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RESEARCH PAPER

# PDE3, but not PDE4, reduces $\beta_1$- and $\beta_2$-adrenoceptor-mediated inotropic and lusitropic effects in failing ventricle from metoprolol-treated patients

Peter Molenaar[1,2*†], Torsten Christ[3*†], Rizwan I Hussain[4,5], Andreas Engel[3], Emanuel Berk[3], Katherine T Gillette[1,2], Lu Chen[1,2], Alejandro Galindo-Tovar[6], Kurt A Krobert[4,5], Ursula Ravens[3], Finn Olav Levy[4,5] and Alberto J Kaumann[7]

[1]*Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia,* [2]*School of Medicine, The University of Queensland, The Prince Charles Hospital, Critical Care Research Group, Brisbane, QLD, Australia,* [3]*Department of Pharmacology and Toxicology, Dresden University of Technology, Dresden, Germany,* [4]*Department of Pharmacology, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo and Oslo University Hospital, Oslo, Norway,* [5]*K.G. Jebsen Cardiac Research Centre and Center for Heart Failure Research, University of Oslo, Oslo, Norway,* [6]*Research Unit of the University Hospital Virgen de la Arrixaca and Department of Pharmacology, University of Murcia, Murcia, Spain, and* [7]*Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK*

**Correspondence**
Dr Alberto Kaumann, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3EG, UK. E-mail: ajk41@hermes.cam.ac.uk; Dr Peter Molenaar, School of Medicine, The Prince Charles Hospital, Chermside, QLD 4032, Australia. E-mail: peter.molenaar@qut.edu.au

*Present address: Instit für Experimetelle Pharmakologie und Toxikologie, Universitätsklinikum, Hamburg Germany.
†PM and TC contributed equally to this work.

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human heart failure; $\beta_1$ and $\beta_2$ adrenoceptors; phosphodiesterases 3 and 4; noradrenaline and adrenaline; inotropism and lusitropism; metoprolol

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## BACKGROUND AND PURPOSE

PDE3 and/or PDE4 control ventricular effects of catecholamines in several species but their relative effects in failing human ventricle are unknown. We investigated whether the PDE3-selective inhibitor cilostamide (0.3–1 μM) or PDE4 inhibitor rolipram (1–10 μM) modified the positive inotropic and lusitropic effects of catecholamines in human failing myocardium.

## EXPERIMENTAL APPROACH

Right and left ventricular trabeculae from freshly explanted hearts of 5 non-β-blocker-treated and 15 metoprolol-treated patients with terminal heart failure were paced to contract at 1 Hz. The effects of (-)-noradrenaline, mediated through $\beta_1$ adrenoceptors ($\beta_2$ adrenoceptors blocked with ICI118551), and (-)-adrenaline, mediated through $\beta_2$ adrenoceptors ($\beta_1$ adrenoceptors blocked with CGP20712A), were assessed in the absence and presence of PDE inhibitors. Catecholamine potencies were estimated from $-\log EC_{50}$s.

## KEY RESULTS

Cilostamide did not significantly potentiate the inotropic effects of the catecholamines in non-β-blocker-treated patients. Cilostamide caused greater potentiation ($P = 0.037$) of the positive inotropic effects of (-)-adrenaline (0.78 ± 0.12 log units) than (-)-noradrenaline (0.47 ± 0.12 log units) in metoprolol-treated patients. Lusitropic effects of the catecholamines were also potentiated by cilostamide. Rolipram did not affect the inotropic and lusitropic potencies of (-)-noradrenaline or (-)-adrenaline on right and left ventricular trabeculae from metoprolol-treated patients.

## CONCLUSIONS AND IMPLICATIONS

Metoprolol induces a control by PDE3 of ventricular effects mediated through both $\beta_1$ and $\beta_2$ adrenoceptors, thereby further reducing sympathetic cardiostimulation in patients with terminal heart failure. Concurrent therapy with a PDE3 blocker and

metoprolol could conceivably facilitate cardiostimulation evoked by adrenaline through $\beta_2$ adrenoceptors. PDE4 does not appear to reduce inotropic and lusitropic effects of catecholamines in failing human ventricle.

**LINKED ARTICLE**

This article is commented on by Eschenhagen, pp 524–527 of this issue. To view this commentary visit http://dx.doi.org/10.1111/bph.12168

**Abbreviations**

β-blocker, β-adrenoceptor blocker (antagonist); CGP20712A, (2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluorometyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide); CR, concentration ratio; ICI118551, (1-[2,3-dihydro-7-methyl-1H-inden-4-yl]oxy-3-[(1-methylethyl)amino]-2-butanol); PKA, cAMP-dependent protein kinase; SR, sarcoplasmic reticulum; $t_{50}$, time to 50% relaxation; TPF, time to peak force

## Introduction

Activation of $\beta_1$ and $\beta_2$ adrenoceptors of human failing ventricle by noradrenaline and adrenaline causes similar inotropic, lusitropic and biochemical effects through the cAMP/cAMP-dependent protein kinase (PKA) pathway (Kaumann *et al.*, 1999). PDEs break down cAMP. At least 21 genes of 11 PDE families are known (Bender and Beavo, 2006). The cAMP-hydrolysing isoenzymes PDE1, PDE2, PDE3, PDE4 and PDE8 are expressed in mammalian heart and PDE3 is particularly highly expressed in human myocardium (Osadchii, 2007). PDE3 is relevant to heart failure in which cardiac cAMP levels and function are depressed (Von der Leyen *et al.*, 1991). By preventing cAMP hydrolysis, PDE3 inhibitors (e.g. milrinone and enoximone) enhance cardiac contractility through activation of cAMP-dependent pathways. Short-lasting infusions or low-dose oral treatment with PDE3 inhibitors have been shown to improve systolic function in chronic heart failure (Van Tassel *et al.*, 2008). In contrast, high-dose chronic treatment with PDE3 inhibitors worsens heart failure and increases mortality, particularly through sudden death (Amsallem *et al.*, 2005). We hypothesize that the PDE3 activity in severe heart failure further reduces harmful cardiostimulation by endogenous catecholamines in β-blocker-treated patients. It is unknown whether blockade of $\beta_1$ adrenoceptors and/or $\beta_2$ adrenoceptors in heart failure (Bristow, 2000) can modify PDE3 activity.

