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RESEARCH PAPER

Treprostinil potentiates the positive inotropic effect of catecholamines in adult rat ventricular cardiomyocytes

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Background and purpose: Prostanoids have been shown to improve exercise tolerance, hemodynamics and quality of life in patients with pulmonary arterial hypertension (PAH). We investigated whether treprostinil exerts direct contractile effects on cardiomyocytes that may explain partly the beneficial effects of these drugs.
Experimental approach: Ventricular cardiomyocytes from adult rats were paced at a constant frequency of 0.5 to 2.0 Hz and cell shortening was monitored via a cell edge detection system. Twitch amplitudes, expressed as percent cell shortening of the diastolic cell length, and maximal contraction velocity, relaxation velocity, time to peak of contraction and time to reach 50% of relaxation were analyzed.
Key results: Treprostinil (0.15 – 15 ng ml^{-1}) slightly increased contractile dynamics of cardiomyocytes at clinically relevant concentrations. However, the drug significantly improved cell shortening of cardiomyocytes in the presence of isoprenaline, a β-adrenoceptor agonist. Treprostinil exerted this effect at all beating frequencies under investigation. Treprostinil mimicked this potentiating effect in a Langendorff preparation as well. The potentiating effect of treprostinil on isoprenaline-dependent cell shortening was no longer seen after phosphodiesterase inhibition. Long-term cultivation of cardiomyocytes with treprostinil did not modify load free cell shortening of these cells, but reduces the duration of contraction.
Conclusions and implications: We conclude that the clinically used prostanoid treprostinil potentiates the positive inotropic effects of catecholamines in adult ventricular cardiomyocytes. This newly described effect may contribute to the beneficial clinical effects of prostanoids in patients with PAH.
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Keywords: prostanoids; cardiomyocytes; cell shortening; cAMP; isoprenaline

Abbreviations: Con-Vel, maximal contraction velocity; EP, PGE_2-activated prostanoid receptor; FP, $PGF_{2\alpha}$- activated prostanoid receptor; IBMX, isobutyl methylxanthine; IP, PGI_2 activated prostanoid receptor; ISO, isoprenaline; LVDP, left ventricular developed pressure; PAH, pulmonary arterial hypertension; R50, time to reach 50% relaxation; Rel-Vel, maximal relaxation velocity; TTP, time to peak

Introduction

The break-through in the treatment of severe pulmonary arterial hypertension (PAH) was the introduction of prostacyclin (epoprostenol) infusion, which led from therapeutic nihilism to hope for the patients. Epoprostenol has been shown to improve haemodynamics, exercise tolerance and survival (Barst *et al*., 1996; McLaughlin *et al*., 2002; Sitbon *et al*., 2002). In the current guidelines, epoprostenol is the drug of choice for PAH patients presenting in WHO functional class IV (Galie *et al*., 2004), although this form of treatment has major drawbacks, due to systemic side effects and the requirement of a permanent central venous catheter. Alternative prostacyclin analogues that do not use a central venous application route have been shown to be efficacious as well. In particular, subcutaneous (s.c.) treprostinil (Simonneau *et al*., 2002) and inhaled iloprost (Olschewski *et al*., 2002) were effective in randomized double-blind controlled trials.

The haemodynamic effect of epoprostenol is characterized by an increase in cardiac output and a decrease in pulmonary and systemic vascular resistance while pulmonary artery pressure does not decrease, on average (Barst *et al*., 1996; McLaughlin *et al*., 2002; Sitbon *et al*., 2002). This led to the speculation that this substance might have a direct positive inotropic effect. Therefore, the first aim of the present study

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was to decide whether treprostinil exerts such direct contractile effects on cardiomyocytes. To investigate this, we performed different sets of experiments using isolated adult rat ventricular cardiomyocytes. We used treprostinil instead of other available prostanoids due to its superior chemical stability (Laliberte *et al*., 2004). The alternative to a direct effect is that prostanoids may increase contractility via a baroreflex activation. The experiments on the isolated cardiomyocytes are able to distinguish between these possibilities.

Several studies have suggested that the haemodynamic effects may be particularly pronounced in severely ill patients. With s.c. trepostinil, the results in NYHA class IV patients were more impressive as compared to class II or III patients (Oudiz *et al*., 2004). The most impressive results with open-label therapy with inhaled iloprost were found in patients with decompensated right heart failure (Olschewski *et al*., 1998, 1999, 2003). A study with intravenous epoprostenol even suggested that despite impressive results in class IV and III patients, there might be no beneficial effect of prostacyclin infusion in class II patients (Higenbottam *et al*., 1998). Prostanoids exert important effects on pulmonary artery smooth muscle cells, endothelial cells, thrombocytes, neutrophils, macrophages and lymphocytes (Olschewski *et al*., 2004). However, this cannot explain why the effects might be so prominent in the subgroup of patients with decompensated right heart function. It is well known that heart failure causes neurohumoral activation with increased catecholamine levels (Nootens *et al*., 1995). This leads to adrenoceptor desensitization. As the beneficial effects of prostanoids are more pronounced in severely ill patients, the second aim of this study was to investigate possible effects of treprostinil on β-adrenoceptor stimulation.

