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Interleukin-1β-induced hyperresponsiveness to [Sar^9,Met(O$_2$)^{11}]substance P in isolated human bronchi

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Abstract

Interleukin-1β has been reported to induce airway hyperresponsiveness in several animal models. In this study, we have investigated whether interleukin-1β was able to potentiate the contractions of human isolated small bronchi (internal diameter $\leq$ 1 mm) provoked by a specific tachykinin NK$_1$ receptor agonist, [Sar^9,Met(O$_2$)^{11}]substance P. Pre-incubation of human isolated small bronchi with interleukin-1β (10 ng/ml, in Krebs–Henseleit solution, at 21°C for 15 h) potentiated the contractile response to [Sar^9,Met(O$_2$)^{11}]substance P. It also increased the [Sar^9,Met(O$_2$)^{11}]substance P-induced release of thromboxane B$_2$, the stable metabolite of thromboxane A$_2$. Indomethacin (10^{-6} M), a non-specific cyclooxygenase inhibitor, or GR 32191 ((1*R*-(1α(*Z*),2β,3β,5α))-(+)-7-(5-(((1,1′-biphenyl)-4-yl)-methoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl)-4-heptenoic acid, hydrochloride) (10^{-6} M), a prostanoid TP-receptor antagonist, blocked the contractions induced by [Sar^9,Met(O$_2$)^{11}]substance P both in control experiments and after interleukin-1β pre-treatment, indicating that prostanoids and thromboxane receptors are directly implicated in the [Sar^9,Met(O$_2$)^{11}]substance P-induced contractile response. The thromboxane mimetic U-46619 (10^{-8}–10^{-6} M) (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F$_{2\alpha}$)-induced contractions of human isolated small bronchi were not enhanced by interleukin-1β pre-treatment, suggesting that no up-regulation of thromboxane receptors occurred. Furthermore, the cyclooxygenase-2 inhibitor CGP 28238 (6-(2,4-difluorophenoxy)-5-methyl-sulfonylamino-1-indanone) (10^{-6} M) had no direct effect on [Sar^9,Met(O$_2$)^{11}]substance P-provoked contractions, but inhibited the interleukin-1β-induced potentiation of [Sar^9,Met(O$_2$)^{11}]substance P response. In conclusion, our results show that interleukin-1β pre-treatment is able to potentiate the contractions of isolated human small bronchi provoked by [Sar^9,Met(O$_2$)^{11}]substance P both by increasing prostanoid synthesis and by inducing a cyclooxygenase-2 pathway. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Isolated bronchus, human; Smooth muscle, airway; Tachykinin; Tachykinin NK$_1$ receptor; Airway hyperresponsiveness; Interleukin-1β

1. Introduction

Airway inflammation, a main feature of asthma, is involved in the pathophysiology of airway hyperresponsiveness. Increased levels of several pro-inflammatory cytokines including interleukin-1β have been found in bronchoalveolar lavage fluid from symptomatic asthmatic patients (Broide et al., 1992; Cembrzynska-Nowak et al., 1993). Among the various endogenous inflammatory mediators and cytokines involved in airway inflammation, interleukin-1β has been shown to induce airway hyperresponsiveness in several models (Hernandez et al., 1991; Van Oosterhout and Nijkamp, 1993; Tsukagoshi et al., 1994; Molimard et al., 1998). The mechanisms of this hyperresponsiveness are unclear but may be mediated in part by prostanoids. Indeed, several studies have shown that prostanoid synthesis may be enhanced by interleukin-1β. Interleukin-1β is able to induce cyclooxygenase-2 production by cultured airway epithelial and smooth muscle cells and subsequently, increases the release of prostanoid into the culture medium (Mitchell et al., 1994; Belvisi et al., 1997; Pang and Knox, 1997). We have recently demon-

