TITLE PAGE

Full title: Resolvin E1, resolvin D1 and resolvin D2 inhibit constriction of rat thoracic aorta and human pulmonary artery induced by the thromboxane mimetic U46619.

Running title: Resolvins and smooth muscle contraction

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ABSTRACT

Background and Purpose: Omega-6 fatty acid-derived lipid mediators such as prostanoids, thromboxane and leukotrienes have well-established roles in regulating both inflammation and smooth muscle contractility. Resolvins are derived from omega-3 fatty acids and have important roles in promoting the resolution of inflammation, but their activity on smooth muscle contractility is unknown. We investigated whether resolvin E1 (RvE1), resolvin D1 (RvD1) and resolvin D2 (RvD2) can modulate contractions of isolated segments of rat thoracic aorta (RTA) or human pulmonary artery (HPA) induced by the α_1-adrenoceptor agonist phenylephrine or the stable thromboxane A_2 mimetic U46619.

Experimental Approach: Contractile responses in RTA and HPA were measured using wire myography. Receptor expression was investigated by immunohistochemistry.

Key Results: Constriction of RTA segments by U46619, but not by phenylephrine, was significantly inhibited by pretreatment for 1 or 24 hours with 10-100 nmol/L RvE1, RvD1 or RvD2. The inhibitory effect of RvE1 was partially blocked by a chemerin receptor antagonist (CCX832). RvE1 at only 1-10 nmol/L also significantly inhibited U46619-induced constriction of HPA segments, and the chemerin receptor, GPR32 and FPR2/ALX were identified in HPA smooth muscle.

Conclusion and Implications: These data suggest that resolvins or their mimetics may prove useful novel therapeutics in diseases such as pulmonary arterial hypertension, which are characterised by increased thromboxane contractile activity.

NON-APPROVED ABBREVIATIONS

ACh, acetylcholine

BLT1, leukotriene B_4 receptor

CPI-17, C-kinase potentiated protein phosphatase-1 inhibitor Mr = 17 kDa

DHA, docosahexaenoic acid

DMEM-F12, Dulbecco's modified Eagle's medium and Ham's F12 nutrient mixture

EPA, eicosapentaenoic acid

FPR2/ALX, Formyl peptide receptor 2/Lipoxin A4 receptor

GPCR, G protein-coupled receptor

GPR18, G protein-coupled receptor 18

GPR32, G protein-coupled receptor 32

HPA, human pulmonary artery

KPSS, potassium physiological salt solution

PDGF, platelet-derived growth factor

PE, phenylephrine

PSS, physiological salt solution

PUFA, polyunsaturated fatty acid

RTA, rat thoracic aorta

RvD1, resolvin D1, 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid

RvD2, resolvin D2, 7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid

RvE1, resolvin E1, 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid

SPM, specialised pro-resolving lipid mediator

TMEM16A, transmembrane protein member 16A

INTRODUCTION

Inappropriate smooth muscle contraction is central to chronic vascular diseases such as pulmonary and systemic hypertension. Many lipid mediators derived from omega-6 polyunsaturated fatty acids (PUFAs) are vasoactive; leukotriene D_4 and thromboxane A_2 are both potent vasoconstrictors, whilst prostaglandin I_2 (prostacyclin) is a vasodilator. Specialised proresolving lipid mediators (SPM) including the resolvins are derived from the omega-3 PUFAs eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) (Serhan et al., 2000, Serhan et al., 2002).

They have important roles in the resolution of inflammation, either *via* their own GPCRs or by modulating GPCRs for omega-6 PUFA (Serhan et al, 2015). For example, resolvin E1 (RvE1) (5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-EPA, Arita et al., 2005) enhances the phagocytosis of apoptotic neutrophils *via* its chemerin receptor (Ohira et al., 2010) and also inhibits the infiltration of neutrophils by antagonising leukotriene B_4 at BLT1 receptors (Arita et al., 2007). Resolvin D1 (RvD1) (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-DHA, Sun et al., 2007) has been shown to bind to two GPCRs, namely the orphan receptor, GPR32, and the lipoxin receptor, ALX (Krishnamoorthy et al., 2010). Evidence that resolvin D2 (RvD2) (7S, 16R, 17S-trihydroxy-4Z, 8E, 10Z, 12E, 14E, 19Z-DHA, Spite et al., 2009) binds to orphan receptor GPR18 expressed on human leukocytes was recently demonstrated, whilst GPR18-knockout mice displayed reduced phagocytotic clearance of bacteria and a lack of resolution (Chiang et al., 2015).

RvE1, RvD1 and RvD2 have been shown to influence vascular smooth muscle cells phenotype, including chemotaxis, proliferation and migration (Ho et al., 2010, Miyahara et al., 2013). More recently, RvD1 loaded into biodegradable wraps was found to reduce neointimal hyperplasia, likely due in part to the reduced proliferation and migration of smooth muscle cells seen *in vitro* (Wu et al., 2017). Importantly, receptors for all three resolvins have been identified in smooth muscle (Ho et al., 2010, Miyahara et al., 2013, Watts et al., 2015, Hiram et al., 2015). However, little is known about whether resolvins can modulate the contractility of vascular smooth muscle. In this study, we investigated whether RvE1, RvD1 and RvD2 can directly modulate the contractility of intact segments of rat thoracic aorta (RTA) and human pulmonary artery (HPA) *in vitro*.

METHODS

Animal tissue retrieval

All housing, care and procedures were carried out in accordance with institutional guidelines. Rats were chosen based on previously published work undertaking successful wire myography experiments with RTA. Rats were housed in standard housing conditions with 0-1 cage companions. Rats (total of 17 male Wistar rats (Charles River, UK or in-house stock), weighing between 200-300 g, aged 6-12 months) were culled via a rising concentration of CO_2 and subsequent cervical dislocation. The RTA was removed and cut into adjacent segments ready for experimentation.

