilol blocks human cardiac β_2-adrenoceptors more than β_1-adrenoceptors, thereby conceivably contributing to the beneficial effects in heart failure. The persistent blockade of β-adrenoceptors is attributed to accumulation of carvedilol in cardiac tissue.
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Keywords: Human heart; β_1- and β_2-adrenoceptors; Heart failure; Noradrenaline; Adrenaline; Carvedilol

1. Introduction

Chronic β-adrenoceptor blockade is effective in the treatment of heart failure [1]. β_1-adrenoceptor-selective blockers metoprolol and bisoprolol as well as carvedilol improve heart function and reduce morbidity and mortality [2–4]. Whether blockade of β_2-adrenoceptors in addition to β_1-adrenoceptors confers benefit to patients with heart failure is a debatable issue that has not been resolved [3–5]. Therefore it is important to know the affinities of clinically used β-blockers at β_1- and β_2-adrenoceptors. However, there is uncertainty about the affinity of carvedilol for human β_1- and β_2-adrenoceptors, with reports claiming moderate β_1-selectivity [6,7], no selectivity [8–10], or slight β_2-selectivity [11–13].

In patients blockade of β-adrenoceptors by carvedilol is longer lasting after withdrawal than with other clinically used β-blockers [14–16]. The blockade of isoprenaline-evoked increase in contractility in human atrial myocardium is irreversible with carvedilol but reversible with metoprolol [16]. Dobutamine-induced cardiostimulation is reduced by carvedilol in both heart failure patients [15] and healthy

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volunteers [16], and not yet completely reversible 44 h after withdrawal of medication [16], despite a reduction of carvedilol plasma concentrations below detectable levels. To account for these findings, it was proposed that carvedilol interacts with β_1-adrenoceptors by persistently binding to an allosteric site [16].

Highly lipophilic carvedilol accumulates in rat heart [17] and human heart [18] and may leak out chronically from cardiac tissues and maintain high occupancy of both β_1- and β_2-adrenoceptors for long periods. It is uncertain through which β-adrenoceptor subtype carvedilol causes more blockade. We therefore determined the affinity of carvedilol for human atrial β_1-and β_2-adrenoceptors from the antagonism of the positive inotropic effects of noradrenaline and adrenaline in human atrial trabeculae. We also compared the persistent blockade by carvedilol of the positive inotropic effects of noradrenaline, mediated through β_1-adrenoceptors, and adrenaline, mediated through β_2-adrenoceptors, in isolated atrial and ventricular trabeculae obtained from non-β-blocker-treated patients and patients chronically treated with carvedilol or β_1-selective blockers. Finally, we compared the influence of chronic treatment with carvedilol or metoprolol on the L-type Ca^{2+} current ($I_{Ca,L}$)-enhancing effects of noradrenaline through β_1-adrenoceptors and adrenaline through β_2-adrenoceptors. The results indicate that carvedilol is selective for human cardiac β_2-adrenoceptors.

2. Methods

2.1. Patients

Human right atrial trabeculae and myocytes were prepared from right atrial appendages of patients undergoing coronary artery bypass surgery at The Prince Charles Hospital, Brisbane, ethics approval numbers EC9876, EC9978, and Gustav Carus Hospital, Dresden Technological University ethics committee (document EK 1140 82202). Right atrial and ventricular trabeculae were prepared from explanted hearts of patients undergoing heart transplantation at The Prince Charles Hospital (ethics approval number EC9876). The investigation conforms to the principles outlined in the Declaration of Helsinki. Patient details are shown in Table 1i–iv.

2.2. Isolated cardiac tissues

Trabeculae from right atrial appendages [19] and ventricle [20] were set up to contract at 1 Hz 37 °C as described. To block both neuronal uptake and α-adrenoceptors the tissues were pretreated for 90 min with 5 μM phenoxybenzamine followed by washout [19,20]. We did not include β_3-adrenoceptor antagonists because all effects of (−)-noradrenaline and (−)-adrenaline in human right atrial and ventricular trabeculae can be explained by an interaction with β_1- and β_2-adrenoceptors [19–21], β_3-adrenoceptor agonists have no effect on contractility of right atrial and ventricular trabeculae in our laboratory [22,23] and pindolol, which is a β_3-agonist at recombinant β_3-adrenoceptors expressed at high density in CHO cells [24] causes positive inotropic effects in human right atrium which are not affected by the β_3-blocker L-748,337 [25].

2.3. Receptor studies in cardiac tissues

β_1-Adrenoceptor-mediated effects were always investigated with noradrenaline in the presence of the β_2-selective blocker ICI118551 (50 nM) and β_2-adrenoceptor-mediated effects were always studied with adrenaline in the presence of the β_1-selective blocker CGP20712 (300 nM) as described [20,21,26]. Serotonin effects were investigated in the presence of (−)-propranolol (200 nM) as described [27,28]. Histamine effects were investigated in the presence of CGP20712 (300 nM).

To investigate the reversibility of receptor blockade by carvedilol, we used right atrial trabeculae from patients without heart failure undergoing coronary artery bypass surgery. Trabeculae were incubated with or without carvedilol 10 nM for 240 min and then a concentration-effect curve was established to noradrenaline or adrenaline. Tissues were washed 10 times at equal intervals over 90 min before another concentration-effect curve was re-established. Experiments carried out on right atrium from patients chronically treated with either metoprolol or atenolol were pooled because we have previously shown that the potency of (−)-noradrenaline for positive inotropic effects is identical in these two groups [29,30].

Equilibrium dissociation constants (K_B) for carvedilol at β_1- and β_2-adrenoceptors were determined in right atrium from patients not in heart failure. Carvedilol (0.3–100 nM) was incubated for a period of 240 min unless otherwise stated and then a concentration-effect curve to noradrenaline or adrenaline was established. Concentration-effect curves were also established to the catecholamines in trabeculae from the same patient not incubated with carvedilol ('control tissues').

