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Lipoxin A_4 reduces house dust mite and TNFα-induced hyperreactivity in the mouse trachea

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ABSTRACT

Lipoxin A_4 (LXA_4) is considered a specialised pro-resolving mediator that decreases inflammation: however, pro-inflammatory effects have been described in the airways. Here, we investigated whether LXA_4 could influence airway hyperreactivity induced in mouse trachea by house dust mite extract (HDM) or TNFα. Intranasal instillation of HDM caused a serotonin (5-HT) mediated airway hyperreactivity *ex vivo* (E_{max}: 78.1 ± 16.2 % *versus* control 12.8 ± 1.0 %) that was reduced by LXA_4 installation one hour prior to HDM (E_{max}: 49.9 ± 11.4 %). Also, in isolated tracheal segments cultured for four days, HDM induced a hyperreactivity (E_{max}: 33.2 ± 3.1 % *versus* control 9.0 ± 0.7 %) that was decreased by LXA_4 (E_{max}: 18.7 ± 1.5 %). One part of the HDM-induced hyperreactivity could be inhibited by the TNFα-inhibitor etanercept. TNFα-induced upregulation of 5-HT responses (E_{max}: 51.3 ± 1.2 % *versus* control 13.9 ± 0.5 %) was decreased by 10 − 1000 nM LXA_4. In precontracted tracheal segments, LXA_4 had no relaxing effect. Overall, LXA_4 was able to decrease airway hyperreactivity induced by both HDM and TNFα, thus having a sub-acute anti-inflammatory effect in airway inflammation.

1. Introduction

Chronic lung inflammation is a prominent feature of airway diseases such as allergic or non-allergic asthma [1]. As acute inflammation progresses, a lipid mediator class switch occurs from pro-inflammatory prostaglandins and leukotrienes to anti-inflammatory and resolution-promoting lipids know as specialised pro-resolving mediators (SPMs) [2]. One hypothesis is that defect resolution of inflammation aggravates the chronic inflammation seen in asthma. In cells recovered from asthmatic patients, decreased production of lipoxins as well as a down-regulated expression of one of its receptors, ALX/FPR2, has been found [3,4]. Furthermore, several studies showing anti-inflammatory effects of lipoxin A_4 (LXA_4; 5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid) have been performed in cells [5,6]. However, initial studies performed in isolated airways point to possible pro-inflammatory functions [7,8]. The role of LXA_4's function in intact airway tissue during inflammation remains unclear. We therefore investigated the function of LXA_4 in isolated airway tissue and hypothesised that treatment with LXA_4 would dampen inflammation-induced airway hyperreactivity.

LXA_4 is a trihydroxylated product of arachidonic acid metabolism that is biosynthesised in reactions involving multiple lipoxygenase (LOX) enzymes from different cell types [9]. LXA_4 is considered to be a part of the SPM family and anti-inflammatory and pro-resolving actions in lung models have been reported [10,11]. One receptor important in conveying LXA_4 signalling is the ALX/FRP2 receptor, and it has been suggested that the docosahexaenoic acid (DHA) derived resolvin-D1 (RvD1; 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) and its aspirin-triggered epimer 17(R)-resolvin D1 (17(R)-RvD1; 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) also signal through this receptor [12]. In addition, LXA_4 binds the aryl hydrocarbon receptor leading to the expression of suppression of cytokine signalling (SOCS-2) protein [13] and has been suggested to displace binding of LTD_4 on the $CysLT_1$ receptor [14]. Lipoxin B_4 (LXB_4, 5S,14R,15S-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid), a constitutional isomer of LXA_4, also has anti-inflammatory actions such as inhibition of TNFα release. However, signalling has been suggested to occur *via* a distinct pathway [5,15,16] thus adding another layer of complexity to SPM-signalling during inflammation and resolution.

House dust mite (HDM) is a common constituent of house dust and

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has been shown to play a role in allergic sensitisation and asthma [17,18]. HDM exposure causes release of the pro-inflammatory cytokine TNFα [19] and inflammation of the airways [20,21]. In mice, TNFα exposure can result in airway hyperreactivity, which is seen as increased serotonin-(5-HT) induced smooth muscle contraction, mediated by the 5-HT_{2a} receptor [22].