Human tissue retrieval

HPA segments were obtained from samples donated by patients with informed consent who were undergoing thoracic surgery at Southampton General Hospital. Samples were obtained following review and approval by the institutional review committee (Ethical permission: Southampton & SW Hants LREC 08/H0502/32 or REC Reference Number 14/SC/0186). HPA were dissected out and cut into adjacent segments ready for experimentation.

General wire myography procedures

Wire myography was carried out using multi wire myograph system 610M from Danish Myo Technology. Segments were mounted on the wire myograph as described previously (Pike et al., 2014). Briefly, segments, which had been cleaned of surrounding tissue, were carefully slid onto pins on the myograph jaw and bathed in physiological salt solution (PSS). Paired adjacent segments from the same animal or human sample were used across the multiple chambers during a single experiment, eliminating the need for sample randomisation. Operator blinding was not carried out since a single individual undertook all experimental work and data are quantitative and not subjective. Based on both published data and preliminary studies in our laboratory, a baseline tension of 1.5 g was set for both RTA and HPA. Tension was permitted to plateau before confirming functional integrity by a contractile response to potassium PSS (KPSS). Concentration-response curves are displayed as a percentage of the KPSS response of that individual tissue segment, whilst reversal of preconstriction experiments are expressed as a

percentage relaxation to account for small differences in segment size and therefore the amount of contractile smooth muscle present.

Resolvin pretreatment and constriction of arteries with U46619 or phenylephrine

Adjacent segments of freshly-isolated RTA or HPA (2 mm length; 800 µm diameter) were incubated in culture plates in DMEM-F12 (+ 10% NCS; + penicillin and streptomycin) with or without RvE1 (0.1-300 nmol/L), RvD1 (1-100 nmol/L) or RvD2 (1-100 nmol/L) for 1 or 24 hours at 37◦C and 5% CO_2. In some experiments, the chemerin receptor antagonist CCX832 (100 nmol/L) (Chemocentryx) or vehicle was added 15 minutes before subsequent resolvin incubation. Segments were mounted on the wire myograph in PSS (described in detail above), and then constricted with cumulative concentrations of the stable thromboxane mimetic U46619 (RTA 1-1000 nmol/L; HPA 0.1-1000 nmol/L) or phenylephrine (PE) (10 nmol/L to 30 µmol/L).

Immunohistochemistry

Segments of HPA were fixed in 10% neutral buffered formalin for 24 hours then processed and embedded in paraffin wax. Sections (4 µm) were immunostained with primary antibodies for chemerin (ab150491, Abcam, UK), GPR32 (ab61429, Abcam, UK) or FRP2/ALX (ab101702, Abcam UK) and visualised with an AEC or DAB chromogen and Mayer's haematoxylin.

Reversal of artery preconstriction

Isolated RTA segments (2 mm length; 800 µm diameter) bathed in physiological salt solution (PSS) were pre-constricted with an 80% submaximal concentration (3 µmol/L) of PE; once a stable contractile plateau had been established, the muscarinic antagonist acetylcholine (ACh) (10 µmol/L) was used to confirm the ability of the constricted segments to relax. Segments were then washed with PSS and constricted with an 80% submaximal concentration of either PE (3 µmol/L) or U46619 (100 nmol/L), then RvD1, RvD2 or RvE1 (100 nmol/L) was applied with changes in tension monitored for the following 10 minutes.

Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). All data were analysed using GraphPad Prism Version 6 (GraphPad Software Inc., La Jolla, CA, USA). The threshold for statistical significance was $p<0.05$. Concentration-response curves are reported as mean±SEM and were analysed using a two-way repeated measures ANOVA with Sidak's multiple comparisons correction, with the exception of data shown in Fig3A. These data were analysed using an ordinary two-way ANOVA owing to some missing values at 0.1 and 0.3 nmol/L U46619 since the cumulative response curve was extended part way through the study to account for the unexpected increased responsive to U46619 in HPA compared to RTA. Reversal experiments are reported as medians and, where appropriate, analysed using a Kruskal-Wallis test with Dunn's multiple comparisons correction. In some experiments (Figure 2A, 2B, 3A, 5A) limited animal availability and time constraints resulted in $n<5$. No statistical analysis has been performed on these data sets. In figure 5A, experimental loss on one occasion has resulted in unequal group sizes. No statistical analysis was performed on this data set.

Materials

Resolvins E1, D1 and D2 were purchased from Cambridge Bioscience (Cambridge, UK). U46619 was purchased from Tocris Bioscience (Abingdon, UK). Acetylcholine and phenylephrine were purchased from Sigma-Aldrich (Dorset, UK). Antibodies against the chemerin and GPR32 receptors were purchased from Abcam (Cambridge, UK).

RESULTS

Pretreatment with RvE1 inhibits U46619-induced constriction of RTA.

The stable thromboxane mimetic U46619 (1-1000 nmol/L) constricted RTA in a concentration-dependent manner (Fig. 1). Pretreatment for 1 hour with RvE1 (10 nmol/L) significantly inhibited U46619-induced constriction of RTA segments, increasing the U46619 EC_{50} by 3.8-fold compared to control (Fig. 1A). Similar inhibition of contractility was seen after pre-treatment with RvE1 (10 nmol/L) for 24 hours, with the U46619 EC_{50} being increased 4.5-fold (Fig 1B). To determine the maximal inhibitory concentration of RvE1, RTA segments were pretreated with RvE1 concentrations from 0.1 to 300 nmol/L for 1 or 24 hours, then constricted with U46619 (1-1000 nmol/L). In each case the inhibitory effect of RvE1 was concentration-dependent, forming bell-shaped response curves with maximal inhibition occurring at 10 nmol/L (Figs. 1C and 1D).

