Phosphodiesterase PDE3, but not PDE4, reduces β₁- and β₂-adrenoceptor-mediated inotropic and lusitropic effects in failing ventricle from metoprolol-treated patients

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Background and purpose  Phosphodiesterases 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 (0.3-1μM) or PDE4 inhibitor rolipram (1-10μM) 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 1Hz. The effects of (-)-noradrenaline, mediated through β1-adrenoceptors (β2-adrenoceptors blocked with ICI118551), and (-)-adrenaline, mediated through β2-adrenoceptors (β1-adrenoceptors blocked with CGP20712A), were assessed in the absence and presence of PDE inhibitors. Catecholamine potencies were estimated from –logEC₅₀s.

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 β₁- and β₂-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 β₂-adrenoceptors. PDE4 does not appear to reduce inotropic and lusitropic effects of catecholamines in failing human ventricle.
Key words Human heart failure - $\beta_1$- and $\beta_2$-adrenoceptors – phosphodiesterases 3 and 4 – noradrenaline and adrenaline – inotropism and lusitropism – metoprolol

Introduction

Activation of $\beta_1$- and $\beta_2$-adrenoceptors ($\beta_1$ARs, $\beta_2$ARs) 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). Phosphodiesterases (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 $\beta$ blocker-treated patients. It is unknown whether blockade of $\beta_1$ARs and/or $\beta_2$ARs 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 (Lehnhart et al., 2005). Therefore, PDE4D3 plausibly may affect catecholamine-evoked contractility. PDE4 controls the inotropic effects and cAMP signals of catecholamines, mediated through β1AR 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 β1AR and/or β2AR.

We now investigated whether the inotropic and lusitropic effects of the catecholamines, mediated through β1AR or β2AR 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 β1ARs and β2ARs. 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 Supplement Table S1A,B. Clinical data from Oslo patients are shown in Supplement 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 Supplement.

Specific activation of $\beta_1$- and $\beta_2$-adrenoceptors

To determine the effects of $\beta_1$AR (Alexander et al., 2011) -selective activation, concentration-effect curves for (-)-noradrenaline were obtained in the presence of ICI118551 (50 nM) to selectively block $\beta_2$ARs. To determine the effects of $\beta_2$AR (Alexander et al., 2011) -selective activation, concentration-effect curves for (-)-adrenaline were determined in
the presence of CGP20712A (300 nM) to selectively block \(\beta_1\)ARs (Kaumann et al., 1999). To assess the influence of the PDE3-selective inhibitor cilostamide (300 nM-1 \(\mu\)M) and the PDE4-specific inhibitor rolipram (1-10 \(\mu\)M) 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 minutes 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 \(\mu\)M) was 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 \(\beta_1\)ARs and \(\beta_2\)-ARs as shown in the representative experiment of 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 \(\mu\)M), administered after a complete concentration-effect curve. The catecholamine concentrations producing a half maximum response, –LogEC\(_{50}\)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 ± S.E.M. of \(n=\) number of patients or trabeculae as indicated. Significance of differences between means were 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).

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 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, 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-1H-inden-4-yl]oxy-3-[(1-methylethyl)amino]-2-butanol) were from Tocris Bioscience (Bristol, UK) or Sigma (Castle Hill, Australia). 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 4-fold and 5-fold respectively in metoprolol-treated (\( P < 0.05 \)) compared to non-\( \beta \)-blocker-treated patients (Figure 1A and B, table 1). The lusitropic effects of (-)-noradrenaline, mediated through \( \beta_1 \)AR, were not significantly
enhanced but the $t_{50}$-abbreviating potency of (-)-adrenaline increased 7-fold ($P<0.001$) by treatment of patients with metoprolol (Supplement Figure 1S A-D, Supplement table S2). These results are consistent with the upregulation of the $\beta_1$AR density and enhanced inotropic responses through these receptors in metoprolol-treated patients (Heilbrunn et al., 1989; Sigmund et al., 1996).

**Cilostamide fails to potentiate the inotropic effects of catecholamines in right ventricular trabeculae from non-$\beta$-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-$\beta$-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 (Figure S2 A,C, table S2) but potentiated the (-)-adrenaline-evoked shortening of $t_{50}$ (Figure S2 D, table S2).