PDE4 is also expressed in human myocardium (Osadchii, 2007) and in particular PDE4D (Johnson *et al.*, 2012), but its functions are not yet clear. PDE4D3 is an integral component of the murine and human cardiac ryanodine RyR2 receptor complex, and it is reduced in murine and human heart failure (Lehnart *et al.*, 2005). Therefore, PDE4D3 plausibly may affect catecholamine-evoked contractility. PDE4 controls the inotropic effects and cAMP signals of catecholamines, mediated through $\beta_1$ adrenoceptors in rodent myocardium (Nikolaev *et al.*, 2006; Rochais *et al.*, 2006; Galindo-Tovar and Kaumann, 2008; Christ *et al.*, 2009) but not in human atrium (Christ *et al.*, 2006a; Kaumann *et al.*, 2007). However, it is unknown whether PDE4 controls human ventricular effects of catecholamines and whether it is through $\beta_1$ adrenoceptors and/or $\beta_2$ adrenoceptors.

We now investigated whether the inotropic and lusitropic effects of the catecholamines, mediated through $\beta_1$ or $\beta_2$ adrenoceptors of ventricular trabeculae from patients with terminal heart failure, are enhanced by PDE3 inhibition with cilostamide and/or PDE4 inhibition with rolipram. We compared results from patients not treated with β-blocker or chronically treated with metoprolol. The results suggest that chronic treatment with metoprolol facilitates PDE3 activity to reduce the inotropic and lusitropic effects of (-)-noradrenaline and (-)-adrenaline, mediated through $\beta_1$ and $\beta_2$ adrenoceptors. PDE4 does not modify the effects of catecholamines.

A progress report of this work was presented to a Biochemical Society Meeting (Christ *et al.*, 2006b).

## Methods

### *Heart transplant patients*

Written informed consent was obtained from all patients. Patients with terminal heart failure underwent heart transplant surgery at The Prince Charles Hospital, Brisbane, ethics approval numbers EC9876, HREC10/QPCH/184, and Gustav Carus Hospital, Dresden Technological University ethics committee (Document EK 1140 82202). Clinical data from Brisbane and Dresden patients are shown in Supporting Information Table S1A,B. Clinical data from Oslo patients are shown in Supporting Information Table S1C. All subjects or next of kin gave written informed consent to participate in the study, which was approved by the ethics committee in South-Eastern Norway Regional Health Authority (#S05172).

### *Isolated ventricular trabeculae from heart transplant patients*

Right or left ventricular trabeculae were dissected, mounted on to tissue electrode blocks and electrically paced at 1 Hz to contract as described (Kaumann *et al.*, 1999). For further details see Supporting Information.

### *Specific activation of $\beta_1$ and $\beta_2$ adrenoceptors*

To determine the effects of $\beta_1$ adrenoceptor (Alexander *et al.*, 2011) selective activation, concentration–effect curves for (-)-noradrenaline were obtained in the presence of ICI118551 (50 nM) to selectively block $\beta_2$ adrenoceptors. To determine the effects of $\beta_2$ adrenoceptor (Alexander *et al.*, 2011) selective activation, concentration–effect curves for (-)-adrenaline were determined in the presence of CGP20712A (300 nM) to selectively block $\beta_1$ adrenoceptors (Kaumann *et al.*, 1999). To assess the influence of the PDE3-selective inhibitor cilosta-

mide (300 nM–1 μM) and the PDE4-specific inhibitor rolipram (1–10 μM) on the effects of the catecholamines, a single concentration–effect curve for a catecholamine was obtained in the absence or presence of a PDE inhibitor. Trabeculae were incubated with PDE inhibitors for 30–45 min prior to commencement of catecholamine concentration–effect curves. At the completion of concentration–effect curves to catecholamines on right ventricular trabeculae, the effects of a maximal concentration of (-)-isoprenaline (200 μM) were determined. Since up to 20 contracting trabeculae were obtained from the same heart, it was often possible to compare the influence of the PDE inhibitors on responses mediated through both $\beta_1$ and $\beta_2$ adrenoceptors as shown in the representative experiment in Figure 3.

### *Analysis and statistics*

Responses of right ventricular trabeculae to catecholamines were expressed as percentage of the response to a maximally effective isoprenaline concentration (200 μM), administered after a complete concentration–effect curve. The catecholamine concentrations producing a half maximum response, $-\log EC_{50}M$ ($pEC_{50}$), were estimated from fitting a Hill function with variable slopes to concentration–effect curves from individual experiments. The data are expressed as mean ± SEM of $n$ = number of patients or trabeculae as indicated. Significance of differences between means was assessed with the use of either Student's *t*-test or ANOVA followed by Tukey–Kramer multiple comparisons *ad hoc* test at $P < 0.05$ using InStat software (GraphPad Software Inc., San Diego, CA, USA).

Concentration–response curves on left ventricular trabeculae from Oslo patients were constructed by estimating centiles ($EC_{10}$–$EC_{100}$) for the receptor-selective effects for each experiment and calculating the corresponding means and the horizontal positioning expressed as $-\log EC_{50}M$. All results are expressed as mean ± SEM and statistical significance was assessed with one-way ANOVA with a *priori* Bonferroni corrections made for multiple comparisons. $P < 0.05$ was regarded as statistically significant.

### *Drugs*

(-)-Adrenaline (+)-bitartrate salt, (-)-noradrenaline bitartrate salt (hydrate), prazosin hydrochloride and atropine sulphate were purchased from Sigma-Aldrich (St. Louis, MO, USA or Castle Hill, Australia). Rolipram, cilostamide, CGP20712A (2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluorometyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide) and ICI118551 (1-[2,3-dihydro-7-methyl-1*H*-inden-4-yl]oxy-3-[(1-methylethyl)amino]-2-butanol) were from Tocris Bioscience (Bristol, UK) or Sigma. Stock solutions were prepared in purified water and kept at –20°C to avoid oxidation. Further dilutions of the drugs were made fresh daily and kept cool (0–4°C) and dark. Repetitive experiments showed that drug solutions treated in these ways are stable.

