Celecoxib dilates guinea-pig coronaries and rat aortic rings and amplifies NO/cGMP signaling by PDE5 inhibition

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Abstract

Objective: Celecoxib carries a smaller cardiovascular risk for myocardial infarction and hypertension than other cyclooxygenase-2 (COX-2)-selective non-steroidal anti-inflammatory drugs NSAIDs ("coxibs") and may ameliorate endothelial dysfunction. We aimed to determine which mechanism possibly accounts for the beneficial effect by investigating its vascular action in different in vitro preparations in comparison with other coxibs and reference phosphodiesterase-5 (PDE5) inhibitors.

Methods: To uncover potential effects on coronary flow, the effects of celecoxib in comparison with other NSAIDs and the PDE5 inhibitors, sildenafil and zaprinast, were investigated in guinea-pig Langendorff heart. This was supported by studies for vasorelaxation, interaction with the NO/cGMP pathway, and measurement of cyclic nucleotide amounts released from rat aortic rings, and inhibition of human PDE5 as well as PDE4 activity.

Results: Bolus injections of sildenafil, celecoxib, and zaprinast (at 100 nmol) into the Langendorff heart increased coronary flow by approximately 100, 65, and 25%, respectively, while rofecoxib, lumiracoxib, parecoxib, and diclofenac, except valdecoxib (>100 nmol), failed to increase coronary flow up to 300 nmol. In rat aorta, sildenafil, celecoxib and zaprinast caused endothelium-dependent relaxation with −log[EC50]M values of 8.90, 6.66 and 5.56, respectively; their rank order of potency corresponds to their coronary dilatory effect. Celecoxib-induced relaxation of aorta was attenuated by the nitric oxide (NO) synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 10⁻⁴ M) and by the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 10⁻⁵ M). In aortic rings, celecoxib (3×10⁻⁵ M) caused a fivefold increase in the cGMP level and potentiated that induced by sodium nitroprusside (5×10⁻⁷ M). Celecoxib and valdecoxib inhibited human PDE5A1 with an IC50 of 1.6×10⁻⁵ and 1×10⁻⁴ M, respectively, whereas other coxibs were without inhibitory effect.

Conclusion: Celecoxib caused coronary vasodilatation in guinea-pig hearts and relaxation of rat aorta and had a potentiating effect on the NO/cGMP signaling pathway in rat aorta through specific blockade of PDE5. These unexpected findings clearly support the notion that celecoxib possesses an as yet undisclosed molecule-specific property that possibly compensates a decrease of prostacyclin-dependent cAMP generation by concomitantly increasing cGMP levels resulting from inhibition of PDE5.

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Keywords: Coronary flow; Celecoxib; NSAID; PDE5; cGMP

1. Introduction

Cyclooxygenase-2 (COX-2) selective inhibitors ("coxibs") increase the incidence of systemic hypertension [1] and thromboembolic complications including myocardial infarction [2]. The observation that cardiovascular side effects are increased (compared to placebo) not only with the methylsulfon group containing drug rofecoxib [3] but also with the arylsulfon group containing drug celecoxib [4] argues for a class-effect. Such an effect appears plausible given that coxibs reduce systemic synthesis of prostacyclin...
Coronary heart disease is 1.5 to 3 times higher in patients on COX-2 selective NSAIDs compared with those of the PDE5 inhibitors sildenafil \[19\] and zaprinast \[20\] or the PDE4 inhibitor rolipram \[21\]. The important protective actions of prostacyclin and its IP receptor involved have clearly been shown in various animal models of cardiovascular disease \[7,8\]. Except from low-dose aspirin however \[9\], alterations of the prostacyclin/thromboxane ratio induced by COX-2 selective NSAIDs have not been linked to cardiovascular risk in humans. In a recent retrospective study, we observed only a weak correlation between systemic thromboxane (but not prostacyclin) formation and systolic blood pressure in patients with congenital hypokalemic salt-losing tubulopathy taking either indomethacin or rofecoxib \[10\]. Finally, unlike coxibs most traditional NSAIDs have not been studied with regard to their cardiovascular side effects in randomized controlled trials (RCT). Thus it remains to be shown in RCT that unselective NSAIDs, which balance lack of prostacyclin by inhibiting thromboxane synthesis are indeed less frequently associated with cardiovascular risk compared to placebo.

