Received: 19 May 2023 | Revised: 17 November 2023 | Accepted: 11 December 2023

DOI: 10.1111/bph.16322

RESEARCH ARTICLE

# Pharmacology of erenumab in human isolated coronary and meningeal arteries: Additional effect of gepants on top of a maximum effect of erenumab

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**Funding information**
Dutch Research Council, Grant/Award Number: Vici grant 09150181910040; Novartis / Amgen

## Abstract

**Background and Purpose:** Multiple drugs targeting the calcitonin gene-related peptide (CGRP) receptor have been developed for migraine treatment. Here, the effect of the monoclonal antibody erenumab on CGRP-induced vasorelaxation was investigated in human isolated blood vessels, as well as the effect of combining erenumab with the small molecule drugs, namely rimegepant, olcegepant, or sumatriptan.

**Experimental Approach:** Concentration–response curves to CGRP, adrenomedullin or pramlintide were constructed in human coronary artery (HCA) and human middle meningeal artery (HMMA) segments, incubated with or without erenumab and/or olcegepant. $pA_2$ or $pK_b$ values were calculated to determine the potency of erenumab in both tissues. To study whether acutely acting antimigraine drugs exerted additional CGRP-blocking effects on top of erenumab, HCA segments were incubated with a maximally effective concentration of erenumab (3 μM), precontracted with KCl and exposed to CGRP, followed by rimegepant, olcegepant, or sumatriptan in increasing concentrations.

**Key Results:** Erenumab shifted the concentration-response curve to CGRP in both vascular tissues. However, in HCA, the Schild plot slope was significantly smaller than unity, whereas this was not the case in HMMA, indicating different CGRP receptor mechanisms in these tissues. In HCA, rimegepant, olcegepant and sumatriptan exerted additional effects on CGRP on top of a maximal effect of erenumab.

**Conclusions and Implications:** Gepants have additional effects on top of erenumab for CGRP-induced relaxation and could be effective in treating migraine attacks in patients already using erenumab as prophylaxis.

KEYWORDS
CGRP, erenumab, gepants, migraine, monoclonal antibodies

**Abbreviations:** AMY1, amylin receptor 1; CGRP, calcitonin gene-related peptide; CLR, calcitonin-like receptor; CTR, calcitonin receptor; HCA, human coronary artery; HMMA, human middle meningeal artery; RAMP1, receptor activity modifying protein 1.

## 1 | INTRODUCTION

Migraine is a highly disabling neurovascular disorder, characterized by a severe headache that is aggravated by physical activity, and is often accompanied by nausea, vomiting, photophobia and phonophobia (Headache Classification Committee of the International Headache Society, 2018). Migraine is classified as the second most disabling disorder worldwide in all age groups, and is the first cause of disability in people under the age of 50 years (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017; Steiner et al., 2018). During a migraine attack, the trigeminovascular system is activated, leading to the release of neuropeptides, including calcitonin gene-related peptide (**CGRP**) (Goadsby et al., 1990; Goadsby & Edvinsson, 1993). CGRP is a potent vasodilator and its infusion has been shown to induce migraine-like headache attacks in migraine patients (Hansen et al., 2010; Lassen et al., 2002). Both CGRP and its receptor have become a target for the development of novel anti-migraine medication.

Recently, two different classes of medication were developed that target CGRP or its receptor: the small-molecule CGRP receptor antagonists called *gepants* and the monoclonal antibodies targeting either CGRP (**eptinezumab**, **fremanezumab**, **galcanezumab**) or the canonical **CGRP receptor** (**erenumab**). All monoclonal antibodies have been designed for prophylactic use and significantly decreased the number of monthly migraine days in clinical trials (Yang et al., 2021). So far, three different gepants, namely **rimegepant**, **ubrogepant** and **atogepant**, have been approved by the Food and Drug Administration (FDA) for acute and/or prophylactic treatment of migraine (AbbVie Inc., 2021; Allergan plc, 2019; Biohaven Pharmaceutical Company Holding Ltd., 2020a).

Of the four monoclonal antibodies, erenumab is the only one designed to target the canonical CGRP receptor (Shi et al., 2016). Subcutaneous monthly administration of 70 mg or 140 mg of erenumab has been shown to be safe and effective for the treatment of migraine (Dodick et al., 2018; Goadsby et al., 2017), including patients who did not respond to two to four other preventive treatments, suggesting that it could be successful in difficult-to-treat patients (Reuter et al., 2018), and is approved by the FDA and European Medicines Agency (EMA) for the preventive treatment of migraine. However, multiple cases of elevated blood pressure were associated with erenumab use during postmarketing surveillance, leading to an amendment of the prescribing information, which now includes a warning for development of hypertension or worsening of pre-existing hypertension (Saely et al., 2021). In line with this warning, a recent study showed blood pressure increases after treatment with erenumab (de Vries Lentsch, van der Arend, et al., 2022). In this study, blood pressure measurements were performed every 3 months in 109 migraine patients treated with erenumab (age 42 ± 13, 85% female). Patients were included if they had eight or more migraine days per month and failed at least four migraine preventatives. Erenumab significantly increased both systolic and diastolic blood pressure at all time points (3–12 months), while no changes in blood pressure were observed in the control group.

### What is already known

- Gepants and erenumab cause efficient blockade of CGRP-induced relaxation of human arteries.
- Both treatments are effective for the acute and/or prophylactic treatment of migraine.

### What does this study add

- Gepants exert additional effects on top of a maximally effective erenumab concentration for CGRP-induced relaxation.
- Erenumab behaves differently in HCA compared with HMMA, based on the Schild plot slope.

### What is the clinical significance

- Gepants could be effective for treating migraine attacks in patients already using erenumab as prophylaxis.
- The safety of combined treatment should be further investigated.

Due to its potent vasodilatory effects, CGRP plays a protective role during both cerebral and cardiac ischemia (MaassenVanDenBrink et al., 2016) and, therefore, the consequences of blocking CGRP signalling should be examined carefully. In line with this protective role, both **olcegepant** and rimegepant were shown to aggravate cerebral ischemia after middle cerebral artery occlusion in mice (Mulder et al., 2020), and olcegepant abolished the protective effects of CGRP in the rat heart during ischemia (Chai et al., 2006).

Apart from potential vascular side effects, another concern is that not all patients respond equally well to treatment with monoclonal antibodies. Recently, **capsaicin**-induced dermal blood flow was assessed in migraine patients before and after erenumab treatment and was used as a measure of trigeminovascular activation (de Vries Lentsch, Al-Hassany, et al., 2022). Patients who showed a <50% response to erenumab showed higher trigeminovascular reactivity compared with patients with ≥50% response, which could suggest that the dose of erenumab used in these patients was not sufficient to block CGRP signalling. One option would be to increase the dose of erenumab in these patients. However, more CGRP blockade also could be achieved by using a gepant on top of the erenumab treatment. Theoretically, this temporary CGRP blockade could be more safe compared with the long-term blockade achieved with the monoclonal antibodies, because this short duration of action would diminish the chance of an ischemic attack occurring at a time when there is full blockade of the CGRP pathway, and

decrease the concern of a mild ischemic event developing into a full-blown infarct (MaassenVanDenBrink et al., 2016).

