Evidence that inward rectifier K+ channels mediate relaxation by the PGI2 receptor agonist cicaprost via a cyclic AMP-independent mechanism

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Abstract

Objective: We investigated the role of the inward rectifier potassium (KIR) channel and the cyclic AMP-dependent pathway in mediating vasorelaxation induced by the prostacyclin analogue cicaprost.

Methods: Small vessel myography was used to assess responses to cicaprost in segments of rat tail artery contracted with phenylephrine. Microelectrode recordings were made from helical strips to assess effects on membrane potential.

Results: Cicaprost caused relaxation and hyperpolarisation that were significantly inhibited by Ba2+ (30–100 mM), a known blocker of KIR channels. Raising extracellular K+ from 5 to 15 mM elicited membrane hyperpolarisation and an endothelium-independent relaxation that was blocked by Ba2+ (30–100 mM), suggesting the existence of functional KIR channels on the smooth muscle. In contrast, neither glibenclamide (10 μM), a blocker of ATP-sensitive K+ channels, nor fluoxetine hydrochloride (100 μM), a blocker of G-protein-gated inward rectifier K+ channels, nor pertussis toxin (PTX; 1 μg/ml), which irreversibly inhibits Gt/Ga, reduced relaxation to cicaprost. Indeed, PTX significantly potentiated responses. Relaxation to cicaprost was not mediated by NO but was partially endothelium-dependent, consistent with a similar inhibition by a combination of charybdotoxin (0.1 μM) and apamin (0.5 μM), blockers of endothelium-derived hyperpolarising factor (EDHF).

Conclusion: We conclude that cicaprost relaxes rat tail artery by activating KIR channels with some involvement from EDHF. The mechanism appears to be largely independent of cyclic AMP and Gi/Go, although the latter appears to counteract relaxation through an unknown pathway and/or receptor.

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Keywords: Prostacyclin analogue; Vascular smooth muscle; Inward rectifier potassium channels; Relaxation; EDHF

1. Introduction

Prostacyclin (PGI2), formed within the vascular endothelium and smooth muscle by the cyclooxygenase (COX) pathway, is a potent inhibitor of vascular tone and platelet aggregation [1]. Due to its short biological half-life and poor selectivity for the PGI2 receptor (IP receptor), research has focussed on the chemically stable PGI2 analogues, which have improved selectivity and potency. Cicaprost is one such analogue that exhibits high affinity binding (Kd ~10–20 nM) for both the mouse and human IP receptor [2]. Studies in mice lacking this receptor provide good evidence that the IP receptor is responsible for the relaxant and hypotensive effects of cicaprost [3] and for cAMP elevation in cultured aortic cells [4]. In species other than mouse, cicaprost has significant agonist activity at the prostaglandin E2 (PGE2) receptor subtype, EP4 [2,5], which couples to Gs. This receptor makes a variable contribution to relaxation induced by cicaprost in pig, rat, rabbit and guinea-pig arteries [6,7].
It is readily assumed that cyclic AMP (cAMP) mediates the major biological activities of PGI2 and its analogues since these agents readily increase cAMP in many different smooth muscle cell types [8–11] and relaxation is commonly potentiated by agents preventing the breakdown of cAMP [12]. In contrast, data from this and other laboratories suggest that cAMP-independent mechanisms underlie a substantial portion of the relaxation induced by the PGI2 analogues, iloprost and beraprost in guinea-pig aorta [12,13]. In addition, cloned IP receptors can couple to multiple G-protein pathways, including Gq and possibly Gi [1,2] suggesting other second messengers could mediate the action of IP agonists. However, examination of the specific role of the IP receptor pathway has been hampered by the lack of selective IP antagonists.