Interestingly, various independent (investigator initiated) studies reported that celecoxib entails a smaller risk of myocardial infarction compared to its main competitor rofecoxib \[11–14\]. Graham and coworkers showed in a nested case–control study that the relative risk for serious coronary heart disease is 1.5 to 3 times higher in patients on rofecoxib versus celecoxib users. Arterial hypertension is more frequently observed in patients taking rofecoxib compared to celecoxib \[15–18\]. Finally, although Solomon et al. showed that celecoxib increased the incidence of cardiovascular fatalities, the overall mortality was identical with both placebo and celecoxib \[4\]. In aggregate, there is increasing cumulative evidence that clinically significant molecule-specific properties may differ considerably among coxibs.

To address potentially different vascular effects between celecoxib and other coxibs we investigated their effects on coronary flow in the isolated, constant-pressure perfused guinea-pig heart. To identify which mechanism may account for the vasorelaxant effect observed by celecoxib in the heart, studies in rat aorta examining the role of endothelium and the NO/cGMP pathway in relaxation were performed, including measurements of cGMP levels alone and in combination with a NO donor. Inhibition of phosphodiesterase type 5 (PDE5), as a potential common mechanism for the effects by celecoxib observed in both vascular tissues, was tested on recombinant human PDE5A1. The vascular effects evoked by celecoxib were compared with those of the PDE5 inhibitors sildenafil \[19\] and zaprinast \[20\] or the PDE4 inhibitor rolipram \[21\].

2. Material and methods

2.1. Reagents

Celecoxib, valdecoxib, parecoxib, rofecoxib and etoricoxib were purchased from Sequio (Oxford, UK). Lumiracoxib and sildenafil citrate were synthesized in the laboratories of ALTANA Pharma AG. Zaprinast, 1H-[1,2,4] oxadiazolo[4,3-α]quinoxalin-1-one (ODQ), sodium nitroprusside (SNP), phenylephrine and arecaidine propargyl ester (APE) were obtained from Tocris (Cologne, Germany). All other drugs and compounds were from Sigma (Deisenhofen, Germany). Cyclic GMP and cAMP enzyme immunoassays were from Amersham (Braunschweig, Germany).

2.2. Constant-pressure perfused guinea-pig Langendorff heart

The investigation conforms with 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) and all experimental protocols were conducted in accordance with German guidelines and approved by respective regulatory authorities for animal care (Reg. Präsidium Freiburg, Germany). Male guinea-pigs (Dunkin Hartley, 400–500 g; Charles River, Sulzfeld, Germany) were killed by cervical dislocation. The heart was rapidly excised and perfused with Krebs–Henseleit solution (mM: NaCl 118, KCl 4.7, CaCl2 1.9, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, and glucose 5.0, at 37 °C gassed with 95% O2/5% CO2) at a constant pressure of 80 cm H2O (62 mm Hg) via retrograde cannulation of the aorta in a Langendorff apparatus. A water-filled balloon catheter connected to a Statham P 23 Db pressure transducer was introduced through an incision in the left atrium into the left ventricle and preloaded to a pressure of 40 mm Hg, mimicking the diastolic pressure \[22\]. Precoronary perfusate flow (CF, at an initial average passive flow of 9–13 μl/min) was measured using an electromagnetic flow meter. The change in left ventricular isovolumetric pressure amplitude (LVP) calculated by subtracting the diastolic from the systolic pressure, the rate of maximal left ventricular pressure rise (dP/dtmax), and the rate of the spontaneously beating heart (HR) were continuously recorded and monitored by a data acquisition system (Notocord, hem version 3.3; F-78290 Croissy). Following at least 1 h of perfusion and stabilization of all cardiac parameters, the test drug was injected as 100 μl bolus (at doses between 0.3 nmol to 0.3 μmol) within 2 s directly into the perfusion tube connected to the aortic inflow and increased threefold after the previous dose had produced its coronary dilatory response and the perfusion flow had returned to pre-drug values again. The drug-induced change in cardiac parameters was expressed as percentage of the initial pre-drug values (mean±SEM).
2.3. Relaxation studies and cGMP content in isolated rat aorta