Previous ex vivo studies showed that erenumab inhibits CGRP-induced relaxation in human isolated arteries, while it did not affect the response to other vasodilators or vasoconstrictors (Ohlsson et al., 2019; Rubio-Beltrán, Labastida-Ramírez, et al., 2019). In the current study, a pharmacological characterization of the inhibition of CGRP-induced relaxation by erenumab was performed in human isolated coronary arteries and human middle meningeal arteries. Moreover, the effect of combining a gepant with erenumab was studied in the same ex vivo model. Finally, the effect of erenumab and/or gepants on the relaxation induced by agonists related to CGRP was studied, because it has been suggested that CGRP may signal via more receptors than the canonical CGRP receptor in trigeminal neuronal tissue (Walker et al., 2015) as well as in human arteries (Gupta, Mehrotra, Villalón, et al., 2006b; Haanes et al., 2016). CGRP, **adrenomedullin** and **amylin** are all part of the same family of peptides and show overlap in the receptors they can activate (Hay et al., 2018). The canonical CGRP receptor consists of calcitonin-like receptor (**CLR**), coupled to receptor activity modifying protein 1 (**RAMP1**), whereas the adrenomedullin 1 (**AM1**) receptor consists of CLR coupled to **RAMP2** and the amylin 1 (**AMY1**) receptor consists of the calcitonin receptor (**CTR**) coupled to RAMP1 (Hay et al., 2018). In the current study, the relaxation to CGRP, adrenomedullin and **pramlintide**, which is a stable amylin analogue, was investigated in the presence of erenumab and/or olcegepant.

## 2 | METHODS

### 2.1 | Patient tissue collection

Distal portions of the left anterior descending coronary artery were isolated from hearts of Dutch heart valve donors. Hearts were provided by ETB-BISLIFE (Heart Valve Department, Beverwijk, The Netherlands), following dissection of the aortic and pulmonary valves for homograft valve transplantation. Hearts were obtained from 33 Dutch postmortem donors with permission for research (20 female and 13 male donors, aged 53 ± 2 years). Donor screening and acceptance was performed by the Dutch Transplant Foundation (Leiden, The Netherlands). Immediately after circulatory arrest, the hearts were harvested, stored at 4°C in a sterile organ-protecting solution and were brought to the laboratory within 24 h of death. Coronary arteries were isolated and stored in oxygenated and carbonated (95% $O_2$/5% $CO_2$) Krebs solution (118-mM NaCl, 4.7-mM KCl, 2.5-mM $CaCl_2$, 1.2-mM $MgSO_4$, 1.2-mM $KH_2PO_4$, 25-mM $NaHCO_3$ and 8.3-mM glucose, pH = 7.4) at 4°C until the start of the experiment, as previously described (Rubio-Beltrán, Chan, et al., 2019).

Human middle meningeal arteries from 11 donors (seven female and four male, aged 53 ± 5 years) were obtained during neurosurgical procedures at the Erasmus Medical Center, Rotterdam, The Netherlands. The arteries were immersed in Medium 199 directly after isolation and stored at 4°C until transport to the lab. Next, all tissue surrounding the artery was removed and the artery was stored in cold oxygenated Krebs solution, as described before (Rubio-Beltrán, Chan, et al., 2019) (119-mM NaCl, 4.7-mM KCl, 1.25-mM $CaCl_2$, 1.2-mM $MgSO_4$, 1.2-mM $KH_2PO_4$, 25-mM $NaHCO_3$ and 11.1-mM glucose, pH = 7.4).

### 2.2 | Wire myography experiments

For functional experiments, 2-mm segments of human coronary arteries and human middle meningeal arteries were mounted in Mulvany myograph organ baths (Danish Myo Technology, Aarhus, Denmark), using Ø 40-µm stainless-steel wires. The organ baths were kept at 37°C and filled with oxygenated Krebs solution. First, the mounted vessel segments were allowed to equilibrate before being stretched to a tension normalized to 0.9 times the estimated diameter at 100-mmHg transmural pressure (Mulvany & Halpern, 1977). The tension was recorded using LabChart data acquisition (AD instruments Ltd, Oxford, UK). Then, 30-mM KCl was added to each vessel segment, after which they were washed and exposed to 100-mM KCl. The normalization phase and exposure to 30-mM KCl and 100-mM KCl was performed in all vessel segments, after which a specific protocol was performed to study (i) the effect of incubation time of erenumab on CGRP-induced relaxations, (ii) the effect of different concentrations of erenumab and (iii) the combined effect of erenumab with gepants, or erenumab with **sumatriptan**.

Vessel segments were incubated with erenumab (1 µM, provided by Amgen Inc., Thousand Oaks, CA, USA) or its vehicle for 15 to 90 min to determine the influence of incubation time, before a concentration–response curve to human αCGRP (0.01 nM–1 µM, half logarithmic steps, PolyPeptide Group) was constructed. The vessel segments were precontracted with 30-mM KCl 15 min before the start of the curve and relaxation responses to CGRP were expressed as a percentage of precontraction. In subsequent experiments a 10-nM, 100-nM, 1-µM, 3-µM or 10-µM concentration of erenumab was studied with a 30-min incubation period before start of the concentration–response curve to αCGRP.

Human coronary artery segments were incubated with 3-µM erenumab or the vehicle for erenumab overnight at 37°C, mimicking physiological conditions, to study the effect of long-term incubation with erenumab and the interaction with gepants. The next day, vessel segments were mounted into the Mulvany myograph system and precontracted with 30-mM KCl, followed by 278-nM CGRP, which is the $EC_{50}$ of CGRP in the presence of 3-µM erenumab, as determined in experiments described above (Figure 2). If vessel segments did not respond substantially to this concentration of CGRP, up to 1 µM of CGRP was added until a clear vasorelaxation could be observed. Next, rimegepant, olcegepant or sumatriptan was added in increasing concentrations to test whether these compounds could exert a direct (rimegepant and olcegepant) or indirect (physiological antagonism, sumatriptan) CGRP-blocking effect on top of erenumab. For rimegepant, first the $C_{max}$ that is reached after a 75-mg dose of rimegepant corrected for the 96% plasma protein binding of this drug (58.7 nM) was tested (Biohaven

Pharmaceutical Company Holding Ltd, 2020b; Conway, Dubowchik, et al., 2019), followed by the $C_{max}$ that was not corrected for plasma protein binding (1.47 μM), followed by a higher concentration of 10 μM. For olcegepant and sumatriptan, the $C_{max}$ (240 and 160 nM, respectively) was not corrected for plasma protein binding, as this is unknown for olcegepant, and for sumatriptan, the free $C_{max}$ (132 nM) is in the same order of magnitude as the $C_{max}$ corrected for plasma protein binding (Grande et al., 2014; Iovino et al., 2004).

To validate the current experimental set-up, we studied the effect of acutely administering 3-μM erenumab after relaxation to CGRP and explored whether adding an additional 3 μM of erenumab to a segment already incubated with 3-μM erenumab could further inhibit the vasodilatory responses to CGRP. After adding erenumab, the first concentration of gepant was added after at least 30 min and only when a stable plateau was reached. Subsequent concentrations were added after a stable plateau was reached as response to the previous compound. Moreover, vessel segments incubated at 4°C, which is the standard method for storing tissues overnight, were included as a control condition to determine the effect of incubation at 37°C on the vessel segments.