Because of earlier findings in airway tissues where we only observed pro-inflammatory effects of LXA_4 [7,8], the focus of this study on LXA_4 was to provide insight into whether or not LXA_4 also displays anti-inflammatory actions. For comparisons, we included RvD1 and 17(R)-RvD1 in the *in vivo* part and LXB_4 in the *in vitro* setting. The influence of LXA_4 on smooth muscle hyperreactivity induced by either HDM or TNFα was studied in isolated mouse tracheal segments.

2. Methods

2.1. Animals and experimental procedures

The study was approved by the regional animal experimentation ethical review board (N55/15). BALB/c mice of 8–14 weeks old (Charles River, Sulzfeld, DE or Envigo, Horst, NL) were used. During *in vivo* challenge, mice were given LXA_4 (20 μL, 0.5 ng/μL) through intranasal instillation, one hour before HDM (20 μL, 2.5 μg/μL) under general anaesthesia (isoflurane, 4%, 250 μl/min for 1 − 2 min). This procedure was repeated for four consecutive days. All mice were killed by cervical dislocation. Whole tracheae were dissected free from connective tissue and along the cartilage rings divided in four equal segments while kept on ice-cold Krebs-Henseleit buffer supplemented with 2.5 mM calcium chloride and 2.1 g/L sodium bicarbonate. Tracheal segments of mice that had received intranasal instillation (*in vivo* exposure) where then assessed for their 5-HT reactivity. For the *in vitro* exposure experiments, tracheal segments from naïve mice were put in a 96 wells culture plate containing 37 °C Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Auckland, NZ) supplemented with 1% penicillin (100 $IU{\cdot}mL^{-1}$) and streptomycin (100 $\mu g{\cdot}mL^{-1}$) under sterile conditions and left to equilibrate for 30 min in a humidified incubator (37 °C, 5% CO_2 in O_2). Compounds were added with one-hour intervals. LXA_4 was added first and TNFα (100 ng/mL) or HDM (1 μg/mL) second. This procedure was repeated every 24 h for a total of 96 h. Every day, segments were moved to fresh medium and compounds added. In the LXA_4-antagonist experiments, WRW4 (1 μM) [23], montelukast (1 μM) [14] or CH223191 (1 μM) [24] were added first, LXA_4 second and TNFα last. In the TNFα inhibition experiments, etanercept (100 μg/mL) was added first and TNFα (100 ng/mL) second. In the 5-HT antagonist experiments, tracheal segments were cultured for four days in the presence of TNFα (100 ng/mL), mounted in the organ bath and ketanserin or atropine was added 30 min before the cumulative addition of 5-HT.

2.2. Functional studies

Tracheal rings were suspended in myographs (DMT, Aarhus, DK) filled with 5 mL Krebs-Henseleit buffer and continuously bubbled with carbogen gas (5% CO_2 in O_2). The force applied was recorded using LabChart 7 (ADInstruments Ltd., Oxford, UK). After suspension, the segments were left to equilibrate for 30 − 60 min. The tension was then stepwise increased to 0.8 mN until a stable baseline was reached [22]. The viability of the segments was tested twice with KCl (60 mM).

A carbachol-(10 μM) induced contraction was acquired after washing. Before further pharmacological investigation, indomethacin (3 μM) was added to prevent release and possible interference of prostaglandins. Then an additional 30 minute equilibration period was completed and was followed by increasing concentrations of 5-HT (1 nM – 10 μM). The experiment ended with a maximal contraction by adding KCl (60 mM) and carbachol (10 μM) on top of the highest 5-HT concentration.

Mice tracheal segments that contracted less than 1.0 mN were excluded. All 'n' refers to the number of segments. The response was normalised against the contraction obtained with carbachol (10 μM) or against the maximal response of the TNFα treated segments in the case of the LXA_4-antagonist experiments.

