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Differential actions of the prostacyclin analogues treprostinil and iloprost and the selexipag metabolite, MRE-269 (ACT-333679) in rat small pulmonary arteries and veins

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ABSTRACT

The prostacyclin (IP) receptor agonists, treprostinil, iloprost and the selexipag metabolite, MRE-269 (ACT-333679) were evaluated in rat distal pulmonary blood vessels. Small pulmonary arteries and veins were pre-contracted with the thromboxane mimetic, U46619 (25 and 100 nM, respectively), and relaxation determined with and without IP receptor antagonists, RO1138452 and RO3244794. In arteries, treprostinil was a more potent vasorelaxant than iloprost, while the efficacy of iloprost was greater. In pulmonary arteries, treprostinil-induced relaxation was essentially abolished by both IP antagonists (1 μM), while responses to iloprost were partially inhibited. Both treprostinil and iloprost were equipotent, prominently relaxing pulmonary veins with responses being similarly and partially sensitive to IP antagonists. In contrast, RO1138452 failed to inhibit relaxations to MRE-269 in either pulmonary arteries or veins, suggesting no involvement of typical IP receptors. Thus, rat pulmonary tissues cannot be considered appropriate to assess classical IP receptors using the proposed highly selective non-prostanoid agonist MRE-269, contrasting with the IP receptor agonism profile of prostacyclin analogues, iloprost and treprostinil.

1. Introduction

The pulmonary pharmacology of prostacyclin remains an area of growing interest because of the use of prostacyclin (epoprostenol) and its stable mimetics in the treatment of pulmonary arterial hypertension (PAH). Two such prostanoid compounds, iloprost and treprostinil which can activate the prostacyclin (IP) receptor, are extensively used in the treatment of this disease [1–3]. Another IP agonist, selexipag, that is chemically not a prostanoid, is in late stage clinical trials [4].

Differences in the efficacy of these agents at IP and other prostanoid receptor types have prompted the need to identify clearly the specific target receptors and mechanisms of action. The multitude of targets for prostacyclin analogues are now known to include prostanoid EP_1, EP_2, EP_3 EP_4, DP_1 FP and TP receptors as well as the IP receptor [5–7]. Furthermore, the importance of signalling through a family of transcription factors called peroxisome proliferator-activated receptors (PPARs) is now widely recognised for both prostacyclin and its stable analogues [8,9]. For selexipag and its active metabolite, MRE-269 (ACT-333679), the IP receptor is considered to be the only significant target [10–12].

The IP, EP_2, EP_4 and DP_1 receptors are vasorelaxant receptors coupled to G_s and therefore elevate intracellular cyclic AMP levels while EP_1, EP_3, FP and TP are contractile receptors coupled to G_i and G_q that either elevate intracellular Ca^{2+} or reduce cyclic AMP [6]. From studies utilising pharmacological agonists and antagonists, it is known that the prostanoid receptors involved in the relaxation of human pulmonary venous preparations in vitro are the IP and DP_1 receptors, and to a lesser extent the EP_4 receptor [13,14]. In human pulmonary artery preparations however, the IP receptor appears to be the key receptor involved in relaxation [13,15]. Rat pulmonary arteries have been reported to express all prostanoid receptors at the message level [12,16], and of those studied so far, IP, EP_3, TP and FP receptors appear to regulate vascular tone [12,17]. Nothing is known about prostanoid receptor expression in rat pulmonary veins.

A recent study has found substantial differences between iloprost and treprostinil with respect to both prostanoid receptor

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binding and biochemical functional activity as a consequence of prostanoid receptor activation [7]. In agreement with previous findings [18], iloprost was shown to have high affinity binding for human EP_1 and IP receptors (K_i 1.1 and 3.9 nM, respectively), lower for FP, EP_3 and EP_4 and very low affinity for DP_1, EP_2 and TP receptors [7]. Likewise, similar binding affinities are described for iloprost against rat EP receptor subtypes [19]. In contrast to iloprost, treprostinil had strong affinity for human DP_1, EP_2 and IP receptor (K_i 4.4, 3.6 and 32 nM, respectively), low affinity for EP_1 and EP_4 receptors and even lower affinity for EP_3, FP and TP receptors. Thus, in addition to their vasodilator IP receptor agonist activity, the preferential binding of iloprost to the vasoconstrictor EP_1 receptor, which contrasts with the binding of treprostinil to the vasodilator DP_1 and EP_2 receptors, is a strong basis for possible differences in their pharmacological profiles [7]. Moreover, using selective prostanoid receptor antagonists, very recent findings using human pulmonary veins suggest roles for both the IP and DP_1 receptor in the vasorelaxant responses to treprostinil [20] (this issue of POLM).

There is as yet, no comparative functional evaluation of the pharmacology of these prostanoid IP receptor agonists in both small pulmonary arteries and small veins, particularly from the rat, the vessels which are of prime importance in the overall determination of pulmonary vascular resistance. Hence in the present study using pressurised small arteries (~0.3 mm luminal diameter) and small veins (~0.5 mm luminal diameter), the vasorelaxant profiles of iloprost and treprostinil in vitro in these pulmonary vessels from the rat has been evaluated and in addition, have been compared with the activity of MRE- 269 in these pulmonary tissues.