Prostanoids exert cell-specific effects via specific receptors. Prostanoid receptors in cardiomyocytes are not found in caveoli-like structures. Ostrom *et al*. (2001) showed that prostanoids do not activate adenylate cylcase in caveolin immunoprecipitated cardiac membranes, but they enhanced cAMP concentrations measured in the supernatant . This would suggest that a supposed interaction between prostanoid effects and β-adrenoceptor stimulation must be mediated on the post-receptor level, that is, by inhibition of phosphodiesterases (PDE). This would allow the cells to accumulate cAMP and enhance the β-adrenoceptor-dependent positive contractile effects in the absence of direct activation of the adenylate cyclase. Therefore, we investigated further whether an inhibition of PDE will modify these responses. In this context we used the nonselective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) and the PDE IV-selective inhibitor rolipram to assess whether or not prostanoids act on PDE inhibition. A PDE IV-specific inhibitor was chosen, as it has recently been demonstrated that PDE IV is the dominant isoform involved in cAMP control in adult cardiomyocytes (Nikolaev *et al*., 2006).

Following the aims of this study, direct and acute effects of treprostinil were analyzed in the first set of experiments. In the second set of experiments, the effect of treprostinil on the well-established positive contractile effect of β-adrenoceptor stimulation was investigated. Experiments were performed in the absence or presence of PDE inhibitors. Thirdly, cardiomyocytes were exposed over-night to treprostinil, to establish possible long-term effects of the drug. In all these experiments load-free cell shortening of isolated cardiomyocytes was analyzed in a beating range from 0.5 to 2.0 Hz. In addition, acute effects of treprostinil were confirmed on a Langendorff-perfused rat heart model. In summary, our results showed that clinically relevant concentrations of treprostinil are able to sensitize adult rat ventricular cardiomyocytes to β-adrenoceptor stimulation in a PDE-dependent manner without having a significant effect of its own.

Methods

Cell preparations

Ventricular heart muscle cells were isolated from male Wistar rats weighing 200 to 250 *g* as described previously in greater detail (Schlüter and Schreiber, 2005). Hearts were excised under deep ether anesthesia, transferred rapidly to ice-cold saline and mounted on the cannula of a Langendorff perfusion system. Heart perfusion and subsequent steps were all performed at 37°C. First, hearts were perfused in the non-circulating mode for 5 min at 10 ml min^{-1} (perfusate in mM: NaCl 110, KH_2PO_4 1.2, KCl 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11, gassed with 5% CO_2–95% O_2). Thereafter, perfusion was continued with recirculation of 50 ml of the above perfusate supplemented with 0.06% (w/v) crude collagenase and 25 μM $CaCl_2$ at 5 ml min^{-1}. After 30 min, ventricular tissue was minced and incubated for 20 min in recirculating medium with 1% (w/v) bovine serum albumin under 5% CO_2-95% O_2. Gentle trituration through a pipette released cells from the tissue chunks. The resulting cell suspension was filtered through a 200-μm nylon mesh. The filtered material was washed twice by centrifugation (3 min, 25 *g*) and resuspended in the above perfusate, in which the concentration of $CaCl_2$ was increased stepwise to 0.2 and 0.5 mM. After further centrifugation (3 min, 25 *g*), the cells in the pellet were suspended in serum-free culture medium (medium 199 with Earle's salts, 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) and plated at a density of 7×10^4 elongated cells per 35 mm culture dish (Falcon, type 3001). The culture dishes had been preincubated overnight with 4% (v/v) fetal calf serum in medium 199. Two hours after plating, cultures were washed with the serum-free medium 199 to remove round and non-attached cells and cell contraction was analysed.

Determination of cell contraction

Cells were allowed to contract at room temperature and analysed using a cell-edge detection system as described previously (Langer *et al*., 2003). Cells were stimulated via two AgCl electrodes with biphasic electrical stimuli composed of two equal, but opposite rectangular 50-V stimuli of 0.5 ms duration. Each cell was stimulated at 1, 0.5 and 2 Hz for 1 min. Every 15 s the next five contractions were averaged.

The mean of these four measurements at a given frequency was used to define the cell shortening of a given cell. Cell lengths were measured at a rate of 500 Hz via a line camera. Data are expressed as cell shortening normalized to diastolic cell length ($dl\,l^{-1}$ (%)). Cell shortening kinetics are expressed as maximal contraction velocity (Con-Vel ($\mu m\,s^{-1}$)), maximal relaxation velocity (Rel-Vel ($\mu m\,s^{-1}$)), time to peak (TTP (ms)) and time to reach 50% relaxation (R50 (ms)).