Effect of the chemerin receptor antagonist CCX832 on inhibition of U46619-induced contractility of RTA and the effect of RvE1 on RTA constriction induced by phenylephrine (PE).

RvE1 does not compete with U46619 for the thromboxane TP receptor (Dona et al., 2008) but it is an agonist for chemerin receptors (Ohira et al., 2010). We therefore explored whether the chemerin receptor antagonist CCX832 (Watts et al., 2013) can block the inhibitory effect of RvE1 on U46619-induced constriction. RTA segments were pretreated with RvE1 (10 nmol/L) in the presence or absence of CCX832 (100 nmol/L) before constriction with U46619 (1-1000 nmol/L). CCX832 reduced the inhibitory effect of RvE1 on U46619-induced constriction (**Fig. 1E**), indicating its dependence on chemerin receptors. To further explore the inhibitory effect of RvE1 on contractility, RTA segments were pretreated with RvE1 (10 nmol/L, 1 hour) before constriction with cumulative concentrations of the alpha-1 adrenoreceptor agonist phenylephrine (PE; 0.01-30 μmol/L). RvE1 pretreatment had no effect on PE-induced constriction (**Fig. 1F**).

D-series resolvins also inhibit U46619-induced constriction of RTA segments.

Experiments were also performed to determine whether the D-series resolvins RvD1 (10 nmol/L) or RvD2 (10 nmol/L) can modulate contractility of RTA segments to U46619 (1-1000 nmol/L). U46619-induced RTA constriction was reduced by 1 hour of pretreatment with either RvD1 or RvD2 (Figs. 2A and 2B respectively).

RvE1 also inhibits U46619-induced constriction of human pulmonary artery (HPA)

In experiments analogous to those in RTA segments, the ability of RvE1 to modulate vascular contractility was investigated in HPA segments (Fig. 3). Pretreatment of HPA with RvE1 (10 nmol/L) for 1 hour significantly impaired HPA constrictions induced by U46619 (0.1-1000 nmol/L) (Fig 3A). The RvE1 inhibitory activity of RvE1 followed a bell-shaped curve with maximal inhibition being an 8-fold increase in U46619 EC_{50} seen at a concentration of 1 nmol/L RvE1 (Fig. 3B).

Expression of resolvin receptors in human pulmonary artery

Isolated HPA immunostained with antibodies against the chemerin receptor, GPR32 and FPR2/ALX demonstrated expression of these receptors in both the vascular endothelium and smooth muscle **(Fig 4).**

Resolvins D1, D2 and E1 do not relax pre-constricted RTA segments.

Having established the inhibitory effect of pretreatment for 1 or 24 hours on RTA and HPA contractility, we next explored whether the addition of resolvins can reverse an 80% submaximal pre-constriction of RTA segments induced by U44619 (100 nmol/L) or PE (3 μmol/L). RvE1, RvD1 and RvD2 (100 nmol/L) each had no effect on RTA segments pre-contracted with either PE (Fig. 4A) or U46619 (Fig. 4B). In contrast, constriction of RTA segments induced by PE were completely reversed within 10 minutes of the addition of acetylcholine (10 μmol/L) (Supporting Fig. 1), probably acting *via* muscarinic receptors.

DISCUSSION AND CONCLUSIONS

Lipid mediators derived from omega-6 fatty acids include highly potent pro-inflammatory and vasoactive mediators such as leukotriene D_4, thromboxane A_2 and prostacyclin. SPMs such as the E-series and D-series Rv derived from omega-3 fatty acids are important mediators in the resolution of inflammation (Serhan et al., 2015), but their ability to modulate contraction of vascular smooth muscle is unknown. In the present study, we investigated the ability of RvE1, RvD1 and RvD2 to prevent or reverse contractions of RTA and HPA segments induced *in vitro* by the stable thromboxane mimetic U46619 and the alpha-1 adrenoceptor agonist PE.

Using wire myography of intact arterial segments, our study shows for the first time that pretreatment of either RTA and HPA segments for only one hour with nanomolar concentrations of RvE1 significantly inhibited constrictions induced by U46619. The effect of RvE1 was concentration-dependent in each tissue with bell-shaped inhibition curves showing maximal inhibition at 10 nmol/L in RTA (Fig. 1) and 1 nM in HPA (Fig. 3), diminishing gradually to zero inhibition at 300 nmol/L. A published study may have failed to detect a direct inhibitory effect of RvE1 on HPA contractility due to their use of a concentration (300 nmol/L) shown to be inactive in our study, although this concentration was reported to inhibit hyperresponsiveness of HPA induced by inflammatory mediators (Hiram et al., 2015). Notably, the authors found RvE1 capable of inhibiting the inflammatory mediator-induced increase in phosphorylation of contractile proteins such as CPI-17 (C-kinase potentiated protein phosphatase-1 inhibitor Mr = 17 kDa I-17). It is possible that the results seen in our study are the result of a decrease in the sensitivity of the smooth muscle contractile proteins. Together these studies suggest that resolvins can directly prevent smooth muscle contraction at low concentrations and prevent the induction of chronic hyperresponsiveness at higher concentrations. Incidentally, bell-shaped concentration-response curves with resolvins have been shown a number of times previously (Spite et al., 2009, Oh et al., 2011, Claria et al., 2012).

The findings that the inhibitory effect of RvE1 in RTA and HPA segments is apparent after only 1 hour of pretreatment, and that it is not enhanced in RTA by longer pretreatment (24 hours), suggest the effect is not dependent on protein synthesis, but is rather a direct action either on the TP_1 receptor activated by U46619 or on the TP signalling pathways leading to contraction. The former is unlikely as RvE1 can inhibit U46619-induced platelet aggregation but does not

displace U46619 from TP_1 receptors, as determined by radioligand binding experiments (Dona et al., 2008). The finding that RvE1 did not inhibit RTA constriction induced by PE (**Fig. 1F**) indicates that it is selective for TP receptor signalling; this may have important implications in the regulation of vascular contractility by thromboxane in cardiovascular disease, including pulmonary hypertension. Further experiments with other lipid and non-lipid contractile agonists will better define the selectivity of resolvin actions on vascular contractility.