Moreover, it is very intriguing that despite the reporting of functional studies on the effects of selexipag and MRE-269 on the rat pulmonary artery [12,17] and on rat platelets [10], no study has yet evaluated the effect of selective IP antagonists on these putative IP responses in the pulmonary vasculature from rat or indeed, the vasculature from any species. Thus the involvement of IP receptor-dependent pathways in mediating the pulmonary relaxant effects of the IP agonists, treprostinil, iloprost and MRE-269, using two selective, but chemically distinct IP receptor antagonists, RO1138452 and RO3244794 [21] has been evaluated in rat small pulmonary arteries and veins.

2. Materials and methods

2.1. Vessel preparation

Animals used in this study were cared for in the central animal facility at University College London as approved by the UK home office department. All experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male (180–250 g) or female Sprague-Dawley rats (250–300 g) were killed by stunning and cervical dislocation. Lungs were removed from and placed in physiological salt solution (PSS) containing (in mM): 112 NaCl, 5, KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 0.5 KH_2PO_3, 0.5 NaH_2PO_3, and 10 glucose (gassed with 95% O_2/5% CO_2 to pH 7.4). Third order pulmonary arteries and veins were cleaned of connective tissue and cut into segments (2 mm long) and mounted on wires in an isometric myograph (500A, JP Trading, Denmark).

2.2. Small vessel myography

Vessels were continuously aerated at 37 °C in PSS and tensioned to an equivalent of ~22 mmHg (3 K Pascal). The normalised luminal diameter of each pressurised segment was obtained as described previously [22] and averaged 339 μm in arteries and 502 μm in veins. An equilibration period of at least 1 h was allowed during which time tissues were contracted with a single application of KCl (90 mM) followed by 2–3 washes. Subsequent to this, blood vessels were contracted with the thromboxane A_2 mimetic U46619, which produced contractions in small veins that were approximately a third less in size (0.85 ± 0.11 mN; n = 52) to arteries contracted with the same dose (100 nM). Thus, in an attempt to assess the effects of the IP agonists against comparable contraction size, arteries were contracted with 25 nM and veins with 100 nM U46619. In some experiments though, arteries and veins were contracted with the same concentration of U46619 (see Fig. 4). It should be noted that distal pulmonary veins did not contract to either phenylephrine (PE; 10 μM) or norepinephrine (10 μM) and barely to serotonin (5-HT; 10 μM). This contrasts to large pulmonary vessels which significantly contract to all these agonists both in veins and arteries [13,23]. Small pulmonary arteries did however contract to PE, but these responses were significantly smaller (P < 0.001, unpaired t-test) compared with U46619, such that responses to 1 μM PE averaged 25% (0.71 ± 0.15 mN; n = 14) of the contractile size to 25 nM U46619 (2.86 ± 0.39 mN; n = 14). For these reasons, the current series of studies on rat small pulmonary vessels were conducted solely using U46619 as a contractile agent. The presence of functional endothelium was assessed by examining responses to the endothelium-dependent vasorelaxant acetylcholine, which at 10 μM gave an average relaxation of contractions induced by U46619 of 55% in pulmonary artery and a 49% in pulmonary vein.

2.3. Experimental protocol

For the majority of experiments 4 vessels were mounted (2 arteries and 2 veins) each day and effects of IP receptor agonists compared in separate vessels from the same animal. Contractions were allowed to plateau, before cumulative concentration–response curves (1–30,000 nM) were constructed for iloprost, treprostinil and MRE-269. In all experiments, prostanoid receptor antagonists were added at least 30 min before the addition of the contractile agonist, U46619. Changes in tone were expressed as the percentage of contractile response induced by U46619 just before the addition of the lowest concentration of the vasorelaxant agonist. Where possible, two concentration–response curves were obtained in the same preparation separated by a washout period of 30–60 min. With this protocol, there was no apparent time-dependent change in the response to any of the IP receptor agonists.

2.4. Data and statistical analysis

Data are presented as mean ± standard error of mean (SEM) of n observations (4–6). Agonist log–concentration curves were constructed and fitted using the sigmoidal fitting routine in Prism 4 (GraphPad Software, La Jolla, CA, USA), where the slope was set to 1. The maximal response (E_{max}) and the concentration of agonist causing 50% of E_{max} (expressed as the mean pEC_{50} value) were extrapolated from the sigmoidal regression curve. Statistical analysis was performed using a non-paired Student t-test or ANOVA with post hoc correction. Values of P < 0.05 were considered statistically significant.

2.5. Drugs

U46619 (Cat No. BML-PG023) was purchased from Enzo life Sciences (Exeter, UK). Treprostinil powder was provided from United Therapeutics. Iloprost (50:50 R/S isomer) and MRE-269 (Cat No. 10010412) were both purchased from Cayman Chemical Company (Ann Arbour, Michigan, USA). The IP antagonists,