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TITLE PAGE

Selective prostacyclin receptor agonist selexipag, in contrast to prostacyclin analogs, does not evoke paradoxical vasoconstriction of rat femoral artery

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RUNNING TITLE PAGE

Running Title: Selexipag and femoral artery

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Abbreviations: PGI_2, prostacyclin; IP receptor, PGI_2 receptor; EP_1 receptor, prostaglandin E receptor 1; EP_3 receptor, prostaglandin E receptor 3; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; QPCR, quantitative polymerase chain reaction; ACT-333679, {4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}acetic acid; selexipag, 2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl)acetamide; DBTSA, (2E)-3-(3',4'-dichlorobiphenyl-2-yl)-N-(2-thienylsulfonyl)acrylamide; SC51322, 8-chloro-2-[3-[(2-furanylmethyl)thio]-1-oxopropyl]hydrazide, dibenz[b,f][1,4]oxazepine-10(11H)-carboxylic acid hydrazide; GR32191B, (4Z)-7-[(1R,2R,3S,5S)-5-([1,1'-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-hetenoic acid.

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ABSTRACT

Selexipag is a selective non-prostanoid prostacyclin (PGI_2) receptor (IP receptor) agonist that is approved for the treatment of pulmonary arterial hypertension (PAH). In contrast to selexipag, PGI_2 analogs used in the clinic are non-selective agonists at prostanoid receptors and can also activate contractile EP_3 receptors. Leg pain is a common side effect in patients receiving treatment with PGI_2 analogs and peripheral vasoconstriction can be responsible for side effects related to muscular ischemia. This study tested the hypothesis that PGI_2 analogs could cause paradoxical vasoconstriction of the femoral artery via EP_3 receptor activation but that only vasorelaxation would be observed in response to selexipag and its active metabolite ACT-333679. Selexipag and ACT-333679 relaxed rings of rat isolated femoral artery contracted with either $PGF_{2\alpha}$ or the α_1AR agonist phenylephrine. ACT-333679 also inhibited contraction of femoral artery to sympathetic nerve stimulation. In contrast, PGI_2 analogs (iloprost, beraprost and treprostinil) caused additional contraction of arterial rings pre-contracted with phenylephrine, which was reverted to relaxation by antagonism of EP_3 receptors. Treprostinil augmented contraction of femoral artery to sympathetic nerve stimulation in an EP_3 receptor-dependent manner. Mechanistically, concomitant EP_3 and α_1AR receptor activation synergistically constricted femoral arteries. It is concluded that selexipag and ACT-333679 are vasorelaxants of the rat femoral artery and, unlike PGI_2 analogs, do not cause paradoxical vasoconstriction via activation of EP_3 receptors. EP_3 receptor-mediated vasoconstriction may contribute to the well-documented peripheral muscle pain reported in PAH patients receiving PGI_2 analogs and leg pain may be less in patients treated with selexipag.

INTRODUCTION

Selexipag (2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl)acetamide) is a selective and orally bioavailable prostacyclin (PGI_2) receptor (IP receptor) agonist (Kuwano et al., 2007) that is approved for the treatment of pulmonary arterial hypertension (PAH). Selexipag lowered the risk of the primary composite end-point of death or a complication related to PAH in newly-treated patients or in patients already treated with one or two other classes of PAH therapies compared to patients who received placebo in the GRIPHON Phase 3 clinical trial (Sitbon et al., 2015). Restoration of IP receptor signaling compensates for the reduced production of PGI_2 in PAH (Christman et al., 1992; Tuder et al., 1999) through mechanisms that include vasodilatation and inhibition of exaggerated vascular smooth muscle cell proliferation (Fetalvero et al., 2007; Smyth et al., 2009). Selexipag and its active metabolite ACT-333679 (previously known as MRE-269) have non-prostanoid structures and possess higher selectivity than PGI_2 analogs for the IP receptor over other prostanoid receptor subtypes in binding and functional cellular assays (Kuwano et al., 2007, Gatfield et al., 2016). In contrast, PGI_2 analogs used in the clinical management of PAH are not selective for the IP receptor and can activate other prostanoid receptor subtypes (Abramovitz et al., 2000; Kuwano et al., 2008; Whittle et al., 2012) as the vasorelaxant efficacy of treprostinil and beraprost, but not selexipag, is reduced via activation of contractile EP_3 receptors in pulmonary artery under conditions associated with PAH (Kuwano et al., 2008; Morrison et al., 2012).