## Results

### *Chronic metoprolol treatment increases the inotropic potencies of catecholamines*

Chronic treatment of patients with metoprolol sensitized right ventricular trabeculae to the inotropic effects of (-)-noradrenaline and (-)-adrenaline. The inotropic potencies of (-)-noradrenaline and (-)-adrenaline were increased fourfold and fivefold, respectively, in metoprolol-treated ($P < 0.05$) compared with non-β-blocker-treated patients (Figure 1A and B, Table 1). The lusitropic effects of (-)-noradrenaline, mediated through $\beta_1$ adrenoceptors, were not significantly enhanced but the $t_{50}$-abbreviating potency of (-)-adrenaline increased sevenfold ($P < 0.001$) by treatment of patients with metoprolol (Supporting Information Fig. S1A–D, Supporting Information Table S2). These results are consistent with the up-regulation of the $\beta_1$ adrenoceptor density and enhanced inotropic responses through these receptors in metoprolol-treated patients (Heilbrunn *et al.*, 1989; Sigmund *et al.*, 1996).

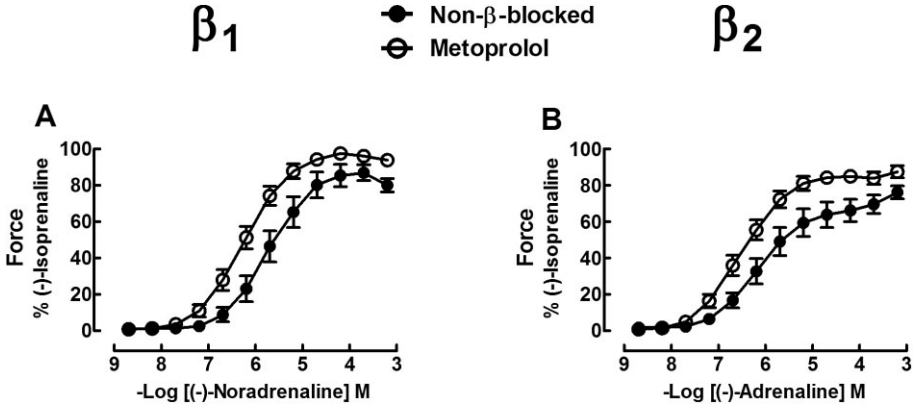



**Figure 1**

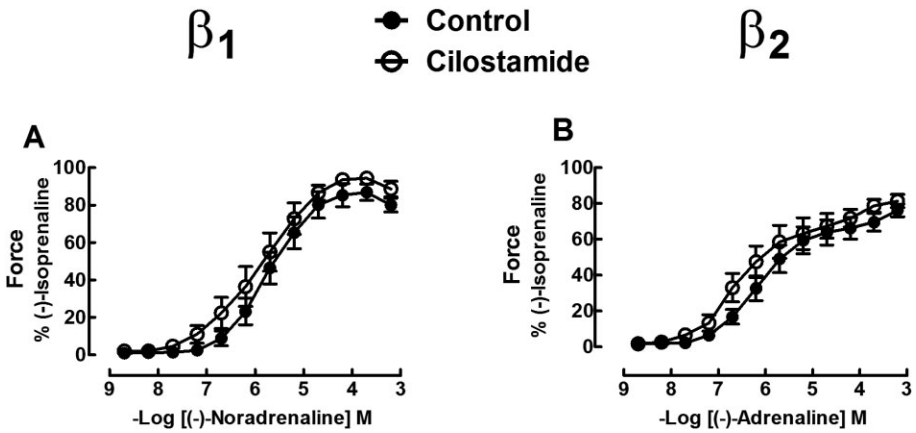
Effects of chronic administration of metoprolol compared with no-β-blocker on inotropic effects of (-)-noradrenaline through activation of $\beta_1$ adrenoceptors (A) and (-)-adrenaline through activation of $\beta_2$ adrenoceptors (B) in right ventricular trabeculae from failing hearts. Note the increased potency of (-)-noradrenaline and (-)-adrenaline for inotropic effects in metoprolol-treated patients. See text and Table 1 for further detail. β adrenoceptor blockade did not significantly increase basal force [$P = 0.07$ for (-)-noradrenaline, $P = 0.095$ for (-)-adrenaline] and maximum force [$P = 0.10$ for (-)-noradrenaline, $P = 0.054$ for (-)-adrenaline]. Data from four [(-)-noradrenaline experiments] or five [(-)-adrenaline experiments] patients with heart failure not treated with a β-blocker and seven patients with heart failure treated with metoprolol.

## Table 1

Inotropic potencies of (-)-noradrenaline and (-)-adrenaline acting through ventricular $\beta_1$ and $\beta_2$ adrenoceptors respectively. Effects of cilostamide (300 nM right ventricle, 1 μM left ventricle) and rolipram (1 μM right ventricle, 10 μM left ventricle) and chronic metoprolol treatment

| | (-)-Noradrenaline | | (-)-Adrenaline | |
|---|---|---|---|---|
| | Non-βB | Metoprolol treated | Non-βB | Metoprolol treated |
| | $pEC_{50}$ (*n*) | $pEC_{50}$ (*n*) | $pEC_{50}$ (*n*) | $pEC_{50}$ (*n*) |
| Right ventricle | | | | |
| Control | 5.65 ± 0.15 (11/4) | 6.25 ± 0.13 (18/7)* | 5.70 ± 0.27 (14/5) | 6.40 ± 0.11 (18/7)* |
| Cilostamide | 5.89 ± 0.24 (10/4) | 6.75 ± 0.17 (17/7) | 6.19 ± 0.27 (12/5) | 7.11 ± 0.16 (13/7)† |
| Rolipram | – | 6.19 ± 0.15 (15/7) | – | 6.50 ± 0.17 (12/7) |
| Left ventricle | | | | |
| Control | – | 6.34 ± 0.16 (7/6) | – | 6.26 ± 0.16 (10/7) |
| Cilostamide | – | 6.77 ± 0.19 (7/6) | – | 6.93 ± 0.12 (8/7)†† |
| Rolipram | – | 6.25 ± 0.10 (7/6) | – | 6.29 ± 0.17 (8/7) |

Non-βB: not treated with β-blockers.
*$P < 0.05$ versus non-βB.
†$P < 0.001$ paired Student's *t*-test for comparison between cilostamide and control (no PDE inhibitor).
††$P < 0.05$ versus control, one-way ANOVA with Bonferroni adjustment for multiple *a priori* comparisons for comparison between cilostamide, rolipram and control.