The chemerin receptor antagonist CCX832 reduced the ability of RvE1 to inhibit RTA constriction induced by U46619 (**Fig. 1E**), indicating that chemerin receptors are required and sufficient for the action of RvE1 in this tissue. As well as the E-series resolvins, we further showed that the ability of RvE1 to suppress U46619-induced vascular contractility is shared by the D-series resolvins RvD1 and RvD2, and that the D-series resolvins were similarly active in the low nanomolar range (Figs. 2A, 2B). D-series resolvins do not act on the chemerin receptor, suggesting that U46619-induced contractility is susceptible to inhibition by multiple resolvin receptor-dependent pathways. RvD1 is an agonist at two GPCRs, the ALX receptor and GPR32[4], and RvD2 may act at the orphan receptor GPR18 (Chiang et al., 2015). Given the ability of all three resolvins to inhibit U46619-induced constriction, it is likely that their corresponding GPCRs have signalling pathways that converge on TP_1 receptor signalling to produce physiological antagonism of vascular contractility. Immunohistochemical experiments confirmed the expression of the chemerin receptor, GPR32 and FPR2/ALX in the vascular endothelium and smooth muscle of HPA sections (Figs. 4A, 4B, 4C), and others have shown that the chemerin receptor is also expressed in RTA tissue (Watts et al., 2013). Interestingly, this latter study also demonstrated chemerin receptor-dependent contraction of RTA by chemerin-9, a nonapeptide derived from chemerin (Wittamer et al., 2003). More recently the same group demonstrated Gαi-dependence of this contraction, with downstream activation of both src and rho kinase (Ferland et al., 2017). Whilst this may seem contradictory to the findings in this study, the activation of the same GPCR to generate opposing effects is demonstrated with the activation of FPR2 by both serum amyloid A and annexin A1 to give proinflammatory and anti-inflammatory actions. It is possible that chemerin and RvE1 are interacting with the chemerin receptor in distinct ways to trigger separate downstream signalling pathways. This concept is explored in the review by Cash et al (2014). Together these studies may reflect a direct effect of resolvins acting at their respective GPCRs on vascular smooth muscle, or perhaps an indirect action mediated by

inhibition of the release of thromboxane or modulation of other vasoactive mediators *via* resolvin GPCRs on endothelial cells. Assays of eicosanoid and other mediator release from endothelium-intact and denuded vessels should be performed to explore these possibilities. The expression of GPR18 was not investigated in this study and to our knowledge it is yet to be investigated in vascular tissues.

Finally, experiments using RTA segments pre-contracted with U46619 or PE showed that RvE1, RvD1 and RvD2 were unable to reverse contractions to these agonists (Fig 5), although contractions were readily reversible with ACh (Supporting Fig 1). This may suggest a mechanism similar to that reported for the ability of RvD1 to prevent, but not reverse, histamine-induced mucin secretion by conjunctival goblet cells, in which GPR32 activation by the resolvin led to inactivation of H_1 histamine receptors due to phosphorylation by intracellular kinases (Li et al., 2013). Intriguingly, previous research has demonstrated the ability of RvE1 to attenuate the phosphorylation of the platelet-derived growth factor-β receptor under both basal and stimulated conditions, providing further evidence of GPCR crosstalk (Ho et al., 2010).

In summary, this study is the first to show that low nanomolar concentrations of RvE1, RvD1 and RvD2 can prevent constriction in rat and human arteries induced by a thromboxane mimetic. Resolvins and stable mimetics of these specialised proresolving mediators may have dual therapeutic activities both to resolve inflammation and to prevent inappropriate vascular contractility in cardiovascular disease.

AUTHOR CONTRIBUTIONS

MJ collected the data, prepared the figures and wrote the main manuscript text. APS, CT and JAW supervised the work and reviewed the manuscript. All authors contributed to experimental design and data interpretation.

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CONFLICTS OF INTEREST STATEMENT

None.

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TABLES, FIGURES AND LEGENDS

One table and five figures are submitted as part of this manuscript. In addition, there is one supporting figure.

TABLES

Table 1: Patient characteristics. A total of 10 samples were used for wire myography. (FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity).

Number of samples	Average age (years)	F / M	Average FEV_1/FVC
10	66.5 ± 0.84	5 / 5	0.64 ± 0.01