Leg pain is a common side effect in patients receiving treatment with PGI_2 analogs and although the effect may be neuropathic in origin (Pagani-Estévez et al., 2017), an additional vascular component can also be considered. Adrenergic activity is increased in the legs of patients with PAH (Velez-Roa et al., 2004) and potent contractile synergy has been reported between α_1-adrenoceptors and EP_3 receptors in preclinical studies (Hung et al., 2006), a phenomenon which could contribute to the peripheral pain reported with PGI_2 analogs.

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The present study tested the hypothesis that selexipag and ACT-333679, unlike non-selective PGI_2 analogs (iloprost, beraprost and treprostinil), cause only relaxation of the femoral artery without paradoxical EP_3 receptor-mediated vasoconstriction.

METHODS

Animals. Original studies in animals have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and were approved by the local Basel-Landschaft cantonal veterinary office (Switzerland). Twelve-week male Wistar rats were obtained from the Biotechnology and Animal Breeding Division (Harlan, Füllinsdorf, Switzerland). All rats were housed in climate-controlled conditions with a 12 h light/dark cycle and had free access to normal pelleted rat chow and drinking water. In certain experiments, pulmonary hypertension (PH) was induced in rats by a single injection of monocrotaline (MCT; 60 mg/kg, i.p.). Vehicle control rats were treated in parallel. Endothelial function was tested 30 days after injection of MCT (Iglarz et al., 2008).

Rat isolated femoral artery. Following euthanasia, rings of femoral artery were prepared from rats using a standard technique. Briefly, the right and left femoral arteries were isolated. Two arterial rings (1.5 mm) were prepared from each artery, and vessels suspended between 40 μm stainless wires in a Mulvany-Halpern myograph system (10 ml) containing modified Krebs-Henseleit buffer of the following composition (mM): NaCl 115; KCl 4.7; $MgSO_4$ 1.2; KH_2PO4 1.5; $CaCl_2$ 2.5; $NaHCO_3$ 25; glucose 10. Care was taken to avoid damage to the endothelium. Bathing solution was maintained at 37°C and aerated with 95% O_2/ 5% CO_2 (pH=7.4). An initial resting force of 3.9 mN was applied to the vessel (Duckles et al., 1985), and changes in force generation were measured using an isometric force recorder (Multi Wire Myograph System Model 610 M Version 2.2, DMT A/S, Aarhus, Denmark) coupled to a EMKA data acquisition system (EMKA Technologies Inc, Paris, France). Viability of the femoral artery was tested by measuring contraction to KCl (60 mM) and the presence of a functional endothelium confirmed by measuring the ability of acetylcholine (10 μM) to relax arterial rings contracted with 9,11-dideoxy-9α,11α-metha-noepoxy

prostaglandin $F_{2\alpha}$ (U46619; 1 μM). Mean relaxation to acetylcholine was 81.6 ± 1.2 % for all rings tested. In certain experiments, contraction of femoral artery to electrical field stimulation (EFS) was measured. Rings of artery were placed between platinum electrodes and stimulated every 5 minutes (17 V, 0.5 ms pulse width, 10 seconds, 4–24 Hz) using an electrical stimulator (EMKA Technologies Inc). Two frequency-contraction curves were obtained in each vessel: an initial control response followed, after a period of recovery, by a second curve in the presence of drug vehicle or test compound (s). Contraction in the presence of test compound was expressed as a percentage of the maximal contraction in the first control response.

Rat isolated pulmonary artery. Rings of extralobar pulmonary artery (EPA) were prepared from rats using standard techniques. Vessels were suspended between stainless wires in a 10 ml tissue bath set-up and processed in a similar manner to that described for the femoral artery. An initial resting force of 4.9 mN was applied to vessels.

Experimental protocols

Relaxation of pulmonary and femoral artery from control and MCT-PH rats: rings of pulmonary and femoral artery were contracted with phenylephrine (1 μM). When the developed force had stabilized, relaxation to acetylcholine (10 μM) was measured.

Relaxation of femoral artery: rings of femoral artery were contracted with either prostaglandin $F_{2\alpha}$ or phenylephrine (3.5 ± 0.9 μM and 3.0 ± 0.5 μM, respectively) to give matched submaximal contraction relative to KCl (60 mM) (50.8 ± 2.5 % and 50.4 ± 2.7 %, respectively). Cumulative concentration-relaxation curves to selexipag, ACT-333679, iloprost, beraprost or treprostinil were obtained when the developed force had stabilized. The interval between additions of higher concentrations of compounds to

the baths was determined by the time required for the response to reach plateau. In experiments that sought to characterize the identity of the receptor mediating responses to test compounds, rings of femoral artery were exposed to either vehicle or receptor antagonists for 30 minutes prior to obtaining cumulative concentration-response curves to agonists. The choice and concentrations of the following receptor antagonists were based on published data: DBTSA (EP_3 receptor; Gallant et al., 2002, Kuwano et al, 2008); SC51322 (EP_1 receptor; McCormick et al., 2010) and GR32191B (TP receptor; Lumley et al., 1989).