The effects of chronic administration of carvedilol were determined in heart tissues from three separate groups, 1. right atrium from patients with non-terminal heart failure undergoing coronary artery bypass surgery, 2. right atrium and 3. right or left ventricle from patients with terminal heart failure (Table 1ii–iii). Comparisons were made with tissues from patients not treated with β-blockers and a combined group of patients chronically treated with metoprolol or atenolol (Table 1ii–iii). In some groups, the effects of serotonin (right atrium, non-terminal heart failure), histamine and dibutyryl cyclic AMP (right ventricular trabeculae) were investigated. The routine use of right ventricular trabeculae in preference to left ventricular trabeculae ensured greater yields of viable trabeculae.

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Table 1

i Summary of details for patients undergoing coronary artery bypass surgery from which right atrial tissues were obtained and used in affinity and washout experiments corresponding to Figs. 1 and 2

n	Aetiology *n*	*Age*	Sex *n*	*EF*	Medication	
47	IHD 47	60±2	M 38	66±2	β-blocker (n, dose mg) Atenolol (26, 54±6) Metoprolol (16, 84±11)	Other (drug n) A29,B26,C4,D31,E11

ii Summary of details for patients with non-terminal heart failure from which right atrial tissue was obtained during coronary artery bypass surgery corresponding to Fig. 3

Combined patient data

Patient Group	*n*	Aetiology *n*	Age	Sex *n*	EF	Medication *n*
Carvedilol Daily dose 22±3 duration 121±54	18	IHD 16 IHD/valve repair 2	65±2	M 15	37±2	A15,B6,C10,D10,E4,F4.

Experimental group	*n*	Age	EF	Daily dose	Duration
Noradrenaline	14	65±3	37±2	23±3	133±66
Adrenaline	16	66±3	37±2	22±3	120±58
Serotonin	9	67±3	39±2	24±4	77±31

Combined patient data

Patient Group	*n*	Aetiology *n*	Age	Sex *n*	EF	Medication *n*
Metoprolol/Atenolol	19	CABG 19	64±2	M 15	37±2	A14,B11,C5,D11,E5,F3

	n	Daily dose
Metoprolol	12	85±12
Atenolol	7	68±9

Experimental group	*n*	Age	*EF*
Noradrenaline	15	67±2	36±2
Adrenaline	17	64±3	37±2
Serotonin	7	71±3	36±4

iii Summary of details for patients with terminal heart failure from which atrial and/or ventricular tissue was obtained corresponding to experiments in Figs. 4 and 5

Combined patient data

Patient group	*n*	Aetiology *n*	Age	Sex	EF	CI	Medication *n*
Non-β-blocked	11	IHD 3 IDC 8	47±5	M 11	24±3	2.5±0.3	A11,B9,C11,D2,F8,G8,H8,I5

Experimental group	*n*	Age	EF	CI
Noradrenaline ventricle	9	47±6	22±2	2.4±0.4
Adrenaline ventricle	10	47±5	22±2	2.4±0.4
Histamine ventricle	5	50±4	19±1	2.2±0.3
Dibutyryl cyclicAMP ventricle	5	48±3	19±1	2.2±0.3
Noradrenaline atrium	7	44±7	26±4	2.8±0.5
Adrenaline atrium	8	46±7	23±2	2.5±0.4

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Table 1 (*continued*)

Combined patient data

Patient Group	*n*	Aetiology *n*	Age	Sex *n*	EF	CI	Medication *n*
Carvedilol	15	IHD 6 IDC 4	48±3	M 14	27±4	2.1±0.2	A12,B9,C11,D6,F8,G9,H6,I6,J2
Daily dose 28±8		RC 1 VD 2					
Duration 220±44		HOCM 1 CS 1					

Experimental group	*n*	Age	EF	CI	Daily dose	Duration
Noradrenaline ventricle	15	48±3	27±4	2.1±0.2	28±8	220±44
Adrenaline ventricle	15	48±3	27±4	2.1±0.2	28±8	220±44
Histamine ventricle	7	45±5	34±6	2.0±0.2	31±13	295±72
Dibutrylyl cAMP ventricle	5	43±3	35±9	2.0±0.2	21±8	286±86
Noradrenaline atrium	9	46±5	30±7	2.1±0.2	31±12	241±64
Adrenaline atrium	10	45±4	28±6	2.1±0.2	33±11	263±61

Combined patient data

Patient Group	*n*	Aetiology *n*	Age	Sex *n*	EF	CI	Medication *n*
Metoprolol	4	IHD 2 IHD/HOCM 1	43±9	M 3	27±8	1.8±0.2	A2,B2,C2,D3,I1
Daily dose 87±24		HOCM 1					
Duration 874±685							

Experimental group		*n*	Age	EF	CI	Daily	Duration
Noradrenaline	ventricle/atrium	4	43±9	27±8	1.8±0.2	87±24	874±685
Adrenaline	ventricle/atrium	4	43±9	27±8	1.8±0.2	87±24	874±685

iv Summary of details for patients with non-terminal heart failure from which right atrial tissue was obtained during coronary artery bypass surgery corresponding to Fig. 6. All patients used for noradrenaline and adrenaline experiments

Patient Group	*n*	Aetiology *n*	Age	Sex *n*	EF	Medication *n*
Carvedilol	5	IHD 2 IHD/valve repair 2	63±6	M 2	40±3	A5,B3,C4,D4,F1
Daily dose 22±2		Valve repair 1				
Duration 98±34						
Metoprolol	4	IHD 2 IHD/valve repair 1	73±5	M 4	39±4	A3,B1,C1,D2
Daily dose 87±44		Valve repair 1				
duration 77± 38.5						

n, Number of patients, age (years), duration (days), dose (mg), CI cardiac index (ml/min/m^2), EF ejection fraction % determined from angiocardiography, IHD ischemic heart disease, IDC idiopathic dilated cardiomyopathy, DC dilated cardiomyopathy, RC restrictive cardiomyopathy, VD valvular disease, HOCM hypertrophic obstructive cardiomyopathy, CS cardiogenic shock.
A angiotensin converting enzyme inhibitor, B nitrate, C diuretic, D hypolipidemic, E L-type Ca^{2+} channel antagonist, F digoxin, G spironolactone, H warfarin, I amiodarone, J AT_1 receptor antagonist (irbesartan).