In addition, full concentration–response curves to CGRP, adrenomedullin and pramlintide, were constructed in both human coronary arteries and human middle meningeal arteries in the presence of erenumab (1–3 μM) and/or olcegepant (1 μM) to investigate whether olcegepant can exert additional effects on top of erenumab for these three different agonists. Full concentration–response curves were performed in the presence of the gepant olcegepant, due to its commercial availability. Subsequently, rimegepant became available and additional experiments were performed using this gepant, because it has been approved for use in patients and, therefore, our results might be more easily translatable to clinical practice.

## 2.3 | Data analysis

For data analysis, sigmoidal curves were constructed using a four parameter (variable slope) nonlinear regression analysis using Prism 8 (GraphPad Software, San Diego, CA, USA), from which a $pEC_{50}$ value was obtained. For incomplete concentration–response curves, the $pEC_{50}$ was calculated by constraining the bottom to zero and the top to the $E_{max}$ of the control curve to the agonist of interest in absence of an antagonist, with a maximum of 100% relaxation. If the calculated $EC_{50}$ was above the highest concentration of agonist used for that experiment, the $pEC_{50}$ value is presented as *smaller than highest concentration of agonist* (<6 or <5.5). Dose ratios were calculated using the $pEC_{50}$ values for the different concentrations of erenumab and its vehicle and plotted in a Schild plot. Linear regression was used to obtain the corresponding $pA_2$ value of erenumab in the human middle meningeal artery or $pK_b$ values for each of the concentrations of erenumab used in human coronary arteries. For a comparison between two or more groups, a paired *t* test (two groups) or one-way ANOVA (more than two groups) was performed, with a post hoc test only if *F* was significant. The threshold of significance was defined as $P < 0.05$. Data and statistical analyses comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis (Curtis et al., 2018). All data is presented as mean ± SEM.

## 2.4 | Materials

Human αCGRP was obtained from PolyPeptide Group (Baar, Switzerland). Rimegepant and olcegepant were purchased from MedChemExpress (Monmouth Junction, USA). Sumatriptan was obtained from Sigma-Aldrich (Saint Louis, USA). Erenumab was provided by Amgen.

## 2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in https://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2021).

# 3 | RESULTS

## 3.1 | Increasing the incubation time of erenumab does not lead to further inhibition of CGRP-induced relaxation

In the first set of experiments, the influence of incubation time of erenumab before starting the concentration–response curve to CGRP was studied, to achieve stable inhibition (equilibrium reached) of the CGRP receptor. For human coronary arteries, incubation times of 15, 30, 45, 60, and 90 min were used. All incubation times led to a similar shift of the concentration–response curve to CGRP and differed significantly from control, based on its $pEC_{50}$ value, while no differences were observed between the different incubation times (Figure 1a; Table 1). For the human middle meningeal artery, a 30-min incubation time, which is the time that is usually used for antagonist incubation, and a 90-min incubation time, the longest in this experiment, were compared. Again, no differences were observed between the $pEC_{50}$ values of a 30-min or 90-min incubation, while both conditions differed significantly from the control (Figure 1b; Table 1). Considering that no differences in shift of the concentration–response curve to CGRP were observed after the different incubation times in each of the assayed tissues for up to 90 min, follow-up experiments were performed using an incubation time of 30 min, which is the standard incubation time for antagonists in these types of experiments.

## 3.2 | Erenumab behaves differently in human coronary arteries versus human middle meningeal arteries

The effect of different concentrations of erenumab was investigated in the different vascular tissues. Distal coronary artery segments of

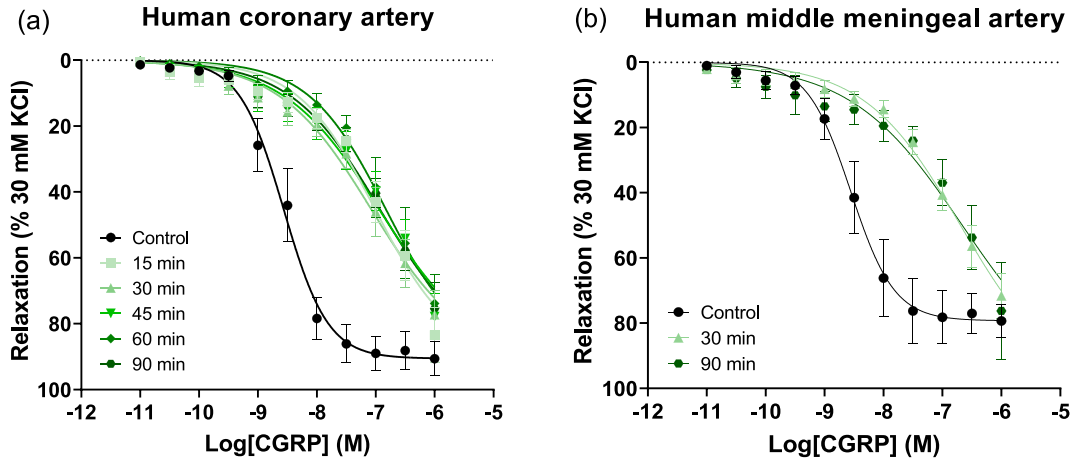


**FIGURE 1** The effect of different incubation times of 1-μM erenumab on the concentration–response curve to CGRP in human isolated arteries. (a) Results in the human coronary artery. (b) Results in the human middle meningeal artery.

**TABLE 1** $pEC_{50}$ values of CGRP-induced relaxation of human coronary arteries after different incubation times of erenumab.

| | Human coronary artery | | Human middle meningeal artery | |
|---|---|---|---|---|
| | $pEC_{50}$ ± SEM | Number | $pEC_{50}$ ± SEM | Number |
| Control | 8.53 ± 0.12 | 8 | 8.49 ± 0.17 | 5 |
| 15 min | 6.77 ± 0.23 | 6 | - | - |
| 30 min | 6.85 ± 0.22 | 8 | 6.74 ± 0.18 | 5 |
| 45 min | 6.77 ± 0.23 | 6 | - | - |
| 60 min | 6.59 ± 0.22 | 6 | - | - |
| 90 min | 6.78 ± 0.11 | 7 | 6.62 ± 0.32 | 4 |

five heart valve donors and middle meningeal artery segments from five individuals were incubated with 10-nM, 100-nM, 1-μM, 3-μM erenumab or vehicle. Each increasing concentration shifted the concentration–response curve to CGRP further to the right in both coronary arteries (Figure 2a; Table 2) and middle meningeal arteries (Figure 2b; Table 2). The $pEC_{50}$ values of the concentration–response curves were used to calculate a dose-ratio between each concentration of erenumab and its vehicle. Subsequently, a Schild plot was constructed, which is often used for characterization of small molecular receptor antagonists, but also has been used for characterization of antibodies targeting a receptor (Issafras et al., 2014; Minter et al., 2013; Ravn et al., 2013). For the human coronary artery, the slope of the Schild plot was significantly smaller than unity (0.61 ± 0.14). Therefore, a $pA_2$ value could not be calculated as an estimate of the potency of erenumab in this tissue. Instead, individual $pK_b$ values were calculated for each of the erenumab concentrations (10 nM: 8.66 ± 0.23, 100 nM: 8.38 ± 0.23, 1 μM: 7.81 ± 0.34, 3 μM: 7.79 ± 0.32). For the human middle meningeal artery, the slope of the Schild plot did not differ from unity (0.86 ± 0.17). Therefore, the $pA_2$ value of 8.20 ± 0.29 provides an accurate estimate of the potency of erenumab in the middle meningeal artery.

