



LUND UNIVERSITY

Modulation of antigen-induced responses by serotonin and prostaglandin E(2) via EP(1) and EP(4) receptors in the peripheral rat lung.

Larsson Callerfelt, Anna-Karin; Dahlén, Sven-Erik; Köhl, Anna-Rebekka; Lex, Dennis; Uhlig, Stefan; Martin, Christian

Published in:
European Journal of Pharmacology

DOI:
[10.1016/j.ejphar.2012.11.039](https://doi.org/10.1016/j.ejphar.2012.11.039)

2013

[Link to publication](#)

Citation for published version (APA):
Larsson Callerfelt, A.-K., Dahlén, S.-E., Köhl, A.-R., Lex, D., Uhlig, S., & Martin, C. (2013). Modulation of antigen-induced responses by serotonin and prostaglandin E(2) via EP(1) and EP(4) receptors in the peripheral rat lung. *European Journal of Pharmacology*, 699(1-3), 141-149. <https://doi.org/10.1016/j.ejphar.2012.11.039>

Total number of authors:
6

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

1 **Modulation of antigen-induced responses by serotonin and prostaglandin E₂ via EP₁**
2 **and EP₄ receptors in the peripheral rat lung**

3

4 Anna-Karin Larsson-Callerfelt^{a,b*}, Sven-Erik Dahlén^b, Anna-Rebekka Kühl^c, Dennis
5 Lex^d, Stefan Uhlig^{c,d} and Christian Martin^{c,d}

6

7 ^{a)} Lung Biology, Department of Experimental Medical Science, Lund University, 22184
8 Lund, Sweden. ^{b)} Experimental Asthma and Allergy Research, The National Institute of
9 Environmental Medicine, Karolinska Institutet, 17177 Stockholm, Sweden. ^{c)} Department
10 of Pulmonary Pharmacology, Borstel Research Center, 23845 Borstel, Germany.

11 ^{d)}Institute for Pharmacology and Toxicology, RWTH Aachen, 52074 Aachen, Germany

12

13 *E-mail addresses:*

14 anna-karin_1.larsson@med.lu.se

15 sven-erik.dahlen@med.lu.se

16 anna-rebekka.kuehl@uni-rostock.de

17 dlex@ukaachen.de

18 suhlig@ukaachen.de

19 chmartin@ukaachen.de

20 **Corresponding author:*

21 Dr. Anna-Karin Larsson-Callerfelt, Unit of Lung Biology, Department of Experimental
22 Medical Science, BMC D12, Lund University, 221 84 Lund, Sweden

23 Phone: +46462229421, E-mail: Anna-Karin_L.Larsson@med.lu.se

24 **Abstract**

25 The cyclooxygenase (COX) pathway and prostanoids may critically contribute to the
26 early allergic airway response. In the rat lung, serotonin (5-HT) is a major mediator of
27 antigen-induced contractions. The aim of this study was therefore to examine the relative
28 role of the COX pathway and serotonin for antigen-induced contractions in the rat lung.
29 Airway responses were studied in rat precision cut lung slices (PCLS). Lung slices were
30 stimulated with ovalbumin or serotonin after pretreatment with COX inhibitors or
31 specific TP or EP receptor antagonists. Changes in airway size (contractions/relaxations)
32 were measured by a digital video camera. The supernatants were analysed for changes in
33 prostaglandin and serotonin release. Airway contractions to ovalbumin were attenuated
34 by the unselective COX inhibitor indomethacin, the selective COX-1 inhibitor FR-
35 122047 and COX-2 inhibitor celecoxib. The EP₁ receptor antagonist ONO-8713 reduced
36 the contractions, whereas the EP₄ receptor antagonist L-161,982 significantly increased
37 the contractile response to ovalbumin. The 5-HT_{2A} receptor antagonist ketanserin
38 completely inhibited the ovalbumin-induced contractions. The different COX inhibitors
39 decreased the production of prostaglandins but did not affect the synthesis of serotonin.
40 The serotonin-induced bronchoconstriction was attenuated by celecoxib and ONO-8713,
41 but not by methacholine. Taken together, our data indicate that PGE₂ is the main
42 prostanoid involved in the early allergic airway response in the rat lung. PGE₂ appears to
43 act both as a primary mediator of antigen-induced airway contraction via the EP₄ receptor
44 and as a downstream modulator of serotonin-induced bronchoconstriction via the EP₁
45 receptor. **Keywords:** Airway smooth muscle,
46 contraction, ovalbumin, precision-cut lung slices, prostaglandins, serotonin

47 **1. Introduction**

48 Airway obstructions play an important role in the development of symptoms associated
49 with remodelling processes and loss of lung function in asthma (Janson, 2010). Standard
50 therapy with anti-inflammatory corticosteroids and bronchodilators does not fully prevent
51 airway obstructions and bronchoconstriction in severe asthma (Holgate and Polosa,
52 2006), requiring new therapeutic approaches to treat this disease state. Prostanoids, such
53 as thromboxane A₂ (TXA₂), prostaglandin D₂ (PGD₂) and prostaglandin E₂ (PGE₂), are
54 involved in various physiological and pathophysiological processes in the lung and play a
55 critical role in asthma (Rolin et al., 2006). Prostanoids are generated from arachidonic
56 acid and converted to PG via cyclooxygenase (COX) (Vane, 1971). The COX enzyme
57 exists in two isoforms; COX-1 (Picot et al., 1994) is constitutively expressed and is
58 involved in regulation of physiological responses and homeostasis, COX-2 (Kurumbail et
59 al., 1996) is mostly inducible and related to inflammation (FitzGerald, 2003). The
60 prostanoids contribute to the asthmatic airway responses in different ways. TXA₂ is
61 involved in allergen-induced asthmatic responses by activation of TP receptors (Manning
62 et al., 1991) and thereby induction of both airway and vascular smooth muscle
63 constrictions (Larsson et al., 2011). TXA₂ may also cause airway hyperresponsiveness
64 (Held and Uhlig, 2000) and contributes to cytokine-induced bronchoconstriction (Martin
65 et al., 2001). PGD₂ is a pro-inflammatory mediator of allergic asthma (Matsuoka et al.,
66 2000), a marker of mast cell activation (Dahlen and Kumlin, 2004) and induces airway
67 and vascular smooth muscle contractions via the TP receptor (Armour et al., 1989;
68 Beasley et al., 1989; Johnston et al., 1995; Larsson et al., 2011; McKenniff et al., 1991).
69 PGE₂ is implicated to have a beneficial role in the lung (Pavord and Tattersfield, 1995;

70 Vancheri et al., 2004), since this prostanoid may maintain airway tone (Tilley et al.,
71 2003) and attenuate allergic airway responses (Hartney et al., 2006; Martin et al., 2002).
72 However, owing to the existence of various EP receptors the potential actions of PGE₂
73 are diverse (Coleman et al., 1994). Recent findings indicate that PGE₂ has its
74 bronchodilatory effect mainly via the EP₄ receptor in man (Benyahia et al., 2012;
75 Buckley et al., 2011). Prostanoids are implicated in the early allergic airway response in
76 different species; rat (Dahlback et al., 1984), guinea pig (Larsson et al, 2005, 2011) and
77 man (Benyahia et al., 2012; Buckley et al., 2011; Ressmeyer et al., 2006). However, in
78 the rat lung, serotonin (5-hydroxytryptamine, 5-HT) is the major mediator released from
79 mast cells granules during an allergen response. The release of serotonin induces potent
80 contractions via the 5-HT_{2A} receptor (Dahlback et al., 1984; Wohlson et al., 2001).
81 Inhibition of the COX pathway enhanced the early allergic response in guinea pig lung
82 (Larsson et al., 2005) and induced airway hyperresponsiveness in murine lung (Peebles et
83 al., 2002). It is unknown whether this modulation is a general mechanism of the early
84 allergic response. The purpose of this study was to examine the contribution of the COX
85 pathway and prostanoids to the early allergic airway response in relation to the effects of
86 serotonin in the rat lung.

87

88

89

90

91

92

93 **2. Methods**

94 *2.1. Animals*

95 Precision-cut lung slices (PCLS) were prepared from 8-week-old Wistar rats (220 ± 20 g)
96 obtained from Charles River (Sulzfeld, Germany) and kept under controlled conditions
97 (22°C , 55% humidity and 12-h day/night rhythm). Animal experiments were approved by
98 the local ethics committee.

99

100 *2.2. Precision-cut lung slices*

101 Rat PCLS were prepared as previously described (Wohlsen et al., 2001). Rats were
102 sacrificed by an overdose of pentobarbital i.p. (60 mg/kg). The trachea was prepared and
103 fixed with a tracheal cannula. The lungs were filled with pre-warmed agarose solution
104 (0.75%) via the trachea and subsequently chilled with ice. The heart-lung package was
105 removed and put on ice to allow the agarose to further cool and solidify. The lung lobes
106 were separated and cut into 5 to 10 mm thick tissue segments from which cores were
107 made along the airways with a coring tool. The cores were cut into 250 ± 20 μm thick
108 slices with a Krumdieck tissue slicer (Alabama Research and Development, Munford,
109 AL). Slices were incubated in minimal essential medium, which was changed every half
110 hour for the first two hours and then every hour for the next two hours to remove the
111 agarose and inflammatory mediators from the airways. For the experiments, slices with
112 airways that had intact surrounding epithelium were moved to 24-well plates and covered
113 with 1 ml of medium. The airways were imaged and digitized using a digital video
114 camera. For measurements, slices with comparable airway size (1.36 ± 0.28 mm^2) were
115 selected, covered with 1 ml of medium and fixed with a nylon thread attached to a

116 platinum wire to avoid movements and allow relaxation of the slice (Schleputz et al.,
117 2011). Images were recorded by an analogue (JAI 2040; JAI Pulnix, Alzenau, Germany)
118 or a digital camera (IRB640; Visitron Systems, Munich, Germany) controlled by the
119 software program Optimas 6.5 (Optimas, Bothell, WA). A control picture was taken
120 before addition of any agonists or antagonists and frames were recorded every 30 sec. A
121 time interval of 5 minutes for cumulatively given doses and 20 minutes for single doses
122 were used. The images were analyzed by the image analysis program Optimas 6.5
123 (Optimas, Bothell, WA).