FIGURE LEGENDS

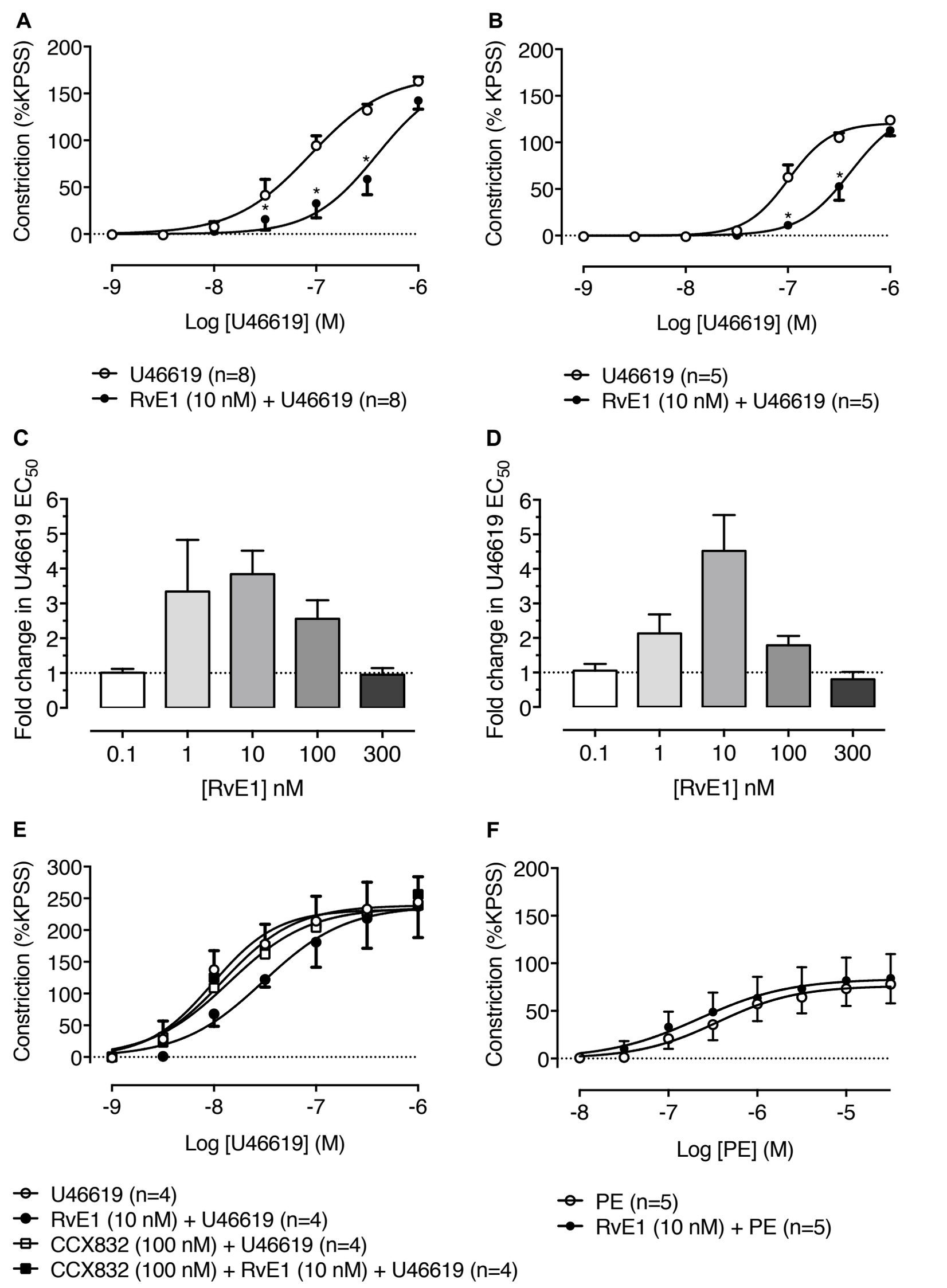
Figure 1: Pretreatment with RvE1 concentration-dependently inhibits U46619-induced constriction of RTA segments. (A) Pretreatment with RvE1 at a concentration of 10 nmol/L for one hour significantly inhibited constriction of RTA segments induced by cumulative concentrations of U46619 (n=8). (B) U46619-induced constriction of RTA segments was also inhibited by RvE1 (10 nmol/L) pretreatment for 24 hours (n=5). (C) Inhibition of U46619-induced constriction was dependent on the concentration of RvE1 (0.1-300 nmol/L) used during pretreatment for one hour or (D) 24 hours. In both instances, the greatest shift in U46619 EC_{50} occurred at 10 nmol/L RvE1. (E) The compound, CCX832, is a novel antagonist of the chemerin receptor, a receptor for RvE1. At 100 nmol/L, CCX832 alone had no effect on U46619-induced constriction of RTA segments. When added 15 minutes before a 1-hour pretreatment with RvE1 (10 nmol/L, n=4), CCX832 reduced the inhibition by RvE1 of U46619-induced constriction at both 10 nmol/L and 30 nmol/L of U46619, suggesting that the inhibitory action of RvE1 is mediated by chemerin receptors. (F) RvE1 (10 nmol/L) pretreatment for one hour did not affect constriction of RTA segments induced by the α_1-adrenoceptor agonist PE, indicating a selective inhibitory activity of RvE1 against the thromboxane mimetic U46619.