### 3.3 | Gepants exert additional effects on top of a maximally effective erenumab concentration for CGRP-induced relaxation

Next, we established that 3 μM of erenumab caused a maximum shift in the concentration–response curve to CGRP. Increasing the erenumab concentration to 10 μM did not provide more inhibition (Figure 3a). Therefore, the concentration of 3-μM erenumab was used to study the interaction of erenumab with gepants, to investigate whether gepants on top of erenumab could provide further inhibition (Figure 3b). Human coronary artery segments were obtained from nine donors. Overnight incubation of erenumab was used as a proxy for prophylactic treatment with erenumab and was combined with the acute administration of a gepant. In these experiments, overnight incubation with 3-μM erenumab led to a decreased response to the concentration of CGRP used for these experiments ($n = 9$; $n = 7$ for 278 nM, which is the $pEC_{50}$ of CGRP in the presence of 3-μM erenumab, and $n = 2$ for 1 μM of CGRP, because two tissues did not show a substantial response to the lower concentration of 278 nM). Rimegepant (Figure 3c), olcegepant (Figure 3d) and sumatriptan (Figure 3e) all reversed the relaxation to CGRP in segments incubated overnight with 3-μM erenumab. The lowest concentration of

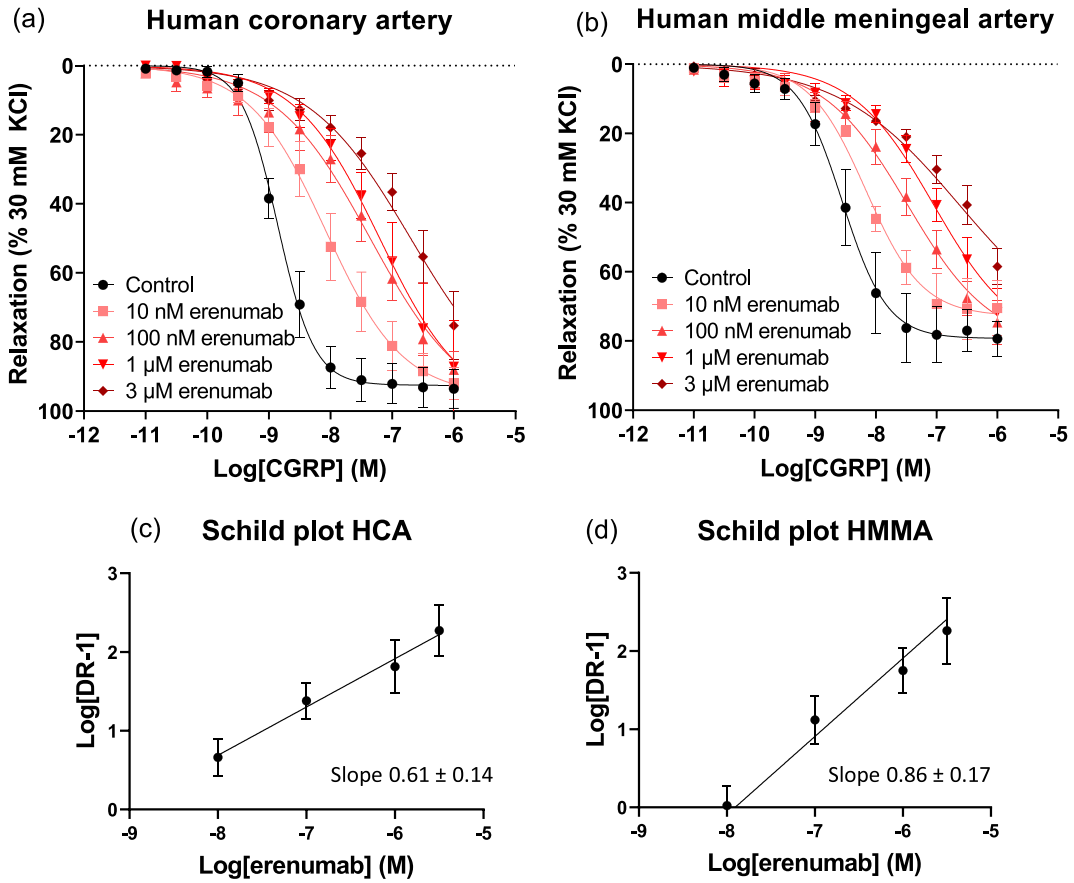


**FIGURE 2** The effect of different concentrations of erenumab on CGRP-induced relaxation of human isolated arteries. Concentration–response curve to CGRP in the presence of 10-nM, 100-nM, 1-μM or 3-μM erenumab or its vehicle in (a) human coronary artery (n = 5) or (b) human middle meningeal artery (n = 5) and their corresponding Schild plots (c and d).

**TABLE 2** $pEC_{50}$ values of CGRP-induced relaxation of human isolated coronary arteries and middle meningeal arteries in the presence of different concentrations of erenumab.

| | Human coronary artery | | Human middle meningeal artery | |
|---|---|---|---|---|
| | $pEC_{50}$ ± SEM | Number | $pEC_{50}$ ± SEM | Number |
| Control | 8.84 ± 0.07 | 5 | 8.50 ± 0.17 | 5 |
| 10 nM | 8.05 ± 0.16 | 5 | 8.22 ± 0.14 | 5 |
| 100 nM | 7.43 ± 0.23 | 5 | 7.31 ± 0.11 | 5 |
| 1 μM | 7.01 ± 0.38 | 5 | 6.74 ± 0.18 | 5 |
| 3 μM | 6.56 ± 0.36 | 5 | 6.23 ± 0.31 | 5 |

rimegepant ($C_{max}$ corrected for plasma protein binding) did not consistently reverse relaxation to CGRP in human tissues that were incubated with 3-μM erenumab (% of contraction to KCl after CGRP: 59 ± 6, and response after 58.7-nM rimegepant: 64 ± 7), while the higher concentrations of rimegepant significantly reversed the relaxation to CGRP (1.47 μM: 86 ± 8 and 10 μM: 85 ± 9). Both concentrations of olcegepant (% of contraction to KCl after CGRP: 58 ± 6; 240-nM olcegepant 86 ± 7; 1-μM olcegepant 90 ± 7) and sumatriptan (CGRP: 51 ± 6; 160-nM sumatriptan: 81 ± 5; 1-μM sumatriptan: 119 ± 7) reversed the relaxation to CGRP in a concentration-dependent manner.