Male rats (Sprague–Dawley, 240–260 g; Charles River, Sulzfeld, Germany) were killed by a blow on the head and exsanguinated. Ring preparations from the thoracic aorta were made in Krebs–Ringer bicarbonate buffer containing (mM) NaCl 120.0, KCl 5.5, CaCl2 2.5, NaH2PO4 1.2, MgCl2 1.2, NaHCO3 20.0 and glucose 11.0. In half of the experiments, the entire length of the thoracic aorta was functionally denuded of the endothelial layer by gently scraping the luminal surface with a 1.5 mm glass rod. The effectiveness of this procedure was tested in each preparation by measuring the vasorelaxation of the precontracted aortic rings to the muscarinic receptor agonist arecaidine propargyl ester (APE, 3 × 10^{-6} M; EC_{100} of its own maximal effect), the response of which in this case remained less than 5%. Alternatively, endothelial integrity was verified by vasorelaxation of more than 80% in response to APE [23]. The aortic preparations were suspended in 10-ml organ baths containing the above-mentioned nutrient solution maintained at 37 °C and continuously equilibrated with 95% O2/5% CO2. The tissues were attached to force displacement transducers, stretched to a resting tension of 1 g and were allowed to equilibrate for 60 min before contraction to a single concentration of phenylephrine (3 × 10^{-7} M; EC_{80–90} of its own maximal effect) was elicited. After stabilization of the response, the drug was cumulatively added to the bath until peak relaxation was achieved. The amount of relaxation (percent values as means±SEM) was quantified on the level of the previously evoked contraction to phenylephrine. The value for half-maximal effect was expressed as-log[EC_{50}]M. When the influence of N^\text{G}-nitro-L-arginine methyl ester (L-NAME) or 1H-[1,2,4] oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) on the celcoxib-induced relaxation was evaluated, L-NAME (10^{-4} M) or ODQ (10^{-5} M) was added to the preparation 30 min prior to phenylephrine.

In the study for measurement of cGMP released from rat aorta, 5 rings/300 μl well were initially pre-incubated in Krebs-Ringer bicarbonate buffer (mentioned above) with 3 × 10^{-7} M phenylephrine for 20 min at 37 °C, after that celecoxib (3 × 10^{-5} M, DMSO), sodium nitroprusside (SNP, 5 × 10^{-7} M, H2O), or celecoxib (3 × 10^{-5} M) plus SNP (5 × 10^{-7} M) was added for additional 20 min incubation at 37 °C. The final DMSO concentration was 0.3%. Thereafter, supernatants were taken following centrifugation and stored at −80 °C. cGMP and cAMP content was determined by a commercially available enzyme immunoassay kit according to the acetylation protocol of the supplier (Amersham Bioscience). Results were calculated via an external standard curve and expressed as released fmol cGMP/aortic ring. The average wet weight of one rat aortic ring amounted to 8.0±0.3 mg (mean±SEM, n=20).
[EC$_{50}$] M values from the concentration–response curves of test compounds for relaxation of rat aorta were performed by fitting their values by a monophasic sigmoidal function by standard program (sigmoidal fit, Origin 7.0). Analysis of significance was performed using Student’s t-test, whereby $P<0.05$ was considered significant.

3. Results

3.1. Effects in perfused guinea-pig Langendorff heart

The average initial values of cardiac parameters measured in constant-pressure perfused guinea-pig Langendorff hearts were as follows: coronary flow (CF) = 11.4 ± 0.8 ml/min; left ventricular pressure amplitude (LVP) = 76 ± 6 mm Hg; rate of maximal left ventricular pressure rise (dP/dt$_{max}$) = 905 ± 54 mm Hg/s; and the rate of the spontaneously beating heart (HR) = 179 ± 6 beats/min (means ± SEM, n = 10).