2.3. Drugs and suppliers

Serotonin hydrochloride, carbachol, KCl, $NaHCO_3$, $CaCl_2$ solution 1 M, indomethacin, Krebs-Henseleit buffer and DMSO were all purchased from Sigma-Aldrich (St. Louis, MO, USA). U-46619, lipoxin A_4 (LXA_4), lipoxin B_4 (LXB_4), resolvin D1 (RvD1) and 17(R)-resolvin D1 (17(R)-RvD1) came from Cayman Chemical (Ann Harbor, MI, USA), murine TNFα from R&D systems (Minneapolis, MN, USA), WRW4 and CH223191 from Tocris Bioscience (Bristol, OXF, GB) and ethanol absolute from VWR (West Chester, PA, USA). 5-HT, carbachol and montelukast stocks were dissolved in demineralised water. TNFα and WRW4 were dissolved in 0.1 % BSA (Sigma-Aldrich) under sterile conditions. DMSO was used to dissolve CH223191 and ethanol absolute for indomethacin and LXA_4. All stocks were stored at − 20 °C, except for LXA_4, which was stored at − 80 °C in amber glass vials. Before usage, stock solutions were diluted in 0.1 % BSA when used in tissue culturing, in PBS for intranasal instillation or buffer during myograph experiments.

2.4. Data and statistics

All data are presented as mean ± SEM. Non-linear regression fit was used to calculate pEC_{50} and E_{max}. One-way ANOVA with Bonferroni correction for multiple testing, comparing other groups with only TNF/HDM treated groups, or student's *t*-test were used to assess statistical significance of the results using GraphPad Prism 8.01 software (GraphPad Software Inc., San Diego, CA). Values of $p < 0.05$ were deemed significant. In the analysis of the antagonist experiments, a one-tailed test was used since an antagonistic effect could only result in increased maximal contraction.

3. Results

3.1. LXA_4 reduces HDM-induced 5-HT responses after in vivo stimulation

To investigate if *in vivo* exposure to LXA_4 could alter HDM-induced airway hyperreactivity, LXA_4 was given *via* intranasal instillation one hour before HDM instillation. RvD1 or 17(R)-RvD1 were also tested to assess the possibility that the effect could be related to ALX/FPR2 activation. In tracheae from mice treated intranasally with PBS, 5-HT caused a concentration-dependent contraction (E_{max} : 12.8 ± 1.0 %, Fig. 1). Daily instillation with HDM (50 μg) for four days increased the maximal contraction of 5-HT (E_{max} : 78.1 ± 16.2 %, $p < 0.05$).

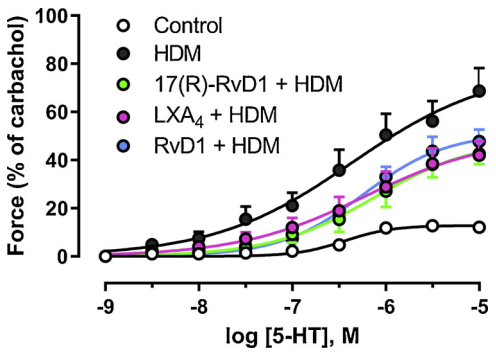

Fig. 1. Concentration-response curves of 5-HT in mouse trachea *ex vivo* after four days of intranasal instillation of selected SPMs (10 ng) one hour before *in vivo* challenge with HDM (50 μg). Data represent mean ± SEM (n = 5-11).

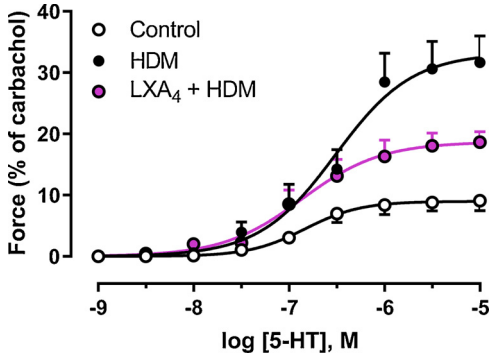

Fig. 2. Concentration-response curves of 5-HT after four days of culturing and exposure to LXA_4 (100 nM) added one hour before HDM (1 μg/mL). Every day, segments were moved to fresh medium and compounds added. Data represent mean ± SEM (n = 5-8).