Langendorff perfusion

Experiments were performed on isolated hearts from male Wistar rats as described previously (Grohé *et al*., 2004). Hearts were rapidly excised and the aorta was cannulated for retrograd perfusion with a 16-gauge needle connected to a Langendorff perfusion system. A polyvinylchloride balloon was inserted into the left ventricle through the mitral valve and held in place by a suture tied around the left atrium. The other end of the tubing was connected to a pressure transducer for continuous measurement of left ventricular pressure. A second transducer connected to the perfusion line just before the heart was used to measure coronary perfusion pressure. The perfusion system consisted of a warmed storage container for perfusate solution, a rotary pump and a temperature-controlled chamber, in which hearts were mounted. Hearts were perfused with a modified Tyrode solution as described earlier (Grohé *et al*., 2004). After attachment to the Langendorff system, the hearts were allowed to stabilize for at least 20 min. The intraventricular balloon was inflated to give a diastolic pressure of 10 mm Hg and balloon volume was held constant thereafter. Hearts received isoprenaline or isoprenaline and treprostinil as indicated in the result sections.

Statistics

Data are expressed as means ± s.e.m. Analysis of variance and the Student–Newman–Keuls test for *post hoc* analysis were used to analyse experiments, in which more than one group was compared. In cases in which two groups were compared, Student's *t*-test for paired samples was employed. $P<0.05$ was regarded as significant.

Materials and solutions

Treprostinil was obtained from Baxter Pharmaceutical solutions LLC (Bloomington, USA) as a stock solution of $10\,mg\,ml^{-1}$ dissolved in sterile water containing 4 mg sodium chloride, 3 mg metacresol and 6.3 mg sodium citrate and stored at 4°C. Working solutions were prepared by dilution with sterile water. Isoprenaline was obtained from Sigma/RBI (Sigma-Aldrich Chemie, Taufkirchen, Germany). A stock solution (10 mM) was prepared in sterile water and stored at −20°C. The working solution was performed by dilution in M199. Rolipram was obtained from Calbiochem (Merck, Darmstadt, Germany). The stock solution (1 mM) was prepared in dimethylsulphoxide (DMSO), stored at −20°C and diluted in M199 as working solution. IBMX was obtained from Calbiochem (Merck, Darmstadt, Germany). The stock solution (10 mM) was prepared in DMSO, stored at −20°C, and diluted in M199 for working solutions. Control cells were treated with the appropriate vehicle solutions.

Results

Acute effects of treprostinil on cell shortening

To determine whether treprostinil exerts direct effects on cell shortening, the drug was added to the culture medium and cell shortening was measured. As a positive control, we also investigated the cardiomyocyte response to isoprenaline, a β-adrenoceptor agonist. Isoprenaline caused a concentration-dependent increase in maximal cell shortening which was more pronounced at higher beating frequencies (Figure 1). In contrast, treprostinil modestly increased cell