Bolus injection of increasing doses of celecoxib (between 1 and 200 nmol) produced a dose-dependent increase in CF, which developed slowly to reach its maximal effect after 2 min and lasted for up to 10 min. At the maximal dose of celecoxib administered (200 nmol), CF was increased by 72 ± 5%. This dose caused a marginal and statistically insignificant ($P>0.05$) increase in HR of 4 ± 3%, whereas LVP and dP/dt$_{max}$ were slightly but significantly ($P<0.05$) increased by maximally 10 ± 4% and 18 ± 8%, respectively (means ± SEM, n = 4) (Figs. 1 and 2). This latter effect appeared to be indirectly mediated by improvement of cardiac perfusion, since no positive inotropic and chronotropic effects were detectable in spontaneously beating right atrial preparations from the same species (data not shown).

The PDE5 inhibitor sildenafil (0.3–300 nmol) induced a much greater increase in CF, maximally amounting to 131 ± 24% above baseline at the highest dose (300 nmol) administered (Fig. 1), thereby also slightly increasing LVP and dP/dt$_{max}$ by 17 ± 17% and 15 ± 4%, respectively, while HR remained unaffected (not shown). In contrast to celecoxib, the time course for its coronary dilatatory effect was rapid to reach the maximal effect (less than 1 min), and then declined within 3 min. The coronary dilatatory effect in response to injected zaprinast (1 to 3000 nmol) was much weaker than that evoked by sildenafil and celecoxib. At 100 nmol zaprinast caused an increase of CF by 25 ± 4%, whereas its maximal effect at 3000 nmol (79 ± 21%), was similar to that elicited by high doses of celecoxib (Fig. 1). For comparison, the selective inhibitor for PDE4 rolipram, only at doses between 100 and 300 nmol caused an increase in coronary flow of maximally 38 ± 8% (mean ± SEM, n = 3), and thus appeared to be the weakest compound of all PDE inhibitors investigated. Based on the doses necessary for an increase of CF by 30%, the rank order of potency for achieving this change was sildenafil > celecoxib > zaprinast > rolipram (−log[ED$_{30}$]mol values 9.05, 7.65, 7.60 and 5.75, respectively).

Next to celecoxib, only valdecoxib at high doses (100 and 300 nmol) led to a small increase in coronary flow by
14 ± 6% and 22 ± 8%, respectively. However, none of the other NSAID tested and administered as bolus injection between 0.3 and 300 nmol (lumiracoxib, parecoxib and rofecoxib) or between 0.3 and 100 nmol (diclofenac) caused a change in CF (Fig. 1), while HR, LVP, and dP/dtmax also remained unchanged (data not shown).

### 3.2. Relaxation studies and cyclic nucleotide content in rat aortic rings

Celecoxib (10⁻⁹–10⁻⁵ M), like the PDE5 inhibitors sildenafil (10⁻¹⁰–10⁻⁶ M) and zaprinast (10⁻⁸–10⁻⁴ M), relaxed phenylephrine-precontracted rat aortic rings in a concentration-dependent manner with the following rank order of potency: sildenafil > celecoxib > zaprinast (Fig. 3). Relaxation by all compounds was endothelium-dependent, as there were enormous differences between the concentration–response curves and the −log[EC₅₀]M values obtained in rings with or without functional endothelium (Fig. 3 and Table 1). Pretreatment of the aortic rings for 30 min with L-NAME (10⁻⁴ M) or ODQ (10⁻⁵ M) attenuated celecoxib-induced relaxation in endothelium intact aortic rings almost the same as endothelium removal did, i.e. both treatments caused an approximate 100-fold shift of the celecoxib concentration–response curve to the right (Fig. 4). Fig. 5 shows the influence of a 20-min treatment by celecoxib and its combination with SNP on extracellular cGMP levels in rat aortic rings previously incubated for 20 min with 3 × 10⁻⁷ M phenylephrine. A high concentration of celecoxib (3 × 10⁻⁵ M) caused an approximately fivefold increase in cGMP (3.78 ± 1.02 fmol/ring) compared to control (0.73 ± 0.14 fmol/ring; *P < 0.05), while a low concentration of SNP (3 × 10⁻⁷ M) increased cGMP level ∼twofold (1.33 ± 0.01 fmol/ring). However, after combining both agents, an approximately ninefold potentiating effect by celecoxib on SNP-induced cGMP content (12.2 ± 1.3 fmol/ring; **P < 0.001) was observed. The increase in cAMP amount released from rat aortic rings after celecoxib (3 × 10⁻⁵ M) was less pronounced (1.6-fold), and did not reach significance (*P > 0.05; data not shown).