2.4. $I_{Ca,L}$ measurement

Atrial myocytes were isolated from non-terminally failing hearts of patients treated with either carvedilol or metoprolol (Table 1iv) and $I_{Ca,L}$ measured as described [31]. Cells were continually superfused via a rapid solution exchange system (inner diameter 310 μm) positioned at a distance of 100–150 μm from the cell under investigation with a flow rate of 100 μl/min allowing intense wash out of remaining drugs. Superfusion was started five minutes before the first catecholamine concentration was applied.

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2.5. Analysis and statistics

Agonist concentrations causing half maximal effects of agonists were estimated as $-\log EC_{50}M$. The antagonism by carvedilol of the catecholamine effects was analyzed with Schild-plots [32] and equilibrium dissociation constants K_B were estimated. Concentration-effect curves were analyzed using GraphPad Prism. One-way ANOVA with post-hoc Bonferroni correction was used to compare multiple sets of data simultaneously.

3. Results

3.1. Carvedilol antagonizes the inotropic effects of adrenaline, mediated through β_2-adrenoceptors more than the effects of noradrenaline, mediated through β_1-adrenoceptors

A single concentration-effect curve to a catecholamine was carried out on 2–6 trabeculae from the same atrium in the absence and presence of carvedilol. Carvedilol antagonized the effects of adrenaline, mediated through β_2-adrenoceptors more than the effects of noradrenaline, mediated through β_1-adrenoceptors (Figs. 1 and 2). Carvedilol (10 nM) shifted the concentration-effect curves by reducing the $-\log EC_{50}$ values for noradrenaline and adrenaline by 1.08 ± 0.12 ($n=6$) and 2.15 ± 0.10 ($n=6$) log units, respectively ($P<0.0001$) (Fig. 1C and D). To inquire whether antagonism by carvedilol was reversible, a second concentration-effect curve to the catecholamines was carried out on carvedilol-treated and non-carvedilol-treated trabeculae. The sensitivity to the catecholamines of the second curve was reduced similarly in non-carvedilol-treated (Fig. 1A and B) and carvedilol-treated (Fig. 1C and D) trabeculae. Thus, the antagonism of the effects of both noradrenaline and adrenaline by 10 nM carvedilol persisted 90 min after carvedilol washout.

In additional experiments, the antagonism was investigated as a function of carvedilol concentrations and Schild-plots determined (Fig. 2). Carvedilol up to 100 nM caused surmountable antagonism of the effects of