### 3.4 | Validation of experimental set-up

For the experiments with a single concentration of CGRP, multiple control conditions were included to validate the experimental set-up (Figure S1B). Similar responses were observed in the segments incubated at 37°C, mimicking physiological conditions, versus 4°C, the standard method of storing tissues overnight, for relaxation to CGRP (Figure S1C and S1D). Moreover, all concentrations of rimegepant significantly reversed the relaxation to CGRP. Furthermore, the effect of acutely administering 3-μM erenumab after relaxation to CGRP was studied, to determine whether the differences that were observed

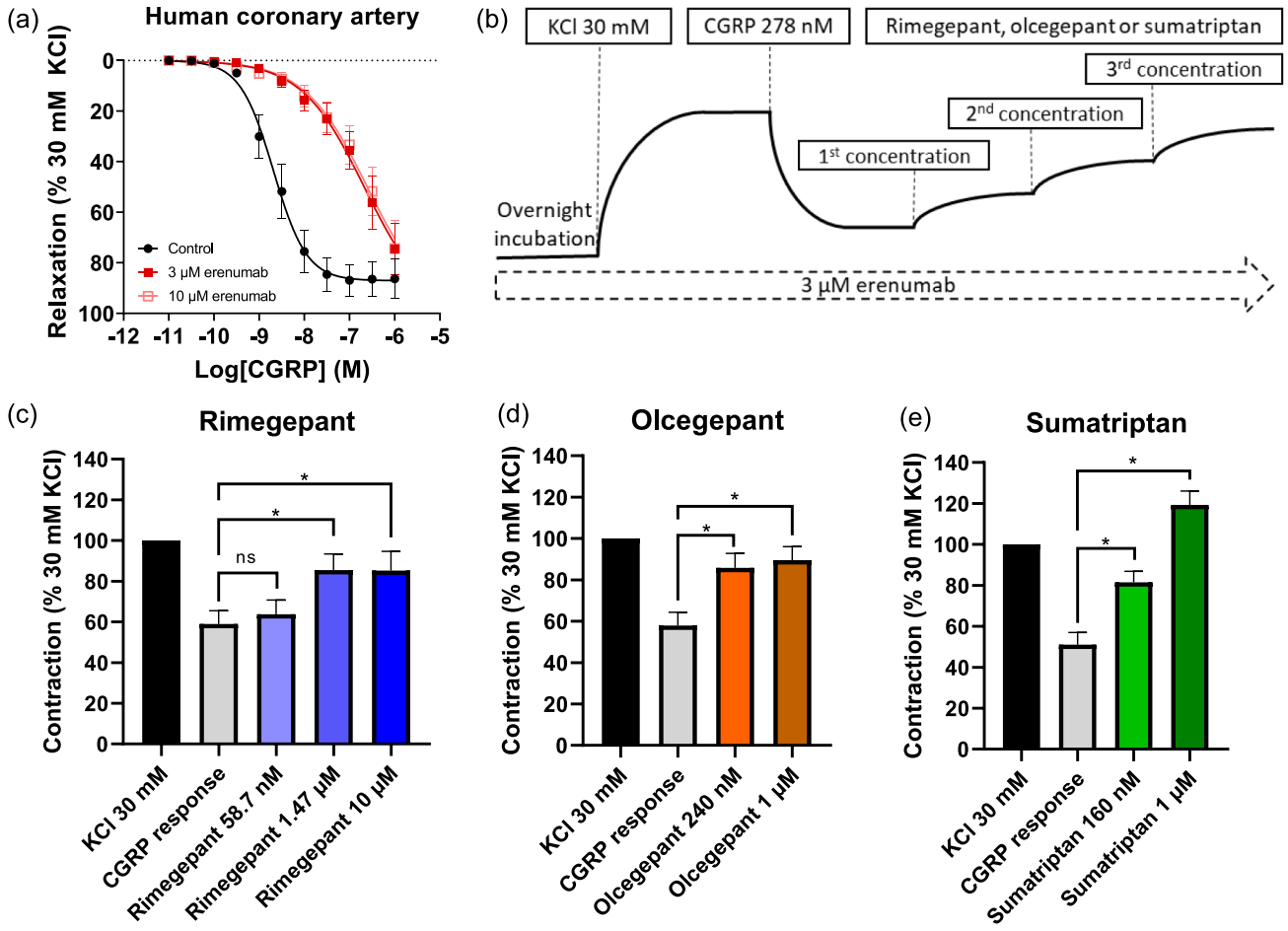


**FIGURE 3** Interaction of erenumab with gepants. (a) 3 μM of erenumab causes a maximal shift in the concentration–response curve to CGRP (n = 5). (b) Schematic example of the changes in force generated by the vessel segment in response to KCl, CGRP and rimegepant, olcegepant or sumatriptan in human coronary arteries incubated overnight with 3-μM erenumab. (c) The response to CGRP and subsequent response to increasing concentrations of rimegepant after overnight incubation with 3-μM erenumab (n = 9). (d) The response to CGRP and subsequent response to increasing concentrations of olcegepant after overnight incubation with 3-μM erenumab (n = 8). (e) The response to CGRP and subsequent response to increasing concentrations of sumatriptan after overnight incubation with 3-μM erenumab (n = 7).

with gepants on top of erenumab were caused by the experimental setup, and to study whether adding an extra 3 μM of erenumab on top of overnight incubation with 3-μM erenumab would be able to further inhibit the vasodilatory responses to CGRP. Using this different experimental setup, rimegepant was still able to exert its additional effect on top of an acute dose of erenumab (Figure S1E) (% of contraction to KCl after CGRP: 19 ± 8; 3-μM erenumab: 55 ± 11; 58.7-nM rimegepant: 58 ± 8; 1.47-μM rimegepant: 76 ± 7; 10-μM rimegepant: 73 ± 6), whereas more erenumab on top of the overnight incubation with 3-μM erenumab only induced a small effect in two out of seven experiments, with no response in the other 5 (Figure S1F) (% of contraction to KCl after CGRP: 54 ± 3; 3-μM erenumab: 53 ± 4; 58.7-nM rimegepant 57 ± 5; 1.47-μM rimegepant: 71 ± 6; 10-μM rimegepant: 72 ± 7).

To confirm that the additional effect of a gepant on top of erenumab was not influenced by incubation time, full concentration–response curves to CGRP were constructed in the presence of 3-μM erenumab and/or 1-μM olcegepant, comparing a 30-min incubation with erenumab with an overnight incubation. The relaxation to CGRP was similar after both incubation times ($pEC_{50}$ 6.34 ± 0.18 and 6.06 ± 0.18 for 30 min and overnight incubation, respectively). In both conditions, olcegepant had significant additional effects on top of erenumab (Figure S1A, $pEC_{50}$ 5.36 ± 0.09 and 5.11 ± 0.27 for 30 min incubation and overnight incubation, respectively).