A large body of evidence suggest that PGI2 analogues inhibit vascular tone through the activation of K+ channels located on the smooth muscle membrane [12]. Depending on the vascular bed, both ATP-sensitive (KATP) and large conductance Ca2+-activated (BKCa) K+ channels have been implicated in relaxation induced by iloprost [13–16] and beraprost [10], presumed to occur in part through protein kinase A (PKA)-mediated phosphorylation [12,16]. In contrast, little is known about the downstream mechanism whereby cicaprost induces relaxation, the most selective IP receptor agonist available. Equally unexplored is the involvement of strong, inwardly rectifying potassium (Kir) channels, which are encoded by the Kir2.0 subfamily. These channels are expressed in arterial smooth muscle, where they contribute to the resting membrane potential [17,18] and are responsible for membrane hyperpolarisation and blood vessel dilation induced by small increases in extracellular K+ [17,19,20]. In some vessels Kir channels are the target for endothelium-derived hyperpolarising factor (EDHF), where K+ liberated via endothelial Ca2+-sensitive K+ channels is thought to activate these channels within the smooth muscle layer [21]. Thus we tested the hypothesis that the IP agonist, cicaprost, relaxes rat tail artery by activating Kir channels, which are known to be expressed in this vessel [22]. Experiments were also designed to investigate the role of the cyclic AMP pathway.

2. Methods

2.1. Tissue preparation

Animals used in this study were cared for in the central animal facility at UCL as approved by the home office. Male Sprague–Dawley rats (180–250 g) were killed by stunning and cervical dislocation. 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). The anterior tail artery was removed and placed in physiological salt solution (PSS) containing (in mM): NaCl 112, KCl 5, CaCl2 1.8, MgCl2 1, NaHCO3 25, KH2PO4 0.5, NaH2PO4 0.5, and glucose 10 and gassed with 95% O2/5% CO2 (pH 7.4). The distal 1/3 of the artery was cleaned and cut into segments (~2 mm long), which were mounted on wires in an isometric myograph (500A JP Trading, Denmark). Vessels were continuously aerated at 37 °C in PSS and pre-tensioned to an equivalent of 100 mmHg. The normalized luminal diameter of segments was obtained as described previously [23] and averaged 687±9 μm (n=96). An equilibration period of at least 1 h was allowed during which time tissues were contracted with phenylephrine (10 μM) and KCl (120 mM) until responses were reproducible. The presence of functional endothelium was assessed using acetylcholine (1 μM) and only segments giving >60% relaxation of phenylephrine-induced contractions (1 μM) were used. Endothelium removal was achieved by rubbing a hair over the luminal surface of the vessel and confirmed by lack of response to acetylcholine.

2.2. Protocol for tension studies

Following equilibration, arteries were precontracted with 1 μM phenylephrine, a concentration producing 70–80% of the maximum response to this agonist. Upon generation of a stable baseline, cumulative concentration–response curves for cicaprost (1–10000 nM) were constructed and relaxation was expressed as percentage of the contraction elicited just before the addition of the lowest dose of cicaprost. Where possible, two dose–response curves to cicaprost were obtained in the same preparation separated by a wash-out period of 30–60 min. Using this protocol, there was no apparent time-dependent change in the response to cicaprost.

2.3. Effect of K+ channel inhibitors

To determine the role of different types of K+ channels, concentration–response curves were constructed for cicaprost in the absence and presence of either BaCl2 (10–100 μM), glibenclamide (10 μM), fluoxetine hydrochloride (100 μM) or a combination of apamin (500 nM) and charybdotoxin (100 nM). Inhibitors were added either prior to phenylephrine (apamin and charybdotoxin) or after contractions had stabilised, in which case inhibitors were left for 15 min before adding cicaprost. The effects of glibenclamide were also assessed against relaxations to KATP channel opener, levocromakalim (1–10,000 nM).

2.4. Functional test for Kir channel expression

In tissues already contracted with phenylephrine, the KCl concentration was increased from 5 to 15 mM and responses were assessed in the absence and presence of BaCl2 (30 or 100 μM). Concentration–response curves were also con-
2.6. Effect of uncoupling G<sub>i</sub>/G<sub>o</sub>

Tail arterial segments were incubated for 18 h at 37 °C in sterile Dulbecco’s Modified Eagle’s Medium (DMEM) containing 1 μg/ml pertussis toxin (PTX), an agent which irreversibly inhibits G<sub>i</sub>/G<sub>o</sub> by ADP-ribosylation [24]. Control tissues from the same animals were incubated in DMEM without PTX. At the end of the incubation period, tissues were washed 3 times with PSS before concentration–response curves were constructed for cicaprost.