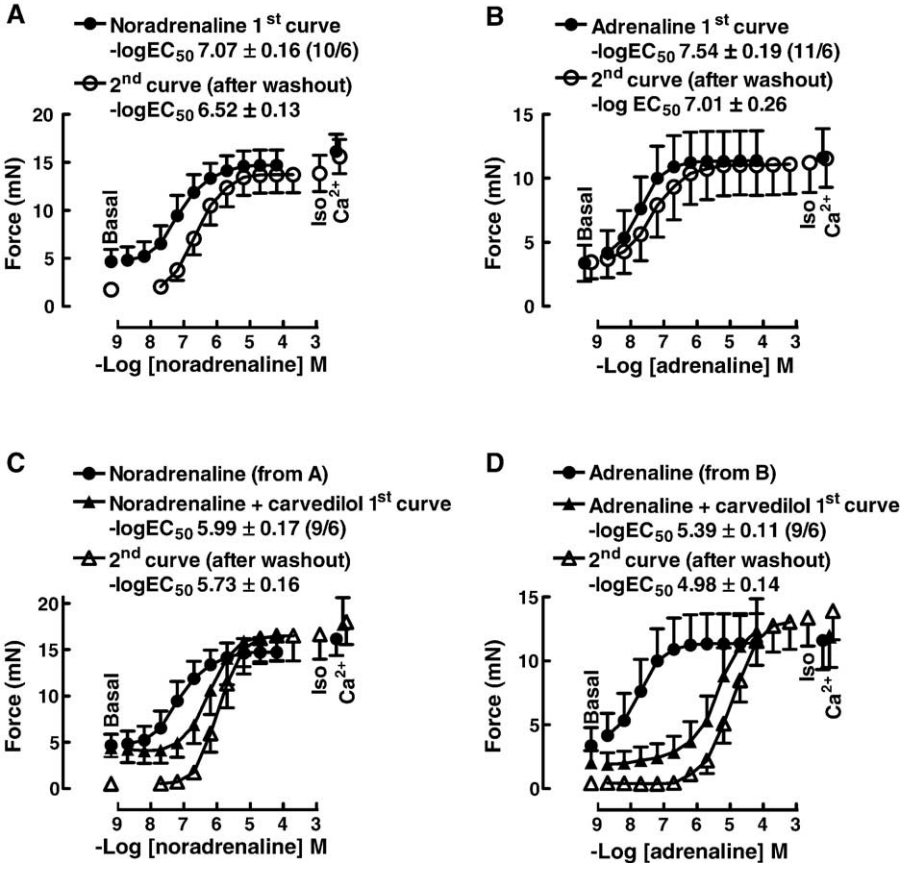

Fig. 1. Greater antagonism of the positive inotropic effects of adrenaline than noradrenaline by 10 nM carvedilol (240 min incubation) on atrial trabeculae from non-failing hearts (Table 1i). Blockade was persistant at both β_1- and β_2-adrenoceptors. Two successive concentration-effect curves for the catecholamines were determined (first curve closed symbols, second curve open symbols), in non-carvedilol-treated trabeculae (A,B) or carvedilol-treated trabeculae in which carvedilol was added exogenously (C,D). The second successive curve was established after a 90-min wash protocol involving 10 changes of incubation buffer which did not include exogenously added carvedilol and shows persistent carvedilol blockade (C,D open symbols) taking the time control (A,B open symbols) into consideration. The first curves in the absence of carvedilol of A and B are also shown for comparison in C and D, respectively. Numbers between parentheses represent trabeculae/patients. Iso, isoprenaline 200 μM; Ca^{2+}, Ca^{2+} 9.25 mM.

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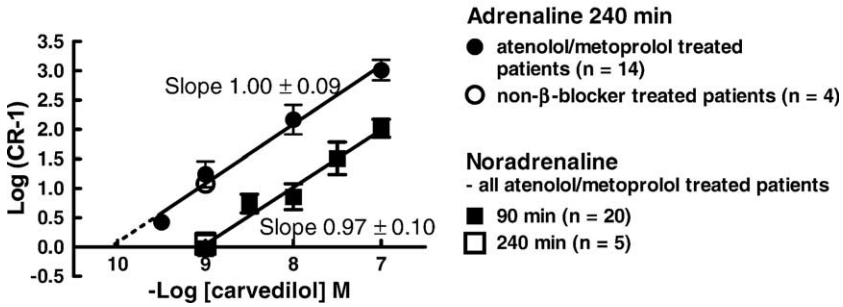

Fig. 2. Schild-plots for carvedilol. $-\log K_B$ values of 10.13±0.08 (n=19 concentration-ratios) and 9.02±0.07 (n=32 concentration-ratios) $P<0.0001$, were estimated from the antagonism vs. adrenaline and noradrenaline, respectively. Carvedilol incubation time was 240 min unless otherwise stated.

noradrenaline and adrenaline. Slopes of Schild-plots were not different from slope one (Fig. 2). Carvedilol was 13-fold more effective in antagonizing the effects of adrenaline through β_2-adrenoceptors than the effects of noradrenaline through β_1-adrenoceptors in atrial trabeculae obtained from patients undergoing coronary artery bypass surgery without heart failure chronically treated with metoprolol or atenolol (Fig. 2). The antagonism of the effects of both noradrenaline and adrenaline by 1 nM carvedilol in atria from β_1-blocker-treated patients was similar to the antagonism observed in atria from 4 patients not treated with β-blockers (Fig. 2). The antagonism of the effects of noradrenaline by 1 nM carvedilol was not different with 90 or 240 min incubation (Fig. 2, open squares). However, the antagonism of the effects of adrenaline by carvedilol (1–100 nM) was consistently

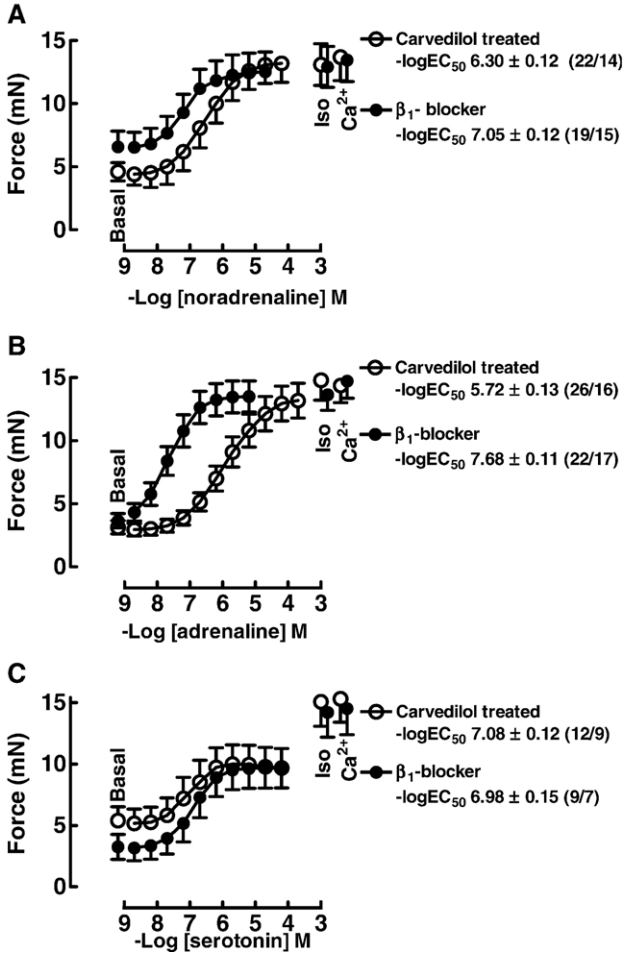

Fig. 3. Greater reduced responsiveness to adrenaline (B) than to noradrenaline (A) of atrial trabeculae obtained from patients with non-terminal heart failure chronically treated with carvedilol (Table 1ii). Comparison with catecholamine responsiveness of atria from patients with non-terminal heart failure chronically treated with β_1-adrenoceptor-selective blockers (Table 1ii). (C) Unchanged effects of serotonin. Numbers between parentheses represent trabeculae/patients. ISO, isoprenaline 200 μM; Ca^{2+}, Ca^{2+} 9.25 mM.