## 3.5 | Additional effects of gepants on top of erenumab are absent for relaxation to adrenomedullin or pramlintide

The effect of 3-μM erenumab and 3-μM erenumab combined with 1-μM olcegepant on the inhibition of the concentration–response curves to CGRP (n = 7), adrenomedullin (n = 9) and pramlintide (n = 7) was assessed in human coronary arteries (Figure 4; Table 3). For CGRP, 3 μM of erenumab significantly shifted the concentration–response curve ($pEC_{50}$: 8.70 ± 0.08 and 6.65 ± 0.12,

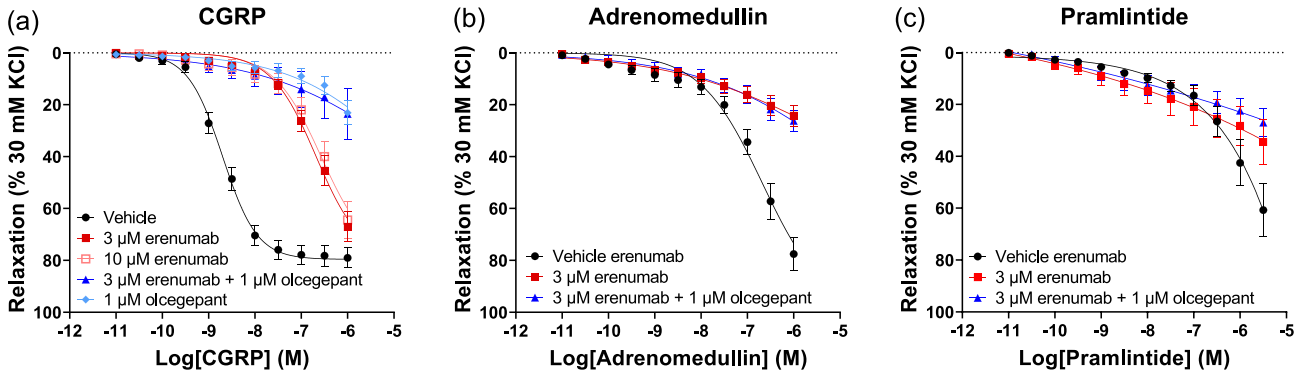


**FIGURE 4** The effect of olcegepant on top of erenumab on CGRP-, adrenomedullin- and pramlintide-induced relaxation of human coronary arteries. Concentration–response curve to (a) CGRP (n = 6–7), (b) adrenomedullin (n = 9) or (c) pramlintide (n = 7), in the presence of vehicle, erenumab and/or olcegepant.

**TABLE 3** $pEC_{50}$ values and $E_{max}$ of CGRP-, adrenomedullin- and pramlintide-induced relaxation of human isolated coronary arteries in the presence of erenumab and/or olcegepant.

| Human coronary arteries | CGRP | | Adrenomedullin | | Pramlintide | |
|---|---|---|---|---|---|---|
| Condition | $pEC_{50}$ ± SEM | Sign dif from vehicle | $pEC_{50}$ ± SEM | Sign dif from vehicle | $pEC_{50}$ ± SEM | Sign dif from vehicle |
| Vehicle erenumab | 8.70 ± 0.08 | - | 6.52 ± 0.25 | - | 5.78 ± 0.33 | - |
| 3-μM erenumab | 6.654 ± 0.12 | * | <6 | * | <5.5 | ns |
| 3-μM erenumab + 1-μM olcegepant | <6 | * | <6 | * | <5.5 | * |
| Condition | $E_{max}$ ± SEM[a] | Sign dif from vehicle | $E_{max}$ ± SEM[a] | Sign dif from vehicle | $E_{max}$ ± SEM[a] | Sign dif from vehicle |
| Vehicle erenumab | 79.01 ± 3.86 | - | 77.55 ± 6.21 | - | 60.71 ± 10.24 | - |
| 3-μM erenumab | 67.04 ± 5.76 | ns | 24.22 ± 3.93 | * | 33.06 ± 7.66 | ns |
| 3-μM erenumab + 1-μM olcegepant | 23.52 ± 9.95 | * | 26.22 ± 3.91 | * | 26.99 ± 5.30 | * |

[a]For $E_{max}$, the response to the highest concentration of agonist (1 μM or 3 μM) is used.

for vehicle and 3-μM erenumab, respectively; Figure 4a). Increasing the concentration of erenumab to 10 μM did not induce a further shift of the concentration–response curve ($pEC_{50}$: 6.56 ± 0.12), while 1 μM of olcegepant on top of 3-μM erenumab significantly shifted the curve further ($pEC_{50}$ < 6), with a $pEC_{50}$ not different from that of olcegepant alone. In contrast, olcegepant did not induce any further shift on top of 3-μM erenumab for adrenomedullin or pramlintide (Table 3). However, 3 μM of erenumab did significantly shift the curve to adrenomedullin and decreased the response to 1 μM of adrenomedullin, the highest concentration used of this agonist (apparent $E_{max}$: 78 ± 6 and 24 ± 4 for vehicle and 3-μM erenumab, respectively, Figure 4b). For pramlintide, 3-μM erenumab seemed to shift the curve to the right, but these results only reached significance for the curves with erenumab and olcegepant ($pEC_{50}$ 5.78 ± 0.33 for vehicle and <5.5 for 3-μM erenumab and 3-μM erenumab + 1-μM olcegepant; Figure 4c). Similarly, only the condition with 3-μM erenumab and 1-μM olcegepant csignificantly decreased the response to the maximum concentration of pramlintide (apparent $E_{max}$: 61 ± 10, 33 ± 8 and 27 ± 5 for vehicle, 3-μM erenumab and 3-μM erenumab + 1-μM olcegepant, respectively). However, the $pEC_{50}$ and maximum response did not differ significantly between the 3-μM erenumab and 3-μM erenumab plus 1-μM olcegepant conditions.

In human middle meningeal arteries obtained from six donors, 3-μM erenumab significantly shifted the concentration–response curve to CGRP ($pEC_{50}$: 8.20 ± 0.05 and 6.78 ± 0.13 for vehicle and 3-μM erenumab, respectively) and olcegepant shifted the curve even further ($pEC_{50}$ < 6, Figure S2A and Table S1); 10-μM erenumab ($pEC_{50}$: 6.60 ± 0.52) did not shift the curve compared with 3-μM erenumab, confirming that 3 μM was causing a maximum shift. The response to the maximum concentration of CGRP used in these experiments (1 μM) was significantly decreased in the segments incubated with 3-μM erenumab (apparent $E_{max}$: 64 ± 9 and 53 ± 9, for vehicle and 3-μM erenumab, respectively) and 1 μM of olcegepant on top of 3-μM erenumab decreased the maximum response even further (apparent $E_{max}$: 29 ± 8). The shift of the concentration–response curve to adrenomedullin by 1 μM of erenumab ($pEC_{50}$: 6.39 ± 0.39 and <6 for vehicle and erenumab, respectively), and the decrease in response to the maximum concentration of adrenomedullin (apparent $E_{max}$: 29 ± 7 and 11 ± 3 for vehicle and erenumab respectively; Figure S2B) did not reach significance. Olcegepant did not have an effect on top of erenumab ($pEC_{50}$ < 6 and apparent $E_{max}$: 14 ± 4). For

pramlintide, experiments using 1 μM of erenumab and 3 μM of erenumab were combined, as olcegepant did not induce a further shift on top of either of these concentrations of erenumab (Figure S2C). However, for the experiments with pramlintide, only two out of six human tissues showed a quantifiable response (>20%) to this agonist. In these two tissues, erenumab shifted the curve to pramlintide, while no further shift was observed after olcegepant. Statistical tests were not performed due to the low number.