## Figure 2

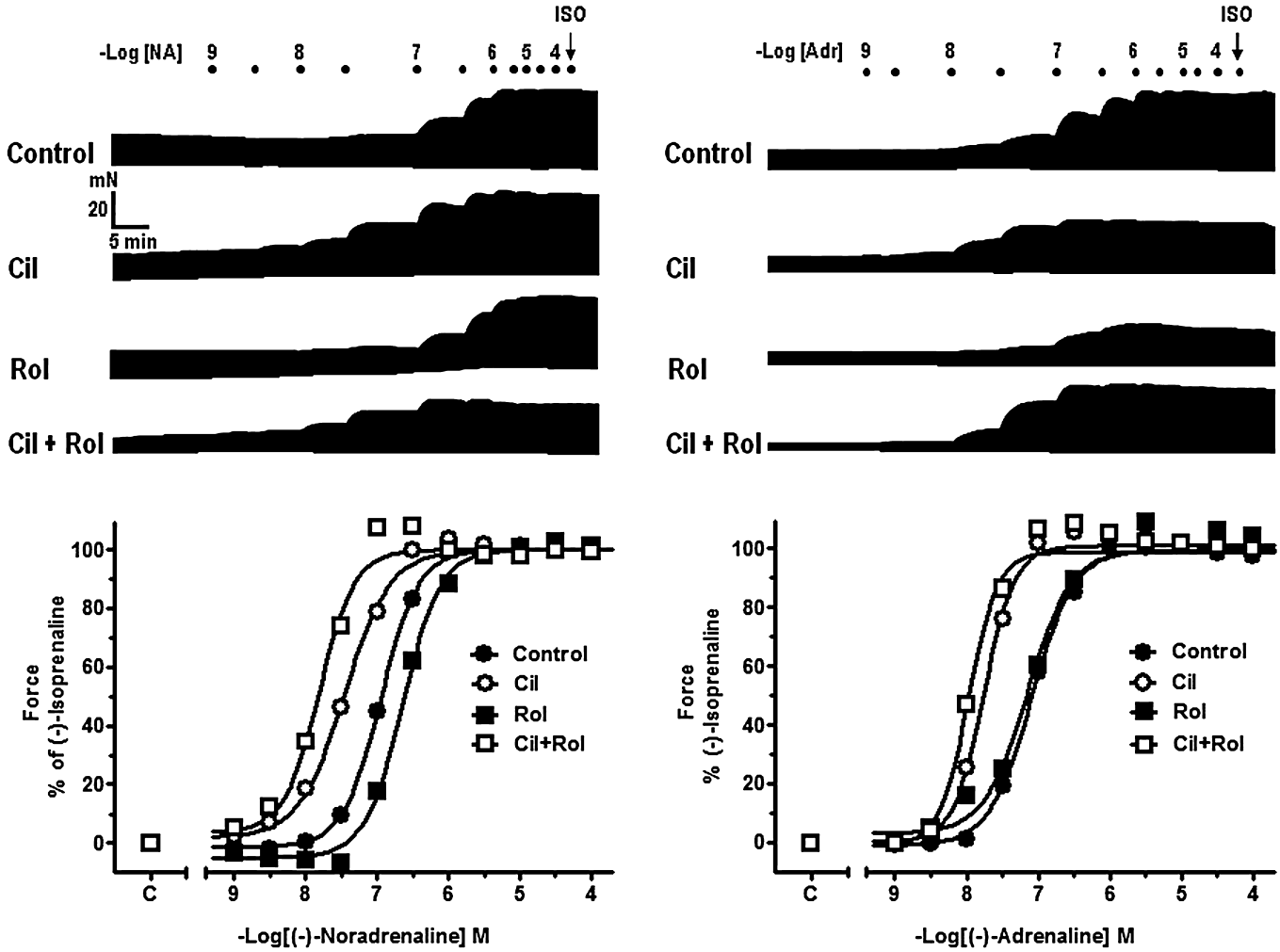
Lack of effect of cilostamide on the inotropic responses of (-)-noradrenaline and (-)-adrenaline in right ventricular trabeculae from four [(-)-noradrenaline experiments] or five [(-)-adrenaline experiments] patients with heart failure not treated with a β-blocker. Shown are concentration–effect curves to (-)-noradrenaline (A) and (-)-adrenaline (B) in the absence or presence of cilostamide (300 nM). Cilostamide did not significantly increase basal force ($P = 0.36$ for the noradrenaline group, $P = 0.46$ for the adrenaline group) or enhance the maximum force caused by (-)-noradrenaline ($P = 0.41$) or (-)-adrenaline ($P = 0.13$).

### *Cilostamide fails to potentiate the inotropic effects of catecholamines in right ventricular trabeculae from non-β-blocker-treated patients*

Cilostamide (300 nM) did not significantly increase contractile force or hasten relaxation in the presence of ICI118551 or CGP20712A in trabeculae from non-β-blocker-treated patients. Cilostamide did not potentiate the positive inotropic effects of (-)-noradrenaline or (-)-adrenaline (Figure 2, Table 1). Cilostamide did not affect the lusitropic effects of (-)-noradrenaline (Supporting Information Fig. S2A,C, Table S2) but potentiated the (-)-adrenaline-evoked shortening of $t_{50}$ (Supporting Information Fig. S2D, Table S2).

### *Cilostamide potentiates more the effects mediated through $\beta_2$ adrenoceptors than $\beta_1$ adrenoceptors in ventricular trabeculae from metoprolol-treated patients*

Cilostamide (300 nM) did not significantly change contractile force in the presence of ICI118551 or CGP20712A on