Contraction of femoral artery to electrical field stimulation: frequency-contraction curves (4–24 Hz) were first obtained in the absence or presence of tetrodotoxin (0.1 μM; 10 minutes) and prazosin (0.1 μM; 10 minutes) to establish that the smooth muscle contraction was neuronal in origin and mediated via activation of α_1ARs (Zachaira et al., 2004). Contraction of femoral artery to EFS was abolished by tetrodotoxin (0.1 μM) and prazosin (0.1 μM) (n=3, data not shown).

In separate experiments, the influence of ACT-333679 or treprostinil (both at 10 μM, 20 minutes' incubation) on EFS-induced contraction was measured. DBTSA (1 μM) was added to the bath 20 minutes prior to addition of treprostinil or beraprost.

Contraction of femoral artery to agonists: cumulative concentration-contraction curves to the $EP_{1/3}$ receptor agonist sulprostone were obtained in rings of femoral artery. The ability of a sub-threshold concentration of sulprostone to contract femoral artery was measured following exposure of the artery to phenylephrine (0.1 μM; 10 minutes), and the role of α_1-adrenoceptors and EP_3 receptors in this response was investigated by prior incubation with either prazosin (0.1 μM; 20 minutes) or DBTSA (1 μM; 20 minutes).

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Materials. Selexipag, ACT-333679 and (2E)-3-(3',4'-dichlorobiphenyl-2-yl)-N-(2-thienylsulfonyl)acrylamide (DBTSA) were synthesized by Nippon Shinyaku Co. Ltd (Kyoto, Japan). Iloprost, beraprost, treprostinil, SC51322 and sulprostone were obtained from Cayman Chemical (Ann Arbor, MI, USA). Acetylcholine, GR32191B, L-NAME, phenylephrine, prostaglandin $F_{2\alpha}$ and U46619 were purchased from Sigma (St Louis, MO, USA).

Analyses of results. Relaxation of rat femoral artery to test compounds is expressed as a percentage of the contraction, and contractile responses are expressed as a percentage of the reference contraction to KCl (60 mM). Results are presented as mean ± S.E.M. In some experiments, the S.E.M. values are smaller than the data symbol. n values refer to the number of animals. Best fit analyses of graphs were performed using GraphPad Prism version 7.02 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). pEC_{50} values are defined as the negative logarithm of the concentration of agonist that evokes half maximal response. The effects of receptor antagonists on responses of femoral artery to analogs of PGI_2 were quantified by comparing calculated areas under the agonist concentration-response curves in the absence and presence of antagonists. Calculation of area under the curve is an integrated analytical method for quantifying the response to an agonist over the whole range of concentrations tested (Hermann et al., 2003; Liang et al., 2010; Morrison et al., 2012). Statistical comparisons between control and treated groups were performed using student's paired t test (two-tailed). Differences were considered significant at $p < 0.05$.

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RESULTS

Endothelial function of pulmonary and femoral artery in MCT-induced PH rats.

Relaxation of extralobar pulmonary and femoral artery to acetylcholine was measured using rings precontracted with the selective α_1AR agonist phenylephrine. Acetylcholine (10 μM) relaxed rings of femoral artery from both control and MCT-PH rats, whereas relaxation of pulmonary artery to acetylcholine was significantly less in arterial rings from MCT-PH rats (**table 1**).

Selexipag and ACT-333679 relax femoral artery.

As femoral arteries from MCT-PH rats displayed a normal endothelial function, the remaining experiments were conducted in femoral arteries from healthy Wistar rats. The effects of selexipag and its metabolite ACT-333679 on isometric force development in rat femoral artery were measured using rings pre-contracted with equi-effective concentrations of either $PGF_{2\alpha}$ or the selective α_1AR agonist phenylephrine. Both selexipag (**figure 1A**) and ACT-333679 (**figure 1B**) relaxed femoral artery. No statistically significant difference in relaxation (area under curves) to either selexipag or ACT-333679 was observed in femoral artery contracted with $PGF_{2\alpha}$ or phenylephrine (**figure 1** and **table 2**). EFS (4-24Hz) contracted femoral artery via endogenously-released norepinephrine (**figure 2**). Maximum contraction under control conditions was 91.1 ± 7.6 % relative to KCl (60 mM). ACT-333679 (10 μM) significantly inhibited contraction of femoral artery to by EFS (4–24Hz; areas under curves: control 1234 ± 135.9, ACT-333679 580.8 ± 69.3; $p < 0.05$, $n = 6$; **figure 2**).