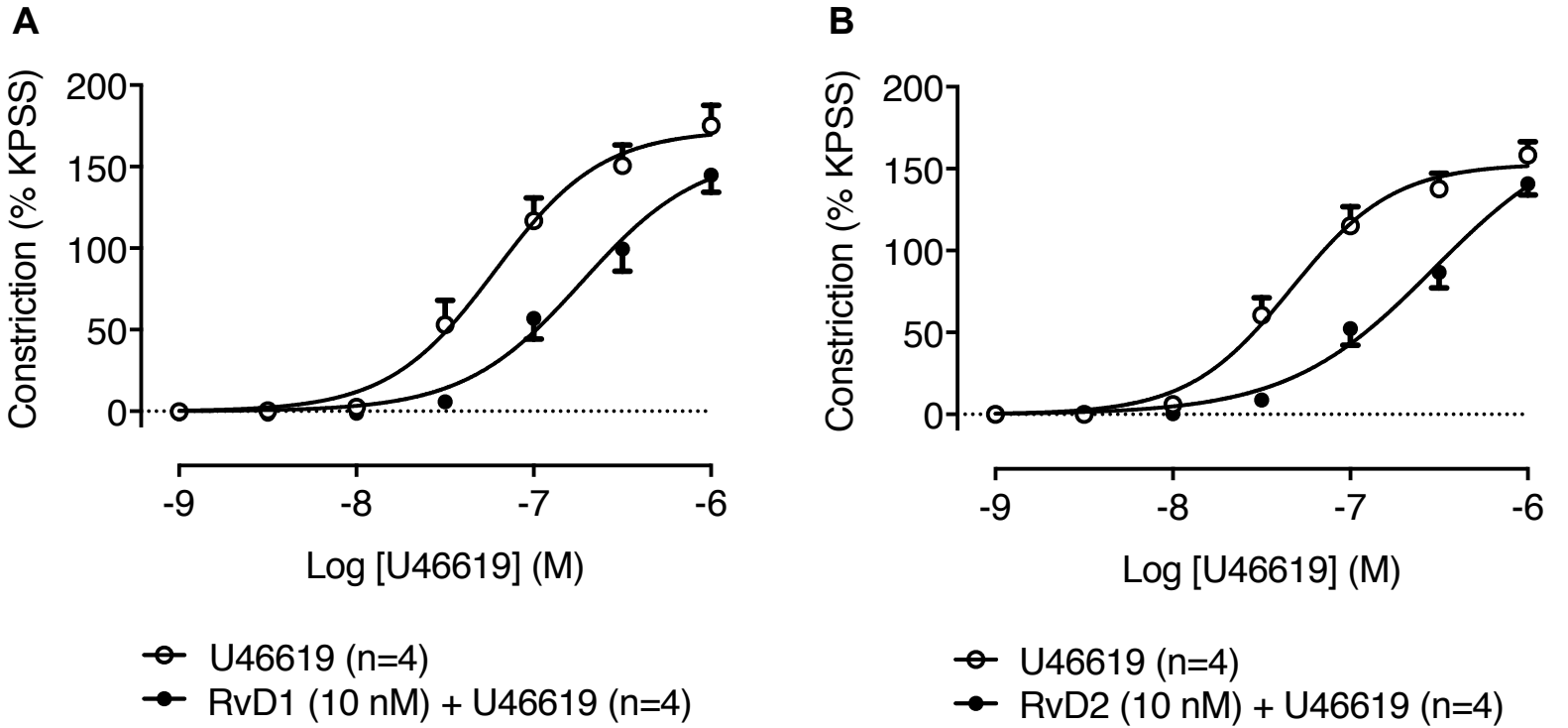
Figure 2: Effect of D-series resolvins on U46619-induced constriction of RTA segments. Pretreatment for one hour with 10 nmol/L concentrations of (A) RvD1 or (B) RvD2 reduced constriction of RTA segments induced by cumulative concentrations of U46619 (n=4).

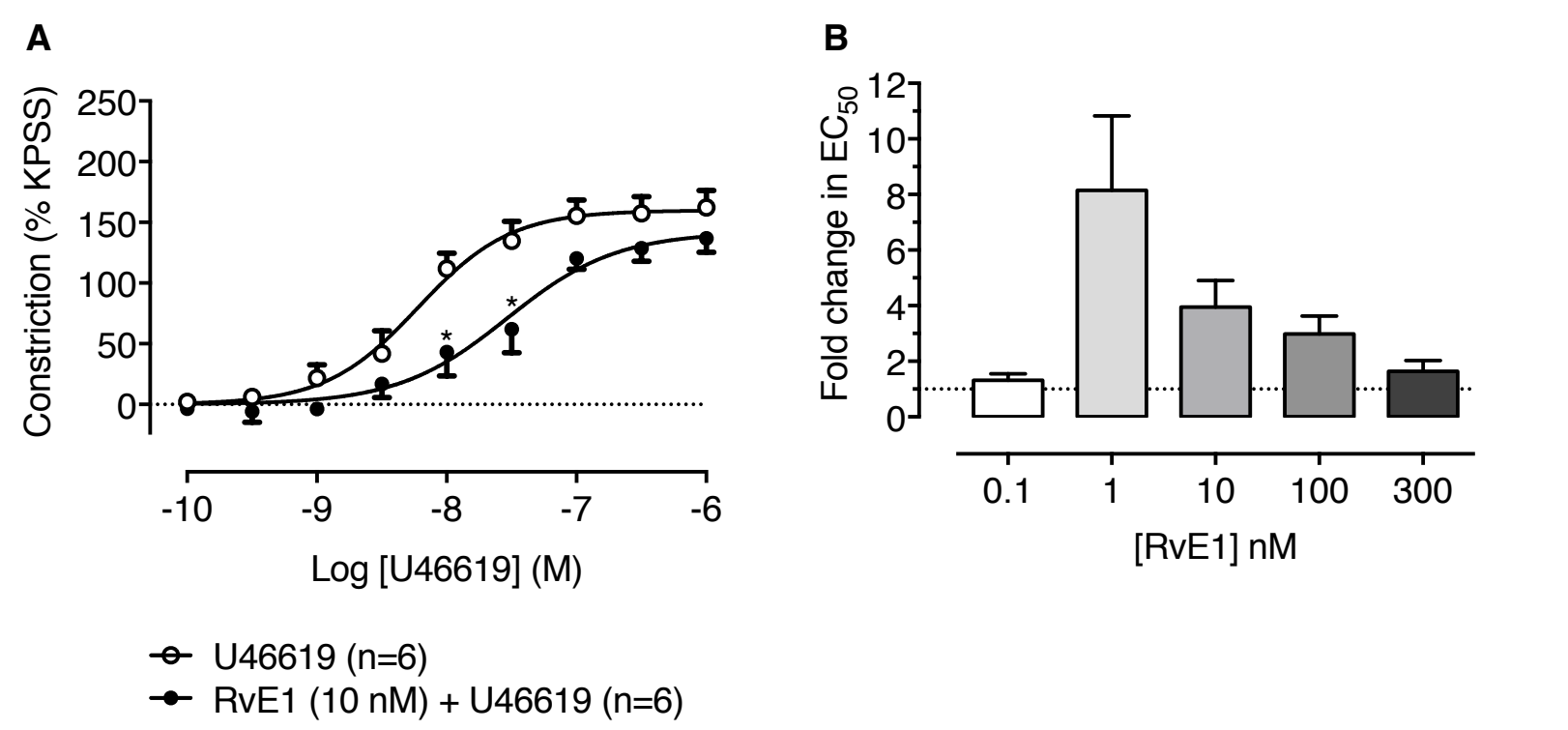
Figure 3: Pretreatment with RvE1 concentration-dependently inhibits U46619-induced constriction of human pulmonary artery (HPA).
(A) Pretreatment with RvE1 at a concentration of 10 nmol/L for one hour significantly inhibited constriction of HPA segments induced by cumulative concentrations of U46619 (n=6). (B) Pretreatment of HPA segments with various concentrations of RvE1 (0.1-300 nmol/L) for one hour significantly inhibited constriction induced by cumulative concentrations of U46619 (1-1000 nmol/L), with the greatest shift in U46619 EC_{50} occurring at 1 nmol/L RvE1, suggesting greater sensitivity of HPA compared with RTA segments.