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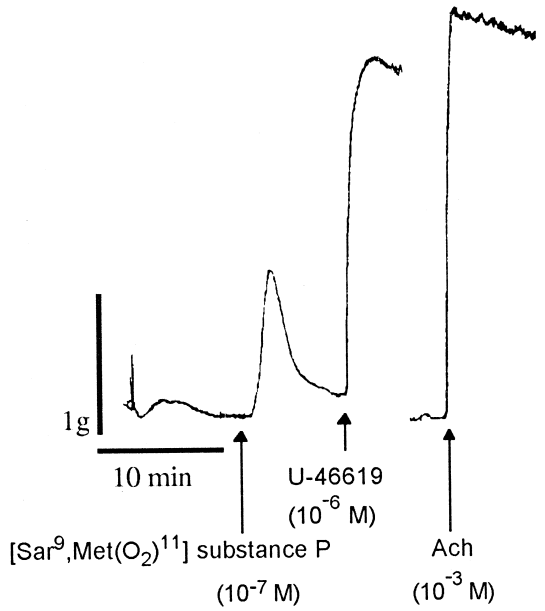

Fig. 1. Representative recording of the effects of [Sar^9,Met(O_2)^{11}]substance P (10^{-7} M), U-46619 (10^{-6} M) and acetylcholine (10^{-3} M) (Ach) after prolonged incubation (during 15 h) in Krebs at room temperature (21°C), in isolated human small bronchi.

strated that bradykinin-induced contraction of isolated human bronchi is enhanced by interleukin-1β through thromboxane synthase induction and subsequent increased thromboxane A_2 release induced by bradykinin (Molimard et al., 1998).

Among contractile mediators involved in asthma, bradykinin exerts a contractile effect on isolated human bronchi indirectly through prostanoid release since it is abolished by the cyclooxygenase inhibitor, indomethacin (Molimard et al., 1994; Naline et al., 1996). A similar release of prostanoid has been described for substance P but its mechanism of action is more complex (Naline et al., 1996). Indeed, substance P has first been reported to induce at high concentrations a contraction of isolated human large bronchi through tachykinin NK_2 receptor stimulation since its effect is abolished by the tachykinin NK_2 receptor antagonist SR 48968 and not by the tachykinin NK_1 receptor antagonist, CP 96345 (Naline et al., 1989; Advenier et al., 1992). We have more recently reported that on isolated human small bronchi (diameter < 1 mm), substance P at low concentrations and some specific tachykinin NK_1 receptor agonists, such as [Sar^9,Met(O_2)^{11}]substance P, produce a concentration-dependent contraction through tachykinin NK_1 receptor stimulation since it is abolished by the tachykinin NK_1 receptor antagonist SR 140333 (Naline et al., 1996). Conversely to tachykinin NK_2 receptor-mediated contraction, the tachykinin NK_1-induced contraction is mediated by prostanoids, since it is abolished by indomethacin (Naline et al., 1996).

Since (1) tachykinin NK_1 receptor stimulation may induce airway smooth muscle contraction through prostanoid release and (2) interleukin-1β may enhance prostanoid release, the aim of this study was to determine whether interleukin-1β induces hyperresponsiveness to a tachykinin NK_1 receptor agonist on human bronchial tissue in vitro and if so, to determine the mechanism of this hyperresponsiveness.

2. Methods

2.1. Human bronchial tissue preparation

Bronchial tissues were removed from 28 patients (25 men and 3 women, mean age 66.2 ± 1.7 years) with lung cancer at the time of the surgical operation. All were previous smokers. None was asthmatic. Just after resection, segments of bronchi were taken as far away as possible from the malignancy and were dissected free of parenchyma. After removal of adhering fat and connective tissues, small rings (inner diameter of 0.5 to 1 mm, 7 mm length) of the same human bronchus were placed in oxygenated Krebs–Henseleit solution (NaCl, 119; KCl, 5.4; $CaCl_2$, 2.5; KH_2PO_4, 0.6; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.7 mM) at room temperature (21°C) for 15 h with or without interleukin-1β (10 ng/ml). Interleukin-1β concentration was chosen in agreement with the work of Hakonarson et al. (1996) and Molimard et al. (1998) and incubation time in agreement with Vigano et al. (1997). After incubation, each set of bronchial rings was suspended under an initial tension of 1.5 g in a 5-ml organ