PGI_2 analogs constrict femoral artery.

The effects of PGI_2 analogs on the rat femoral artery were compared to that of selexipag and ACT-333679 in rings pre-contracted with equi-effective concentrations of either $PGF_{2\alpha}$ or phenylephrine. Although iloprost, beraprost and treprostinil evoked

concentration-dependent relaxation of femoral artery contracted with $PGF_{2\alpha}$ (**figure 3**), these PGI_2 analogs did not cause vasorelaxation but rather induced further contraction in femoral arterial rings pre-contracted with phenylephrine (**figure 3**). Maximum contraction to iloprost, beraprost and treprostinil was 44.4 ± 15.1 %, 78.4 ± 9.8 % and 34.6 ± 12.1 %, respectively. Differences in areas under curves for responses to iloprost and beraprost (over full range of concentrations tested) were statistically significant ($p < 0.05$, n = 6; **figure 3A** and **B**), whereas areas under curves for responses to treprostinil were significantly different only at concentrations above 1μM ($p < 0.05$, n = 6; **figure 3C**).

Iloprost, beraprost and treprostinil caused weak vasorelaxation of femoral artery contracted with phenylephrine in the presence of the EP_3 receptor antagonist (DBTSA, 1 μM) (**figure 4)**. Relaxation to iloprost, beraprost and treprostinil was 40.8 ± 5.6 %, 51.8 ± 9.3 % and 37.6 ± 7.4 %, respectively. Differences in areas under curves for responses to all PGI_2 analogs tested in the absence and presence of DBTSA were statistically significant ($p < 0.05$, n = 5; **figure 4**). Antagonism of EP_1 (SC51322, 1 μM) and TP (GR32191B, 0.1 μM) receptors did not significantly modulate the reactivity of femoral artery to PGI_2 analogs (**table 3**).

In direct contrast to ACT-333679, treprostinil (10 μM) significantly increased contraction to EFS (maximum contraction: control 94.5 ± 10.2 % versus treprostinil 188.6 ± 9.2 %, $p < 0.05$; n = 4; **figure 5**), and this augmented contraction was significantly reduced by the EP_3 receptor antagonist DBTSA (area under curves: control 1398 ± 162.5, treprostinil 2586 ± 199.6, $p < 0.01$; treprostinil and DBTSA 1512 ± 376.8, $p < 0.05$ versus treprostinil alone; n = 4; **figure 5**). Antagonism of TP receptors with GR32191B (1 μM) did not significantly inhibit the effect of treprostinil on contraction to EFS (area under curves: control 1398 ± 162.5, treprostinil 2470 ± 182.3, $p < 0.01$; treprostinil and GR32191B 1954 ± 374.5, $p > 0.05$ versus treprostinil alone; n= 4).

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α_1ARs and EP_3 receptors act synergistically in femoral artery.

Since reactivity of the femoral artery to PGI_2 analogs was only modulated during α_1AR stimulation, the potential pharmacological interaction between contractile EP_3 receptors and α_1ARs was investigated. The $EP_{1/3}$ receptor agonist sulprostone caused concentration-dependent contraction of rat femoral artery (**figure 6A**; pEC_{50} value 6.4 ± 0.3, E_{max} 140.6 ± 15.6 %). Sulprostone at a concentration that did not by itself cause contraction, (sub-threshold concentration of 1 nM), was able to contract femoral artery in the presence of phenylephrine (0.1 μM) (**figure 6B**). Next, the identity (ies) of the receptor subtype (s) involved in the exaggerated contraction to sulprostone in the presence of phenylephrine was determined. The EP_3 receptor antagonist DBTSA (1 μM) and prazosin (0.1 μM; selective α_1AR antagonist) significantly reduced sulprostone-evoked contraction (**figure 6B**). The selective EP_1 receptor antagonist SC51322 (1 μM) did not inhibit contraction to sulprostone (control, 47.4 ± 10.5 %, treated, 38.5 ± 6.9 %; $P > 0.05$, $n = 4$).

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DISCUSSION

The results of this study demonstrate the functional impact of selectivity of selexipag and its metabolite for the IP receptor over other prostanoid receptors. Relaxation of femoral artery to selexipag and ACT-333679 is not modulated by co-activation of contractile EP_3 receptors, nor is it dependent on the nature of the contractile agent used to raise vascular tone. In contrast, PGI_2 analogs activate EP_3 receptors to contract femoral artery in the presence of phenylephrine, and treprostinil augments contraction to nerve-released norepinephrine.