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>With functional endothelium</th>
<th>Without functional endothelium</th>
</tr>
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<tbody>
<tr>
<td>Celecoxib</td>
<td>6.66 ± 0.07 (48)</td>
<td>4.27 ± 0.04 (8)</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>8.90 ± 0.04 (12)</td>
<td>6.68 ± 0.08 (11)</td>
</tr>
<tr>
<td>Zaprinast</td>
<td>5.56 ± 0.03 (8)</td>
<td>4.43 ± 0.04 (8)</td>
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Given are −log[EC₅₀]M values for a half-maximal effect (mean ± SEM for a number n aortic rings indicated in brackets).

Fig. 5. Changes in extracellular cGMP levels in isolated rat aortic rings by a single treatment with either celecoxib (3 × 10⁻⁵ M) or SNP (3 × 10⁻⁷ M) alone or by their combination added 20 min prior to phenylephrine. Levels of cGMP (expressed as fmol/aortic ring) were measured with a commercially available kit (see Section 2). Given are means ± SEM for a number of n = 3–8 rings. *P < 0.05 compared with untreated control. **P < 0.001 compared with celecoxib or SNP alone.

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Fig. 4. Effects of L-NAME (10⁻⁴ M) and ODQ (10⁻⁵ M) on celecoxib-induced relaxation of the intact rat aorta precontracted by phenylephrine. L-NAME or ODQ was added 30 min before the addition of phenylephrine. Given are means ± SEM of n = 37 rings for the relaxant effect of celecoxib alone and n = 12 for those in the presence of either L-NAME or ODQ.

Fig. 6. Concentration–response curve for celecoxib to inhibit recombinant human PDE5A1 (mean ± SEM, n = 3). Given are also the calculated IC₅₀ values for the tested reference coxibs and PDE5 inhibitors.
3.3. Effect on recombinant human PDE5A1 and PDE4 activity

The effects of coxibs on phosphodiesterase type 5 (PDE5A1) were tested in vitro using recombinant human enzyme. In contrast to all other coxibs, only celecoxib and to a much lesser extent valdecoxib inhibited PDE5A1 with IC$_{50}$ values of $1.6 \times 10^{-5}$ M and $\sim 1 \times 10^{-4}$ M, respectively (Fig. 6). Furthermore, celecoxib inhibited PDE4 activity (IC$_{50}$ values of $1 \times 10^{-5}$ M) but had no additional effect on the PDE1, 2 and 3 isoenzymes (data not shown). However, inhibition of PDE4 is not unique to celecoxib as valdecoxib also inhibited PDE4 activity, albeit at higher concentrations (IC$_{50}$ values of $7 \times 10^{-4}$ M). The other coxibs were without inhibitory effect.

4. Discussion

4.1. General considerations

Celecoxib is a selective COX-2 inhibitor [24]. However, to date no evidence has been obtained that celecoxib exerts additional pharmacological actions as a specific PDE5 inhibitor, an effect we incidentally observed in search for various chemical compounds as potential inhibitors of PDE isoenzymes. In the present study, we thus examined this property for possible relevance (a) in the isolated, constant-pressure perfused guinea-pig Langendorff heart because of the known role of PDE5 in the regulation of coronary flow [25,26] and (b) in precontracted rat aortic rings [27,28], two suitable tissues responding with increase in coronary flow and endothelium-dependent vasorelaxation, respectively, upon administration of selective PDE5 inhibitors.