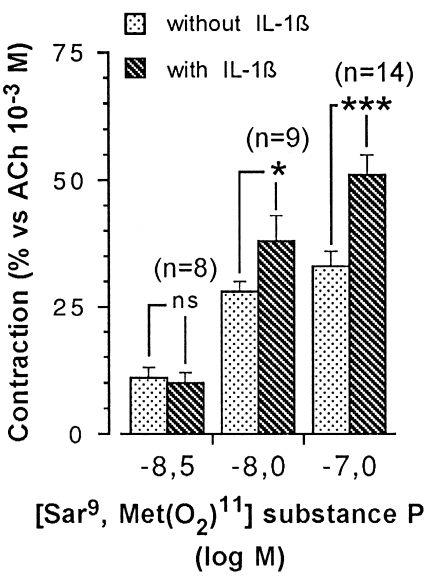

Fig. 2. Effects of noncumulative addition of [Sar^9,Met(O_2)^{11}]substance P (3×10^{-9}, 10^{-8} and 10^{-7} M) after a prolonged incubation with or without interleukin-1β (10 ng/ml, 15 h, 21°C). Contractions are expressed as percentage of the effect induced by acetylcholine 10^{-3} M added at the end of the experiments. Results are reported as mean ± S.E.M. of 8 to 14 experiments. Significant differences from studies without and after pre-treatment with interleukin-1β are shown by: $^*P < 0.05$; $^{***}P < 0.001$.

Table 1
Effect of interleukin-1β (IL-1β) pre-treatment (10 ng/ml, 15 h, 21°C) on basal tone, [Sar^9,Met(O$_2$)^{11}]substance P- or acetylcholine-induced contraction of isolated human bronchi (n = 8–14)

	n	Tension (mg) (without IL-1β)	Tension (mg) (with IL-1β)	Difference of tension after pre-treatment (mg), increase (+) or decrease (−)
Basal tone (mg)	8	2650 ± 160	2618 ± 180	
Increase of tension induced by [Sar^9,Met(O$_2$)^{11}] substance P (3×10^{-9} M)	8	192 ± 53	162 ± 35	(−) 30 ± 56
Increase of tension induced by acetylcholine (10^{-3} M)	8	1537 ± 152	1667 ± 179	(+) 131 ± 128
Basal tone (mg)	9	2716 ± 240	2738 ± 270	
Increase of tension induced by [Sar^9,Met(O$_2$)^{11}] substance P (10^{-8} M)	9	477 ± 68	683 ± 90	(+) 205 ± 37[b]
Increase of tension induced by acetylcholine (10^{-3} M)	9	1674 ± 229	1822 ± 192	(+) 147 ± 116
Basal tone (mg)	14	2950 ± 140	2964 ± 110	
Increase of tension induced by [Sar^9,Met(O$_2$)^{11}] substance P (10^{-7} M)	14	480 ± 95	758 ± 109	+280 ± 90[a]
Increase of tension induced by acetylcholine (10^{-3} M)	14	1407 ± 174	1467 ± 155	+60 ± 14

[a] $P < 0.01$, significant difference of tension after pre-treatment with IL-1β.
[b] $P < 0.001$, significant difference of tension after pre-treatment with IL-1β.

bath containing Krebs–Henseleit solution, bubbled with 95% O$_2$–5% CO$_2$ and maintained at 37°C according to our previous works ((Naline et al., 1996). The tissue was allowed to equilibrate over a 1-h time period during which time the Krebs–Henseleit solution was changed every 15 min. Changes in contraction force were measured isometrically with UF1 strain gauge transducers and displayed on I.O.S.-Moise 3 recorder (Dei Lierre, Mitry Mory, France).