We established that endothelial function was preserved in femoral, but not pulmonary artery from MCT-PH rats demonstrating the vascular selectivity of this PH model. Further experiments using the femoral artery were therefore performed in the presence of a functional vascular endothelium. The femoral artery was chosen for investigation since its occlusion contributes to leg pain in patients with peripheral artery disease (Beard 2000). This artery is also predominantly used in preclinical models of leg ischemia (Krishna et al., 2016; Queme et al., 2017).

Vasorelaxation of femoral artery to selexipag and ACT-333679 was similar in rings pre-contracted with either $PGF_{2\alpha}$ or the α_1AR agonist phenylephrine. These data are in good agreement with previous findings in the pulmonary artery (Kuwano et al., 2008; Morrison et al., 2012). Reactivity to analogs of PGI_2 was markedly different from that measured in response to selexipag and ACT-333679. PGI_2 analogs relaxed femoral artery pre-contracted with $PGF_{2\alpha}$, but caused further contraction of femoral artery pre-contracted with phenylephrine. This augmented contraction to PGI_2 analogs might be caused by activation of contractile EP_3 receptors since antagonism of EP_3 receptors revealed modest relaxation to all PGI_2 analogs tested. The contraction of femoral artery to PGI_2 analogs measured during α_1AR activation contrasted with the weak relaxation observed under the same conditions in pulmonary artery (Morrison et al., 2012). These

data suggest an important synergy between EP_3 receptors and the adrenergic system in the femoral artery.

Differential effects of ACT-333679 and analogs of PGI_2 were also observed following transmural sympathetic nerve stimulation. ACT-333679, at a concentration that evoked maximal relaxation of the femoral artery, inhibited arterial contraction to EFS. This inhibitory effect of ACT-333679 is considered to be mediated via post-synaptic IP receptors in a manner similar to that observed for PGI_2 in rabbit mesenteric artery (Armstrong et al., 1979). The same concentration of treprostinil, however, significantly augmented contraction to EFS in an EP_3 receptor-dependent manner. Sensitivity of EFS-induced contraction to tetrodotoxin and prazosin confirmed the nerve origin and critical involvement of α_1ARs in this response (Zacharia et al., 2004). Thus, the ability of treprostinil to augment contraction of femoral artery to endogenously-released norepinephrine is consistent with post-synaptic interplay between α_1ARs and EP_3 receptors.

Marked contractile synergy between EP_3 receptors and α_1ARs has been described in the rat femoral artery (Hung et al., 2006). This artery receives a dense sympathetic innervation and possess a high content of norepinephrine (Todd 1980; Duckles et al., 1985; Stassen et al., 1998). Thus, the femoral artery is suitable for study of the potential pharmacological interplay between EP_3 receptors and α_1ARs and its effect on vascular responsiveness to selexipag and analogs of PGI_2. Synergy between α_1ARs and EP_3 receptors in femoral artery was further supported by the observations that a sub-threshold concentration of the $EP_{1/3}$ receptor agonist sulprostone evoked significant contraction of femoral artery only in the presence of phenylephrine. Activation of both EP_3 and α_1ARs receptors was required since contraction to sulprostone was abolished by either DBTSA or prazosin. The contractile synergy between femoral EP_3 receptors and α_1ARs described here and by others (Hung et al., 2006) may contribute to the well-documented peripheral muscle pain (myalgia)

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reported in patients with PAH receiving treatment with PGI_2 analogs (Tapson et al., 2012, 2013, Pagani-Estévez et al., 2017). Involvement of other lower limb arteries which are under adrenergic control, e.g. popliteal artery (Sada et al., 1985) cannot be excluded. Although the development of pain is considered to arise from IP and EP_3 receptor-dependent sensitization of sensory afferent neurons (Nakae et al., 2005, Southall and Vasko, 2001), our data support an additional vascular mechanism. Leg ischemia is commonly associated with pain in the calf and thigh muscles while walking due to restriction of blood flow through the femoral artery (Beard 2000). In addition, reduced blood flow and tissue oxygenation, as occur following exaggerated vasoconstriction, promote the production of metabolism-derived pain mediators (Queme et al., 2017).

The high selectivity of selexipag and its metabolite for the prostacyclin IP receptor precludes EP_3 receptor-mediated vasoconstriction and sensitization of afferent neurons, which might translate into improved tolerability over PGI_2 analogs in patients with PAH. In conclusion, differences in the pharmacology of the selective prostacyclin IP receptor agonists selexipag and ACT-333679, and non-selective analogs of PGI_2 are described in rat femoral artery. Selexipag and ACT-333679 relax femoral artery, whereas EP_3 receptor-mediated contraction to PGI_2 analogs was exacerbated during α_1AR stimulation.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: K.M., M.I., M.C.

Conducted experiments: R.E., F.H.