124

125 *2.3. Sensitization for antigen studies*

126 For antigen studies with ovalbumin, the lung slices were incubated over night with cell
127 culture medium containing 1% serum from actively sensitized rats, as previously done
128 (Wohlsen et al., 2001). The medium was not changed until the following day. All other
129 lung slices were maintained in standard cell culture medium. Control studies were
130 performed to verify that 1% serum (of sensitized rats) did not interfere with responses
131 induced by other agonists and that ovalbumin did not show any effect in non-sensitized
132 slices.

133

134 *2.4. Study design*

135 Airway contractions to ovalbumin, PGD₂, PGE₂, the thromboxane receptor analogue
136 u46619, serotonin and methacholine were studied in rat PCLS. Effects of selective COX
137 inhibitors and selective EP₁, EP₂, EP₃, EP₄ and TP receptor antagonists on airway tone
138 were assessed. The release of serotonin and prostanoids after ovalbumin-stimulation were

139 analysed in the supernatant. A single concentration of ovalbumin (10 µg/ml) was used for
140 antigen-induced contractions. This concentration was selected from a cumulative
141 concentration-response curve (0.01-1000 µg/ml of ovalbumin) (Fig 1A). The single dose
142 (10 µg/ml) of ovalbumin produced a strong, stable and reproducible bronchoconstriction
143 with the same maximum airway contraction as generated by cumulative challenge of
144 ovalbumin (Fig 1B).

145

146 *2.5. Measurements of released mediators*

147 Supernatant (0.5 ml) of six incubated PCLS (weight 0.03 g/slice) was collected and
148 immediately frozen at -80°C. The samples were taken at three different time points. First
149 from unchallenged slices to obtain initial mediator release, thereafter, 20 minutes from
150 pre-treatment with the different drugs and finally 20 minutes after the slices were
151 challenged with 10 µg/ml ovalbumin. Enzyme immunoassays of TXA₂, PGD₂, PGE₂ and
152 serotonin were performed according to the manufacturer's instructions. TXA₂ was
153 measured as the stable metabolite TXB₂ and PGD₂ as PGD₂-mox. The assay detection
154 limits for the different mediators were 7.8 pg/ml for TXB₂, PGD₂, PGE₂ and 50 pg/ml for
155 serotonin. The enzyme immunoassay specificity for the different mediators to interfere
156 with each other was less than 0.01% for PGE₂, PGD₂-mox and serotonin, whereas the
157 antibody tracer for TXB₂ cross reacted with PGD₂ (0.53%) and with PGE₂ (0.09%).

158

159 *2.6. RT-PCR analysis*

160 PCLS were snap-frozen and pounded in liquid nitrogen. Total RNA was isolated from 30
161 mg lung powder with a NucleoSpin RNA II Kit (Machery Nagel GmbH & Co. KG,

162 Düren, Germany) automated on a QIAcube roboter (QIAGEN GmbH, Hilden, Germany).
163 RNA was quantified in buffered 10 mM TRIS-HCl, pH 7.5, using a NanoDrop 1000
164 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham (MA), USA). For reverse
165 transcription 274 ng of total RNA was added to 1 µl of oligo(dT)₁₅ Primer (0.5 µg/µl)
166 (Invitrogen, Karlsruhe, Germany) and water to a total volume of 12 µl. Samples were
167 incubated for 10 minutes at 65°C to linearize the RNA. 4 µl buffer (5x), 2 µl dNTP (10
168 mM), 1 µl Rnasin (40U/µl) and 1 µl M-MLV RT (H⁻) (200U/µl) (all substances from
169 Promega GmbH, Mannheim, Germany) were added and RNA was reverse transcribed for
170 90 minutes at 40°C. This was followed by a 2 minutes heat-inactivation step at 95°C. 20
171 µl of water was added afterwards to a final volume of 40 µl per sample. All incubation
172 steps were performed on a Biometra UNO II Thermocycler (Biometra GmbH, Göttingen,
173 Germany). For real-time qPCR 1 µl of cDNA was incubated as template with 0.5 µl
174 forward primer (6.25 µM) (Eurofins MWG GmbH, Ebersberg, Germany), 0.5 µl reverse
175 primer (6.25 µM), 5 µL SYBR-Green I Mastermix (Roche-Diagnostics GmbH,
176 Mannheim, Germany) and 3 µL water according to manufacturer's instructions in a
177 LightCycler 480 (Roche-Diagnostics GmbH). Following primer pairs were used for 5-
178 HT_{2A}-receptor (gene symbol: Htr2a, NCBI Reference Sequence: NM_017254.1): sense
179 5'-CCA CCA ACT ATT TCC TGA TGT C-3' antisense 5'-GCA CAT CCA GGT AAA
180 TCC AG-3' and for Beta-2-microglobulin (gene symbol: B2m, NCBI Reference
181 Sequence: NM_012512.2): sense 5'-CCG TGA TCT TTC TGG TGC TTG TCT-3'
182 antisense 5'-ATC GGT CTC GGT GGG TGT GAA T-3'. Quantification after real-time
183 qPCR was performed with Cp values, acquired via the Second Derivative Maximum
184 method. Advanced relative quantification was performed with the LightCycler 480

185 Software 1.5 SP3 (Roche-Diagnostics GmbH) and efficiency-corrected by in-run
186 standard curves using the Roche Applied Science E-Method (Tellmann, 2006). Data were
187 referenced first to the correspondent housekeeping gene B2m and normalized to the mean
188 of the experimental control. Real-time qPCR quality control was performed by in-run
189 negative controls, Melting Curve profiles using the LightCycler 480 Software and
190 product separation in agarose gels.

191

192 2.7. Drugs

193 Indomethacin, ovalbumin (chicken egg albumin, grade V), serotonin, ketanserin and
194 dimethylsulfoxid (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). 4-[5-(4-
195 methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide (Celecoxib;
196 Celebrex[®]) was obtained from Pfizer (CA). 1-[4,5-bis(4-methoxyphenyl)-2-
197 thiazoyl]carbonyl]-4-methylpiperazine hydrochloride (FR-122047), 3R-[[4-
198 fluorophenyl)sulfonyl]amino]-1,2,3,4-tetrahydro-9H-carbazole-9-propanoic acid
199 (BAYu3405, Ramatroban) was purchased from Bayer AG (Wuppertal, Germany). PGD₂,
200 PGE₂, 9,11-dideoxy-9 α ,11 α -methanoepoxy PGF_{2 α} (U46619), 9-oxo-6-propan-2-
201 yloxyxanthene-2-carboxylic acid (AH6809) and N-[[4'-[[3-butyl-1,5-dihydro-5-oxo-1-[2
202 (trifluoromethyl)-phenyl]-4H-1,2,4-triazol-4-yl]methyl][1,1'-biphenyl]-2-yl]sulfonyl]-3-
203 methyl-2-thiophenecarboxamide (L-161,982) were bought from Cayman Chemical (Ann
204 Arbor, MI, USA). (E)-3-[4-[[2-(furan-2-ylsulfonyl)-(2-methylpropyl)amino]-5-
205 (trifluoromethyl)phenoxy]methyl]phenyl]prop-2-enoic acid (ONO-8713) (Norel et al.,
206 2004) was a generous gift from ONO Pharmaceutical CO. LTD (Osaka, Japan). (E)-N-(5-
207 bromo-2-methoxyphenyl)sulfonyl-3-[2-(naphthalen-2-ylmethyl)phenyl]prop-2-enamide

208 (L-798,106) were bought from Tocris Bioscience, Bristol, UK. The EIA kits for TXB₂,
209 PGD₂-mox and PGE₂ were obtained from Cayman Chemicals (Ann Arbor, MI). ELISA
210 kit for serotonin was purchased from IBL-Hamburg (Hamburg, Germany). FR-122047,
211 ONO-8713, BAYu3405 and celecoxib were dissolved in DMSO. The final concentration
212 of DMSO or ethanol never exceeded 0.3% (v/v) and did not influence the induced
213 contractions. The other drugs were dissolved and diluted in distilled water. Dilutions of
214 drugs were freshly made from the stocks for each experiment. The drugs were present in
215 the medium fluid during the remaining experiment. Pretreatments were given 20 minutes
216 before the addition of ovalbumin, serotonin or methacholine.