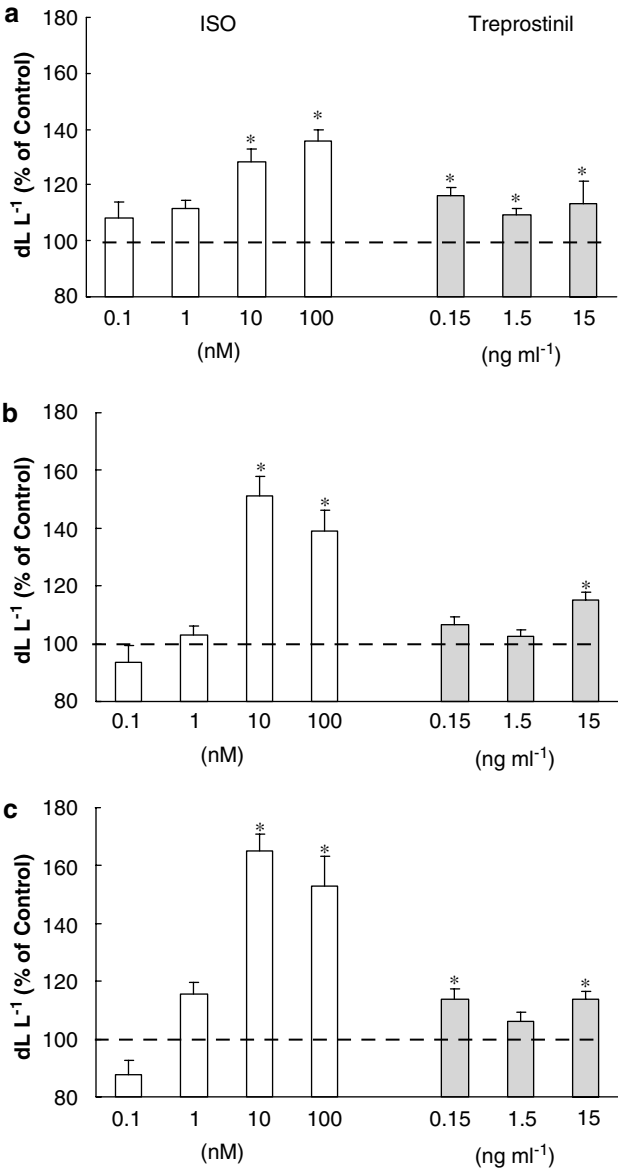

Figure 1 Effect of treprostinil and isoprenaline on cell shortening in cardiomyocytes. Cells were incubated for 5 min with either isoprenaline (ISO) or treprostinil at the indicated concentrations. Cell shortening was analyzed at 0.5 (**a**), 1.0 (**b**) and 2.0 Hz (**c**). Cell shortening is expressed as percent cell shortening of the diastolic cell length and normalized to vehicle treated control cells. Cell shortening of control cells was 10.19 ± 0.32%, 8.82 ± 0.21% and 7.09 ± 0.29% at 0.5, 1, and 2 Hz, respectively. Data are given as means ± s.e.m. from 60 cells. *$P<0.05$ vs untreated controls.

shortening mainly at low beating frequencies. No clear concentration–response relationship was observed (Figure 1). At the clinically relevant concentrations of treprostinil (1.5 ng ml^{-1}; Laliberte *et al*., 2004), there were only very small effects evoked by treprostinil. As indicated in Table 1, isoprenaline increased contraction or relaxation velocities by 53%. However, treprostinil did not increase these parameters. Similar findings hold for TTP and R50 values. These two parameters decreased in the presence of isoprenaline, but not in the presence of treprostinil.

Effect of treprostinil on isoprenaline-dependent effects

In the next set of experiments, we investigated the effect of treprostinil on the isoprenaline-dependent increase in cell shortening. Treprostinil, in a clinically relevant concentration of 1.5 ng ml^{-1}, potentiated the effect of 0.1–10 nM isoprenaline (Figure 2). The effect was significant at all beating frequencies tested but most impressive at higher beating frequencies. Representative cell-shortening tracings are given in Figure 3 as absolute cell shortening (Figure 3a–c) and upon normalization to peak shortening (Figure 3d). In the latter case, the effect of isoprenaline and the combination of isoprenaline and treprostinil on cell-shortening dynamics can be seen. These experiments suggested that treprostinil potentiated the effects of isoprenaline.

We hypothesized that treprostinil improves isoprenaline action not by direct activation of an adenylate cyclase but indirectly by an inhibition of phosphodiesterases (PDE). Therefore, we repeated these experiments in the absence or the presence of IBMX, a non-selective phosphodiesterase inhibitor, and in the presence of rolipram, a PDE IV-specific inhibitor. As expected, IBMX alone increased isoprenaline-induced cell shortening in a similar way to treprostinil, but treprostinil did not further increase cell shortening (Figure 4). Similarly, rolipram alone increased isoprenaline-induced cell shortening in a similar way to treprostinil, but no additive effect was observed in the presence of both drugs (Figure 4).

Finally, we investigated whether the effect of treprostinil on the isoprenaline-dependent increase in cell shortening could be observed in the whole heart. For that reason, a Langendorff preparation was used and the left ventricular function was analyzed. First, the effect of treprostinil alone was recorded. Tresprostinil alone (1.5 ng ml^{-1}) did not change left ventricular developed pressure (LVDP, 70.0±2.0 vs 66.7±0.7 mm Hg, $n=4$, n.s.), dP dt^{-1}_{max} (2982±83 vs 2852±95 mm Hg), or dP dt^{-1}_{min} (1991±40 vs 1917±86 mm Hg). Heart rates were also not altered (236±39 vs 257±25 beats per minute). Left ventricular diastolic pressure remained unchanged (12.3±1.9 vs 11.5±1.3 mm Hg). The effects of isoprenaline and isoprenaline in the presence of treprostinil were studied next. In the presence of treprostinil, the effect of isoprenaline on LVDP, dP dt^{-1}_{max} and dP dt^{-1}_{min} was significantly higher than in the absence of the drug (Figure 5a, c and d). The positive chronotropic effect of isoprenaline,

Table 1 Acute effect of isoprenaline and treprostinil on cell shortening dynamics

	n	*Con-Vel* (μm s^{-1})	*Rel-Vel* (μm s^{-1})	*TTP* (ms)	*R50* (ms)
Control	30	205±9	170±8	102±7	152±12
Isoprenaline (10 nM)	30	291±13*	147±9*	76±5*	118±9*
Control	60	193±12	171±12	82±13	120±17
Treprostinil (1.5 ng ml^{-1})	60	185±7	177±7	80±7	118±9

Data are means±s.e.m. *$P<0.05$ vs the corresponding control. Data are from independent sets of experiments. Cells were paced at 2 Hz.