Performed data analysis: K.M., R.E., F.H.

Wrote or contributed to the writing of the manuscript: K.M., M.I., M.C.

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LEGENDS FOR FIGURES

Figure 1 Relaxation to selexipag (A) and ACT-333679 (B) in rat femoral artery contracted with equi-effective concentrations of either $PGF_{2\alpha}$ or phenylephrine; n = 6/group.

Figure 2 Effect of ACT-333679 (10 μM) on contraction of rat femoral artery to EFS; * $p < 0.05$, ** $p < 0.01$; n = 6/group.

Figure 3 Responses to iloprost (A), beraprost (B) and treprostinil (C) in rat femoral artery contracted with equi-effective concentrations of either $PGF_{2\alpha}$ or phenylephrine; * $p < 0.05$ for comparison of area under curves at concentrations above 1 μM, ** $p < 0.01$ for comparison of area under curves over full range of concentrations tested; n = 6/group.

Figure 4 Effects of the EP_3 receptor antagonist DBTSA (1 μM) on responses of rat femoral artery to iloprost (A), beraprost (B) and treprostinil (C). Rings of artery were contracted with phenylephrine; * $p < 0.05$ for comparison of area under curves; n = 5/group.

Figure 5 Effect of treprostinil (10 μM) on contraction of rat femoral artery to EFS in the absence and presence of the EP_3 receptor antagonist DBTSA; ** $p < 0.01$, *** $p < 0.001$; n = 6/group.

Figure 6 Contraction of rat femoral artery to sulprostone in the absence or presence of phenylephrine. Sulprostone causes concentration-dependent contraction of femoral artery (A). A subthreshold concentration of sulprostone (1 nM) contracts femoral artery in presence of phenylephrine (0.1 μM) (B). DBTSA (1 μM; B) and

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prazosin (0.1 μM; B) inhibit contraction to sulprostone in the presence of phenylephrine; * $p < 0.05$, ** $p < 0.01$; n = 6/group.

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TABLES

Table 1. Relaxation to acetylcholine (10 μM) in pulmonary and femoral artery from control and MCT-PH rats.

	pulmonary artery	femoral artery
Control	81.2 ± 4.2 %	82.9 ± 3.4 %
MCT-PH	25.3 ± 6.9 %****	90.7 ± 2.3 %

**** $p < 0.0001$ compared to control pulmonary artery

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Table 2. Relaxation of femoral artery to selexipag and ACT-333679.

	selexipag	ACT-333679
pEC_{50}		
$PGF_{2\alpha}$	5.4 ± 0.1	5.5 ± 0.1
phenylephrine	5.5 ± 0.1	5.6 ± 0.1
E_{max}		
$PGF_{2\alpha}$	113.3 ± 5.4	126.9 ± 7.0
phenylephrine	116.6 ± 6.6	121.0 ± 6.6

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Table 3. Effect of SC51322 and GR32191B on responses of femoral artery to PGI_2 analogs (area under curve).

	iloprost	beraprost	treprostinil
Control	684.7 ± 50.7	742.5 ± 44.2	822.5 ± 47.7
SC51322	704.1 ± 44.5	769.1 ± 25.6	841.0 ± 43.7
Control	752.4 ± 21.2	729.4 ± 17.3	870.0 ± 27.0
GR32191B	724.6 ± 28.3	756.7 ± 14.0	857.2 ± 12.5

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Figure 1.

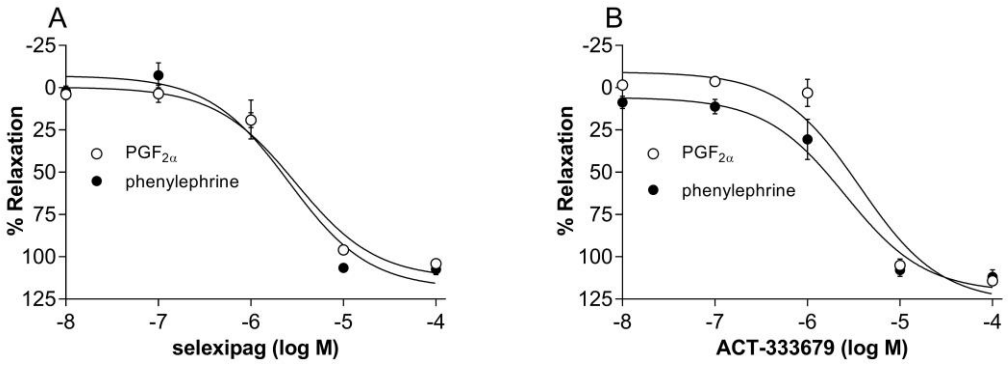

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Figure 2.