Intranasal exposure to LXA_4 (10 ng) one hour prior to each instillation of HDM reduced the maximal 5-HT contraction (E_{max} : 49.9 ± 11.4 %, $p < 0.05$). In the same way, intranasal pre-treatment with 17(R)-RvD1 (10 ng) reduced the HDM response (E_{max} to 48.9 ± 8.8 % ($p < 0.05$). Treatment with RvD1 (10 ng) numerically reduced the E_{max} to 51.3 ± 4.7 % ($p = 0.07$). The potency (pEC_{50}) of 5-HT was unaffected by all treatments.

3.2. LXA_4 reduces HDM-induced 5-HT responses in vitro

To test whether LXA_4 would have the same effect in an *in vitro* HDM model, cultured tracheal segments were exposed to LXA_4 (100 nM) and HDM (1 μg/mL). After four days of culturing, 5-HT caused a concentration-dependent contraction (E_{max} : 9.0 ± 0.7 %, Fig. 2). Daily exposure of HDM to isolated segments increased the maximal contraction of 5-HT (E_{max} : 33.2 ± 3.1 %, $p < 0.05$). Addition of LXA_4 one hour before HDM reduced the maximal contraction (E_{max} : 18.7 ± 1.5 %, $p < 0.05$). The pEC_{50} was unaffected by all treatments.

3.3. HDM-induced upregulation of 5-HT response is partly mediated by TNFα

Because previous studies have shown that LXA_4 can block the release of TNFα and dampen its effect [5,25], it was investigated if HDM-induced hyperreactivity was mediated by TNFα release and thus could be blocked by a TNFα inhibitor.

We first established that the TNFα inhibitor etanercept could block the hyperreactivity-inducing effect of TNFα. Etanercept (100 μg/mL) was added one hour before TNFα (100 ng/mL) during four-day culture (Fig. 3A). 5-HT caused a concentration-dependent contraction in control segments (E_{max} : 21.4 ± 1.4 %). TNFα increased the maximal contraction of 5-HT (E_{max} : 76.8 ± 1.2 %, $p < 0.05$). When adding etanercept before TNFα, the effect of TNFα was completely inhibited (E_{max} : 21.5 ± 1.1 %). Treatment with etanercept was not different from control (E_{max} 16.3 ± 1.1 %).

We next cultured tracheal segments with etanercept (100 μg/mL) and HDM (1 μg/mL) to assess if etanercept could inhibit HDM-induced hyperreactivity (Fig. 3B). After four days of culture, 5-HT caused a concentration-dependent contraction (E_{max} : 17.3 ± 2.0 %). When exposed for four days to HDM, the maximal contraction of 5-HT was enhanced (E_{max} : 29.8 ± 1.0 %, $p < 0.05$). Etanercept added one hour before HDM reduced the maximal contraction (E_{max} : 21.5 ± 1.3 %, $p < 0.05$). Etanercept alone had no effect (E_{max} : 16.3 ± 1.1 %) and none of the potencies of 5-HT were affected by the treatments.

3.4. LXA_4 and LXB_4 reduce TNFα-induced 5-HT responses in vitro

To investigate if LXA_4 could inhibit TNFα-induced airway hyperreactivity, segments were exposed to LXA_4 before TNFα. After four days of culturing, control segments displayed a concentration-dependent contraction (E_{max} : 13.9 ± 0.7 %, Fig. 4A–C). When culturing with TNFα (100 ng/mL), the maximal contraction increased (E_{max} : 51.3 ± 0.7 %, $p < 0.05$) (Fig. 4A–C). Exposure to LXA_4 (10, 100 and 1000 nM) decreased the TNFα-induced hyperreactivity (E_{max} : 24.4 ± 1.3 %, 34.2 ± 1.7 % and 32.0 ± 3.7 %, respectively, $p < 0.05$). The dampening effect of LXA_4 did not differ significantly between the concentrations tested. LXA_4 alone had an no effect with respect to the concentrations investigated.