## Figure 3

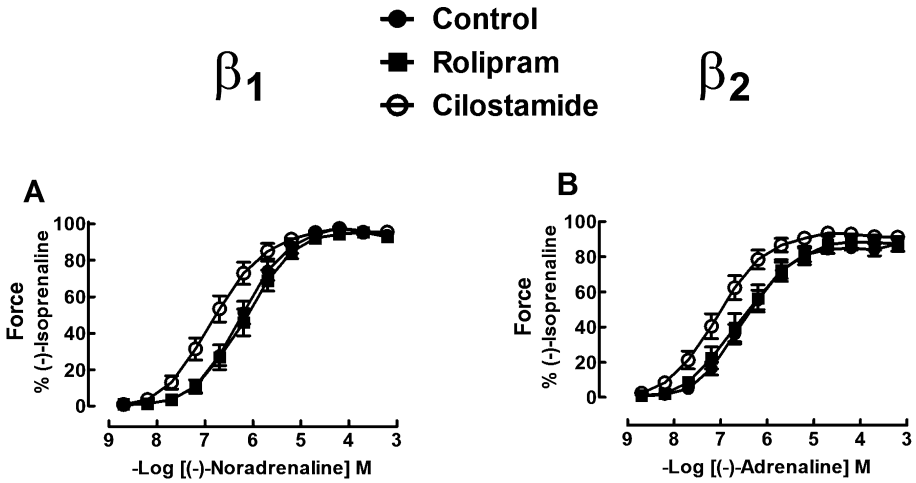
Representative experiment carried out on right ventricular trabeculae obtained from a 48-year-old male patient with ischaemic heart disease, left ventricular ejection fraction 25 %, chronically administered metoprolol 142.5 mg daily. Shown are original traces for (-)-noradrenaline and (-)-adrenaline in the absence or presence of cilostamide (Cil, 300 nM), rolipram (Rol, 1 μM), or Cil + Rol, followed by (-)-isoprenaline (ISO, 200 μM). The bottom panels show the corresponding graphical representation with non-linear fits. Note the clear potentiation of inotropic effects of both (-)-noradrenaline and (-)-adrenaline in the presence of cilostamide but the lack of potentiation by rolipram.

right ventricular trabeculae. Cilostamide caused leftward shifts of the inotropic concentration–effect curves of (-)-noradrenaline and (-)-adrenaline as shown in the representative experiment in Figure 3. Inotropic results from right ventricular trabeculae of seven patients are shown in Figure 4. Cilostamide almost significantly increased the inotropic potency of (-)-noradrenaline ($P$ = 0.06) (Figure 4A, Table 1). When data from right ventricular trabeculae of two additional metoprolol-treated Oslo patients (results not shown) were pooled with the data from seven Brisbane–Dresden patients, cilostamide significantly ($P$ < 0.02, $n$ = 9) potentiated the inotropic effects of (-)-noradrenaline. Cilostamide (300 nM) potentiated the effects of (-)-adrenaline on force (fivefold, $P$ < 0.05, Figure 4B, Table 1). Cilostamide potentiated threefold the effects of (-)-noradrenaline on $t_{50}$ ($P$ < 0.05) but not time to peak force (TPF) (Supporting Information Fig. S3A,C, Table S2). Cilostamide potentiated the effects of (-)-adrenaline on TPF (threefold) and $t_{50}$ (fourfold) respectively (both $P$ < 0.05, Supporting Information Fig. S3B,D, Table S2).

Cilostamide (1 μM) caused a non-significant ($P$ < 0.07) leftward shift of the concentration–effect curve for the inotropic effects of (-)-noradrenaline on left ventricular trabeculae ($P$ < 0.07, Figure 5A, Table 1), but potentiated the inotropic effects of (-)-adrenaline fivefold ($P$ < 0.05, Figure 5B, Table 1).

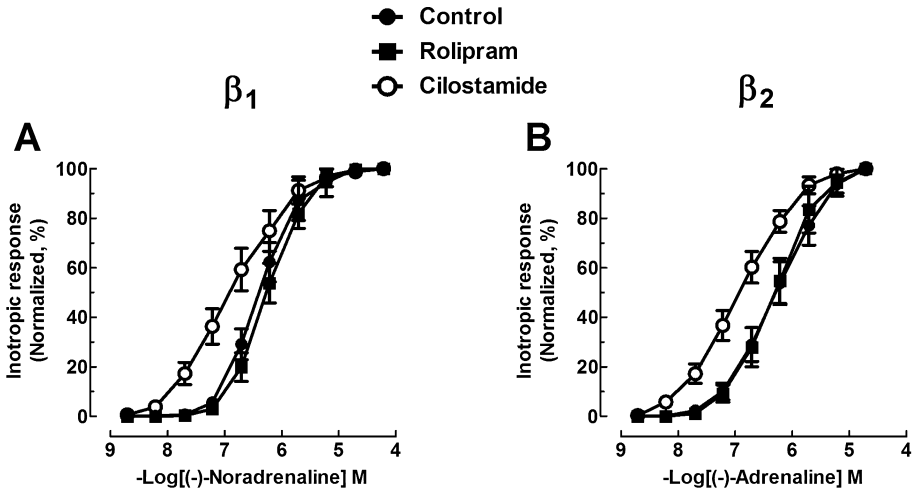
Cilostamide did not potentiate the TPF effects of (-)-noradrenaline or (-)-adrenaline on left ventricular trabeculae but potentiated the effects on time to reach 80% relaxation fourfold and fivefold respectively (both $P$ < 0.05, Supporting Information Fig. S4, Table S3).

Cilostamide caused a non-significant trend of greater potentiation of the inotropic effects of (-)-adrenaline through $\beta_2$ adrenoceptors (0.80 ± 0.11 log units, $n$ = 9) than (-)-noradrenaline through $\beta_1$ adrenoceptors in right ventricular




## Figure 4

Potentiation of the inotropic effects of (-)-adrenaline by cilostamide ($P < 0.05$) in right ventricular trabeculae from seven patients from Brisbane/Dresden with heart failure chronically administered with metoprolol (B). In the same hearts, cilostamide caused a leftward shift of the inotropic effects of (-)-noradrenaline (A) which was not quite significant ($P = 0.06$). Rolipram had no effect on the inotropic effects of (-)-noradrenaline or (-)-adrenaline. See text for further explanation. Shown are concentration–effect curves to (-)-noradrenaline (A) and (-)-adrenaline (B) in the absence or presence of cilostamide (300 nM) or rolipram (1 μM).




## Figure 5

Cilostamide potentiates the inotropic effects of (-)-adrenaline in left ventricular trabeculae from seven [(-)-noradrenaline experiments] or eight Oslo patients [(-)-adrenaline experiments] with heart failure and chronically administered with metoprolol. Shown are concentration–effect curves to (-)-noradrenaline (A) and (-)-adrenaline (B) in the absence or presence of cilostamide (1 μM) or rolipram (10 μM). Inotropic data are normalized as a percentage of the maximal response to (-)-noradrenaline or (-)-adrenaline.

trabeculae from metoprolol-treated patients (0.48 ± 0.18 log units, $n = 9$, Brisbane/Dresden/Oslo hearts) ($P = 0.14$, paired Student's $t$-test). However, when all right and left ventricular inotropic data from metoprolol-treated patients were pooled, cilostamide (0.3–1 μM) potentiated significantly more the $\beta_2$-adrenoceptor-mediated effects of (-)-adrenaline (0.78 ± 0.12 log units, $n = 15$) than the $\beta_1$-adrenoceptor-mediated effects of (-)-noradrenaline (0.47 ± 0.12 log units, $n = 15$) ($P = 0.037$). These results suggest that PDE3 limits more the inotropic responses through $\beta_2$ adrenoceptor than $\beta_1$ adrenoceptor.