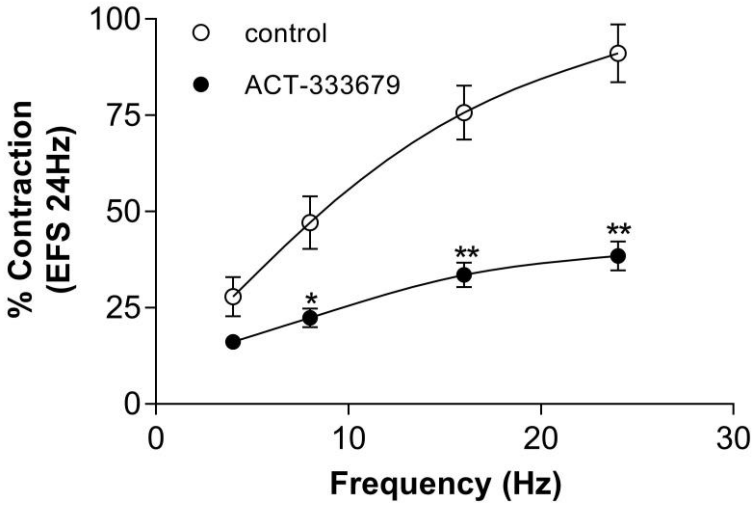

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Figure 3.

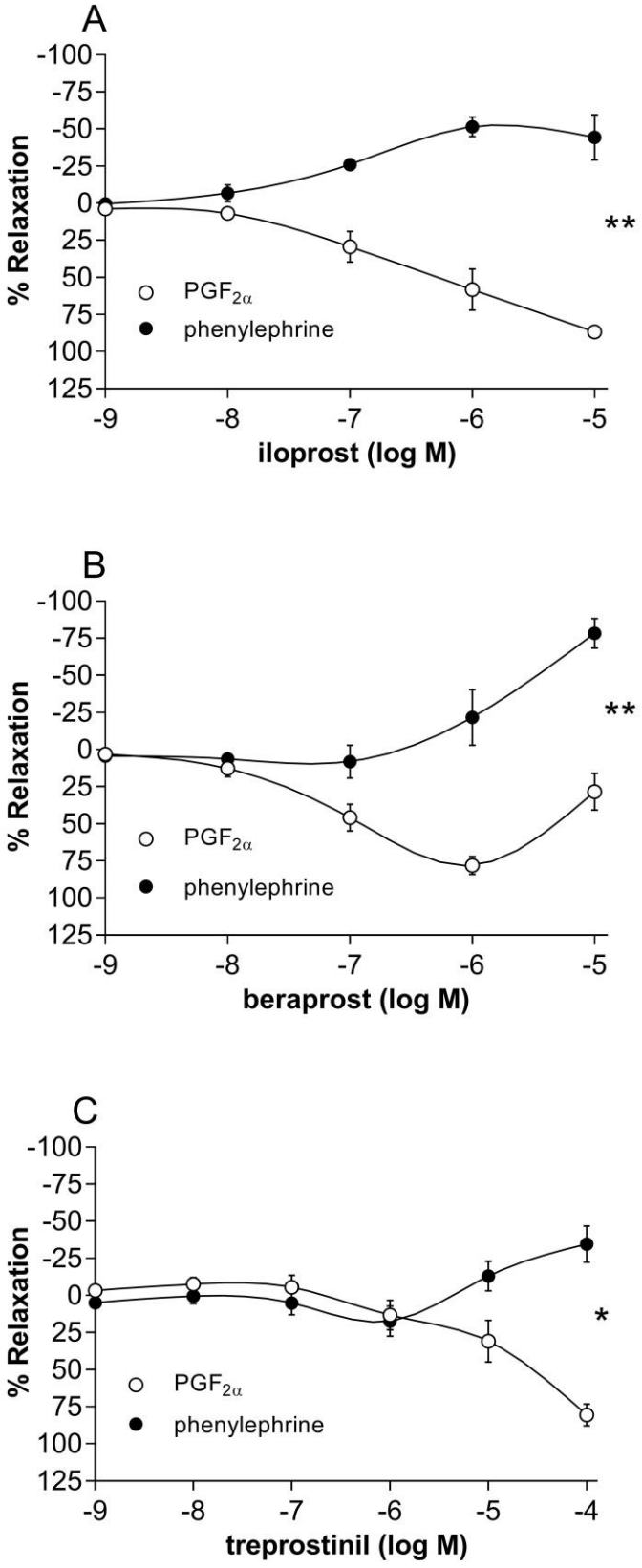

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Figure 4.