2.7. Membrane potential studies

Membrane potential in helical arterial strips of rat tail artery was recorded as described previously [25]. Strips were pre-tensioned to an equivalent of 1 g and perfused continuously with PSS at 2.5 ml/min. Sharp microelectrodes, which had resistance 60–100 MΩ when filled with 3 M KCl, were used to impale smooth muscle cells. Measurements were made with a high-impedance pre-amplifier (Biologic) and data were recorded on an oscilloscope (DSO 420, Gould Nicolet, Essex, UK) and chart recorder (Easygraf TA 240S, Gould Nicolet, Essex, UK). The abrupt drop in voltage followed by a sharp return to baseline on exit was deemed a successful impalement. The effect of cicaprost (1 μM) or 15 mM K<sup>+</sup> was examined in the absence of contractile agonists. In some experiments, a second application of cicaprost was examined in the presence of Ba<sup>2+</sup> (30 μM) following a 30-min washout period.

2.8. Drugs/chemicals

Cicaprost was a gift from Schering AG (Berlin, Germany) and levocromakalim was kindly donated by Smithkline Beecham (Harlow, UK). Phenylephrine, glibenclamide, acetylcholine and BaCl<sub>2</sub> were all purchased from Sigma (Poole, Dorset, UK). PTX and forskolin were obtained from Calbiochem-Novachem (Beeston, Nottingham, UK). SQ22536 and DDA were purchased from Alexis Corporation (Bingham, Nottingham, UK). Rp-cAMPs and its inactive analogue were purchased from BIOLOG Life Sciences Institute (Bremen, Germany) and fluoxetine was from Tocris Cookson Ltd (Avonmouth, UK). Levocromakalim and forskolin were dissolved in 100% dimethylsulphoxide and glibenclamide was dissolved in 50% v/v dimethylsulphoxide/polyethylene glycol; these were stored as 10 mM stock solutions at 4 °C. Cicaprost was stored at 4 °C at a concentration of 0.5 mg/ml in a buffer containing (in mM): 9.9 Tris, 152 NaCl and 176 ethanol. All other stock solutions were made up in distilled water. When required, stock solutions were diluted in physiological salt solution. Final solvent concentrations did not exceed 0.1% and, under these circumstances, they had no discernible effects on responses.

3. Data and statistical analysis

Data are presented as mean±standard error of mean (SEM) of n observations and fitted, where appropriate, using a variable slope sigmoidal fitting routine in GraphPad Prism4 (San Diego, CA, USA). The concentration of agonist causing a 50% relaxation of the maximal response (E<sub>max</sub>) is expressed as the mean pEC<sub>50</sub> value, with individual pEC<sub>50</sub> values obtained from single experiments. Statistical analysis was performed as indicated in text using either the Student’s t-test (pair or unpaired) or ANOVA with post-hoc correction for pairwise comparisons (Student–Newman–Keuls) or against control (Bonferroni t-test). P values <0.05 were considered statistically significant.

4. Results

4.1. Effect of Ba<sup>2+</sup> on cicaprost-induced relaxation

Cicaprost caused a concentration-dependent relaxation of tail arterial segments precontracted with 1 μM phenylephrine, giving a mean pEC<sub>50</sub> value of 7.16±0.07 and an E<sub>max</sub> of 65.3±2.8% (n=20). A typical response to cicaprost is shown in Fig. 1A. This agent caused substantial, albeit partial, relaxation at highest dose used (1 μM), whereas almost full relaxation (93±1.5%; n=4) was obtained by the addition of 1 μM forskolin. To examine the role of K<sub>R</sub> channels, we tested the effects of Ba<sup>2+</sup> on responses to cicaprost. Although Ba<sup>2+</sup> is a non-specific blocker of K<sup>+</sup> channels, it is relatively selective for K<sub>R</sub> channels at low micromolar (30 μM) concentrations [26]. As expected, Ba<sup>2+</sup> on its own caused a contraction suggesting that K<sup>+</sup> channels contribute to vascular tone (Fig. 1B). Moreover, we found that cicaprost failed to produce discernible relaxation in tissues pre-treated with 100 μM Ba<sup>2+</sup>, despite the substantial relaxation to forskolin obtained in the same vessel (Fig. 1B). Indeed, in some but not all tissues, cicaprost caused a small