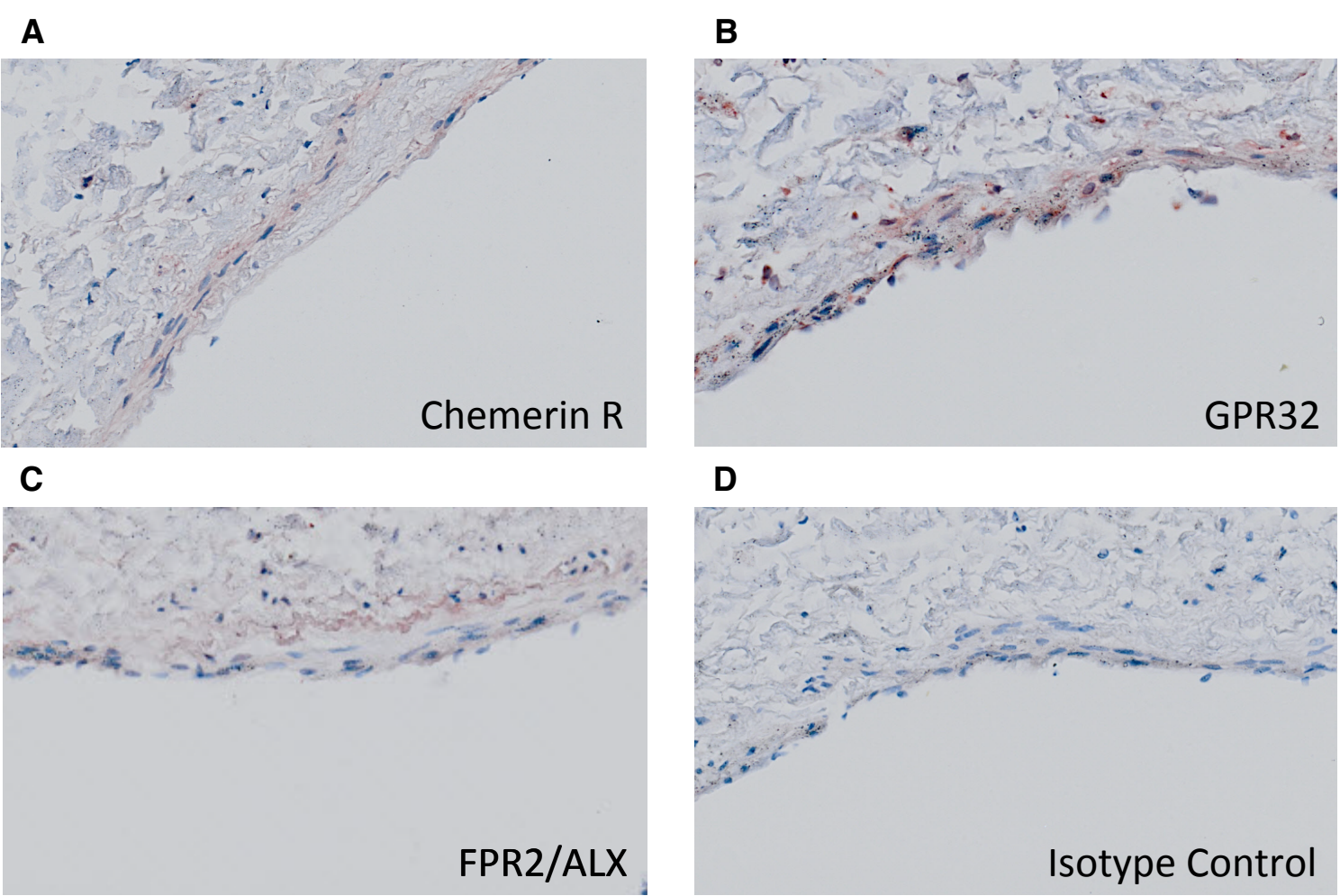
Figure 4: Expression of resolvin receptors in HPA. (A) Immunohistochemistry of formalin-fixed paraffin sections showed expression of (A) the chemerin receptor (RvE1 receptor) (B) RvD1 receptor GPR32 and (C) RvD1 receptor FPR/ALX in the vascular endothelium and smooth muscle of HPA segments. (D) **HPA isotype** control.