### *Rolipram does not modify inotropic and lusitropic potencies of (-)-noradrenaline and (-)-adrenaline*

Rolipram did not significantly modify force, TPF and $t_{50}$ or $t_{80}$ in right and left ventricular trabeculae incubated with ICI118551 or CGP20712A. The inotropic and lusitropic effects of (-)-noradrenaline and (-)-adrenaline were not significantly changed by rolipram (1 μM) in right ventricular trabeculae (inotropic: Figures 3 and 4, Table 1; lusitropic: Supporting Information Fig. S3, Table S2) and rolipram (10 μM)

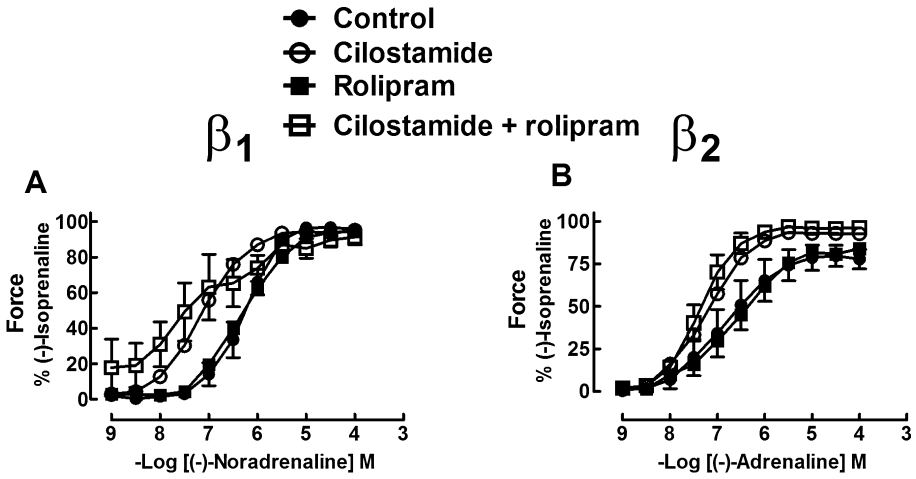



**Figure 6**

Effects of the combination of cilostamide and rolipram on the inotropic responses of (-)-noradrenaline (A) and (-)-adrenaline (B) in right ventricular trabeculae from three patients with heart failure and chronically administered with metoprolol. While the combination of cilostamide and rolipram potentiated the inotropic responses of (-)-noradrenaline and (-)-adrenaline, the degree of potentiation did not differ from that caused by cilostamide alone.

in left ventricular trabeculae (inotropic: Figure 5, Table 1; lusitropic: Supporting Information Fig. S4, Table S3).

The effects of the combination of cilostamide (300 nM) and rolipram (1 μM) on the inotropic and lusitropic potencies of (-)-noradrenaline and (-)-adrenaline were investigated in three metoprolol-treated patients. Cilostamide + rolipram potentiated the inotropic [(-)-noradrenaline $P < 0.05$; (-)-adrenaline $P < 0.05$] and lusitropic [(-)-noradrenaline TPF, $t_{50}$ both $P < 0.05$; (-)-adrenaline TPF, $t_{50}$ both $P < 0.05$] effects of both (-)-noradrenaline and (-)-adrenaline, but the degree of potentiation did not significantly ($P > 0.05$) differ from the potentiation caused by cilostamide alone (inotropic: Figure 6; lusitropic: Supporting Information Fig. S5).

## Discussion

Our work revealed two important aspects of the control by PDEs of the inotropic effects of catecholamines. Chronic treatment of heart failure patients with metoprolol induced PDE3 to reduce the inotropic responses more through $\beta_2$ adrenoceptors than $\beta_1$ adrenoceptors. PDE4 appears not to be involved in the inotropic and lusitropic control at all.

### *Control by PDE3 of the function of $\beta_1$ and $\beta_2$ adrenoceptors in heart failure patients treated with metoprolol*

PDE3 activity is stimulated by activation of $\beta_1$ adrenoceptors and $\beta_2$ adrenoceptors, which in turn causes a negative feedback by hydrolysing cAMP and thereby reducing inotropic and lusitropic effects. A tonic receptor activation by endogenous catecholamines increases cAMP and PKA activity in a compartment that allows the latter to phosphorylate and activate PDE3 (Gettys *et al.*, 1987), which in turn hydrolyses cAMP. This effect is likely to be more important for $\beta_2$ adrenoceptors, at least in human heart, because these receptors are more efficient than $\beta_1$ adrenoceptors at activating $G_s$ and stimulating ventricular adenylyl cyclase (Kaumann and Lemoine, 1987), as verified with recombinant receptors (Levy *et al.*, 1993). Therefore, inhibition of PDE3 may potentiate $\beta_2$-adrenoceptor-mediated responses more than $\beta_1$-adrenoceptor-mediated responses, as shown here for human failing ventricle and previously for non-failing atrial myocardium from patients without heart failure (Christ *et al.*, 2006a).

A reduction in the expression and activity of PDE3 has been reported in heart failure patients (Silver *et al.*, 1990; Ding *et al.*, 2005a,b). Treatment with isoprenaline causes sustained down-regulation of PDE3A (Ding *et al.*, 2005b), as also observed in human heart failure and animal heart failure models (Ya and Abe, 2007), presumably due to the high catecholamine plasma levels. A down-regulation of PDE3A would be expected to increase cAMP levels in heart failure so that inhibition of the enzyme would conceivably affect the effects of catecholamines less. The lack of significant potentiation by cilostamide of the inotropic and lusitropic effects of both (-)-noradrenaline through $\beta_1$ adrenoceptors and marginal potentiation of the effects of adrenaline through $\beta_2$ adrenoceptors in our five non-β-blocked patients is consistent with this expectation. In contrast, chronic treatment of heart failure patients with metoprolol revealed robust potentiation of the inotropic and lusitropic effects of the catecholamines through $\beta_1$ and $\beta_2$ adrenoceptors. We speculate that this effect of metoprolol is due to chronic β adrenoceptor blockade, thereby preventing the suppressing effects of endogenous catecholamines on PDE3 activity.