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contraction in the presence of Ba\textsuperscript{2+} at the higher doses. Significant inhibition (P < 0.01, n = 6; 2-way ANOVA) of cicaprost-induced relaxation was also observed at 30 μM Ba\textsuperscript{2+}, which shifted the concentration–response curve to the right (pEC\textsubscript{50} 6.2 ± 0.31; Fig. 1C). However, no significant effect on relaxation was observed with 10 μM Ba\textsuperscript{2+}. Similarly, Ba\textsuperscript{2+} (100 μM) blocked relaxant responses to iloprost, significantly reducing E\textsubscript{max} from 57 ± 6.1% to 15 ± 5.8% (P = 0.002; n = 5; 2-way ANOVA). In contrast to PGI\textsubscript{2} analogues, Ba\textsuperscript{2+} (100 μM) only weakly affected the concentration–response curve to forskolin causing a small but significant (P = 0.023, paired t-test) shift in the pEC\textsubscript{50} value from 6.62 ± 0.14 to 6.13 ± 0.11 (n = 5; Fig. 1D), although E\textsubscript{max} was not reduced.

4.2. Role of K\textsubscript{ATP} and GIRK channels

It is conceivable that K\textsubscript{ATP} channels contribute to the mechanism of cicaprost-induced relaxation since these channels are sensitive to inhibition by BaCl\textsubscript{2}, (EC\textsubscript{50} 100 μM; [26]) and appear to contribute to iloprost-induced relaxation in tail artery [16]. Experiments were therefore carried out in the presence of the K\textsubscript{ATP} channel inhibitor, glibenclamide (10 μM). Under these conditions, concentration–response curves for cicaprost were superimposable (Fig. 2A) whereas glibenclamide almost fully inhibited relaxation to levomakalim over the entire concentration range (P < 0.001, n = 4; 2-way ANOVA; Fig. 2B).

Evidence suggests that IP receptors can under certain circumstances couple to G\textsubscript{i} [1]. This in turn may activate GIRK channels, which are themselves sensitive to block by Ba\textsuperscript{2+} [18]. We therefore investigated this possibility using the GIRK inhibitor, fluoxetine [27]. In the presence of 100 μM fluoxetine, the concentration–response curve for cicaprost (0.001–1 μM) was essentially identical to that of the control (Fig. 2C).

4.3. Effect on membrane potential

Resting membrane potential in untreated tail arteries averaged −55.4 ± 0.7 mV (n = 13). Application of 1 μM cicaprost caused a significant hyperpolarisation (Fig. 3A) that was significantly reduced by 30 μM Ba\textsuperscript{2+} (P < 0.01; paired t-test), which by itself caused a small membrane depolarisation (4.3 ± 1.9 mV; n = 3). On average, cicaprost caused a 6.1 ± 1.0 mV hyperpolarisation to −61.1 ± 0.8 mV (n = 9).

4.4. Functional expression of K\textsubscript{IR} channel in rat tail artery

We wished to test for the presence of functional K\textsubscript{IR} channels in our preparation by examining the effect of...
extracellular K⁺ (7.5–20 mM) on tension. Raising extracellular K⁺ caused relaxation of phenylephrine contractions, peaking at 15 mM and becoming smaller thereafter (Fig. 4A). In the presence of 100 μM a²⁺, KCl no longer caused relaxation (Fig. 4B). Instead small contractions were observed. In a series of experiments, 15 mM K⁺ relaxed tail arteries to ~35%, an effect independent of functional endothelium (Fig. 4C and D). Relaxations were also significantly attenuated by 30 μM BaCl₂, with a greater inhibition seen at 100 μM. In separate experiments, we found that 15 mM K⁺ caused a 5.4±1.4 mV (n=5) hyperpolarisation in resting arteries.

4.5. Role of the endothelium, NO and EDHF

We tested the possibility that endothelial KIR channels or factors released from the endothelium might contribute to relaxation. Indeed, removal of the endothelium did significantly attenuate (P<0.001, n=18; 2-way ANOVA) responses to cicaprost, reducing the relaxant response at 1 μM by 36%, although in its absence, substantial relaxation to cicaprost still remained (Fig. 5A). In contrast, blockade of endothelial-derived NO with L-NAME (300 μM) had no effect on responses to cicaprost (Fig. 5B). We also examined the effect of combined treatment with the intermediate and small conductance calcium-dependent K⁺ channel blockers charybdotoxin and apamin, sensitivity to which is the hallmark of relaxation through EDHF [21]. Our results showed that charybdotoxin and apamin did produce a significant reduction (P<0.01, n=8; 2-way ANOVA) in the relaxant response of cicaprost, the magnitude of which approached that observed by endothelium removal.