217

218 *2.8. Calculations and Statistics*

219 Airway area before addition of any drug was defined as 100%. Airway contractions were
220 expressed as the percentage decrease in airway area compared to the initial airway area.
221 All data are presented as mean \pm standard error of the mean (S.E.M.). In all experiments
222 the numbers of n represent animals and not the number of slices. Time courses were
223 analyzed by the area under the curve (AUC). Enzyme immune assay measurements and
224 allergen-induced bronchoconstriction curves were analyzed by Student t-test (two curves
225 or bars) or by analyses of variances (ANOVA, more than two curves or bars) followed by
226 Bonferroni's post-hoc test. Concentration-response curves to methacholine and serotonin
227 were analyzed by comparison of sigmoid curves with Prism 5 (Graphpad, San Diego,
228 USA). A p-value of less than 0.05 was considered significant. The Statistic program JMP
229 5.1 (Cary, NC, USA) was used to calculate the power of the experiments.

230

231 **3. Results**

232 *3.1. Effect of the COX pathway on ovalbumin-induced contractions*

233 Ovalbumin (10 µg/ml) generated a stable and reproducible airway bronchoconstriction
234 (Fig 1A). The antigen-induced contractions to ovalbumin (10 µg/ml) were significantly
235 attenuated by the unselective COX inhibitor indomethacin (10 µM; P=0.012; Fig 2A), the
236 selective COX-1 inhibitor FR-122047 (5 µM; P=0.016; Fig 2B) and the selective COX-2
237 inhibitor celecoxib (10 µM; P=0.044; Fig 2C). PGD₂, PGE₂ and TXA₂ analogue u46619
238 were tested on non-sensitized slices to evaluate the attenuated bronchoconstriction
239 induced by the different COX-inhibitors. PGD₂ (10 µM; n=3) and PGE₂ (10 µM; n=3)
240 did not induce any bronchoconstriction or dilatatory effects in the rat PCLS (*Table 1*), nor
241 did the specific EP receptor agonists for EP₁, EP₂ and EP₄ (*data not shown*), whereas the
242 TP receptor analogue u46619 (50 µM; n=3) induced airway contractions that were
243 completely blocked by the TP receptor antagonist BAYu3405 (10 µM; n=3; P<0.05;
244 *Table 1*). In line, only the TP receptor agonist u46619 induced contractions in the lung
245 slices. However, both EP and TP receptor activation has been shown to be involved in
246 allergen-mediated airway contractions. PGE₂ may have diverse roles acting on both
247 contractile EP receptors (mainly EP₁, but also EP₃) and relaxant EP receptors (EP₂ and
248 EP₄) (Buckley et al., 2011). EP₁ receptor antagonist ONO 8713 (Norel et al., 1999), EP₂
249 receptor antagonist AH6809, EP₃ receptor antagonist L-798,106 and EP₄ receptor
250 antagonist L-161,982 and the TP receptor antagonist BAYu3405 were therefore tested on
251 ovalbumin-induced contractions to further characterise the contractile response after
252 COX inhibition. The EP₁ receptor antagonist ONO-8713 (10 µM) significantly reduced
253 the ovalbumin-induced contractions (P=0.004; Fig 3A), whereas the EP₄ receptor

254 antagonist L-161,982 (0.5 μ M) significantly increased the ovalbumin-induced
255 contractions ($P=0.042$; Fig 3D) whereas pre-treatment with EP₂ receptor antagonist
256 AH6809 (5 μ M) or EP₃ receptor antagonist L-798,106 (0.5 μ M) or TP receptor
257 antagonist BAYu3405 (10 μ M), had no significant effect (Fig 3B, C, E). Since serotonin
258 is known as a major mediator of this particular ovalbumin model, the inhibitory effect of
259 the COX inhibitors and the EP receptor antagonists was compared with the effect of the
260 5-HT_{2A} receptor antagonist ketanserin. Ketanserin (0.1 μ M; $P=0.005$) completely
261 inhibited the contractile response to ovalbumin (Fig 3F).

262

263 *3.2. Synthesis of serotonin and prostanoids after challenge with ovalbumin*

264 The supernatant was analysed to verify if there were any changes in generation of
265 serotonin and prostaglandins after ovalbumin stimulation and COX inhibition. The
266 medium contained relatively high levels of PGE₂, TXB₂ and PGD₂ that were not changed
267 after stimulation by ovalbumin. The COX inhibitors indomethacin (10 μ M) and celecoxib
268 (10 μ M) significantly decreased the formation of PGE₂, TXB₂ and PGD₂, whereas the
269 COX-1 inhibitor FR-122047 (5 μ M) significantly reduced the synthesis of TXB₂ (Fig 4C)
270 and PGD₂ (Fig 4D), but not PGE₂ (Fig 4B). The formation of serotonin was significantly
271 increased after addition of ovalbumin 10 μ g/ml ($P=0.032$). Notably, its synthesis or
272 release was not affected by the different COX inhibitors (Fig 4A). In addition, pre-
273 incubation with COX inhibitors or EP receptor antagonists did not change the initial
274 airway size in PCLS, indicating no direct effect on airway tone.

275

276 *3.3. Effect of the COX pathway on serotonin-induced contractions*

277 Since serotonin was the major contractile mediator of the antigen-induced response, the
278 effect of COX and prostanoids on serotonin-induced contractions was also evaluated to
279 investigate potential downstream modulations. Indomethacin (10 μ M) significantly
280 attenuated the airway contraction induced by serotonin (0.01-10 μ M; $P=0.02$; Fig 5A)
281 and shifted the concentration-response to the right (pEC_{50} : 6.22 ± 0.01 vs control pEC_{50} :
282 6.40 ± 0.05 ; $P=0.008$). FR-122047 (5 μ M) had no effect on serotonin-induced
283 bronchoconstriction (ns; Fig 5B), whereas celecoxib (10 μ M) decreased the response to
284 serotonin ($P=0.001$; Fig 5C) and shifted the concentration-response curve to the right
285 (pEC_{50} : 5.89 ± 0.08 vs control pEC_{50} : 6.12 ± 0.07 ; $P=0.03$). Pretreatment with the EP₁
286 receptor antagonist ONO-8713 (10 μ M) attenuated the contractile response to cumulative
287 doses of serotonin ($P=0.01$; Fig 5D) and shifted the concentration-response to the right
288 (pEC_{50} : 5.66 ± 0.07 vs control pEC_{50} : 6.27 ± 0.11 ; $P=0.003$). The EP₂ receptor antagonist
289 AH6809 (5 μ M) also attenuated the contractile response to cumulative doses of serotonin
290 and shifted the concentration-response to the right, and the bottom of the concentration-
291 response curves was unequal ($P=0.02$; Fig 5E), showing differences in potency and
292 efficacy. Also the EP₄ receptor antagonist L-161982 (0.5 μ M) shifted the concentration-
293 response curve to the right (pEC_{50} : 5.89 ± 0.07 vs control pEC_{50} : 6.15 ± 0.06 ; $P=0.004$;
294 Fig 5G). Neither the EP₃ receptor antagonist L-798,106 (0.5 μ M; Fig 5F) nor the TP
295 receptor antagonist BAYu3405 (10 μ M; Fig 5H) had any significant effects on the
296 contractions induced by serotonin. Analysis of the 5-HT_{2A} receptor expression indicated
297 that COX-inhibition with indomethacin or celecoxib enhanced the expression of the 5-
298 HT_{2A} receptor after 4h (Fig 6).

299

300 *3.4. Effect of COX inhibition and EP₁ receptor antagonism on methacholine-induced*
301 *contractions*

302 To determine if the effect of COX inhibition and EP₁ receptor antagonism was specific to
303 serotonin rather than a general property of rat airways, methacholine-induced
304 bronchoconstriction was evaluated in the presence and absence of the COX inhibitor
305 indomethacin (10 μM) or the EP₁ receptor antagonist ONO-8713 (10 μM). The
306 contractions to methacholine were not altered by either indomethacin or ONO-8713 (Fig
307 7).

308

309 **4. Discussion**

310 In this study, we present evidence that in the early allergic airway response in rat;
311 especially PGE₂ may act both as a primary mediator of antigen-induced airway
312 contraction via COX and the EP₁ and EP₄ receptors and as a downstream modulator of
313 serotonin-induced bronchoconstriction via COX-2 and the EP₁ receptor after antigen
314 challenge. Previous studies have indicated that prostanoids and serotonin are involved in
315 the early allergic airway response in the rat (Dahlback et al., 1984; Hele et al., 2001;
316 Nagase et al., 1996; Wohlsen et al., 2001). The relative contributions of these mediators,
317 however, remained uncertain. In the rat PCLS, the antigen-induced contractions to
318 ovalbumin were significantly attenuated by selective COX-1 and COX-2 inhibitors,
319 suggesting a role for both isoenzymes in the peripheral rat lung during the early allergic
320 airway response. Both COX-1 and COX-2 have been shown to be constitutively
321 expressed in the normal rat lung (Ermert et al., 1998b) with high enzyme activity (Baber
322 et al., 2003; Ermert et al., 1998a), suggesting a crucial role for COX isoenzymes in the
323 regulation of pulmonary responses. The beneficial effect of COX inhibition during the
324 antigen response in the rat PCLS was somewhat surprising and opposite to other studies,
325 since in other models COX inhibition resulted in airway hyperresponsiveness and
326 increased contractility (Larsson et al., 2005; Peebles et al., 2002; Watts and Cohen,
327 1993). On the other hand, leukotrienes, the major mediators in COX-sensitive asthma,
328 play only a minor role in rat PCLS (Wohlsen et al., 2001), which may explain the
329 influence on the AHR and contractility. In addition, passively sensitized PCLS represents
330 a mast-cell dependent model to study mainly early allergic airway responses (Ressmeyer
331 et al., 2006; Wohlsen et al., 2001).