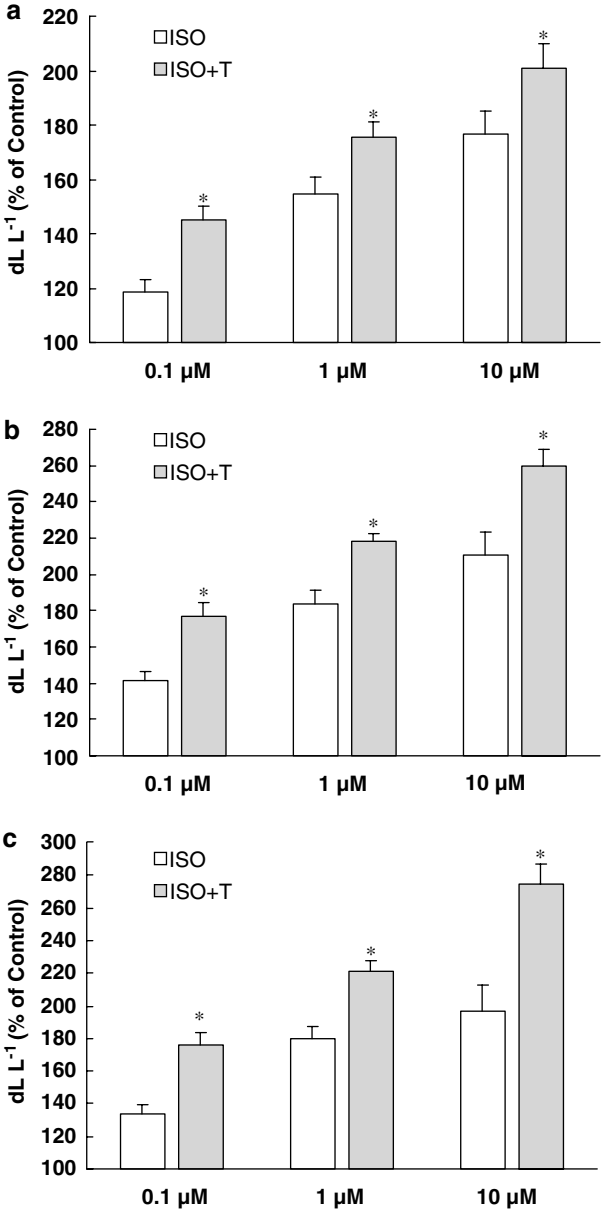

Figure 2 Effect of treprostinil on isoprenaline-dependent cell shortening. Cells were incubated for 5 min with either isoprenaline (ISO) at the indicated concentrations or ISO and treprostinil (T; 1.5 ng ml^{-1}). Cell shortening was analyzed at 0.5 (**a**), 1.0 (**b**) and 2.0 Hz (**c**). Cell shortening is expressed as percent cell shortening of the diastolic cell length and normalized to vehicle treated control cells. Cell shortening of control cells was 7.19±0.18%, 5.82±0.18% and 5.51±0.14% at 0,5, 1 and 2 Hz, respectively. Data are given as means±s.e.m. from 93 cells. *$P<0.05$ vs untreated controls.

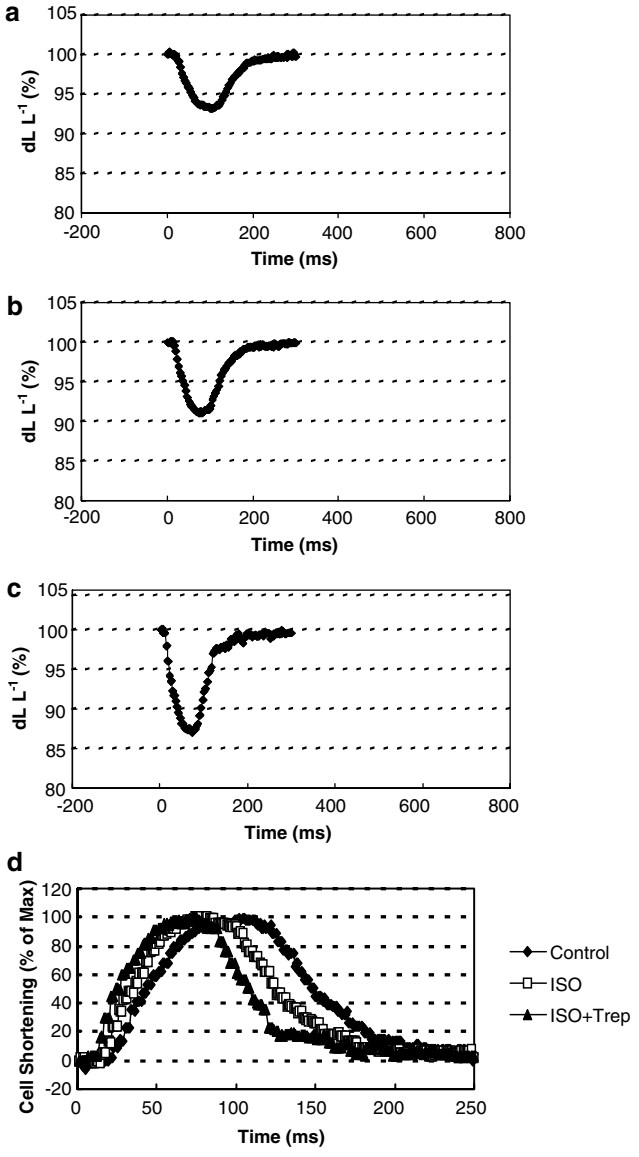

Figure 3 Representative single cell recordings of cardiomyocytes paced at 0.5 Hz normalized to the diastolic cell lengths set as 100%. (**a**) Control; (**b**) Cell incubated in the presence of isoprenaline (10 nM); (**c**) Cell incubated in the presence of isoprenaline (10 nM) and treprostinil (1.5 ng ml^{-1}). (**d**) Comparison of cell shortening shown under (**a–c**) and normalized to the maximal shortening.