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

Cilostamide (300 nM) did not significantly change contractile force in the presence of ICI118551 or CGP20712 on right ventricular trabeculae. Cilostamide caused left-ward shifts of the inotropic concentration-effect curves of (-)-noradrenaline and (-)-adrenaline as shown in the representative experiment of Figure 3. Inotropic results from right ventricular trabeculae of 7 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 7 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 (5-fold, P < 0.05, Figure 4B, table 1). Cilostamide potentiated 3-fold the effects of (-)-noradrenaline on t50 (P < 0.05) but not TPF (Figure S3A,C, table S2). Cilostamide potentiated the effects of (-)-adrenaline on TPF (3-fold) and t50 (4-fold) respectively (both P < 0.05, Supplement Figure S3B,D, table S2).

Cilostamide (1 µM) caused a non-significant (P < 0.07) leftward shift of the concentration-effects 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 5-fold (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 4-fold and 5-fold respectively (both P < 0.05, Figure 4S, table S3).

Cilostamide caused a non-significant trend of greater potentiation of the inotropic effects of (-)-adrenaline through β2AR (0.80±0.11 log units, n=9) than (-)-noradrenaline through β1AR in right ventricular 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 β2AR-mediated effects of (-)-adrenaline (0.78±0.12 log units, n=15) than the β1AR-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 β2AR than β1AR.
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 $\mu$M) in right ventricular trabeculae (inotropic, Figures 3 and 4, table 1; lusitropic Figure S3, table S2) and rolipram (10 $\mu$M) in left ventricular trabeculae (inotropic Figure 5, table 1; lusitropic Figure S4, table S3).

The effects of the combination of cilostamide (300 nM) and rolipram (1 $\mu$M) on the inotropic and lusitropic potencies of (-)-noradrenaline and (-)-adrenaline were investigated in 3 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 Figure 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 $\beta_2$ARs than $\beta_1$ARs. PDE4 appears not to be involved in the inotropic and lusitropic control at all.

_Control by PDE3 of the function of $\beta_1$ARs and $\beta_2$ARs in heart failure patients treated with metoprolol._
PDE3 activity is stimulated by activation of $\beta_1$ARs and $\beta_2$ARs, 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 hydrolys cAMP. This effect is likely to be more important for $\beta_2$AR, at least in human heart, because these receptors are more efficient than $\beta_1$AR at activating Gs and stimulating ventricular adenylyl cyclase (Kaumann and Lemoine, 1987), as verified with recombinant receptors (Levy et al., 1993). Therefore inhibition of PDE3 may potentiate $\beta_2$AR-mediated responses more than $\beta_1$AR-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, 2005b). 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 $\beta_1$AR and marginal potentiation of the effects of adrenaline through $\beta_2$AR in our 5 non-$\beta$-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 $\beta_1$ARs and $\beta_2$ARs. We speculate that this effect of metoprolol is due to chronic $\beta$AR
blockade, thereby preventing the suppressing effects of endogenous catecholamines on PDE3 activity.

The increased \( \beta_2 \)AR-mediated ventricular inotropic and lusitropic effects in ventricular trabeculae caused by metoprolol-treatment of heart failure patients agrees with a similar (6-fold) enhancement of the \( \beta_2 \)AR-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 \( \beta_2 \)AR appears to be the result of chronic \( \beta_1 \)AR blockade. Experimental long-lasting exposure to catecholamines elicits upregulation of \( G_{ia} \) (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 \( G_{ia} \) increased (Neumann et al., 1988). Human \( \beta_2 \)ARs can couple to and activate \( G_{ia} \), in addition to \( G_{sa} \), when they are stimulated by a very high isoprenaline concentration in human atrium (Kilts et al., 2000). Through chronic \( \beta_1 \)AR blockade of patients by treatment with metoprolol or possibly atenolol, the noradrenaline-induced elevation of \( G_{ia} \) ceases, \( G_{ia} \) levels are reduced (Sigmund et al., 1996), conceivably thereby favouring coupling of \( \beta_2 \)AR to \( G_{sa} \) to allow enhanced inotropic and lusitropic effects of adrenaline through \( \beta_2 \)AR. This hypothesis requires future research.

Our results suggest that chronic \( \beta \)-adrenoceptor-blockade facilitates the control by PDE3s of catecholamine effects, particularly through \( \beta_2 \)ARs. 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 \( \beta_2 \)AR, more than the effects of noradrenaline, mediated through
β₁AR, regardless of whether or not patients had been treated with β₁AR-selective blockers (Christ et al., 2006a). Changes of ventricular β₂AR 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 β₁AR and β₂AR function after chronic βAR blockade.