The increased $\beta_2$-adrenoceptor-mediated ventricular inotropic and lusitropic effects in ventricular trabeculae caused by metoprolol treatment of heart failure patients agree with a similar (sixfold) enhancement of the $\beta_2$-adrenoceptor-mediated inotropic potency of (-)-adrenaline in human atria obtained from patients without heart failure chronically treated with atenolol (Hall *et al.*, 1990). The increased cardiac

responsiveness to adrenaline through $\beta_2$ adrenoceptors appears to be the result of chronic $\beta_1$ adrenoceptor blockade. Experimental long-lasting exposure to catecholamines elicits up-regulation of $G_{i\alpha}$ (Eschenhagen *et al.*, 1992). A similar situation occurs in heart failure in which the sympathetic nervous system is hyperactive (Cohn, 1989), plasma noradrenaline levels are increased (Thomas and Marks, 1978) and ventricular $G_{i\alpha}$ increased (Neumann *et al.*, 1988). Human $\beta_2$ adrenoceptors can couple to and activate $G_{i\alpha}$, in addition to $G_{s\alpha}$, when they are stimulated by a very high isoprenaline concentration in human atrium (Kilts *et al.*, 2000). Through chronic $\beta_1$ adrenoceptor blockade of patients by treatment with metoprolol or possibly atenolol, the noradrenaline-induced elevation of $G_{i\alpha}$ ceases, $G_{i\alpha}$ levels are reduced (Sigmund *et al.*, 1996), conceivably thereby favouring coupling of $\beta_2$ adrenoceptor to $G_{s\alpha}$ to allow enhanced inotropic and lusitropic effects of adrenaline through $\beta_2$ adrenoceptors. This hypothesis requires future research.

Our results suggest that chronic β adrenoceptor blockade facilitates the control by PDE3s of catecholamine effects, particularly through $\beta_2$ adrenoceptors. However, the generality of this argument has to be restricted to heart failure, or it runs afoul because in atrial myocardium obtained from patients without heart failure we observed that cilostamide potentiated the effects of adrenaline, mediated through $\beta_2$ adrenoceptors, more than the effects of noradrenaline, mediated through $\beta_1$ adrenoceptors, regardless of whether or not patients had been treated with $\beta_1$ adrenoceptor-selective blockers (Christ *et al.*, 2006a). Changes of ventricular $\beta_2$ adrenoceptor function in heart failure (Nikolaev *et al.*, 2010) and profound anatomical differences between ventricle and atrium (Bootman *et al.*, 2011) may be relevant to account for the different consequences of PDE3 control in the two tissues with respect to $\beta_1$ adrenoceptor and $\beta_2$ adrenoceptor function after chronic β adrenoceptor blockade.

The lusitropic (Supporting Information Figs S3–5, Tables S2, S3) effects mediated through $\beta_1$ and $\beta_2$ adrenoceptors were usually potentiated by cilostamide to a similar extent as the corresponding inotropic effects in trabeculae from β-blocker-treated patients (Figures 4–6, Table 1). PDE3 activity in human ventricle is associated with membrane vesicle-derived t-tubules and junctional sarcoplasmic reticulum (SR), causing hydrolysis of cAMP in the vicinity of phospholamban (PLB) (Movsesian *et al.*, 1991; Lugnier *et al.*, 1993). In ventricular myocardium from failing hearts, noradrenaline and adrenaline produce similar increases in PKA-catalysed phosphorylation of the proteins mediating myocardial relaxation, PLB (at Ser16), troponin-I (TnI) and cardiac myosin-binding protein-C (Kaumann *et al.*, 1999). Our lusitropic results are consistent with an increased phosphorylation of PLB, TnI and myosin-binding protein-C by isoprenaline in the presence of the PDE3 inhibitor pimobendan in human failing myocardium (Bartel *et al.*, 1996).

### *PDE4 inhibition does not affect the inotropic and lusitropic effects of catecholamines*

PDE4 isoenzymes, their subtypes and splicing variants, are equally expressed in rodent and human ventricle but murine hearts have a considerably higher PDE4 activity than human hearts (Richter *et al.*, 2011). Inhibition of PDE4 causes potentiation of the positive inotropic effects mediated through rodent $\beta_1$ adrenoceptors (Kaumann, 2011). In contrast, our results from human failing ventricle demonstrate that inhibition of PDE4 with rolipram did not potentiate the positive inotropic and lusitropic effects of (-)-noradrenaline and (-)-adrenaline, mediated through $\beta_1$ and $\beta_2$ adrenoceptors respectively. It could be argued that we were unable to demonstrate a potentiating effect of rolipram because PDEs, including PDE4s, are down-regulated in heart failure (Ding *et al.*, 2005a,b; Lehnart *et al.*, 2005). However, we have also reported for human atrial myocardium, obtained from non-failing hearts, that rolipram failed to potentiate the positive inotropic effects of (-)-noradrenaline and (-)-adrenaline mediated through $\beta_1$ and $\beta_2$ adrenoceptors (Christ *et al.*, 2006a; Kaumann *et al.*, 2007). Our findings are consistent with an early report demonstrating that cilostamide but not rolipram inhibited SR-associated PDE activity in human ventricle from heart failure patients (Movsesian *et al.*, 1991).

Taken together, our present results and a critical appraisal of the literature make it unlikely that PDE4s modulate human inotropic and lusitropic effects of catecholamines, mediated through both $\beta_1$ and $\beta_2$ adrenoceptors in non-failing and failing hearts. Moreover, extrapolation of results from the PDE4 function in mouse and rat hearts to human inotropic and lusitropic effects of physiological catecholamines can actually be misleading. However, PDE4s can reduce the occurrence of catecholamine-evoked arrhythmias in murine ventricle (Galindo-Tovar and Kaumann, 2008; Lehnart *et al.*, 2005) and apparently in human atrium (Molina *et al.*, 2012). However, clinical trials with a PDE4 inhibitor, roflumilast, have not provided evidence for cardiovascular side effects in approximately 1500 roflumilast-treated patients compared with 1500 placebo patients (Calverley *et al.*, 2009). A comparison between human and other species of the control of $\beta_1$-adrenoceptor and $\beta_2$-adrenoceptor-mediated inotropy and lusitropy by PDE3 and PDE4, as well as protection against arrhythmias, is summarized in Supporting Information Table S4.