332 To understand the attenuated antigen-induced bronchoconstriction after COX-
333 inhibition, the effect of the prostanoids PGD₂, PGE₂ and thromboxane was investigated
334 on rat airway tone, where only the TP receptor agonist u46619 induced some
335 contractions. Prostanoid receptors show considerable versatility and may mediate
336 bronchoconstriction via both TP and EP₁ receptors (Lydford and McKechnie, 1994) and
337 bronchodilatory effects through DP₁, EP₂ and EP₄ receptors (Hartney et al., 2006; Norel
338 et al., 2004; Tilley et al., 2003). Therefore, to further investigate the reduced contractile
339 response after COX-inhibition, we focused on the prostanoid receptors EP₁₋₄ and the TP
340 receptor. Interestingly, the EP₁ receptor antagonist ONO-8713 attenuated the antigen-
341 induced airway contraction, whereas the EP₄ receptor antagonist L-161,982 potently
342 increased the contractions to ovalbumin. This data implicate that PGE₂ may modulate the
343 early allergic airway response in rat lungs in two ways, mainly *via* activation of relaxant
344 EP₄ receptors but also in part *via* activation of contractile EP₁ receptors. Recent findings
345 indicate that PGE₂ has its bronchodilatory effect mainly via the EP₄ receptor in man
346 (Benyahia et al., 2012; Buckley et al., 2011). Notably, the beneficial relaxant effect of
347 PGE₂ via EP₄ receptor in this study correlated with the results obtained in human.
348 Focusing on the different EP receptors on mast cells, there is little information about the
349 distribution on mast cells. Feng and colleagues (Feng et al., 2006) have characterized the
350 EP receptors on human mast cells. Interestingly they only found expression of EP_{1, 2, 3}
351 receptor mRNA. From their view of EP receptor activity, increasing cAMP via EP₂ and
352 EP₄, seems to be important, whereas the role of EP₃, which acts via increase of calcium,
353 is only minor. Also in our study the EP₃ receptor does not appear to have a direct or
354 indirect effect on mast cell activation, which is again in line with the findings of Feng et

355 al, who found different EP₃-receptor subtypes, which can either be coupled to the G-
356 protein G_s or G_i (Feng et al., 2006).

357 In addition, also the TP receptor antagonist partly affected the antigen-response,
358 indicating that TP receptors may contribute as well. Both TXA₂ and PGD₂ are known to
359 mediate airway contractions via the TP receptor (Larsson et al., 2011; McKenniff et al.,
360 1991), whereas PGD₂ may also cause bronchodilation via the DP₁ receptor (Larsson et
361 al., 2011; Norel et al., 1999). Despite the pharmacological evidence of prostanoid
362 involvement in the early allergic airway response, supernatant levels of TXB₂, PGD₂ or
363 PGE₂ were not increased after antigen challenge. COX inhibition significantly but not
364 completely reduced the generation of the prostanoids in the rat PCLS. The release of
365 serotonin was significantly increased after addition of ovalbumin and remained
366 unaffected by the different COX inhibitors. These data raised the hypothesis that
367 prostanoids may act as modulators of airway responsiveness. Since the 5-HT receptor
368 antagonist ketanserin completely blocked the antigen-induced contractions in the rat
369 PCLS, we hypothesised that serotonin is the main mediator of EAR and that the COX
370 metabolites may modulate the serotonin response. We observed that serotonin-induced
371 bronchoconstriction was attenuated by selective COX-2 inhibition and EP₁ antagonism,
372 whereas in contrast to the antigen-induced response, COX-1 inhibition had no effect. The
373 EP₂ and EP₄ receptor antagonist may also affect the serotonin-induced constriction. This
374 may result is probably depending on the fact that the EP₂ receptor antagonist has similar
375 affinity to the EP₁ receptor (Buckley et al., 2011). However, apart from the EP₃ receptor
376 antagonist, the other EP receptor antagonists shifted the concentration- response curve to
377 the right. This is in line with the dilatory response via EP₂ and EP₄ on smooth muscle

378 cells. For EP₁ antagonism, the strongest effect was found, when both EP₂ and EP₄
379 receptors were triggered by endogenous PGE₂ produced by the PCLS during challenge.
380 In cases where either the EP₂ or EP₄ receptor was blocked this relaxation was reduced.
381 Again the EP₃ receptor seems to play a minor role also in smooth muscle cells.
382 Interestingly, the EP_{2,4} receptors have been found on human smooth muscle cells (Mori et
383 al., 2011). From our data, we would assume that the EP₁ receptor must have a role on
384 smooth muscle cells or mast cells, maybe only in the rat species.

385 Our data support the notion that the generation of PGE₂ was due to COX-2, as the
386 potent COX-1 inhibitor FR-122047 (Ochi and Goto, 2002) showed no effect on PGE₂
387 production in this study. In line with this, PGE₂ has been described to be generated in
388 high amounts by the COX-2 pathway in alveolar epithelium cells and airway smooth
389 muscle cells (Belvisi et al., 1997). The present findings suggest that during the EAR,
390 serotonin-induced bronchoconstriction is enhanced by COX-2 derived PGE₂ acting on
391 EP₁ receptors in the rat lung. As the methacholine-induced bronchoconstriction was not
392 altered by either COX inhibition or EP₁ antagonism, the interaction between serotonin
393 and PGE₂ appears to be specific for serotonin. Similar findings have been reported from
394 other disease models (Sato et al., 2000; Xie et al., 2003), that also implicated that 5-HT
395 responses may in part be mediated by the release of prostaglandins and associated with
396 COX-2 expression. It is possible that PGE₂, formed either in response to allergen or 5-HT
397 receptor activation, interacts at the cellular signalling level with 5-HT_{2A} receptor-induced
398 responses (Berg et al., 1998; Selbie and Hill, 1998). This hypothesis is supported by the
399 present finding that COX-inhibition enhanced 5-HT_{2A} receptor expression in the rat
400 PCLS.

401 Thus, while there is some evidence that the COX-2-derived prostanoids might at
402 least to some extent have been produced in epithelial and smooth muscle cells, it is
403 tempting to speculate that the effect of the COX-1 inhibitor was occurring in mast cells
404 that contain both COX-1 and COX-2 (Ermert et al., 1998b). This speculation is based on
405 our observation that the COX-1 inhibitor had no effect on serotonin-induced
406 bronchoconstriction or 5-HT₂AR expression in the present study, and on the finding that
407 COX-2 inhibitors had no effect on antigen-induced release of PGD₂ from rat mast cells
408 (Lau and Stenton, 1998). Unfortunately, high basal levels of PGE₂, TXB₂ and PGD₂ in
409 the supernatant of the lung slices, made it difficult to interpret the findings in Fig 4,
410 although the reduced levels of TXB₂ and PGD₂ in FR122047-treated slices could be
411 explained by the inhibition of COX-1 in mast cells. By note, high levels of PGE₂ are
412 typical in asthmatic situation, where increased levels of PGE₂ have been measured in
413 lung tissue and bronchoalveolar lavage fluid (Aggarwal et al., 2010; Krawiec et al.,
414 2001), representing the pathophysiological situation of asthma.

415

416 **5. Conclusions**

417 The major aim of this study was to evaluate the role of COX isoenzymes and prostanoids
418 in antigen-induced airway contractions of the peripheral rat lung. Since the preparation of
419 PCLS is essentially the same in all species; this model also provides the opportunity to
420 compare the early allergic airway response in different species. In guinea pig and human
421 PCLS, both prostanoids and histamine contribute to the allergen-induced
422 bronchoconstriction (Ressmeyer et al., 2006). In the rat PCLS, where the allergen-
423 induced bronchoconstriction is almost exclusively mediated by serotonin, the antigen-

424 response appears to be modulated by locally formed prostanoids, in particular by PGE₂,
425 derived from COX-2 and to some extent from COX-1. Apparently, the mechanisms by
426 which prostanoids contribute to the early allergic airway response differ among species.
427 In guinea pigs and humans, prostanoids are primary mediators of the antigen-induced
428 bronchoconstriction (Larsson et al., 2005; Ressmeyer et al., 2006; Wohlsen et al., 2003),
429 whereas in the rat lung prostanoids, and especially PGE₂, act both as primary mediators
430 of the antigen-induced airway contraction and modulate the serotonin-induced
431 bronchoconstriction. Interestingly, the EP₄ receptor has a bronchoprotective role during
432 antigen exposure in this model which correlate with the bronchodilatory results obtained
433 in man (Benyahia et al., 2012; Buckley et al., 2011), suggesting that the rat may be a
434 promising test model for asthma therapy with EP₄ agonists.

435

436 **Competing interests**

437 The authors declare that they have no competing interests.

438

439 **Acknowledgements**

440 The authors are grateful for financial support from the Swedish Heart Lung Foundation,
441 the Swedish Research Council in Medicine and Health, The Crafoord Foundation, the
442 Royal Physiographic Society in Lund, the Alfred Österlund Foundation, European
443 Respiratory Society, the Medical Faculty of Lund University and Karolinska Institutet.

444 The authors would like to express their gratitude to Jürgen Sarau at Borstel Research
445 Center, Hanna Czajkowska at RWTH Aachen and Margareta Andersson at Karolinska
446 Institutet for excellent technical assistance.