4.2. Effect of celecoxib in perfused guinea-pig Langendorff heart

The principle finding of this study is that celecoxib increases coronary flow (CF) in perfused guinea-pig heart up to 72% at the highest dose administered (200 nmol). Using the same dose, the PDE5 inhibitors sildenafil and zaprinast led to more than 100% and less than 35% increase of CF, respectively. To exclude the possibility, that celecoxib’s effect on coronary flow may result from co-inhibition of PDE4, we investigated the potent and selective PDE4 inhibitor rolipram [21]. At a comparable dose rolipram caused only a 15% increase of CF, suggesting that concomitant inhibition of PDE4 by celecoxib, as a possible additional mechanism contributing to vasodilatation in guinea-pig coronaries, can be disregarded. As heart rate and force of contraction were not decreased but rather slightly enhanced upon exposure to the highest dose of celecoxib, we conclude that this dose of celecoxib does not provoke any cardiac depressant or unspecific toxic effects, which may underlay the observed coronary vasodilatation. Except valdecoxib at high doses (100–300 nmol), neither diclofenac, which exhibits a similar degree of COX-2 selectivity as celecoxib [29], nor any other coxib (lumiracoxib, parecoxib and rofecoxib) increased coronary flow, thus clearly demonstrating a molecule-specific property of celecoxib obviously unrelated to COX-inhibition.

4.3. Effect of celecoxib on relaxation and NO/cGMP pathway in rat aorta

In intact rat aorta celecoxib caused relaxation, which was 170-fold weaker compared to the strong PDE5 inhibitor sildenafil, but 10 times more potent than with the PDE5 inhibitor zaprinast. The rank order of potency for vasorelaxation in rat aorta (sildenafil>celecoxib>zaprinast) is identical to that observed when analyzing coronary flow where celecoxib was roughly 25-fold weaker than sildenafil, but 10-fold more potent than zaprinast. Similarly to our studies in perfused guinea-pig heart, the PDE5 inhibitor rolipram has been shown to be roughly 10-fold weaker than zaprinast as a relaxant in rat aorta with functional endothelium [27]. Thus, the rank order of potency in guinea-pig heart and rat aorta can be extended to sildenafil>celecoxib>zaprinast>rolipram. As expected from previous studies on rat aorta, the PDE5 inhibitors sildenafil and zaprinast exerted endothelium-dependent relaxation of rat aorta, a property surprisingly also shown by celecoxib. This is in contrast to the endothelium-independent relaxation by PDE3 inhibitors, and the weak and only partially endothelium-dependent relaxation by PDE4 inhibitors, thus making inhibitors acting selectively on PDE5 functionally discernible from those on PDE3 and on PDE4 in this model [27]. Particularly, in sildenafil, which was 165-fold weaker in relaxing endothelium-denuded rat aorta compared to a functionally intact one, the difference in potency for celecoxib amounted to a factor of more than 200, whereas that of zaprinast, in agreement with previous studies was less than factor of 20, probably due to its reduced solubility at high concentrations [27].

Interestingly endothelium-dependent vasodilatation by celecoxib has already been reported: Widlanski et al. observed an increased brachial artery vasodilatation function in hypertensive patients treated with celecoxib compared to placebo [30]. Correspondingly, celecoxib but not rofecoxib (nor diclofenac) enhanced endothelium-dependent vasodilatation in rats with salt sensitive hypertension [31]. Likewise, rofecoxib did not alter endothelial function/dysfunction in either healthy subjects [32] or patients with coronary artery disease [33,34]. Chenerved et al. reported that celecoxib, given to patients with coronary artery disease at 200 mg bid for 2 weeks improved endothelium-dependent flow-mediated vasodilatory response in the brachial artery test following reactive hyperemia [35]. This group viewed this phenomenon as a consequence to reduced oxidative stress and inflammation. Although not formally excluded, this mechanism appears unlikely to underlay the acute coronary dilatation and
relaxation of rat aorta with celecoxib in the present in vitro study given the immediate onset of action.