In addition, LXB_4 was added to investigate if the ameliorating effect was selective for LXA_4 within the lipoxin family. Control segments in Fig. 4D reached an E_{max} of 15.4 ± 3.0 %. Four-day exposure to TNFα (100 ng/mL) increased the maximal contraction (E_{max} : 60.8 ± 3.0 %, $p < 0.05$). LXB_4 (0.1 μM) reduced the maximal contraction (E_{max} : 44.0 ± 4.6 %, $p < 0.05$). Additionally, LXB_4 (1 μM) also reduced the maximal contraction (E_{max} : 50.8 ± 4.0 %), though this did not reach statistical significance ($p = 0.18$). Moreover, none of the potencies were affected by the treatments.

3.5. The ALX/FPR2 receptor partly mediates the anti-inflammatory effect of LXA_4

We next addressed which receptor mediated the effect of LXA_4. The ALX/FPR2 receptor antagonist WRW4 (1 μM) reduced the effect of LXA_4 partly whereas the aryl hydrocarbon receptor antagonist CH223191 (1 μM) or the $CysLT_1$ receptor antagonist montelukast (1 μM) had no significant effect on LXA_4-mediated inhibition of airway hyperreactivity (Table 1).

3.6. LXA_4 does not relax tracheal smooth muscle

To determine whether the effect of LXA_4 could be related to relaxation of tracheal smooth muscle, tracheal segments from naïve mice

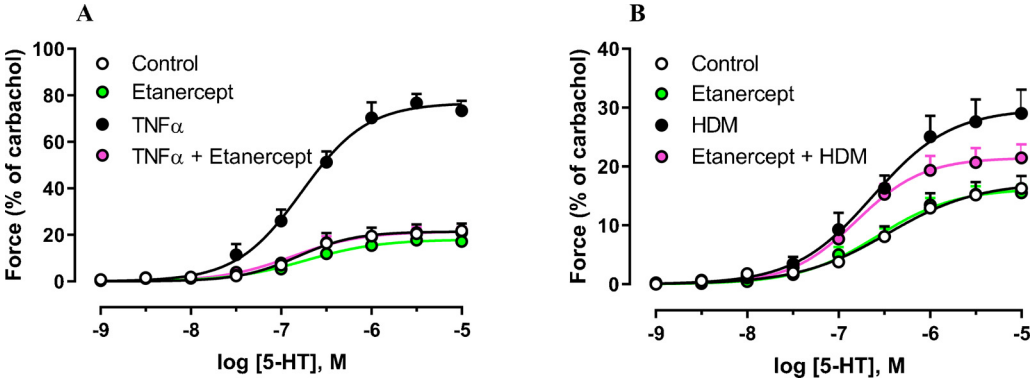

Fig. 3. A, concentration-response curves of 5-HT after four days of culturing and exposure to etanercept (100 μg/mL) added one hour before TNFα (100 ng/mL) (n = 6-8). B, concentration-response curves of 5-HT after four days of culturing and exposure to etanercept (100 μg/mL) added one hour before HDM (100 ng/mL). Data represent mean ± SEM (n = 6-11).