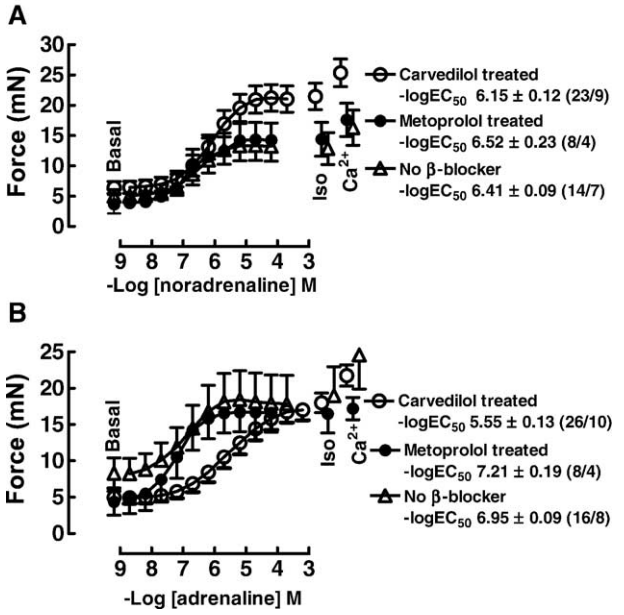

Fig. 4. Greater reduced responsiveness to adrenaline (B) than to noradrenaline (A) in atrial trabeculae from patients with terminal heart failure treated with carvedilol (Table 1iii). Comparison with catecholamine responsiveness of atria from patients with terminal heart failure not treated with β-blockers (Table 1iii) or chronically treated with metoprolol (Table 1iii). Note that the maximum noradrenaline and Ca^{2+} responses in the 3 groups of A were not significantly different (P=0.14 and P=0.13, respectively, one way ANOVA). The numbers between parentheses represent trabeculae/patients. ISO, isoprenaline 200 μM; Ca^{2+}, Ca^{2+} 9.25 mM.

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smaller with 90 min incubation (data not shown) than 240 min incubation.

3.2. Persistent and selective blockade of β_2-adrenoceptors in atrial trabeculae from patients with non-terminal heart failure chronically treated with carvedilol

Atrial appendages were obtained from patients with non-terminal heart failure chronically treated with carvedilol or β_1-selective blockers (atenolol or metoprolol) with matching ejection fractions (Table 1ii). The positive inotropic effects of noradrenaline, mediated through β_1-adrenoceptors, and adrenaline, mediated through β_2-adrenoceptors, were compared. Atrial trabeculae from carvedilol-treated patients responded 5.6-fold less to noradrenaline than trabeculae from β_1-blocker-treated patients (Fig. 3A). Responsiveness to adrenaline in trabeculae from carvedilol-treated patients was 91.2-fold less than in trabeculae from β_1-selective blocker-treated patients (Fig. 3B).

To investigate whether carvedilol also altered the function of other receptors coupled to G_s protein we compared the effects of serotonin, mediated through 5-HT_4 receptors [27,28] in the 2 groups (Table 1ii). The inotropic potency and efficacy of serotonin was not significantly different in atrial trabeculae from patients treated with carvedilol or β_1-selective blockers (Fig. 3C).

3.3. Chronic carvedilol treatment of patients with terminal heart failure causes persistent blockade of β_2-adrenoceptors of atrial myocardium

Atrial trabeculae from explanted hearts in terminal failure of carvedilol-treated patients responded 1.8-fold and 2.3-fold less to noradrenaline than atrial trabeculae from non-β-blocker-treated and metoprolol-treated patients, respectively (Fig. 4A). Responsiveness to adrenaline in atrial trabeculae of explanted hearts from carvedilol-treated patients was 25.1-fold and 45.7-fold less than in trabeculae from non-β-blocker-treated and metoprolol-treated patients, respectively (Fig. 4B).

3.4. Chronic carvedilol treatment of patients with terminal heart failure causes persistent selective blockade of β_2-adrenoceptors of ventricular myocardium

Right ventricular trabeculae from explanted hearts in terminal failure of carvedilol-treated patients responded 3.9-