however, was not altered (Figure 5b). Left ventricular diastolic pressures did not change in the presence of isoprenaline, irrespective of whether treprostinil was present or not (ISO: -0.9 ± 1.1 mm Hg; ISO + T: -1.6 ± 1.3 mm Hg).

Long-term effects of treprostinil on cardiomyocyte performance
In the last set of experiments, we investigated whether treprostinil changes cardiomyocyte cell shortening when the cells were chronically exposed to the drug. At the clinically relevant concentration (1.5 ng ml^{-1}), treprostinil did not modify cell length (72.5 ± 7.1 vs 70.9 ± 8.3 μm), cell width (18.0 ± 1.1 vs 17.8 ± 2.2 μm), or cell volume ($18\,440\pm797$ vs $17\,634\pm1.315$ μm^3, each $n=40$, n.s.). Treprostinil induced a

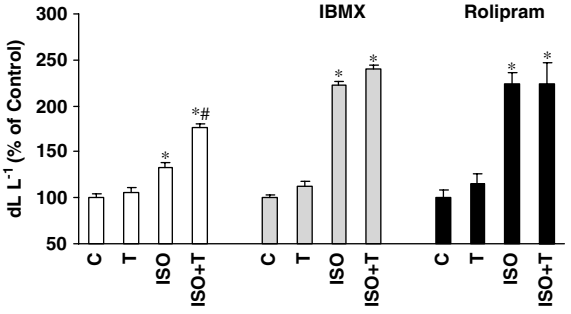

Figure 4 Effect of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) on the potentiating effect of treprostinil on the isoprenaline-dependent cell shortening. Cells were incubated for 5 min with either isoprenaline (ISO, 1 nM), treprostinil (Trep, 1.5 ng ml^{-1}), IBMX (1 mM) and rolipram (1 μM) or in combinations of these drugs as indicated. Cells were paced at 2 Hz. Cell shortening is expressed as percent cell shortening of the diastolic cell length. Cell shortening of control cells was 7.93 ± 0.12% (basal), 6.84 ± 0.17% (IBMX) and 6.86 ± 0.31% (Rolipram). Data are given as means$\pm$s.e.m. from 47 cells $^*P<0.05$ vs control; $^{\#}P<0.05$ vs ISO.

shortening in the duration of individual twitches, as indicated by an increased contraction velocity and reduced TTP and R50 (Table 2). A slight reduction in cell shortening *per se* was observed at the lowest beating frequency (Figure 6). The stimulation to isoprenaline was not altered.

Discussion

Main findings
The present study investigated whether a clinically well-characterized prostacylin analogue has direct effects on the contractility of ventricular cardiomyocytes. We used treprostinil in a concentration range that has been used for treatment of PAH. We applied the prostanoid to freshly isolated cardiac ventricular myocytes to exclude any reflex mechanisms that could have an effect on cardiac contractility. We confirmed the main findings in a Langendorff preparation.

The main finding of this study is that treprostinil, without showing strong effects on contractile responsiveness of its own, strongly potentiated the β-adrenoceptor-dependent cell shortening and increases of contraction and relaxation velocities. These conclusions are based on the following experiments: First, acute addition of treprostinil to cardiomyocytes had a minor effect on the parameters of contractility, when compared to isoprenaline. This became particularly evident at higher beating frequencies that are nearer to the physiological heart rate of rats. Second, in the presence of isoprenaline, treprostinil induced a significant increase in contractility that was much more impressive at higher beating frequencies. Third, in the Langendorff preparation, the presence of treprostinil significantly augmented the isoprenaline-induced increases in left ventricular developed pressure, $dP\,dt^{-1}_{max}$ and $dP\,dt^{-1}_{min}$. Interestingly, it did not augment the positive chronotropic effect of isoprenaline. When cells were exposed to treprostinil for a longer time, no hypertrophic effect was found but shortening dynamics were improved.