447 **References**

- 448 Aggarwal, S., Moodley, Y.P., Thompson, P.J., Misso, N.L., 2010. Prostaglandin E2 and
449 cysteinyl leukotriene concentrations in sputum: association with asthma severity
450 and eosinophilic inflammation. *Clin Exp Allergy* 40, 85-93.
- 451 Armour, C.L., Johnson, P.R., Alfredson, M.L., Black, J.L., 1989. Characterization of
452 contractile prostanoid receptors on human airway smooth muscle. *Eur J*
453 *Pharmacol.* 165, 215-222.
- 454 Baber, S.R., Champion, H.C., Bivalacqua, T.J., Hyman, A.L., Kadowitz, P.J., 2003. Role
455 of cyclooxygenase-2 in the generation of vasoactive prostanoids in the rat
456 pulmonary and systemic vascular beds. *Circulation.* 108, 896-901.
- 457 Beasley, R.C., Featherstone, R.L., Church, M.K., Rafferty, P., Varley, J.G., Harris, A.,
458 Robinson, C., Holgate, S.T., 1989. Effect of a thromboxane receptor antagonist on
459 PGD₂- and allergen-induced bronchoconstriction. *J Appl Physiol.* 66, 1685-1693.
- 460 Belvisi, M.G., Saunders, M.A., Haddad el, B., Hirst, S.J., Yacoub, M.H., Barnes, P.J.,
461 Mitchell, J.A., 1997. Induction of cyclo-oxygenase-2 by cytokines in human
462 cultured airway smooth muscle cells: novel inflammatory role of this cell type. *Br*
463 *J Pharmacol.* 120, 910-916.
- 464 Benyahia, C., Gomez, I., Kanyinda, L., Boukais, K., Danel, C., Leseche, G., Longrois,
465 D., Norel, X., 2012. PGE(2) receptor (EP(4)) agonists: potent dilators of human
466 bronchi and future asthma therapy? *Pulm Pharmacol Ther* 25, 115-118.
- 467 Berg, K.A., Maayani, S., Clarke, W.P., 1998. Interactions between effectors linked to
468 serotonin receptors. *Ann N Y Acad Sci.* 861, 111-120.

469 Buckley, J., Birrell, M.A., Maher, S.A., Nials, A.T., Clarke, D.L., Belvisi, M.G., 2011.
470 EP4 receptor as a new target for bronchodilator therapy. *Thorax* 66, 1029-1035.

471 Coleman, R.A., Smith, W.L., Narumiya, S., 1994. International Union of Pharmacology
472 classification of prostanoid receptors: properties, distribution, and structure of the
473 receptors and their subtypes. *Pharmacol Rev* 46, 205-229.

474 Dahlback, M., Bergstrand, H., Sorenby, L., 1984. Bronchial anaphylaxis in actively
475 sensitized Sprague Dawley rats: studies on mediators involved. *Acta Pharmacol*
476 *Toxicol (Copenh)*. 55, 6-17.

477 Dahlen, S.E., Kumlin, M., 2004. Monitoring mast cell activation by prostaglandin D2 in
478 vivo. *Thorax*. 59, 453-455.

479 Ermert, L., Ermert, M., Althoff, A., Merkle, M., Grimminger, F., Seeger, W., 1998a.
480 Vasoregulatory prostanoid generation proceeds via cyclooxygenase-2 in
481 noninflamed rat lungs. *J Pharmacol Exp Ther*. 286, 1309-1314.

482 Ermert, L., Ermert, M., Goppelt-Struebe, M., Walmrath, D., Grimminger, F., Steudel, W.,
483 Ghofrani, H.A., Homberger, C., Duncker, H., Seeger, W., 1998b. Cyclooxygenase
484 isoenzyme localization and mRNA expression in rat lungs. *Am J Respir Cell Mol*
485 *Biol*. 18, 479-488.

486 Feng, C., Beller, E.M., Bagga, S., Boyce, J.A., 2006. Human mast cells express multiple
487 EP receptors for prostaglandin E2 that differentially modulate activation
488 responses. *Blood* 107, 3243-3250.

489 FitzGerald, G.A., 2003. COX-2 and beyond: Approaches to prostaglandin inhibition in
490 human disease. *Nat Rev Drug Discov*. 2, 879-890.

491 Hartney, J.M., Coggins, K.G., Tilley, S.L., Jania, L.A., Lovgren, A.K., Audoly, L.P.,
492 Koller, B.H., 2006. Prostaglandin E2 protects lower airways against
493 bronchoconstriction. *Am J Physiol Lung Cell Mol Physiol.* 290, L105-113.

494 Held, H.D., Uhlig, S., 2000. Mechanisms of endotoxin-induced airway and pulmonary
495 vascular hyperreactivity in mice. *Am J Respir Crit Care Med.* 162, 1547-1552.

496 Hele, D.J., Birrell, M.A., Webber, S.E., Foster, M.L., Belvisi, M.G., 2001. Mediator
497 involvement in antigen-induced bronchospasm and microvascular leakage in the
498 airways of ovalbumin sensitized Brown Norway rats. *Br J Pharmacol.* 132, 481-
499 488.

500 Holgate, S.T., Polosa, R., 2006. The mechanisms, diagnosis, and management of severe
501 asthma in adults. *Lancet* 368, 780-793.

502 Janson, C., 2010. The importance of airway remodelling in the natural course of asthma.
503 *Clin Respir J* 4 Suppl 1, 28-34.

504 Johnston, S.L., Freezer, N.J., Ritter, W., O'Toole, S., Howarth, P.H., 1995. Prostaglandin
505 D2-induced bronchoconstriction is mediated only in part by the thromboxane
506 prostanoid receptor. *Eur Respir J.* 8, 411-415.

507 Krawiec, M.E., Westcott, J.Y., Chu, H.W., Balzar, S., Trudeau, J.B., Schwartz, L.B.,
508 Wenzel, S.E., 2001. Persistent wheezing in very young children is associated with
509 lower respiratory inflammation. *Am J Respir Crit Care Med* 163, 1338-1343.

510 Kurumbail, R.G., Stevens, A.M., Gierse, J.K., McDonald, J.J., Stegeman, R.A., Pak, J.Y.,
511 Gildehaus, D., Miyashiro, J.M., Penning, T.D., Seibert, K., Isakson, P.C.,
512 Stallings, W.C., 1996. Structural basis for selective inhibition of cyclooxygenase-
513 2 by anti-inflammatory agents. *Nature.* 384, 644-648.

514 Larsson, A.K., Back, M., Hjoberg, J., Dahlen, S.E., 2005. Inhibition of nitric-oxide
515 synthase enhances antigen-induced contractions and increases release of
516 cysteinyl-leukotrienes in guinea pig lung parenchyma: nitric oxide as a protective
517 factor. *J Pharmacol Exp Ther.* 315, 458-465.

518 Larsson, A.K., Hagfjard, A., Dahlen, S.E., Adner, M., 2011. Prostaglandin D induces
519 contractions through activation of TP receptors in peripheral lung tissue from the
520 guinea pig. *Eur J Pharmacol* 669, 136-142.

521 Lau, H.Y., Stenton, G.R., 1998. Effects of non-steroidal anti-inflammatory drugs and
522 cyclooxygenase-2 specific inhibitors on mediator release from rat peritoneal mast
523 cells. *Inflamm Res.* 47 Suppl 1, S22-23.

524 Lydford, S.J., McKechnie, K., 1994. Characterization of the prostaglandin E2 sensitive
525 (EP)-receptor in the rat isolated trachea. *Br J Pharmacol* 112, 133-136.

526 Manning, P.J., Stevens, W.H., Cockcroft, D.W., O'Byrne, P.M., 1991. The role of
527 thromboxane in allergen-induced asthmatic responses. *Eur Respir J.* 4, 667-672.

528 Martin, C., Uhlig, S., Ullrich, V., 2001. Cytokine-induced bronchoconstriction in
529 precision-cut lung slices is dependent upon cyclooxygenase-2 and thromboxane
530 receptor activation. *Am J Respir Cell Mol Biol.* 24, 139-145.

531 Martin, J.G., Suzuki, M., Maghni, K., Pantano, R., Ramos-Barbon, D., Ihaku, D., Nantel,
532 F., Denis, D., Hamid, Q., Powell, W.S., 2002. The immunomodulatory actions of
533 prostaglandin E2 on allergic airway responses in the rat. *J Immunol.* 169, 3963-
534 3969.

535 Matsuoka, T., Hirata, M., Tanaka, H., Takahashi, Y., Murata, T., Kabashima, K.,
536 Sugimoto, Y., Kobayashi, T., Ushikubi, F., Aze, Y., Eguchi, N., Urade, Y.,

537 Yoshida, N., Kimura, K., Mizoguchi, A., Honda, Y., Nagai, H., Narumiya, S., 2000.
538 Prostaglandin D2 as a mediator of allergic asthma. *Science*. 287, 2013-2017.

539 McKenniff, M.G., Norman, P., Cuthbert, N.J., Gardiner, P.J., 1991. BAY u3405, a potent
540 and selective thromboxane A2 receptor antagonist on airway smooth muscle in
541 vitro. *Br J Pharmacol*. 104, 585-590.

542 Mori, A., Ito, S., Morioka, M., Aso, H., Kondo, M., Sokabe, M., Hasegawa, Y., 2011.
543 Effects of specific prostanoid EP receptor agonists on cell proliferation and
544 intracellular Ca(2+) concentrations in human airway smooth muscle cells. *Eur J*
545 *Pharmacol*. 659, 72-78.

546 Nagase, T., Dallaire, M.J., Ludwig, M.S., 1996. Airway and tissue behavior during early
547 response in sensitized rats: role of 5-HT and LTD4. *J Appl Physiol*. 80, 583-590.