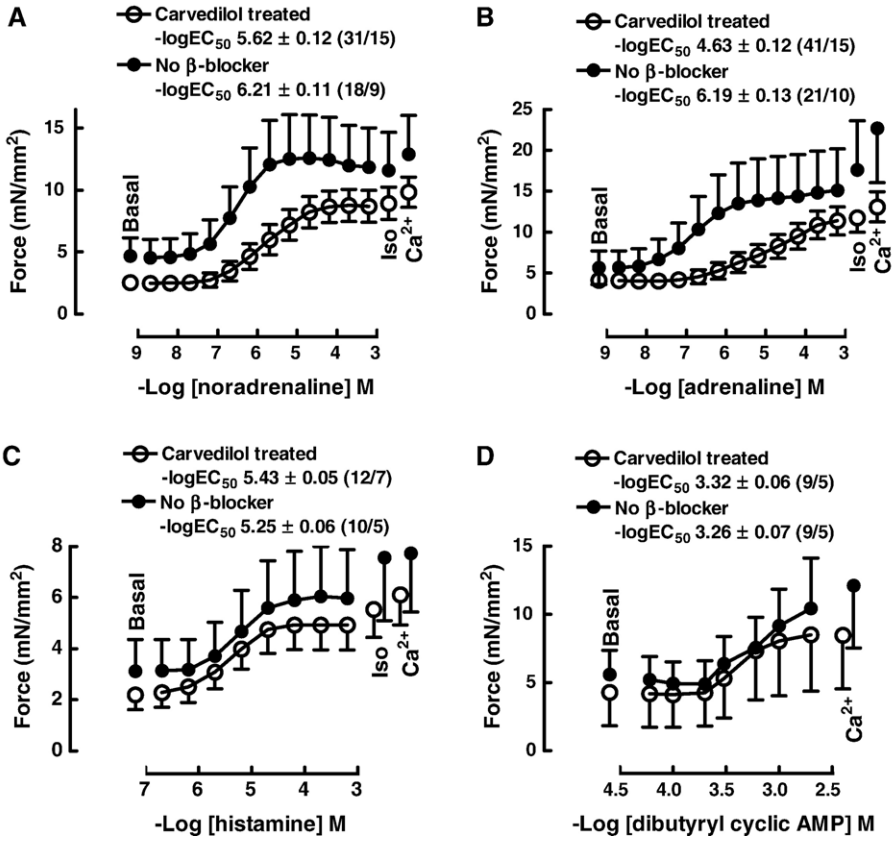

Fig. 5. Greater reduced responsiveness to adrenaline (B) than to noradrenaline (A) of right ventricular trabeculae from patients with terminal heart failure treated with carvedilol (Table 1iii). Unchanged responses to histamine (C) and dibutyryl cyclic AMP (D). Comparison with catecholamine responsiveness of ventricular trabeculae from patients with terminal heart failure not treated with β-blockers (Table 1iii). Numbers between parentheses represent trabeculae/patient. ISO, isoprenaline 200μM; Ca^{2+}, Ca^{2+} 9.25 mM.

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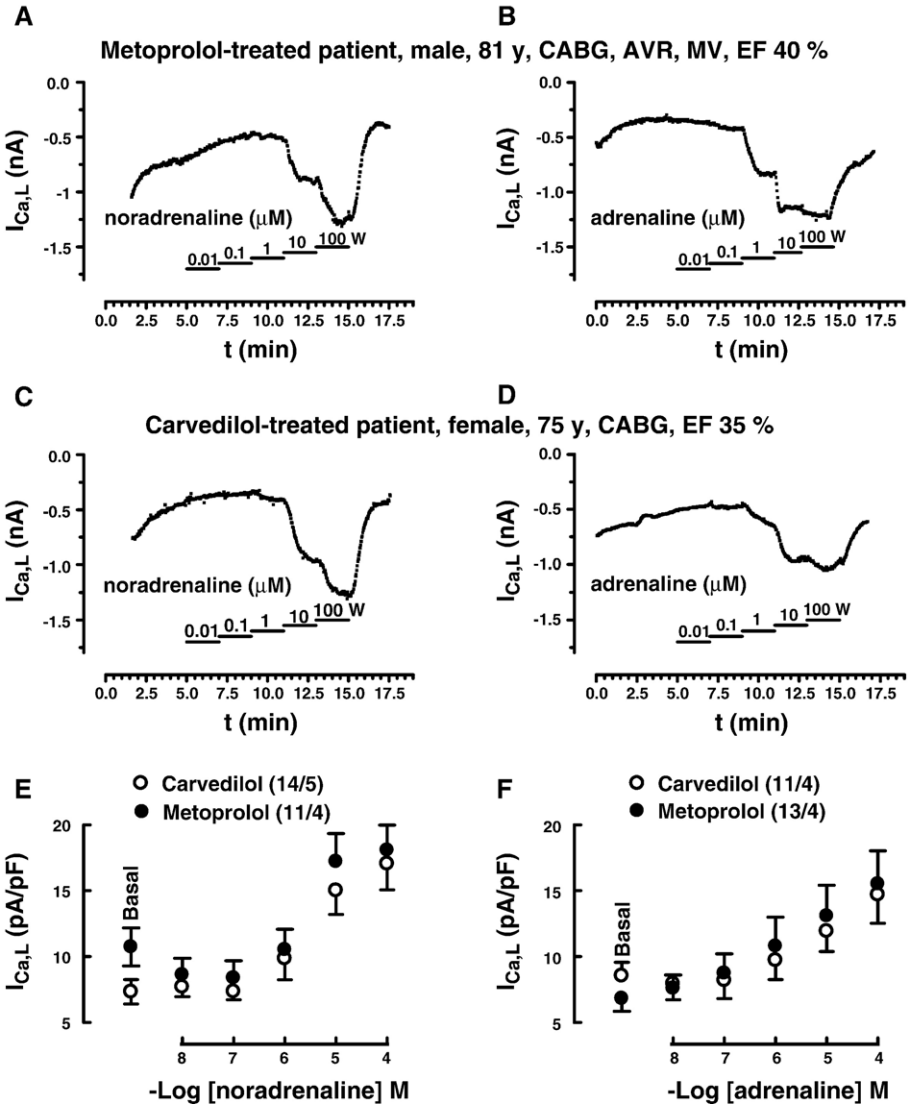

Fig. 6. Increases of $I_{Ca,L}$ current by noradrenaline through β_1-adrenoceptors (A,C,E) and adrenaline through β_2-adrenoceptors (B,D,F) in atrial myocytes obtained from patients with non-terminal heart failure chronically treated with carvedilol (Table 1iv) or metoprolol (Table 1iv). Representative experiments are shown in myocytes from a metoprolol-treated patient (A, B) and carvedilol-treated patient (C, D). The effects of 10 μM and 100 μM noradrenaline were significant in both myocytes from both carvedilol-treated ($P<0.001$) and metoprolol-treated ($P<0.001$) patients. The effects of 10 μM adrenaline was significant ($P<0.05$) in carvedilol-treated patients; the effects of 100 μM adrenaline was significant ($P<0.05$) in both carvedilol-treated and metoprolol-treated patients. Numbers between parentheses in Figures E and F are myocytes/patients.

fold less to noradrenaline than trabeculae from non-β-blocker-treated patients (Fig. 5A). Responsiveness to adrenaline in trabeculae from explanted hearts of carvedilol-treated patients was 36.3-fold less than in trabeculae from non-β-blocker-treated patients (Fig. 5B).

We also tested left ventricular trabeculae from 3 carvedilol-treated and 5–6 non-β-blocker-treated patients. Noradrenaline was 6.9-fold less potent in carvedilol-treated patients ($-\log EC_{50}$ carvedilol-treated 5.13±0.41; non-β-blocker group 5.97±0.11, $P=0.045$) and adrenaline was 190.5-fold less potent ($-\log EC_{50}$ carvedilol-treated 4.06±0.14; non-β-blocker group 6.34±0.09, $P<0.001$).