## 4 | DISCUSSION

Erenumab induced a rightward shift of the concentration–response curve to CGRP, with higher concentrations of erenumab up to 3 μM inducing a larger shift. A Schild plot was constructed for both vascular tissues used, resulting in a Schild plot slope significantly smaller than unity in the coronary arteries and a slope not different from one in meningeal arteries. Apart from methodological errors, a slope different from unity can be caused by (i) hemiequilibrium conditions, (ii) noncompetitive type of antagonism or (iii) involvement of multiple receptors (Kenakin, 1982). No differences were observed between 30 and 90 min, or overnight incubation with erenumab, and therefore equilibrium conditions are expected to be reached at the start of the concentration–response curve to CGRP. If erenumab was a noncompetitive antagonist, a decrease in maximum response ($E_{max}$) would have been expected, at least for higher concentrations of antagonist, when the receptor reserve is bound as well. Here, the $E_{max}$ did not differ between the conditions with or without increasing concentrations of erenumab. Thus, erenumab was assumed to act like a competitive antagonist, as suggested before (Ohlsson et al., 2019). However, it is important to note that the relaxation curves to CGRP did not reach a maximum response in the presence of erenumab, so the assumption that the $E_{max}$ is not affected by erenumab is based on the nonlinear regression model fits, which show an equal or even higher $E_{max}$ compared with the control curve, in contrast to a decrease in $E_{max}$ that would be expected for noncompetitive antagonism. Another explanation for the Schild plot slope different from one is the involvement of multiple receptors. This possibility implied that different receptors are expressed in these different vascular beds, for example, the co-expression of the canonical CGRP receptor with amylin-1 (AMY1) receptor in human coronary arteries, as has been suggested before (Haanes et al., 2016). CGRP is equipotent with amylin at the AMY1 receptor (Hay et al., 2018); thus, the vasodilatory responses to CGRP could be mediated via the activation of AMY1 receptors. In line with this hypothesis, a Schild plot slope <1 in human coronary arteries has been observed for some of the gepants (i.e., atogepant, rimegepant and olcegepant) (Haanes et al., 2016; Mulder et al., 2020; Rubio-Beltrán, Chan, et al., 2019) and both competitive and noncompetitive antagonism of olcegepant were observed depending on the vascular bed that was studied (Edvinsson et al., 2002; Gupta, Mehrotra, Villalón, et al., 2006b; Sheykhzade et al., 2004).

In the current series of experiments, the potency of erenumab in human middle meningeal arteries and human coronary arteries did not differ, because the $pA_2$ value for human middle meningeal arteries is within the range of $pK_b$ values of human coronary arteries, with the smallest potency difference between the two tissues around 100 nM of erenumab, which is a clinically relevant concentration ($C_{max}$ 15.8 $\mu g \cdot ml^{-1}$, equal to 105 nM, after 140-mg erenumab SC; Amgen Inc, 2021). Ideally, a CGRP receptor blocker should be more potent in the meningeal vasculature compared with the coronary circulation, so that it effectively reduces migraine headaches while limiting cardiovascular side effects. Some of the gepants, specifically atogepant, olcegepant and rimegepant, have been shown to be more potent in human middle meningeal arteries compared with coronary arteries when $pK_b$ or $pA_2$ values obtained from experiments in these tissues were compared (Gupta, Mehrotra, Avezaat, et al., 2006; Gupta, Mehrotra, Villalón, et al., 2006b; Mulder et al., 2020; Rubio-Beltrán, Chan, et al., 2019).

Interestingly, a maximum effect of erenumab on inhibiting CGRP-induced relaxation is observed for concentrations of 3 μM or higher. This observation does not match the classical pharmacology of competitive antagonists. Therefore, two major explanations remain, namely, that erenumab is not actually a competitive antagonist, which seems unlikely because the $E_{max}$ is not affected by increasing the concentration of erenumab, or that experimental limitations are causing this phenomenon of a maximum effect of erenumab on CGRP-induced relaxation. For example, monoclonal antibodies have been described to form aggregates in a concentration-dependent manner, possibly limiting therapeutic effects at high concentrations (van der Kant et al., 2017). Increased exposure to air-liquid interfaces, which can be induced by continuous bubbling as is done in our experimental set-up, also results in increased aggregation (Sreenivasan et al., 2021). Theoretically, high concentrations of monoclonal antibody could result in increased aggregation. However, no visible aggregation was present for concentrations up to 10 μM of erenumab in our experimental setup. In addition, considering that the slope of the Schild plot does not differ from unity in human middle meningeal arteries, we do not believe possible aggregation interferes with our results up to 3 μM of erenumab, although we cannot exclude this for concentrations higher than 3 μM. Alternatively, whereas erenumab is assumed to behave as a competitive antagonist because the $E_{max}$ is not affected and the Schild plot slope in meningeal artery does no differ from unity, the nature of antagonism might change at higher concentrations. Possibly, the high concentrations of CGRP that are used to induce relaxation in the presence of high concentrations of erenumab might result in nonspecific relaxations that are mediated via other receptors, which are not inhibited by erenumab. Then, the simple competitive antagonism between an agonist and antagonist competing for a single receptor would change due to activation of a second receptor by the agonist. In the case of CGRP, it is 1.5–2.5 log units less potent at other receptors within the CGRP receptor family, such as the AM1, **AM2**, CTR, **AMY2** or **AMY3** receptors, compared with the potency of CGRP at the canonical CGRP receptor or AMY1 receptor (Hay et al., 2018), and could thus mediate the effects of CGRP at high concentrations. Increasing the concentration of erenumab further would then not result in more inhibition, because CGRP can induce vasodilation via other receptors that are not blocked.

Sumatriptan significantly reversed the vasodilatory responses to CGRP when administered on top of erenumab, which was expected because it activates different receptors (**5-HT$_{1B}$**), resulting in vasoconstriction (Chan et al., 2014; MaassenVanDenBrink et al., 1998; Nilsson et al., 1999), and was included in the current study as a positive control. Rimegepant and olcegepant lack this vasoconstrictor potential (Conway, Croop, et al., 2019; Petersen et al., 2005), but both exert additional effects on top of erenumab and achieve more CGRP blockade. Theoretically, their effects should be similar, as they both target the canonical CGRP receptor at the CGRP binding site. Erenumab binds to 18 residues of CLR and five residues of RAMP1, of which seven overlap with binding sites of CGRP (Garces et al., 2020). The small-molecule gepants bind to less residues at the CGRP receptor, which largely overlap with the binding sites of erenumab, including six of eight binding sites of telcagepant (Garces et al., 2020). Even though the interface between erenumab and the CGRP receptor is much larger compared with the gepants, this does not necessarily result in a higher affinity of erenumab for the canonical CGRP receptor. When comparing the dissociation constant $K_d$, erenumab seems to have a higher affinity compared with telcagepant ($K_d = 56$ pM for erenumab, $K_d = 1.9$ nM for telcagepant), whereas it does not differ much from olcegepant ($K_d = 45$ pM) (Bussiere et al., 2019; Moore et al., 2009; Schindler & Doods, 2002).