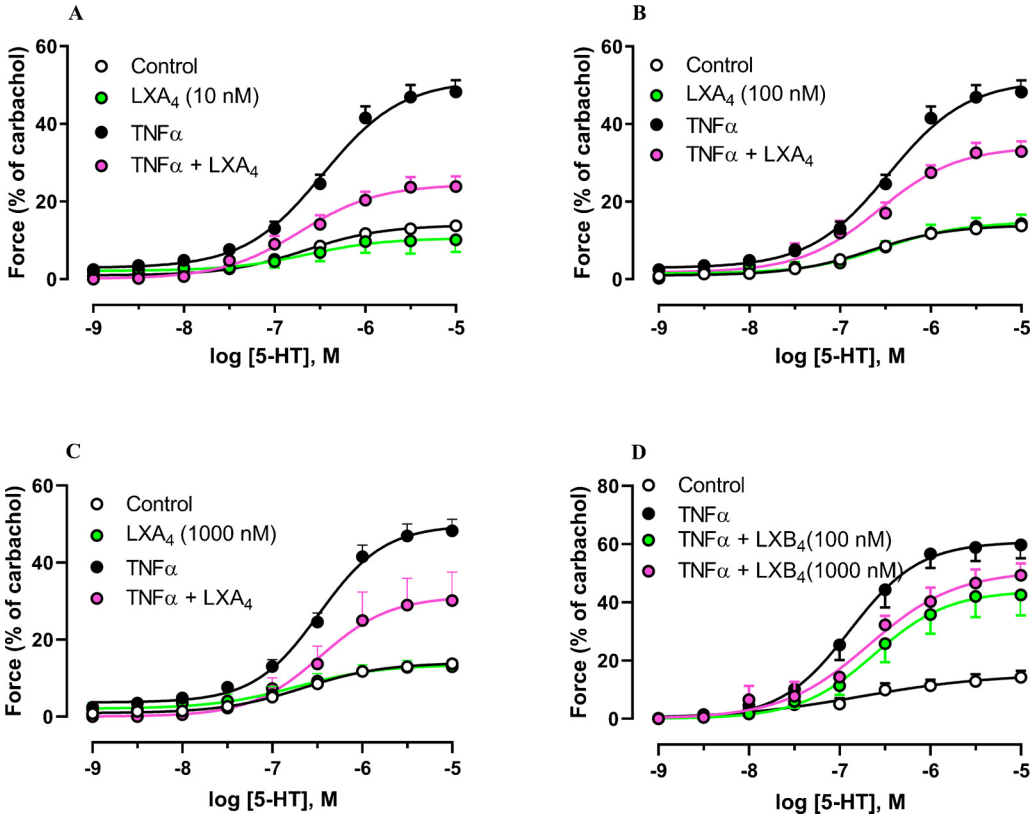

Fig. 4. Concentration-response curves of 5-HT after four days of culturing and exposure to different concentrations LXA_4 and LXB_4. As experiments were done in paired segments, the same TNFα (n = 21) and control segments (n = 20) are used in A, B and C. LXA_4 (10-1000 nM) was added one hour before TNFα (100 ng/mL) (n = 5-9) or LXA_4 (10-1000 nM) alone (*n* = 4-12). D, LXB_4 (100 or 1000 nM) was added one hour before TNFα (100 ng/mL) (*n* = 7-13), control (n = 9), TNFα (n = 10). Data represent mean ± SEM.

Table 1
Effects of WRW4 (ALX/FPR2 receptor antagonist), montelukast ($CysLT_1$ receptor antagonist) or CH223191 (aryl hydrocarbon receptor antagonist) on 5-HT-induced contractions after four days of culture and exposure to TNFα (100 ng/mL) and LXA_4 (100 nM).

	LXA_4	WRW4 (1 μM)	Montelukast (1 μM)	CH223191 (1 μM)
n	7	6	9	8
mean	61.6 ± 3.8 %	81.3 ± 6.8 %	70.9 ± 8.5 %	76.0 ± 12.8 %
p	n.a.	0.04	0.57	0.53

Data represent mean ± SEM, normalised against the maximal response of the TNFα treated segments (*n* = 6–17). n.a. = not applicable.

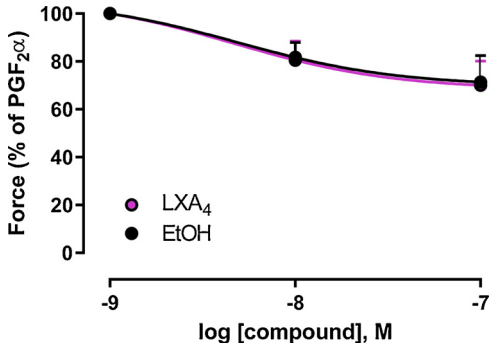

Fig. 5. Concentration-response curves of LXA_4 or ethanol added after submaximal precontraction with $PGF_{2\alpha}$ (3 μM). Data represent mean ± SEM (*n* = 7-10).

were submaximally precontracted with $PGF_{2\alpha}$ (3 μM). Two concentrations of LXA_4 (10 nM and 100 nM) or vehicle (ethanol) were then cumulatively added (Fig. 5). No smooth muscle effect of LXA_4 could be observed.