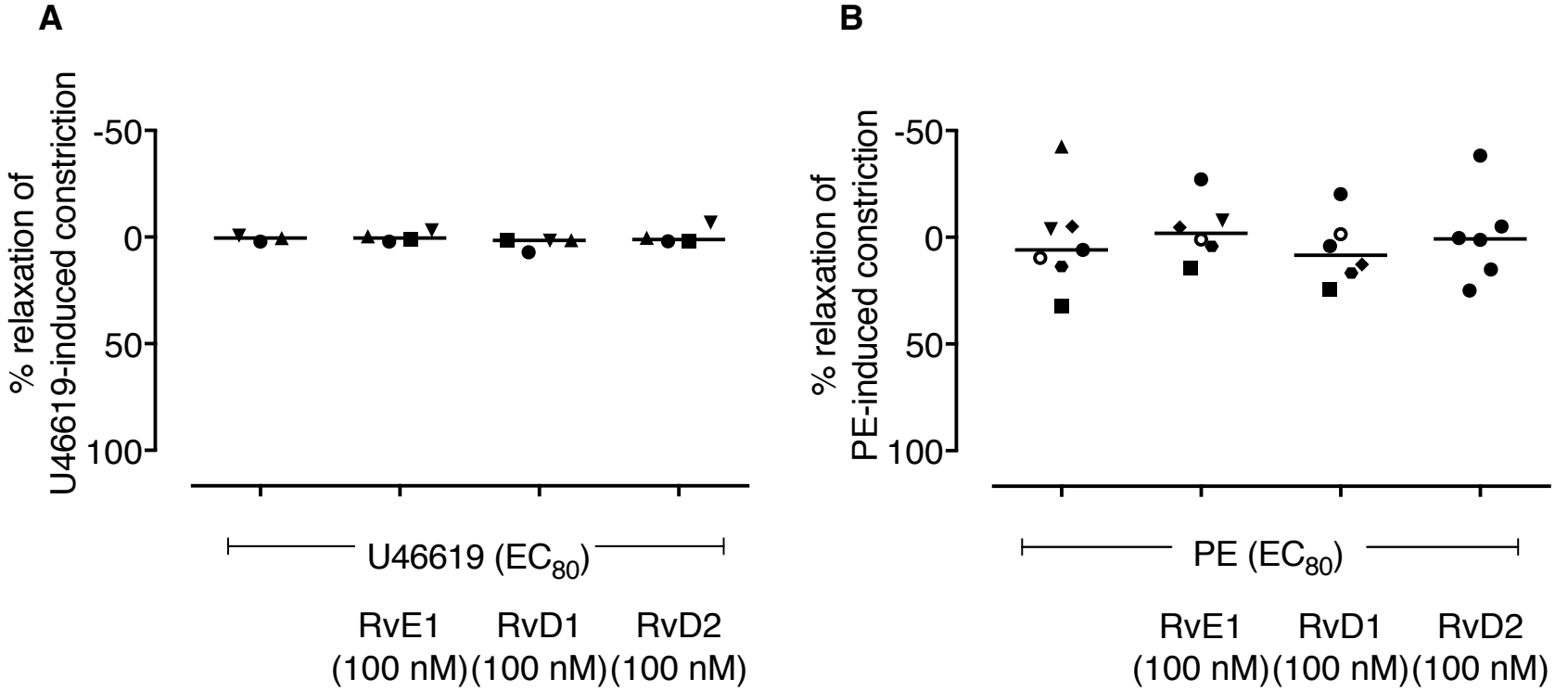
Figure 5: Resolvins E1, D1 and D2 do not reverse constriction of rat thoracic aorta (RTA). RTA segments were preconstricted with a submaximal (EC80) concentration of agonist and then treated with RvD1, RvD2 or RvE1 (100 nmol/L). The resolvins had no vasodilatory effect on RTA preconstricted with either (A) U46619 or (B) PE.


A
Constriction (%KPSS)
200
150
100
50
0
-9
-8
-7
-6
Log [U46619] (M)
*
*
*
U46619 (n=8)
RvE1 (10 nM) + U46619 (n=8)
B
Constriction (% KPSS)
200
150
100
50
0
-9
-8
-7
-6
Log [U46619] (M)
*
*
U46619 (n=5)
RvE1 (10 nM) + U46619 (n=5)
C
Fold change in U46619 EC50
6
5
4
3
2
1
0
0.1
1
10
100
300
[RvE1] nM
D
Fold change in U46619 EC50
6
5
4
3
2
1
0
0.1
1
10
100
300
[RvE1] nM
E
Constriction (%KPSS)
300
250
200
150
100
50
0
-9
-8
-7
-6
Log [U46619] (M)
U46619 (n=4)
RvE1 (10 nM) + U46619 (n=4)
CCX832 (100 nM) + U46619 (n=4)
CCX832 (100 nM) + RvE1 (10 nM) + U46619 (n=4)
F
Constriction (%KPSS)
200
150
100
50
0
-8
-7
-6
-5
Log [PE] (M)
PE (n=5)
RvE1 (10 nM) + PE (n=5)


A
Constriction (% KPSS)
200
150
100
50
0
-9
-8
-7
-6
Log [U46619] (M)
U46619 (n=4)
RvD1 (10 nM) + U46619 (n=4)
B
Constriction (% KPSS)
200
150
100
50
0
-9
-8
-7
-6
Log [U46619] (M)
U46619 (n=4)
RvD2 (10 nM) + U46619 (n=4)


A
Constriction (% KPSS)
250
200
150
100
50
0
-10
-9
-8
-7
-6
Log [U46619] (M)
*
*
U46619 (n=6)
RvE1 (10 nM) + U46619 (n=6)
B
Fold change in EC50
12
10
8
6
4
2
0
0.1
1
10
100
300
[RvE1] nM


A
Chemerin R
B
GPR32
C
FPR2/ALX
D
Isotype Control


A
% relaxation of U46619-induced constriction
-50
0
50
100
U46619 (EC80)
RvE1 (100 nM)
RvD1 (100 nM)
RvD2 (100 nM)
B
% relaxation of PE-induced constriction
-50
0
50
100
PE (EC80)
RvE1 (100 nM)
RvD1 (100 nM)
RvD2 (100 nM)