Celecoxib-induced vasorelaxation in rat aorta was attenuated not only by endothelium removal, but also by pretreatment with the nitric oxide synthase inhibitor L-NAME, or with ODQ, an inhibitor of the soluble guanylate cyclase, clearly indicating that relaxation depends on NO released from the endothelium. To gather additional independent evidence that celecoxib enhances NO/cGMP signaling, we exposed rat aortic rings pretreated for 20 min with phenylephrine to celecoxib with or without the NO donor SNP, and quantified extracellular cGMP released from the aortic rings. Celecoxib at a concentration \( (3 \times 10^{-5} \text{ M}) \) threefold higher than necessary to cause maximal vasorelaxation \((10^{-5} \text{ M})\), significantly elevated the cGMP level fivefold over control. Only a twofold increase in cGMP content from the tissue was observed compared to untreated controls, possibly due to the low concentration of SNP used \((3 \times 10^{-7} \text{ M})\), and consequently, a low cGMP amount released from smooth muscle into the extracellular space, or due to the fact that other PDEs (predominantly PDE3, but also PDE1 and 5) are localized in rat aorta\([27,36]\) that may hydrolyse cGMP and keep cGMP level relatively low. However, co-incubation of celecoxib and SNP increased cGMP level ninefold over that evoked by SNP alone. This suggests that celecoxib amplifies vascular NO/cGMP signaling as the underlying mechanism to cause vasorelaxation of a precontracted tissue like intact rat aorta, in which NO, synthesized in and released from the endothelium, diffuses into smooth muscle cells to stimulate cGMP in order to counteract vasoconstriction. In contrast, the effect of celecoxib to concomitantly inhibit PDE4 and to increase cAMP levels in rat aortic rings was weak and statistically not significant (data not shown). Thus, the results obtained from relaxation and biochemical experiments on rat aorta clearly indicate that the vasodilatory properties of celecoxib most likely result from PDE5 inhibition.

4.4. Inhibition of PDE5 by celecoxib

Given the overlapping roles in vascular dilatory responses to activation and integrity of endothelial prostacyclin synthase and endothelial nitric oxide synthase, whose biological effects are enhanced through inhibition of PDE5, we tested whether celecoxib directly inhibits PDE5-enzymatic activity. Using recombinant human PDE5A1 we could show that celecoxib inhibits enzymatic activity with an \( IC_{50} \) value of \( 1.6 \times 10^{-5} \text{ M} \), whereas, with the exception of valdecoxib at high concentrations, all other coxibs investigated were without inhibitory effect. Compared to sildenafil however, the \( IC_{50} \) of celecoxib is approximately 1000 times higher in vitro\([37]\). Especially the potency of the hydrophilic compound sildenafil to elicit coronary dilatation in guinea-pig Langendorff heart may be underestimated due to its short-lived effect as compared to the longer lasting effect observed with the more lipophilic celecoxib. The result of the present study using bolus injections of coxibs into the perfusion stream of guinea-pig heart is certainly difficult to compare with existing pharmacokinetic data in humans. It is noteworthy, however, that the dose of celecoxib which is required to achieve a 30% increase in coronary flow is only 25 times higher than that of sildenafil, thus arguing for a specific effect. In the functionally intact rat aorta, where steady state conditions for cumulative drug administrations are achieved more easily, concentrations of celecoxib to evoke half-maximal relaxation of the precontracted vessel were 170-fold higher than those necessary for sildenafil, but may also be influenced by different cellular penetration.

4.5. Summary and conclusion

In conclusion, we show that celecoxib is unique in causing dilatation of guinea-pig coronary vasculature in vitro. Inhibition of PDE5 may be the mechanism by which celecoxib exerts this effect, because (a) an identical rank order of potency was observed for celecoxib in comparison with other PDE5 inhibitors, namely sildenafil > celecoxib > zaprinast, to evoke coronary dilatation and to cause relaxation of intact rat aorta, (b) its relaxant effect in aorta, typically for PDE5 inhibitors, disappeared after endothelium removal or inhibition of the NO/cGMP pathway, and (c) of its ability to potentiate the effect of a NO donor on GMP levels, and (d) of its unique property to inhibit human PDE5A1. If these \textit{in vitro} results can also be extended to humans, beneficial effects on the cardiovascular adverse events otherwise observed with the coxibs, e.g. increased risk of myocardial infarction and stroke, may be expected, in that celecoxib may functionally compensate a decrease of prostacyclin-dependent cAMP generation by increasing cGMP levels mediated through moderate inhibition of PDE5. As hemodynamic studies suggest that the PDE5 inhibitor sildenafil is a modest vasodilator with the potential to increase coronary flow and coronary flow reserve\([38]\), it remains to be shown whether this mechanism could also account for celecoxib to sufficiently explain the smaller risk of myocardial infarction.

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