548 Norel, X., de Montpreville, V., Brink, C., 2004. Vasoconstriction induced by activation
549 of EP1 and EP3 receptors in human lung: effects of ONO-AE-248, ONO-DI-004,
550 ONO-8711 or ONO-8713. *Prostaglandins Other Lipid Mediat*. 74, 101-112.

551 Norel, X., Walch, L., Labat, C., Gascard, J.P., Dulmet, E., Brink, C., 1999. Prostanoid
552 receptors involved in the relaxation of human bronchial preparations. *Br J*
553 *Pharmacol* 126, 867-872.

554 Ochi, T., Goto, T., 2002. Differential effect of FR122047, a selective cyclo-oxygenase-1
555 inhibitor, in rat chronic models of arthritis. *Br J Pharmacol*. 135, 782-788.

556 Pavord, I.D., Tattersfield, A.E., 1995. Bronchoprotective role for endogenous
557 prostaglandin E2. *Lancet*. 345, 436-438.

558 Peebles, R.S., Jr., Hashimoto, K., Morrow, J.D., Dworski, R., Collins, R.D., Hashimoto,
559 Y., Christman, J.W., Kang, K.H., Jarzecka, K., Furlong, J., Mitchell, D.B., Talati,

560 M., Graham, B.S., Sheller, J.R., 2002. Selective cyclooxygenase-1 and -2
561 inhibitors each increase allergic inflammation and airway hyperresponsiveness in
562 mice. *Am J Respir Crit Care Med.* 165, 1154-1160.

563 Picot, D., Loll, P.J., Garavito, R.M., 1994. The X-ray crystal structure of the membrane
564 protein prostaglandin H2 synthase-1. *Nature.* 367, 243-249.

565 Ressmeyer, A.R., Larsson, A.K., Vollmer, E., Dahlen, S.E., Uhlig, S., Martin, C., 2006.
566 Characterisation of guinea pig precision-cut lung slices: comparison with human
567 tissues. *Eur Respir J.* 28, 603-611.

568 Rolin, S., Masereel, B., Dogne, J.M., 2006. Prostanoids as pharmacological targets in
569 COPD and asthma. *Eur J Pharmacol.* 533, 89-100.

570 Sato, K., Li, J., Metais, C., Bianchi, C., Sellke, F., 2000. Increased pulmonary vascular
571 contraction to serotonin after cardiopulmonary bypass: role of cyclooxygenase. *J*
572 *Surg Res* 90, 138-143.

573 Schleputz, M., Uhlig, S., Martin, C., 2011. Electric field stimulation of precision-cut lung
574 slices. *J Appl Physiol* 110, 545-554.

575 Selbie, L.A., Hill, S.J., 1998. G protein-coupled-receptor cross-talk: the fine-tuning of
576 multiple receptor-signalling pathways. *Trends Pharmacol Sci.* 19, 87-93.

577 Tellmann, G., 2006. The E-Method: a highly accurate technique for gene-expression
578 analysis. *Nature Methods* 3, i - ii.

579 Tilley, S.L., Hartney, J.M., Erikson, C.J., Jania, C., Nguyen, M., Stock, J., McNeisch, J.,
580 Valancius, C., Panettieri, R.A., Jr., Penn, R.B., Koller, B.H., 2003. Receptors and
581 pathways mediating the effects of prostaglandin E2 on airway tone. *Am J Physiol*
582 *Lung Cell Mol Physiol.* 284, L599-606.

583 Vancheri, C., Mastruzzo, C., Sortino, M.A., Crimi, N., 2004. The lung as a privileged site
584 for the beneficial actions of PGE2. *Trends Immunol.* 25, 40-46.

585 Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for
586 aspirin-like drugs. *Nat New Biol.* 231, 232-235.

587 Watts, S.W., Cohen, M.L., 1993. Time-dependent improvement in guinea pig tracheal
588 contractility: modification by cyclooxygenase inhibitors. *J Pharmacol Exp Ther*
589 266, 950-957.

590 Wohlsen, A., Martin, C., Vollmer, E., Branscheid, D., Magnussen, H., Becker, W.M.,
591 Lepp, U., Uhlig, S., 2003. The early allergic response in small airways of human
592 precision-cut lung slices. *Eur Respir J.* 21, 1024-1032.

593 Wohlsen, A., Uhlig, S., Martin, C., 2001. Immediate allergic response in small airways.
594 *Am J Respir Crit Care Med.* 163, 1462-1469.

595 Xie, G., Wang, Y., Sharma, M., Gabriel, A., Mitchell, J., Xing, Y., Meuser, T., Palmer,
596 P.P., 2003. 5-Hydroxytryptamine-induced plasma extravasation in the rat knee
597 joint is mediated by multiple prostaglandins. *Inflamm Res* 52, 32-38.

598

599

600

601

602

603

604

605

606 **Figure legends**

607 **Fig 1.** Airway contractions to ovalbumin in rat PCLS. A) Contractions to cumulative
608 concentrations of ovalbumin (0.01-1000 $\mu\text{g/ml}$, n=5). B) Contractions to a single dose of
609 ovalbumin (10 $\mu\text{g/ml}$, n=5). Contractions are expressed as the decrease of airway area
610 (%) compared to the initial airway area. Data are presented as mean \pm S.E.M.

611

612 **Fig 2.** Effect of COX inhibition on ovalbumin-induced bronchoconstriction. Before
613 airway contractions were induced by ovalbumin (10 $\mu\text{g/ml}$), the lung slices were
614 pretreated with A) the unselective COX inhibitor indomethacin (10 μM , n=15; control
615 n=16), B) the selective COX-1 inhibitor FR-122047 (5 μM , n=7; control n=7), C) the
616 selective COX-2 inhibitor celecoxib (10 μM , n=8; control n=8). Control slices are shown
617 in black, experiments with the inhibitors in gray. Bronchoconstriction is expressed as the
618 decrease of airway area (%) compared to the initial airway area. Data are presented as
619 mean \pm S.E.M. *, P<0.05.

620

621 **Fig 3.** Effect of EP, TP and 5-HT_{2A} receptor antagonists on contractions to ovalbumin (10
622 $\mu\text{g/ml}$). Before airway contractions were induced by ovalbumin (10 $\mu\text{g/ml}$), the lung
623 slices were pretreated with A) the EP₁ receptor antagonist ONO-8713 (10 μM , n=6;
624 control n=6), B) the EP₂ receptor antagonist A6809 (5 μM , n=6; control n=6) C) the EP₃
625 receptor antagonist L-798,106 (0.5 μM , n=6; control n=6) D) the EP₄ receptor antagonist
626 L-161,982 (0.5 μM , n=6; control n=6), E) the TP receptor antagonist BAYu3405 (10 μM ,
627 n=5; control n=5) or F) the 5-HT_{2A} receptor antagonist ketanserin (0.1 μM , n=5; control
628 n=5). Control slices are shown in black, experiments with the inhibitors in gray.

629 Bronchoconstriction is expressed as the decrease of airway area (%) compared to initial
630 airway area. Data are presented as mean \pm S.E.M. **, P<0.01.

631

632 **Fig 4.** The synthesis of A) serotonin, B) PGE₂, C) TXB₂, and D) PGD₂ was measured and
633 compared with initial release in the medium supernatant after pretreatment with selective
634 and unselective COX inhibitors and exposure to 10 μ g/ml ovalbumin. Control: Medium;
635 Control: Ovalbumin 10 μ g/ml; Indomethacin: Indomethacin 10 μ M + ovalbumin 10
636 μ g/ml; FR122047: FR-122047 5 μ M + ovalbumin 10 μ g/ml; Celecoxib: Celecoxib 10
637 μ M + ovalbumin 10 μ g/ml. Data are expressed as the mean \pm S.E.M of 5 independent
638 experiments *, P<0.05; **, P<0.01; ***, P<0.001.

639

640 **Fig 5.** Effect of COX inhibition, EP and TP antagonists on contractions induced by
641 cumulative doses of serotonin (0.01-10 μ M). Before airway contractions were induced by
642 serotonin, the lung slices were pretreated with A) the COX inhibitor indomethacin (10
643 μ M, n=9; control n=9), B) the COX-1 inhibitor FR-122047 (5 μ M, n=5; control n=5), C)
644 the COX-2 inhibitor celecoxib (10 μ M, n=5; control n=5), D) the EP₁ antagonist ONO-
645 8713 (10 μ M, n=5; control n=5), E) the EP₂ receptor antagonist A6809 (5 μ M, n=5;
646 control n=6) F) the EP₃ receptor antagonist L-798,106 (0.5 μ M, n=6; control n=6), G) the
647 EP₄ receptor antagonist L-161,982 (0.5 μ M, n=6; control n=6) or H) the TP antagonist
648 BAYu3405 (10 μ M, n=6; control n=6). Control slices are shown in black, experiments
649 with the inhibitors in gray. Bronchoconstriction is expressed as the decrease of airway
650 area (%) compared to initial airway area. Data are presented as mean \pm S.E.M. *, P<0.05;
651 **, P<0.01; ***, P<0.001.

652

653 **Fig 6.** Receptor expression of 5-HT_{2A}R. An incubation of PCLS with the EP₁ antagonist
654 ONO-8713 (10 μ M), the COX-1 inhibitor FR-122047 (5 μ M), the unselective COX-
655 inhibitor indomethacin (10 μ M) and the COX-2-inhibitor celecoxib (10 μ M) for 4h
656 resulted in the change of the 5-HT_{2A} receptor. Data were referenced to the housekeeping
657 gene B2m and normalized to the mean of the experimental control. Data (n=5) are
658 presented as mean \pm S.E.M. *, P<0.05.