4.6. Effect of high extracellular K⁺ concentration

Relaxation associated with EDHF-induced activation of KIR channels has commonly been reported to be significantly attenuated by 30 mM KCl [28,29] or by membrane depolarisation elicited by high concentrations of vasoconstrictors [21]. Likewise, we found that there was a significant (P<0.01; 2-way ANOVA) attenuation in the relaxation induced by cicaprost in the presence of 30 mM K⁺ (Fig. 6). Relaxation to the maximum dose of cicaprost (1 μM) was 32% in the presence of 30 mM KCl compared to 65% in its absence.

4.7. Effect of inhibitors of adenylyl cyclase and PKA

Experiments were carried out in the presence of inhibitors of both adenylyl cyclase and PKA to determine...
the involvement of the cAMP pathway in the relaxation induced by cicaprost. The results presented in Fig. 7 show that the concentration–response curves for cicaprost (0.001–1 \( \mu \text{M} \)) were unaffected by the two adenylyl cyclase inhibitors SQ22536 (100 \( \mu \text{M} \)) or DDA (100 \( \mu \text{M} \)). In addition, Rp-cAMPs (100 \( \mu \text{M} \) ) had no effect when compared to its inactive control, Na butyrate (100 \( \mu \text{M} \)).

4.8. Role of Gi/Go

Since the inhibitors of cyclic AMP pathway did not alter the relaxation induced by cicaprost, we investigated the possibility that relaxation was through PTX-sensitive GTP-binding proteins, as has been demonstrated for PGI₂-

Fig. 4. Raising extracellular K⁺ produces relaxation of rat tail artery that is blocked by Ba²⁺. Tracings showing K⁺-induced relaxation of an arterial segment exposed to increasing doses of extracellular K⁺ (in 2.5 mM increments) in the absence (A) or presence (B) of 100 \( \mu \text{M} \) Ba²⁺. The mean inhibitory effect of Ba²⁺ on the relaxation induced by 15 mM K⁺ is shown in the presence (C) or absence of the endothelium (D). Data is shown as the mean of at least 4 experiments. *: \( P<0.01 \) or **: \( P<0.001 \) when compared to control (one-way ANOVA).

Fig. 5. Role of endothelium on relaxation induced by cicaprost. Cumulative concentration–response curves were constructed for cicaprost in the absence or presence of the endothelium (A), the absence or presence of L-NAME (B) or the absence and presence of apamin and charybdotoxin (C). In C, the concentration of phenylephrine used was lowered to give the same contraction as the control. Data are presented as mean±SEM from at least 6 experiments.

Fig. 6. The effect of 30 mM K⁺ on the relaxation induced by cicaprost. Tissues were precontracted with either 1 \( \mu \text{M} \) phenylephrine (control) or 30 mM KCl and phenylephrine combined. Cumulative concentration–response curves were constructed for cicaprost and data presented are mean±SEM from at least 4 experiments.
mediated dilation in the pig cerebral circulation [30]. Following treatment with 1 μg/ml PTX for 18 h the relaxant response to cicaprost was significantly (P<0.001, n=5; 2-way ANOVA) enhanced by this treatment over the whole concentration range (Fig. 8), with the pEC50 value increasing from 6.00±0.18 to 6.65±0.14 and Emax from 75.6±5.5 to 103.5±3.0.

5. Discussion

The results of this study are consistent with the hypothesis that cicaprost relaxes rat tail arteries through a cAMP-independent activation of KIR channels. Ba2+ at a concentration (30 μM) considered relatively selective for the KIR channel [26] significantly inhibited both hyperpolarisation and relaxation induced by cicaprost. A contribution from other types of Ba2+-sensitive K+ channels could be ruled out since inhibitors of KATP and GIRK channels were without effect. Moreover, the cAMP pathway is unlikely to mediate relaxation, as adenylyl cyclase and PKA inhibitors failed to reverse the effects of cicaprost. However, relaxation was partially dependent on the endothelium and inhibited similarly by a combination of apamin and charybdotoxin, suggesting a contribution from EDHF to KIR activation. Our results therefore represent the first evidence for the involvement of KIR channels in the action of an IP receptor agonist.