The concentration of erenumab that was used to study the combined effect with gepants was around 30 times higher compared with the $C_{max}$ after subcutaneous administration of 140 mg (Amgen Inc., 2021). However, local drug concentrations could differ from systemic concentrations and therefore this concentration could still be clinically relevant. For the gepants, multiple concentrations around the $C_{max}$ were included.

Beside possible experimental limitations, an explanation for the effect of the gepants on top of erenumab could be that gepants target additional receptors. Rimegepant and olcegepant show only poor affinity for the adrenomedullin 1 (CLR-RAMP2) and adrenomedullin 2 (CLR-RAMP3) receptors (Hay et al., 2003, 2006; Pan et al., 2020), which are part of the **calcitonin receptor family** (Hay et al., 2018). However, rimegepant antagonism at the AMY1 receptor (CTR-RAMP1) was only 17- to 30-fold lower compared with at the CGRP receptor (CLR-RAMP1), as measured in transfected Cos7 cells (Pan et al., 2020), and clinically relevant concentrations of rimegepant are in the range of AMY1 receptor binding in vitro whereas for olcegepant antagonism at the AMY1 receptor was approximately 150-fold lower compared with at the CGRP receptor (Hay et al., 2006), suggesting that gepants could exert an effect on receptors other than the canonical CGRP receptor. Emerging data suggest that erenumab can target the AMY1 receptor (Bhakta et al., 2021; Garelja et al., 2022), although the exact potency for inhibition of CGRP should be confirmed in future studies.

A second explanation for the additional effect of the gepants on top of erenumab could be that gepants target the CGRP receptor in a different manner, for example after it has been internalized. The CGRP receptor internalizes after binding of CGRP (Gingell et al., 2020; Kuwasako et al., 2000; Manoukian et al., 2019), and endosomal signalling of CGRP is suggested to mediate pain transmission (Yarwood et al., 2017). A hypothesis could be that the gepants, as small-molecule antagonists, target the CGRP receptor when it is already in the endosomes, and can thereby provide additional inhibition on top of erenumab. Indeed, rimegepant was shown to have a high passive permeability (190–320 nm·s$^{-1}$) as measured in a PAMPA assay (Luo et al., 2012) and is in class 2 of the Biopharmaceutics Classification System (BCS), suggesting low solubility and high permeability (European Medicines Agency, 2022). However, future research should further investigate this hypothesis.

The different receptors in the calcitonin family of receptors show distinct internalization patterns as demonstrated by cell culture experiments. Whereas the canonical CGRP receptor clearly internalizes, the AMY1 receptor shows limited internalization after stimulation with CGRP (Gingell et al., 2020). However, erenumab induces internalization of both the CGRP receptor and the AMY1 receptor, even in the absence of CGRP (Bhakta et al., 2021). It is currently unknown if and how gepants affect receptor internalization. Further research should elucidate the exact mechanism of how gepants exert their additional effect on CGRP blockade on top of erenumab.

Interestingly, in the current study, olcegepant did not induce additional effects on top of erenumab for the agonists adrenomedullin and pramlintide, whereas it induced larger inhibition of CGRP when administered on top of erenumab. However, because both adrenomedullin and pramlintide are less potent than CGRP, the concentration–response curves did not reach a plateau at the highest concentration used. Higher concentrations would be needed to draw definitive conclusions on whether gepants could have additional effects on top of erenumab for the agonists adrenomedullin and pramlintide, which is unfortunately not feasible in our experimental set-up. If an additional effect is indeed not present for adrenomedullin or pramlintide, this could suggest that different receptor populations mediate responses to CGRP, adrenomedullin and pramlintide in human isolated coronary arteries. In human middle meningeal arteries, the shift of the concentration–response curves to pramlintide and adrenomedullin by erenumab did not reach significance. This could be explained by limitations in the experimental set-up, in which the maximum concentration of agonists used did not induce a large enough relaxation. Moreover, only two out of six human tissues showed a quantifiable response to pramlintide. Experiments were not repeated with higher concentrations of agonists in meningeal arteries due to scarcity of the tissue. It cannot be concluded whether olcegepant exerts additional effects on top of erenumab in this tissue.

Because gepants can exert an effect on top of erenumab, their combined use could be beneficial for migraine treatment. However, combined use also could result in additional safety concerns, considering the protective role of CGRP during ischemia (MaassenVanDenBrink et al., 2016). Recently, the safety of combining ubrogepant with monoclonal antibodies erenumab or galcanezumab was investigated (Jakate et al., 2021). The pharmacokinetic profile of ubrogepant was not affected, and no safety concerns were observed after combining the two different types of drugs, with comparable adverse events as observed in studies with the individual drugs and no effect on blood pressure, ECGs or other vital signs. However, the follow-up was short and no participants with cardiovascular disease or hypertension were

enrolled in the study. Another study showed that rimegepant use in addition to preventive treatment with erenumab, galcanezumab or fremanezumab was well tolerated and did not lead to an increase in adverse events (Berman et al., 2020).

## 5 | CONCLUSIONS

The experiments performed in this study have resulted in two main conclusions. First of all, blockade of CGRP-induced relaxation by erenumab differs between human coronary arteries and human middle meningeal arteries, based on the slope of the Schild plot. Interestingly, a similar phenomenon is observed for some of the gepants. However, at a clinically relevant concentration, erenumab seems equally potent in both tissues. Second, both rimegepant and olcegepant can exert additional effects on top of a maximum shifting concentration of erenumab and achieve more CGRP blockade, which could also be relevant for combining gepants and erenumab for clinical use.

## DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

### CONFLICT OF INTEREST STATEMENT

TdV, ERB, AvdB, RD and AHJD declare no conflict of interest. JS is employed by Novartis. JB is employed by Amgen. AMvdB received personal fees (fees as advisor or speaker, consultancy, or any other) from Allergan-Abbvie, Lilly, Novartis and Teva. She received research support from Novartis, Satsuma and Tonix, as well as independent research support from the Dutch Research Council and the Netherlands Organisation for Health Research and Development.

### AUTHOR CONTRIBUTIONS

**Tessa de Vries:** Conceptualization; formal analysis; investigation; visualization; writing—original draft. **Eloísa Rubio-Beltrán:** Investigation; writing—review and editing. **Antoon van den Bogaerdt:** Resources; writing—review and editing. **Ruben Dammers:** Resources; writing—review and editing. **Alexander A.H. Danser:** Conceptualization; writing—review and editing. **Josefin Snellman:** Conceptualization; writing—review and editing. **Jeanine Bussiere:** Conceptualization; writing—review and editing. **Antoinette MaassenVanDenBrink:** Conceptualization; funding acquisition; supervision; writing—review and editing.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**How to cite this article:** de Vries, T., Rubio-Beltrán, E., van den Bogaerdt, A., Dammers, R., Danser, A. H. J., Snellman, J., Bussiere, J., & MaassenVanDenBrink, A. (2024). Pharmacology of erenumab in human isolated coronary and meningeal arteries: Additional effect of gepants on top of a maximum effect of erenumab. *British Journal of Pharmacology*, 1–14. https://doi.org/10.1111/bph.16322