The lusitropic (Figures S3-5, tables S2, S3) effects mediated through β₁AR and β₂AR were usually potentiated by cilostamide to a similar extent as the corresponding inotropic effects in trabeculae from β-blocked-treated patients (Figures 4-6, table 1). PDE3 activity in human ventricle is associated with membrane vesicle-derived t-tubules and junctional SR, causing hydrolysis of cAMP in the vicinity of phospholamban (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, phospholamban (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 β₁ARs (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 \( \beta_1 \)AR and \( \beta_2 \)AR respectively. It could be argued that we were unable to demonstrate a potentiating effect of rolipram because PDEs, including PDE4s, are downregulated in heart failure (Ding et al., 2005a; 2005b; Lehnhardt 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 \( \beta_1 \)AR and \( \beta_2 \)AR (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 \( \beta_1 \)AR and \( \beta_2 \)AR 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; Lehnhardt 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 1,500 roflumilast-treated patients compared to 1,500 placebo patients (Calverley et al., 2009). A comparison between human and other species of the control of \( \beta_1 \)AR and \( \beta_2 \)AR-mediated inotropy and lusitropy by PDE3 and PDE4, as well as protection against arrhythmias, is summarised in 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 β₁ARs and β₂ARs 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 catecholamines through β₁ARs only by 2.5-fold more than β₂ARs (Supplement Figure 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 β₂ARs. 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] × K_B⁻¹). The therapeutic plasma level of 100 ng.ml⁻¹ (310 nM) metoprolol (Kindermann et al., 2004) which hardly binds to plasma proteins, using K_B values of 40 nM for β₁AR and 98 nM for β₂AR (Supplement Figure S6) would produce CR values of 8.8 for β₁ARs and 4.2 for β₂ARs. The 5-fold potentition of the inotropic effects of (-)-adrenaline by cilostamide suggests that endogenous increases in plasma (-)-adrenaline could conceivably surmount the β₂AR 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 β₁ARs and β₂ARs respectively, plausibly by restoring the decreased activity and expression of PDE3 in heart failure. Quantitative considerations, based on differences in the affinity profile metoprolol for β₁ARs and β₂ARs, suggest that treatment with a PDE3-selective inhibitor could potentially
facilitate adverse stress-induced adrenaline effects through $\beta_2$ARs in patients treated with metoprolol. PDE4 does not control the inotropic and lusitropic effects mediated through $\beta_1$ARs and $\beta_2$ARs in human heart.

**Conflict of Interest:** none declared.

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

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<th>(-)-Noradrenaline</th>
<th>(-)-Adrenaline</th>
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<tr>
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<td>Non-βB</td>
<td>Metoprolol treated</td>
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<td><strong>Right Ventricle</strong></td>
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<td>Control</td>
<td>5.65 ± 0.15 (11/4)</td>
<td>6.25 ± 0.13 (18/7)*</td>
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<td>Cilostamide</td>
<td>5.89 ± 0.24 (10/4)</td>
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<td>Rolipram</td>
<td>6.19 ± 0.15 (15/7)</td>
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<td><strong>Left Ventricle</strong></td>
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<td>Control</td>
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<td>6.26 ± 0.16 (10/7)</td>
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<tr>
<td>Cilostamide</td>
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<td>6.93 ± 0.12 (8/7)††</td>
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<tr>
<td>Rolipram</td>
<td>6.25 ± 0.10 (7/6)</td>
<td>6.29 ± 0.17 (8/7)</td>
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Non-β: not treated with β-blockers

*P < 0.05 vs non-βB
†P < 0.001 Paired Student’s t-test for comparison between Cilostamide and Control (no phosphodiesterase inhibitor)
†† P < 0.05 vs. control, One-way ANOVA with Bonferroni adjustment for multiple a-priori comparisons for comparison between cilostamide, rolipram and Control.
Figure legends

**Figure 1** Effects of chronic administration of metoprolol compared to no-β-blocker on inotropic effects of (-)-noradrenaline through activation of β₁-adrenoceptors (A) and (-)-adrenaline through activation of β₂-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. βAR-blockade did not significantly increase basal force (P=0.07 for (-)-noradrenaline, P=0.095 for (-)-adrenaline) and E_{max} (P=0.10 for (-)-noradrenaline, P=0.054 for (-)-adrenaline). Data from 4 ((-)noradrenaline experiments) or 5 ((-)adrenaline experiments) patients with heart failure not treated with a β-blocker and 7 patients with heart failure treated with metoprolol.

**Figure 2** Lack of effect of cilostamide on the inotropic responses of (-)-noradrenaline and (-)-adrenaline in right ventricular trabeculae from 4 ((-)noradrenaline experiments) or 5 ((-)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).

**Figure 3** Representative experiment carried out on right ventricular trabeculae obtained from a 48 year old male patient with ischemic 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.

**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 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 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.

**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 3 patients with heart failure and chronically administered metoprolol. Whilst 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.