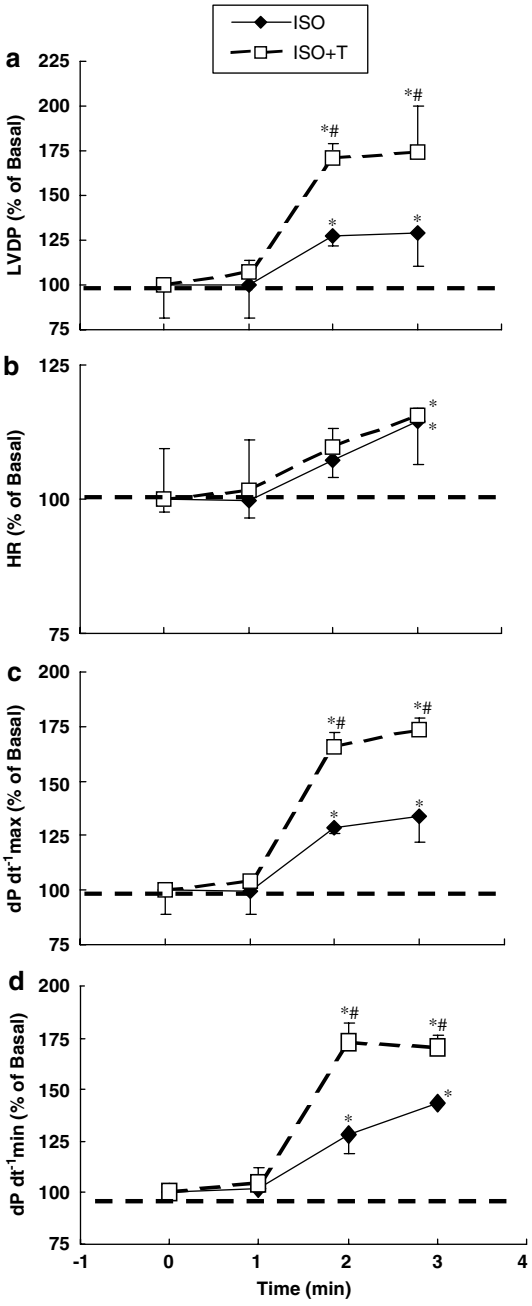

Figure 5 Effect of treprostinil on the left ventricular function of isolated perfused rat hearts. Response to isoprenaline (ISO, 10 nM) and ISO plus treprostinil (T; 1.5 ng ml^{-1}). (**a**) Changes in left ventricular developed pressure (LVDP). Basal LVDP was 96±7 mm Hg. (**b**) Changes in heart rate (HR). Basal heart rate was 257±12. (**c**) Changes in $dP\,dt^{-1}_{max}$. Basal values were 3130±481 mm Hg s^{-1}. (**d**) Changes in $dP\,dt^{-1}_{min}$. Basal values were 2090±164 mm Hg s^{-1}. Data are given as means±s.e.m. from four hearts $^{*}P<0.05$ vs basal; $^{\#}P<0.05$ vs ISO.

Table 2 Chronic effects of treprostinil on cell shortening dynamics

	n	*Con-Vel* (μm s^{-1})	*Rel-Vel* (μm s^{-1})	*TTP* (ms)	*R50* (ms)
Control	60	151±5	142±5	103±3	156±4
Treprostinil (1.5 ng ml^{-1})	60	170±5*	154±7	82±2*	122±3*

Data are means±s.e.m. $^{*}P<0.05$ vs the corresponding control. Data are from independent experiments, in which the cells were incubated with treprostinil for 24 h. Cells were paced at 2 Hz.

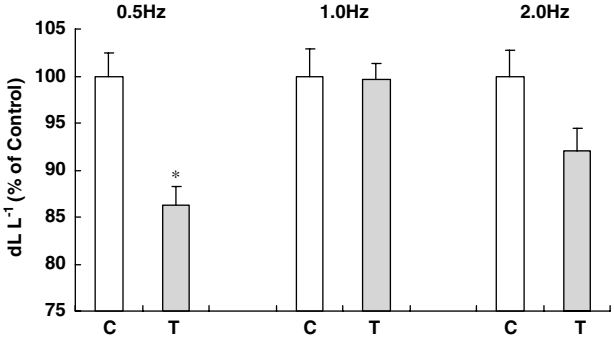

Figure 6 Effect of treprostinil (T, 1.5 ng ml^{-1}) on cell shortening of cardiomyocytes treated with the agonist for 24 h. Cell shortening is expressed as percent cell shortening of the diastolic cell length (dL L^{-1}) and normalized to vehicle treated control cells. Cell shortening of control cells was 12.17±0.41% (0.5 Hz), 9.66±0.33% (1.0 Hz) and 8.57±0.39% (2 Hz). Data are means±s.e.m. from 60 cells. $^{*}P<0.05$ vs control cells.

Cardiomyocyte-directed effects of prostanoids

In the present study we hypothesized that prostanoids may directly influence cardiomyocyte contractility. This hypothesis was based on previous findings that ligands of prostanoid receptors can exert such an effect. The best characterized pathway is probably through the prostaglandin FP receptor, that mediates the effect of prostaglandin $F_{2\alpha}$ on intracellular pH, intracellular calcium, cell shortening and L-type calcium currents in rat cardiomyocytes (Yew *et al.*, 1998). A direct positive hypertrophic effect has also been demonstrated for this type of receptors (Pönicke *et al.*, 2000). We did not find such an effect for treprostinil, suggesting that it does not act via these receptors. Stimulation of cardiac IP receptors, the PGI_2 receptor family, has been shown to prevent cardiomyocyte hypertrophy via cAMP-dependent signaling (Ritchie *et al.*, 2004). Cross talks between classical pro-hypertrophic pathways and cAMP-dependent pathways have been described before (Schäfer *et al.*, 2001). As treprostinil probably acts via these IP receptors, one may not expect a pro-hypertrophic effect of prostanoids and indeed we did not find such an effect. However, the observation period may have been too short to see such an effect.