Chronic carvedilol treatment did not modify the positive inotropic responses to histamine (Fig. 5C), mediated through H_2 receptors [33], or dibutyryl cyclic-cAMP (Fig. 5D) compared to non-β-blocker-treated patients.

3.5. Similar $I_{Ca,L}$ responses to noradrenaline, mediated through β_1-adrenoceptors, and to adrenaline, mediated through β_2-adrenoceptors, in atrial myocytes from non-terminal heart failure patients treated with carvedilol or metoprolol

Noradrenaline (10 nM–100 μM) in the presence of ICI118551 (50 nM) caused similar increases of $I_{Ca,L}$ in myocytes from patients treated with metoprolol or carvedilol (Fig. 6). Adrenaline (10 nM–100 μM) in the presence of

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CGP20712 (300 nM) caused similar increases in $I_{Ca,L}$ in myocytes from carvedilol-treated and metoprolol-treated patients (Fig. 6).

4. Discussion

We demonstrated in human cardiac tissues that carvedilol was consistently β_2-selective. Carvedilol, administered to isolated atrial trabeculae, antagonized 13-fold more the positive inotropic effects of adrenaline, mediated through β_2-adrenoceptors, than the effects of noradrenaline mediated through β_1-adrenoceptors on atrial trabeculae. Persistent greater blockade of the effects of adrenaline through β_2-adrenoceptors than of noradrenaline through β_1-adrenoceptors was also detected in the myocardium obtained from heart failure patients chronically treated with carvedilol compared to metoprolol-treated or non-β-blocker-treated patients. In contrast, the $I_{Ca,L}$-enhancing responses to noradrenaline through β_1-adrenoceptors and to adrenaline through β_2-adrenoceptors were not different in atrial myocytes from carvedilol-treated and metoprolol-treated patients.

4.1. Carvedilol causes selective β_2-adrenoceptor blockade

Persistent β-adrenoceptor blockade in atria from carvedilol-treated patients has been previously observed and attributed to an allosteric effect on β_1-adrenoceptors, using isoprenaline as agonist [16]. We confirm the persistent β-adrenoceptor blockade in both atria and ventricular trabeculae obtained from patients chronically treated with carvedilol, but demonstrate that the residual blockade is one order of magnitude greater for β_2-adrenoceptors, activated by adrenaline, than β_1-adrenoceptors, activated by noradrenaline. Chronic carvedilol-treatment reduced the inotropic potency of adrenaline through β_2-adrenoceptors more than of noradrenaline through β_1-adrenoceptors in atria from non-terminal heart failure compared to atenolol/metoprolol-treated patients (16.3-fold, Fig. 3), atria from terminal heart failure compared to non β-blocker-treated- (13.8-fold, Fig. 4) or metoprolol-treated patients (19.9-fold, Fig. 4) and ventricle compared to non β-blocker-treated-patients (9.3-fold, Fig. 5). This evidence of preferential residual β_2-adrenoceptor blockade is consistent with the 13-fold β_2-adrenoceptor-selectivity, estimated from our experiments (Figs. 1 and 2) when atrial trabeculae from the same patients were incubated with or without carvedilol 10 nM in vitro. Data from Schild-plot analysis makes it unlikely that carvedilol modifies β_1-adrenoceptors through an allosteric mechanism [16]. Alternatively, we propose that hyporesponsiveness to isoprenaline observed in isolated atria from carvedilol-treated patients [16] is mainly due to selective blockade of β_2-adrenoceptors.

As observed previously in atria from non-failing hearts [21,29,34], atria from moderately failing hearts of patients chronically treated with the β_1-adrenoceptor-selective atenolol or metoprolol exhibited a 4.3-fold greater sensitivity to adrenaline, acting through β_2-adrenoceptors, than to noradrenaline, acting through β_1-adrenoceptors (Fig. 3A and B). The mechanism of this selective β_2-hyperresponsiveness is still elusive, but not due to residual antagonism of β_1-adrenoceptors [29]. In contrast, atria from carvedilol-treated patients showed a 3.8-fold lower sensitivity to adrenaline than to noradrenaline (Fig. 3A and B). The mechanism of the β_2-adrenoceptor hyporesponsiveness caused by carvedilol is due to the persistently greater blockade of β_2- than β_1-adrenoceptors.

4.2. Mechanisms of the persistent myocardial β-adrenoceptor hyporesponsiveness in isolated myocardium from carvedilol-treated patients

Carvedilol is concentrated in the rat heart but the free fraction of drug is only 1% [17]. Carvedilol also accumulates in failing human ventricle to 0.07 ng/mg wet weight [18], equivalent to 170 nM, if all the carvedilol is freely available. If this were the concentration in the extracellular fluid the potency of noradrenaline and adrenaline should be decreased 2 and 3 orders of magnitude, respectively, as expected from our pK_B estimates 9.0 and 10.1, respectively. However, the residual carvedilol-induced hyposensitivity was only between approximately 0.5 and less than 2 orders of magnitude for noradrenaline and adrenaline, respectively. Therefore, in line with a previous suggestion [14], lipophilic carvedilol [35] appears to accumulate at sites distinct from β-adrenoceptors and may conceivably leak out from lipophilic stores and maintain a relatively high concentration in the biophase thereby causing β-adrenoceptor occupancy. This may in turn result in the persistent β-adrenoceptor hyporesponsiveness detected by us and others [16] and shown herein to be greater through β_2- than β_1-adrenoceptors.