The existence of K+-induced relaxation attenuated by Ba2+ is widely accepted as evidence for the presence of KIR channels [17,21]. This is based on a number of observations, including sensitivity to low micromolar Ba2+ but not to other K+ channel inhibitors [31,32] and absence of dilation in cerebral arteries from Kir2.1−/− mice [20]. Smooth muscle KIR channels are likely to be the target since most blood vessels dilate to K+ in the absence of the endothelium [29,31,32], though not always [33]. We demonstrated the presence of functional KIR channels in our preparation by recording a Ba2+-sensitive, K+-induced relaxation that was endothelium-independent and associated with smooth muscle membrane hyperpolarisation. Moreover, electrophysiological studies in rat tail artery have shown the existence of KIR currents in isolated myocytes with strong antibody staining for Kir2.1 observed in the medial layer of this vessel but with little or no protein evident in the endothelium for this or other Kir2.0 subunits [22]. Thus, we believe that smooth muscle KIR channels are responsible for the effects of K+ ions in tail artery. This contrasts results in rat mesenteric artery where endothelial KIR channels and Na+–K+–ATPase located on smooth muscle cells appear to account for K+-induced dilation [34].

The IC50 for Ba2+ block of K+-induced membrane hyperpolarisation and dilation in small coronary and cerebral arteries is ~3–8 μM [31], as is the Ba2+ block of Kir2.1 currents at ~60 mV [19]. We found that higher concentrations of Ba2+ (100 μM) were required to fully block cicaprost- and K+-induced relaxation as reported for dilations associated with KIR channels in middle cerebral artery [32]. This could simply reflect the voltage-dependent nature of the Ba2+ block meaning that higher concentrations are needed to inhibit the channel at depolarised membrane potentials [17]. Thus, the effective Ba2+ concentration required would be dependent on the level of vasoconstrictor tone. However, we cannot rule out the possibility that other smooth muscle K+ channels might also contribute to

![Fig. 8](https://example.com/fig8.png)

**Fig. 8.** The effect of pertussis toxin (PTX) treatment on the relaxation induced by cicaprost. Arterial segments were incubated for 18 h in DMEM in the absence (control) and presence of 1 μg/ml PTX. Concentration–response curves to cicaprost were constructed in tissues precontracted with 1 μM phenylephrine. Data shown as means±SEM from 5 separate experiments.
Moreover, in the presence of SQ22536, the highly specific inhibitors, SQ22536 and dideoxyadenosine as well as the PKA inhibitor Rp-2-cAMPs failed to suppress responses to cicaprost. Likewise, in guinea-pig aorta relaxant responses to iloprost or beraprost were not inhibited by SQ22536, despite these agents inhibiting the rise in cAMP [9,10]. The relative lack of effect of Ba2+ on forskolin-induced relaxation that we observed in rat tail artery is also consistent with this interpretation. Furthermore, forskolin does not mimic responses to PGI2 analogues in coronary or aortic smooth muscle, although in these studies other K+ channels appear to mediate relaxant effects [13,14]. More recently, the NO donor, sodium nitroprusside, has been reported to activate Kir2 channels in pressurised coronary arterioles [36]. In this respect, it is worth noting that bradykinin relaxation of isolated arteries is associated with substantial release of PGI2 and, unlike vasorelaxant responses to acetylcholine, is sensitive to block by NO inhibitors [37,38]. Thus, it is possible that PGI2 could contribute to Kir activation, either directly through an unknown mechanism or indirectly through the release of EDHF. Whether this represents a more widespread physiological mechanism of vasodilatation remains to be determined but could in part underlie the therapeutic benefits of PGI2 analogues, which are currently being used to treat pulmonary hypertension and peripheral vascular diseases, including critical limb ischemia and Raynaud’s phenomenon [1,12].

In summary, this study provides the first evidence for the involvement of Kir channels by IP receptor agonists. Our results raise the intriguing possibility that PGI2 might make a greater contribution than previously thought to EDHF, which in some blood vessels is clearly mediated by Kir channels [21]. The mechanism of action of cicaprost appears to be largely independent of cAMP and NO and future experiments will be required to determine the pathway whereby this agent activates K+ channels to cause relaxation.

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