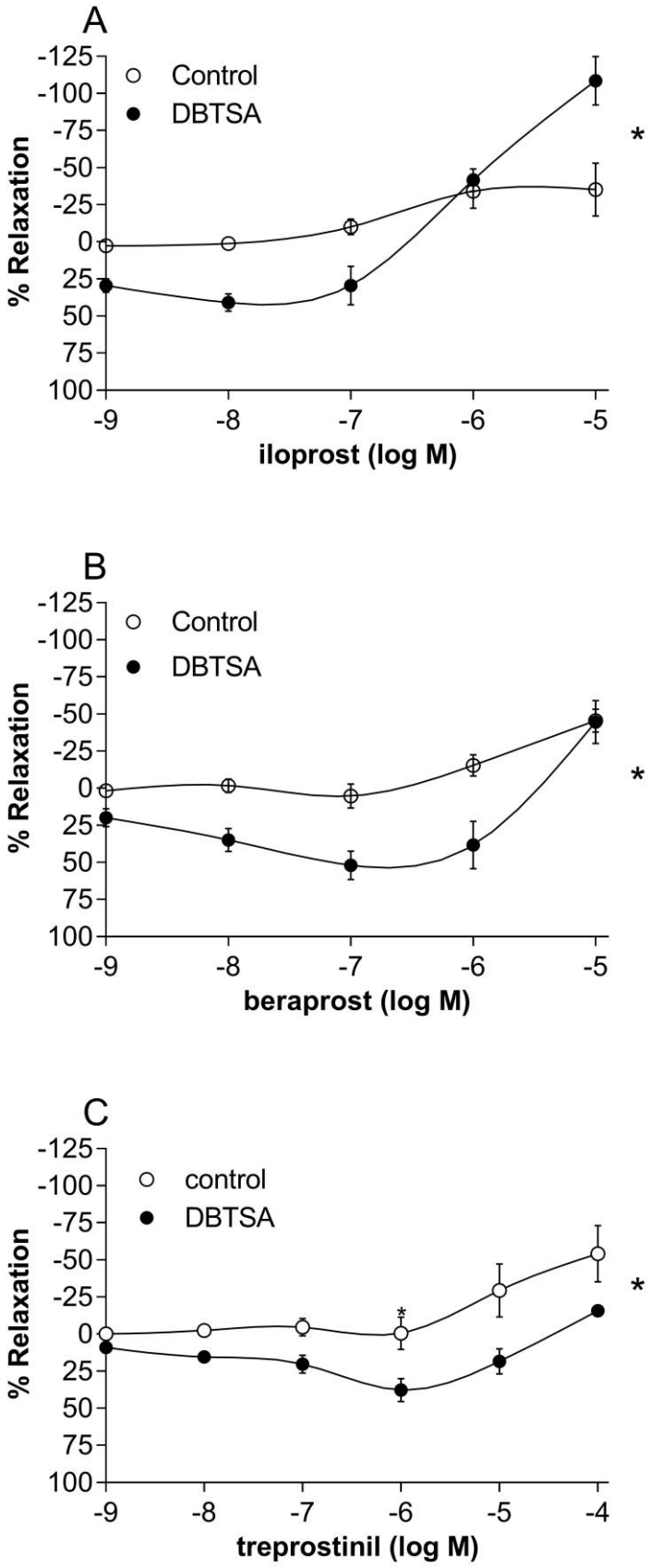

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Figure 5.

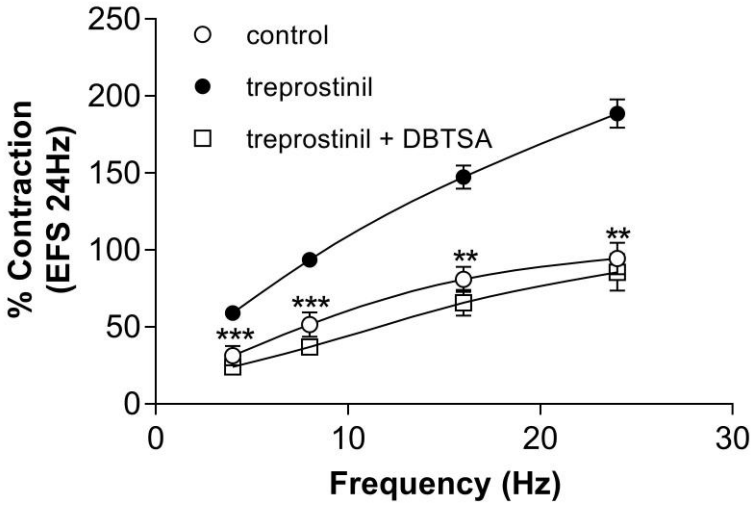

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Figure 6.