3.7. 5-HT induced contractions are predominantly mediated by the 5-HT_{2a} receptor on tracheal smooth muscle

As 5-HT causes both direct contraction through the 5-HT_{2a} receptor expressed on smooth muscle cells [22] and indirect contraction through neuronal release of acetylcholine (ACh) [26], we tested which pathway was predominant in our model. After four days of culture and exposure to TNFα (100 ng/mL), atropine (1 μM), added half an hour before the cumulative addition of 5-HT, only slightly right-shifted the concentration-response curve (pEC_{50} = 6.46, $p < 0.05$) compared to untreated, TNFα cultured segments (pEC50 = 6.88), without affecting the maximal contraction (E_{max}: 64.8 ± 3.5 % *vs.* E_{max} : 63.1 ± 3.5 %, Fig. 6). In contrast, the 5-HT_{2a} receptor antagonist ketanserin (0.1 μM and 1 μM), added half an hour before 5-HT, concentration-dependently right-shifted the curve to such an extent that pEC_{50} values could not be determined, since the maximal responses were decreased (E_{max} : 31.4 ± 8.2 % and 12.6 ± 4.2 %, $p < 0.05$).

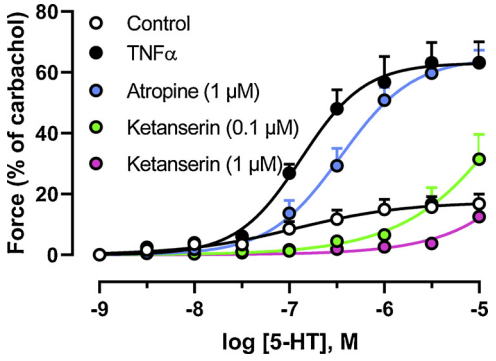

Fig. 6. Concentration-response curves of 5-HT after four days of culturing and exposure to TNFα (100 ng/mL) with atropine (1 μM) or ketanserin (0.1 μM or 1 μM) added 30 min before addition of 5-HT. Data represent mean ± SEM (*n* = 7-9).

4. Discussion

Using both *in vivo* and *in vitro* challenge, it was found that LXA_4 can reduce HDM- and TNFα-induced 5-HT hyperreactivity in mouse trachea. The hyperreactivity induced by HDM was partially mediated through the release of TNFα. One part of the effect on LXA_4 was exerted *via* the ALX/FPR2 receptor. Moreover, LXA_4 did not have an intrinsic smooth muscle effect. The 5-HT-induced contractions were predominantly mediated by $5\text{-}HT_{2a}$ receptors on the smooth muscle.

First, intranasal instillation of LXA_4, RvD1 and 17(R)-RvD1 before the instillation of HDM in mice led to a decrease of HDM-induced hyperreactivity to 5-HT in mice. A protective effect of LXA_4 has also been shown in a mouse model of cockroach allergen-induced hyperresponsiveness [27]. In that study, oral ingestion of stable analogues of LXA_4 could reduce allergen-induced airway inflammation and hyperresponsiveness measured by plethysmography and methacholine challenge under anaesthesia. It is therefore unclear if that protective effect was due to a systemic effect or a direct effect on the airways. Our current study however documents that the tested SPMs directly inhibit airway hyperreactivity in the airways. Thus, *in vivo* administration of selected SPMs had a protective effect on antigen-induced airway hyperreactivity.

As *in vivo* HDM exposure can induce a systemic reaction involving IgE activation and infiltration of cells, we next investigated if HDM induces airway hyperreactivity regardless of systemic inflammation. In the four-day *in vitro* protocol, HDM caused a marked increase of 5-HT-mediated constrictions that could be dampened by LXA_4. Thus, both the increase for the 5-HT contraction and the inhibitory action of LXA_4 in this setting were obtained without systemic influence. This implicates that the structural unit within the airways has intrinsic immunological and inflammatory properties. However, this does not preclude that systemic effects also may take place *in vivo*. This question will be addressed in future studies.