659

660 **Fig 7.** Effect of COX inhibition and EP₁ receptor antagonist on airway contractions to
661 cumulative doses of methacholine (0.01-10 μ M). Before airway contractions were
662 induced by methacholine, the lung slices were pretreated with indomethacin (10 μ M,
663 n=4, gray) or ONO-8713 (10 μ M, n=4, gray dashes) compared to control (n=6, black).
664 Bronchoconstriction is expressed as the decrease of airway area (%) compared to initial
665 airway area. Data are presented as mean \pm S.E.M.

1 **Table 1.** Contractile responses to PGD₂, PGE₂ and U46619 in ratPCLS

Agonists	Contractions (%)	SEM
PGD ₂ (10 μM)	0	0.8
PGE ₂ (10 μM)	0	1.1
U46619 (50 μM)	16	2.1
U46619 (50 μM) + BAYu3405 (10 μM) ^a	0	0.1

2

3 Airway contractions to PGD₂ (10 μM, n=3), PGE₂ (10 μM, n=3), TP receptor agonist

4 U46619 (50 μM, n=3) and U46619 (50 μM) in combination with the TP receptor

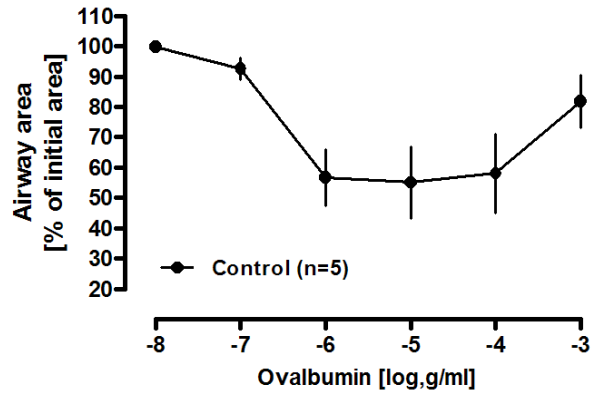
5 antagonist BAYu3405 (10 μM, n=3, ^aP<0.05) in rat PCLS. Contractions are expressed as

6 the decrease of airway area (%) compared to the initial airway area. Data are presented as

7 mean ± S.E.M.

8

Fig 1A



1B

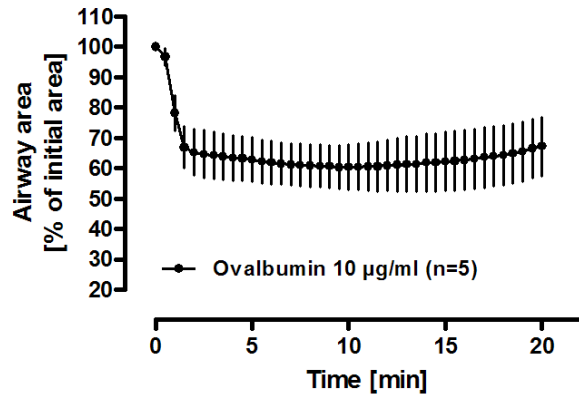
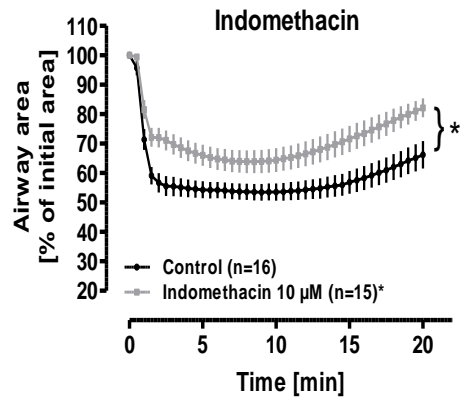
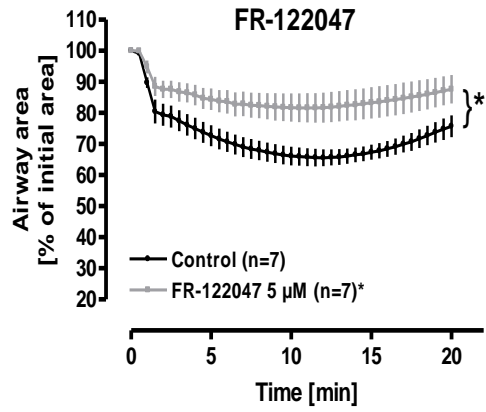