It is well established that an elevation of cardiomyocyte cAMP levels increases cell shortening, as well as contraction and relaxation velocities. Indeed, isoprenaline known to act via cAMP evoked such effects. However, treprostinil showed little or no effects of its own. This makes it unlikely that treprostinil *per se* increases the cAMP content of cardiomyocytes. It is also in line with these suggestions, that in a previous study, PGI_2 increased the maximal contraction amplitude in rat atria to a lesser extent than $PGF_{2\alpha}$ (Metsa-Ketela, 1981). $PGF_{2\alpha}$ is known to act on adenylate cyclase via FP receptors but these are unlikely involved in the effect of

treprostinil as discussed above. Vice versa, PGI_2 acts on IP receptors, but does not act on adenylate cyclase. Adult rat cardiomyocytes exposed to cicaprost, characterized as a 'pure' IP receptor agonist, nevertheless show some cAMP accumulation. In the presence of IBMX, however, cicaprost only modestly increased cAMP levels (Ritchie *et al*., 2004). Collectively, these data and the newly performed experiments in our study suggest that prostanoids acting on IP receptors are able to increase cAMP levels in cardiomyocytes but not by direct activation of adenylate cyclase. An inhibition of PDE is the more likely effect. All the effects exerted by treprostinil described in this study would fit this conclusion.

Non-specific PDE inhibitors have been shown to improve the activity of aerolized prostacyclin to induce pulmonary vasodilation (Schermuly *et al*., 2001). This may be in contrast to our new finding on cardiomyocytes. However, in the earlier study, the PDE inhibitors did neither specifically nor in general inhibit PDE IV. Therefore, the importance of different PDEs in different tissues may explain the divergent outcome. It should be noted that even in the experiments presented here, the combination of isoprenaline and treprostinil did not evoke an additive effect in regard to heart rate, suggesting that potentiation as shown for cardiomyocytes, does not occur in pacemaker cells.

We demonstrated that treprostinil potentiates the isoprenaline-dependent inotropic effects. The drug acutely improved cell shortening and shortening dynamics of isoprenaline-treated cells. This was particularly impressive at higher, and thus more physiological, beating frequencies. We have previously shown that cell shortening at higher beating frequencies, in comparison to low beating frequencies, depends more on the activity of SERCA2 (Anwar *et al*., 2005). Increased SERCA2 activity increases relaxation velocity, shortens time to 50% relaxation and improves cell shortening. Indeed, isoprenaline activates SERCA2 activity via phosphorylation of phospholamban in a cAMP-dependent way.

Comparison of the in vitro results to the clinical situation
As mentioned in the Introduction, this study was aimed to further study potential mechanisms of the beneficial effects of prostanoids in patients with PAH. Our finding that treprostinil potentiates the cardiac effect of β-adrenoceptor stimulation might explain why patients with markedly impaired right ventricular function profit most from prostanoids. It is well known that heart failure causes neurohumoral activation with increased catecholamine levels (Nootens *et al*., 1995) that leads to adrenoceptor desensitization. The observed prostanoid effect may counterbalance this clinical problem.

Limitations of the study
Basically, this is an experimental study on isolated cardiomyocytes under near-physiological conditions but with apparent modifications. For example, the experiments were performed at room temperature within a frequency range of 0.5–2.0 Hz (corresponding to 30–120 beats per minute) while the physiological heart rate of the donor animals is 260 beats per minute. On the other hand, we have previously shown that no major differences occur at higher beating rates in this set up (Langer *et al*., 2003). It must be pointed out that the main result, that is the effect of treprostinil on isoprenaline-induced cell shortening was confirmed in the Langendorff heart preparation. Under these conditions, hearts were allowed to beat freely at 4–5 Hz and at 37°C. Thus, we do not expect that the differences between the *in vitro* experiments and the *in vivo* situation will lead to faulty conclusions. Nevertheless, the results require further investigation. A main problem not solved in this study is the lack of experimental evidence for the mechanism by which the effects of treprostinil can be explained. Likewise, it is an open question if the same effects would be found in the right ventricle, in human cardiomyocytes and in diseased hearts of patients with PAH.

Conclusion

Our study provides evidence that treprostinil significantly augments the positive inotropic effects of catecholamines on the heart without significant effects of its own. This finding partly explains the beneficial effects of prostanoids in decompensated right heart failure but further studies are needed to elucidate the underlying mechanisms and their relevance to patients with PAH.

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Conflict of interest

The authors state no conflict of interest.

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