As it previously has been found that TNFα causes an increased contraction to 5-HT [22], we investigated if the observed HDM-induced hyperreactivity was mediated by TNFα. By blocking TNFα with etanercept, it was shown that the effect of HDM on the 5-HT contractility was partly reduced. This is in line with previous results where it was shown that intratracheal instillation of HDM in mice leads to an increased release of TNFα [19] as also shown in human airway epithelial cells [28]. Previous findings also show that the tumour necrosis factor 1 receptor (TNFR1) is expressed on tracheal epithelium and that TNFR1 expression is increased after stimulation with IL-1β in both tracheal epithelium and smooth muscle [29]. As there was not a complete reduction of airway hyperreactivity by etanercept, release of additional mediators presumably accounts for a residual part of the hyperreactive response to HDM.

Because HDM caused release of TNFα and LXA_4 showed a protective effect on HDM-induced hyperreactivity, we hypothesised that LXA_4 could ameliorate TNFα-induced hyperreactivity *in vitro*. That LXA_4 may inhibit TNFα has previous been shown in human pulmonary endothelial cells, where oxidised 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) caused release of LXA_4, which inhibited NF-κB signalling induced by TNFα. The same study also showed that intratracheal treatment with OxPAPC diminished the TNFα concentration in bronchoalveolar lavage (BAL) fluid after LPS exposure in C57BL/6 mice [11]. In the present study, a protective effect of lipoxins on the TNFα-induced hyperreactivity was indeed shown for LXA_4 and further substantiated by the finding that the constitutional isomer LXB_4 showed the same effect.

Pharmacologically, the effects of SPMs have sometimes been described to exert a bell-shaped dose- or concentration-response curve by multiple receptors [13,14,30,31]. However, in the concentration range used in this study, no bell-shape response was observed. When investigating the known receptors for LXA_4 signalling, the ALX/FPR2 antagonist WRW4 partly antagonised the response, whereas antagonists for the aryl hydrocarbon receptor [13] and the $CysLT_1$ receptor [14,31] had no inhibitory effect. Similarly, in a model of lung inflammation, the inhibitory effect by LXA_4 was only partly mediated through the ALX/FPR2 receptor [11]. When Barnig et al. used this antagonist to block the inhibitory effect of LXA_4 on IL-13 secretion by ILC2 cells, they observed also a partial block with a large standard error [6], thus pointing at the need for a better antagonist to establish the receptors involved. The finding that RvD1 and 17(R)-RvD1 both could reproduce the effect of LXA_4 after intranasal instillation suggests a common pathway, signalling *via* the ALX/FPR2 receptor as these SPMs are proposed agonists of this receptor [12,32]. That the ALX/FPR2 receptor is expressed in mouse lung tissue, including the epithelial lining, has been shown in several studies [33–36]. As with LXA_4, LXB_4 can reduce allergic airway inflammation [15], suggesting also a possible shared pathway. In the present study, we thus provide some evidence for the involvement of the ALX/FPR2 receptor, but we acknowledge that additional receptors may be involved and would like to point out the need for a better antagonist of this receptor to establish the role of LXA_4.

In conclusion, our experiments show that specific SPMs such as LXA_4, 17-(R)-RvD1 and RvD1 have a marked anti-inflammatory effect that can prevent *in vivo* HDM-induced upregulation of 5-HT airway hyperreactivity in mouse trachea. This protective function is also replicated by LXA_4 in an *in vitro* model with HDM and TNFα and could be replicated by the structural isomer LXB_4. This effect is not due to smooth muscle relaxation. One part of the protective effect by LXA_4 is mediated by the ALX/FPR2 receptor, but future studies are needed to clarify the receptors involved. For the lipoxins, these results show a new pharmacological function and add to the increasing evidence of the predominant anti-inflammatory and pro-resolution functions of this lipid mediator family.

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