Hyporesponsiveness of β_1- and β_2-adrenoceptor mediated responses in heart tissues from patients chronically treated with carvedilol is unlikely to be due to down-regulation of β-adrenoceptors. Furthermore, the selective hyporesponsiveness of β_2-adrenoceptor mediated responses is also unlikely to be due to selective down-regulation of β_2-adrenoceptors. No change in β-adrenoceptor density in right ventricular septal endomyocardial biopsies from heart failure patients treated with carvedilol for 4 months was observed [36]. Moreover, the lack of hyporesponsiveness observed in myocytes for both β_1- and β_2-adrenoceptor mediated effects observed in this study is also consistent with no change in receptor density.

As shown previously with isoprenaline [16], the antagonism by carvedilol (10 nM) of the effects of noradrenaline and adrenaline through both β_1- and β_2-adrenoceptors persisted after washout of the β-blocker. To account for persistent blockade, an allosteric mechanism at β_1-adrenoceptors was proposed [16]. The persistent blockade by carvedilol could also be due to covalent binding. However, blockade by carvedilol was surmounted by higher catechol-

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amine concentrations (Fig. 1), and slopes of one of Schild-plots were consistent with competitive antagonism (Fig. 2), ruling out covalent binding and making an allosteric mechanism unlikely.

We discussed that the accumulation of carvedilol and continuous leakage from sites of storage in cardiac tissues [17,18] could account for the persistent blockade of catecholamine effects through both β_1- and β_2-adrenoceptors. It is, however, unlikely that binding of carvedilol to a myocyte, subsequently isolated and not surrounded by other cells, would continue to exhibit persistent blockade of the catecholamine effects because carvedilol would slowly dissociate (2–4 h) from the receptors and other binding sites. As expected, whole cell patch clamping experiments in isolated myocytes revealed that the $I_{Ca,L}$ increases caused by noradrenaline through β_1-adrenoceptors and by adrenaline through β_2-adrenoceptors were not blunted in myocytes obtained from carvedilol-treated patients compared to myocytes from metoprolol-treated patients. We propose that intense washing during the cell isolation procedure and the subsequent application of rapid solution exchange during the experiment may lead to much more effective washout of carvedilol than in experiments with intact tissue.

4.3. Chronic treatment with carvedilol does not reduce the function of serotonin 5-HT$_4$ receptors and histamine H$_2$ receptors

Chronic carvedilol treatment did not affect the atrial responses to serotonin, compared to atria from β_1-blocker-treated patients. Chronic treatment with β_1-selective blockers sensitizes 5-HT$_4$ receptors to the arrhythmic [37] and inotropic effects of serotonin [28]. The serotonin potency and efficacy in atria from carvedilol-treated patients was similar to that observed in atrial myocardium from patients treated with atenolol or metoprolol [28], suggesting that carvedilol also sensitizes the 5-HT$_4$ receptor system.

Carvedilol treatment failed to affect the ventricular responses to histamine, mediated through H_2 receptors [33], and to dibutyryl cyclic AMP. Thus, the persistent β_1- and β_2-adrenoceptor blockade caused by carvedilol does not result in cross talk with ventricular H_2 receptors or modify signals downstream of adenylyl cyclase.

4.4. Possible clinical implications

Noradrenaline and adrenaline are equieffective as inotropic stimulants through β_1- and β_2-adrenoceptors, respectively, of isolated ventricular myocardium from patients with terminal heart failure [20]. β_2-adrenoceptors mediate arrhythmias in human atrial myocardium [38] and ventricular fibrillation in the myocardium of dogs with experimental myocardial infarction and ischemia [39]. It is therefore plausible that patients with ischemic heart disease and heart failure may suffer β_2-adrenoceptor-mediated arrhythmias, including fatal ventricular fibrillation, during surges of adrenaline in situations of stress and cardiac surgery. Unlike β_1-selective blockers, β_2-selective carvedilol would prevent such arrhythmias, a property that may contribute to the beneficial effects in heart failure and to life prolongation. Recent evidence is consistent with this proposal. Carvedilol reduces the incidence of both atrial and ventricular arrhythmias in patients with myocardial infarction and already treated with ACE inhibitors, as found in a retrospective but blinded analysis of the CAPRICORN [40] trial [41]. Carvedilol markedly reduced the incidence of transient atrial fibrillation, compared to β_1-selective metoprolol or atenolol, in patients that underwent coronary bypass grafting [42]. High adrenaline plasma levels have been measured during [43] and after cardiopulmonary bypass [44] which may trigger transient atrial fibrillation. The greater effectiveness of carvedilol than β_1-selective blockers in preventing transient atrial fibrillation is probably due to blockade of β_2-adrenoceptors.

4.5. Study limitations

Several aetiologies of terminal heart failure patients were combined together with male/female patients. Nevertheless, our observations that carvedilol antagonized the effects of adrenaline more than noradrenaline were consistent across all aetiologies and both sexes. We reported studies on right ventricular trabeculae however clinical data concerning right heart status could not be obtained. During the course of our studies, where possible, we also used left ventricular trabeculae from 3 patients chronically treated with carvedilol. Patients chronically treated with carvedilol also showed greater reductions in the potency of adrenaline compared to noradrenaline. Finally, the patient's timing for surgery was not determined by this study and therefore we did not have control over the duration of treatment of β-blockers. Nevertheless, the selective reduction in potency of adrenaline versus noradrenaline was a consistent finding in all chronic carvedilol treated hearts.

4.6. Conclusion

We conclude that carvedilol antagonizes more the effects of adrenaline through β_2-adrenoceptors than the effects of noradrenaline through β_1-adrenoceptors. In contrast to β_1-selective blockers that only prevent deleterious effects of catecholamines mediated through β_1-adrenoceptors, carvedilol may preferentially prevent harmful effects of adrenaline, including arrhythmias, mediated through β_2-adrenoceptors, thereby contributing to its beneficial effects in heart failure.

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