## Clinical implications

Although we did not detect a direct inotropic change with cilostamide, this PDE3 inhibitor potentiated the inotropic effects of the endogenous catecholamines mediated through ventricular $\beta_1$ and $\beta_2$ adrenoceptors of metoprolol-treated patients, consistent with PDE3 inhibition. The induction of PDE3 activity in metoprolol-treated patients could further reduce cardiostimulation by endogenous catecholamines.

We found on human atrium that metoprolol blocks the effects of catecholamines through $\beta_1$ adrenoceptors only by 2.5-fold more than $\beta_2$ adrenoceptors (Supporting Information Fig. S6). We predict that heart failure patients under therapy with a PDE3 inhibitor + metoprolol could be at risk of not being protected against adverse stress-induced surges of adrenaline, acting through $\beta_2$ adrenoceptors. From simple competitive inhibition, the concentration ratio (CR) of a catecholamine in the presence and absence of metoprolol can be calculated from $CR = 1 + ([metoprolol] \times K_B^{-1})$. The therapeutic plasma level of 100 $ng \cdot mL^{-1}$ (310 nM) metoprolol (Kindermann *et al.*, 2004) which hardly binds to plasma proteins, using $K_B$ values of 40 nM for $\beta_1$ adrenoceptors and

98 nM for $\beta_2$ adrenoceptors (Supporting Information Fig. S6), would produce CR values of 8.8 for $\beta_1$ adrenoceptors and 4.2 for $\beta_2$ adrenoceptors. The fivefold potentiation of the inotropic effects of (-)-adrenaline by cilostamide suggests that endogenous increases in plasma (-)-adrenaline could conceivably surmount the $\beta_2$ adrenoceptor blockade caused by metoprolol in patients also treated with a PDE3 inhibitor.

## Conclusions

Treatment with metoprolol induces the control by PDE3 of the ventricular inotropic and lusitropic effects of (-)-noradrenaline and (-)-adrenaline through $\beta_1$ adrenoceptors and $\beta_2$ adrenoceptors, respectively, plausibly by restoring the decreased activity and expression of PDE3 in heart failure. Quantitative considerations, based on differences in the affinity profile of metoprolol for $\beta_1$ and $\beta_2$ adrenoceptors, suggest that treatment with a PDE3-selective inhibitor could potentially facilitate adverse stress-induced adrenaline effects through $\beta_2$ adrenoceptors in patients treated with metoprolol. PDE4 does not control the inotropic and lusitropic effects mediated through $\beta_1$ and $\beta_2$ adrenoceptors in human heart.

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## Conflict of interest

None declared.

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1** Effects of chronic administration of metoprolol compared with no-β-blocker on lusitropic effects [time to peak force and time to 50% relaxation ($t_{50}$)] of (-)-noradrenaline through activation of $\beta_1$ adrenoceptors (A,C) and (-)-adrenaline through activation of $\beta_2$ adrenoceptors (B,D) in right ventricular trabeculae from failing hearts. Data from four [(-)-noradrenaline experiments] or five [(-)-adrenaline experiments] patients with heart failure not treated with a β-blocker and seven patients with heart failure treated with metoprolol.

**Figure S2** Effect of cilostamide on the lusitropic responses of (-)-noradrenaline and (-)-adrenaline in right ventricular trabeculae from four [(-)-noradrenaline experiments] or five [(-)-adrenaline experiments] patients with heart failure not treated with a β-blocker. Shown are concentration–effect curves to (-)-noradrenaline (A,C) and (-)-adrenaline (B,D) in the absence or presence of cilostamide (300 nM). Cilostamide potentiated the (-)-adrenaline-evoked hastening of relaxation (shortening of $t_{50}$).

**Figure S3** Cilostamide, but not rolipram, potentiates the lusitropic effects of (-)-adrenaline and (-)-noradrenaline in right ventricular trabeculae from seven patients with heart failure chronically administered with metoprolol. Shown are concentration–effect curves to (-)-noradrenaline (A,C) and (-)-adrenaline (B,D) in the absence or presence of cilostamide (300 nM) or rolipram (1 μM).

**Figure S4** Cilostamide, but not rolipram, potentiates the relaxant effects of (-)-noradrenaline and (-)-adrenaline in left ventricular trabeculae from seven [(-)-noradrenaline experiments] or eight Oslo patients [(-)-adrenaline experiments] with heart failure chronically administered with metoprolol. Shown are concentration–effect curves to (-)-noradrenaline (A,C) and (-)-adrenaline (B,D) in the absence or presence of cilostamide (1 μM) or rolipram (10 μM). See Supporting Information Table S3 for analysis.

**Figure S5** Effects of the combination of cilostamide (300 nM) and rolipram (1 μM) on the lusitropic responses of (-)-noradrenaline and (-)-adrenaline in right ventricular trabeculae from three patients with heart failure chronically administered with metoprolol. While the combination of cilostamide and rolipram potentiated the lusitropic responses of (-)-noradrenaline and (-)-adrenaline, the degree of potentiation did not differ from that caused by cilostamide alone.

**Figure S6** Determination of the affinity of metoprolol at $\beta_1$ and $\beta_2$ adrenoceptors in human right atrium. Shown are cumulative concentration–effect curves for (-)-noradrenaline at $\beta_1$ adrenoceptors (A) and (-)-adrenaline at $\beta_2$ adrenoceptors (B) in the absence and presence of metoprolol. Numbers in parentheses are (trabeculae/patients). The corresponding Schild plots are shown in (C).

**Table S1** Summary of patients.

**Table S2** Lusitropic potencies of (-)-noradrenaline and (-)-adrenaline acting through right ventricular $\beta_1$ and $\beta_2$ adrenoceptors respectively. Effects of cilostamide (300 nM) and rolipram (1 μM) in non-β-blocker-treated (non-βB) and chronic metoprolol-treated patients.

**Table S3** Lusitropic potencies of (-)-noradrenaline and (-)-adrenaline acting through left ventricular $\beta_1$ and $\beta_2$ adrenoceptors of metoprolol-treated patients. Effects of cilostamide (1 μM) and rolipram (10 μM).

**Table S4** Reduction of inotropic and lusitropic responses as well as protection against arrhythmias, mediated through myocardial $\beta_1$ and $\beta_2$ adrenoceptors, by PDE3 and PDE4 in different species.