Fig 2A



2B



2C

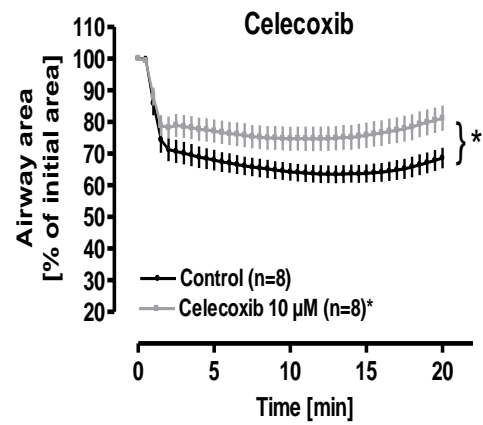


Figure 3

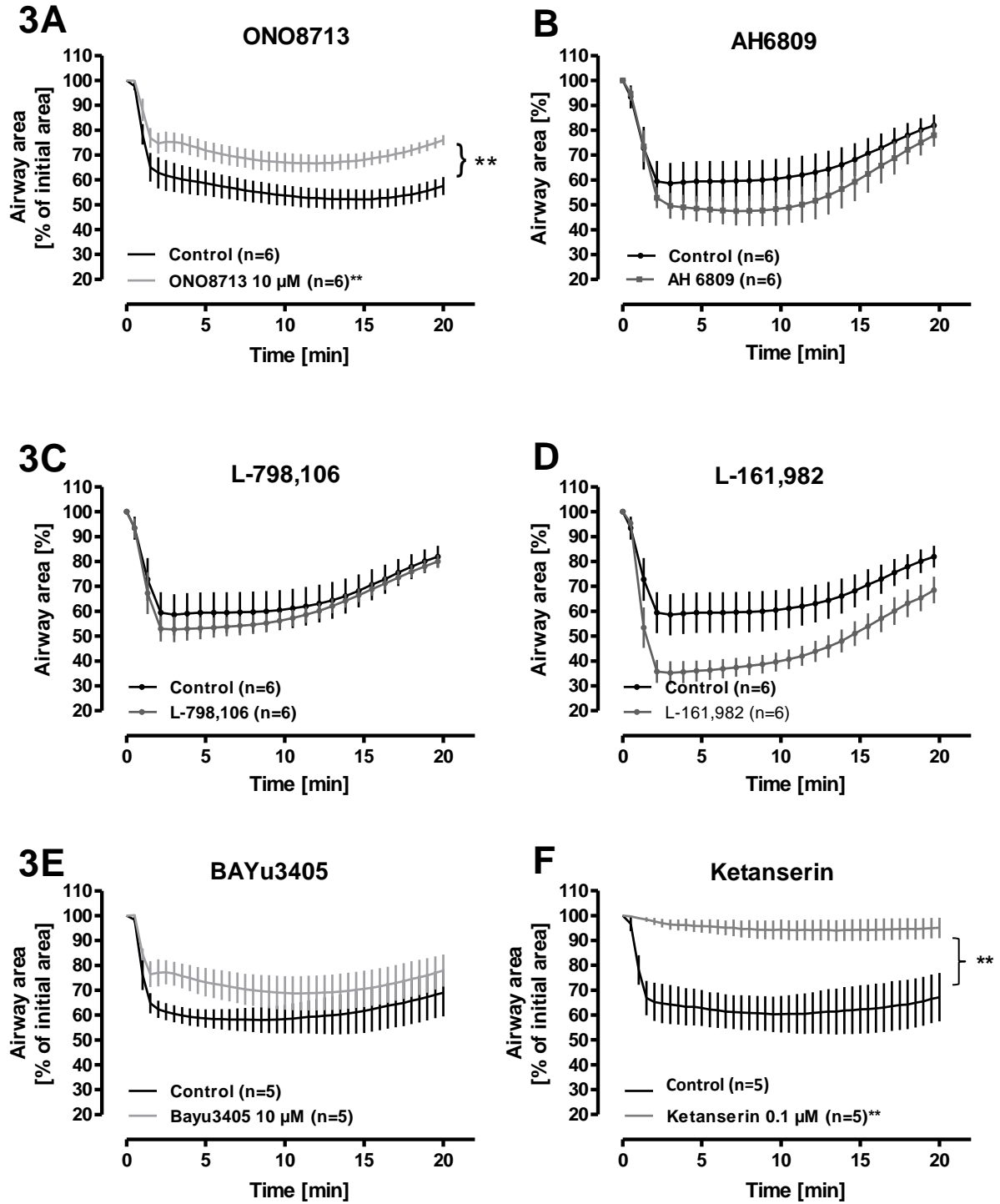


Fig 4

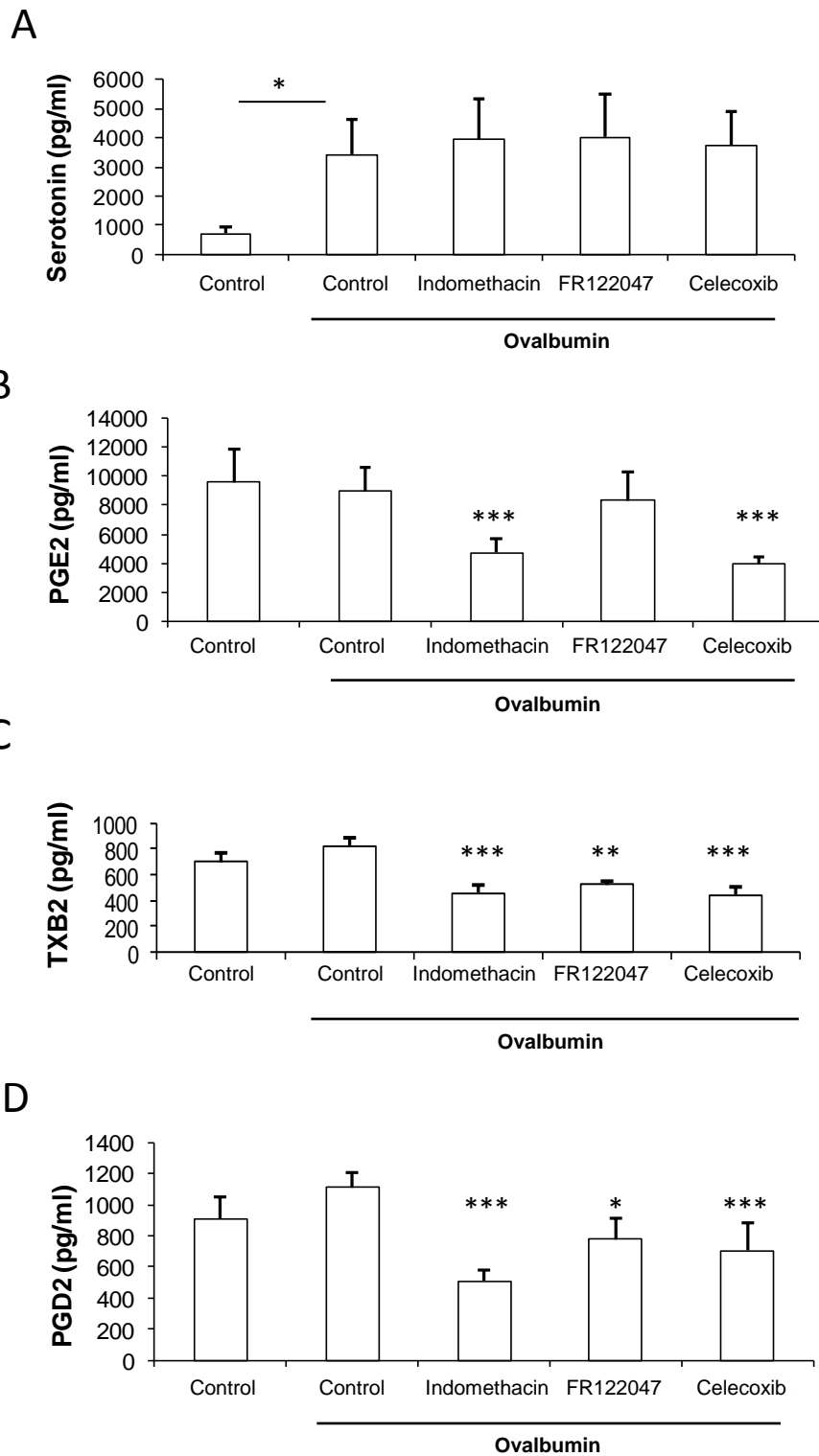


Figure 5

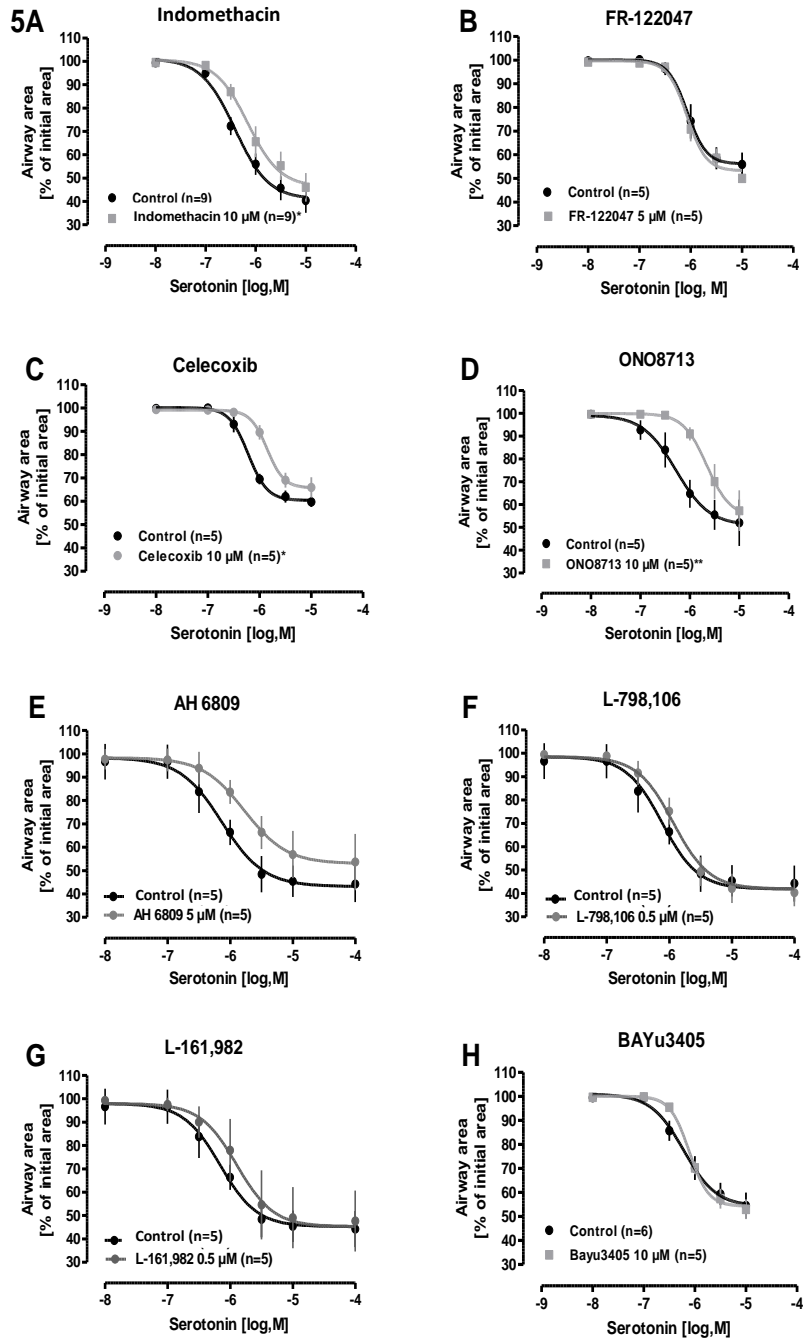


Fig 6

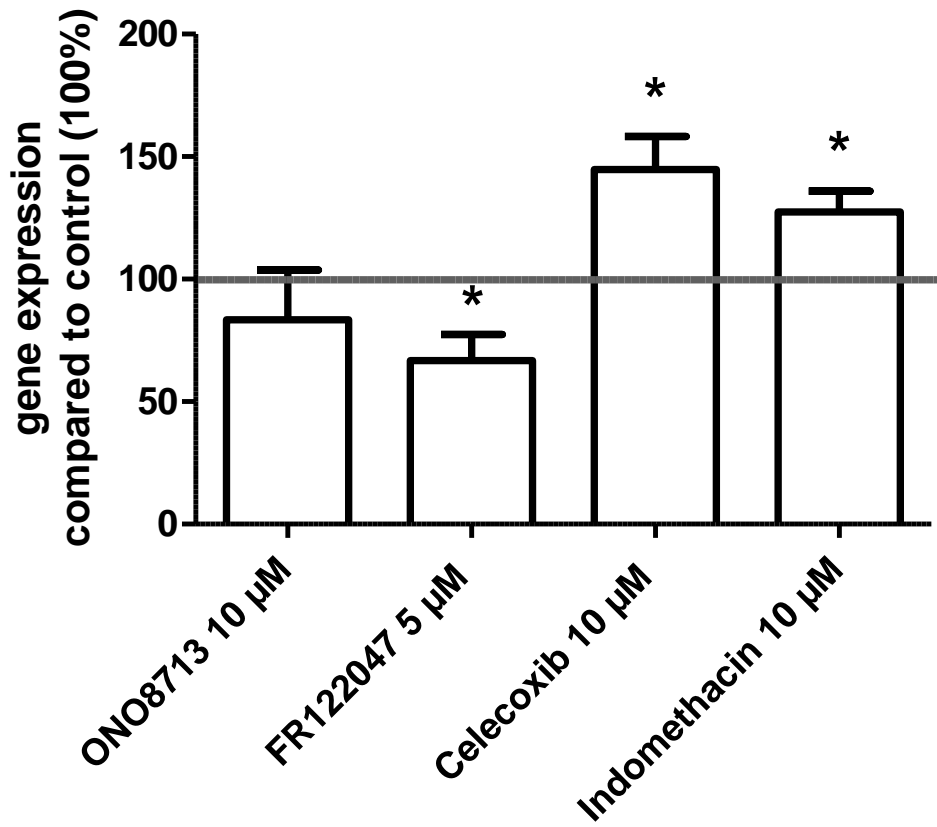


Fig 7