Experiments were conducted on parallel groups of four to eight rings, with one ring serving as control.

2.2. Functional procedures

Only one concentration–response curve was recorded for each ring. Concentration–response curves for [Sar^9, Met(O$_2$)^{11}]substance P (3×10^{-9}, 10^{-8}, and 10^{-7} M) were recorded by applying non-cumulative concentrations of the drug to avoid the rapid desensitization of the tachykinin NK$_1$ receptor which we have demonstrated (Naline et al., 1996). Cumulative concentration–response curves for acetylcholine (10^{-7} to 10^{-3} M) or for the thromboxane mimetic U-46619 (10^{-8} to 10^{-6} M) were recorded by applying increasing concentrations of drugs in logarithmic increments.

Pre-treatments before addition of the contractile agent ([Sar^9,Met(O$_2$)^{11}]substance P 10^{-7} M) with the prostanoid TP-receptor antagonist GR 32191 (10^{-6} M, 1 h) or with indomethacin (10^{-6} M, 1 h) were performed according to our previous works (Molimard et al., 1995, 1998). The concentration of the cyclooxygenase-2 inhibitor CGP 28238 (10^{-6} M, 1 h) was chosen for a maximal inhibition of

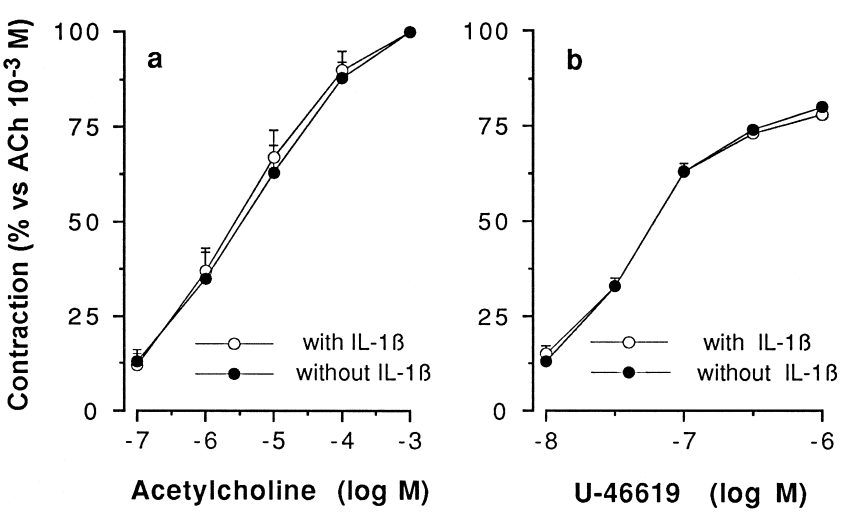

Fig. 3. Cumulative concentration–response curves (a) for acetylcholine (10^{-7} to 10^{-3} M) and (b) for the thromboxane mimetic U-46619 (10^{-8} to 10^{-6} M) after a prolonged incubation with or without interleukin-1β (10 ng/ml, 15 h, 21°C). Results are reported as mean ± S.E.M. of seven to nine experiments. The differences from studies without and after pre-treatment with interleukin-1β are not significant.

cyclooxygenase-2 without inhibition of cyclooxygenase-1 (Klein et al., 1994).

Contractile responses were expressed from baseline in grams or as percentage of the effect induced by the highest concentration of acetylcholine (10^{-3} M) added at the end of the experiments.

2.3. Biochemical procedures

The bronchial segments were pre-treated (or not for control) for 1 h with GR 32191 10^{-6} M, indomethacin 10^{-6} M or the tachykinin NK_1 receptor antagonist, SR 140333 10^{-10} M, according to our previous works (Naline et al., 1989; Molimard et al., 1995, 1998). Measurement of prostanoid release by airway preparation was performed by determination of thromboxane B_2, the stable metabolite of thromboxane A_2, and 6 keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostaglandin I_2 in the organ bath fluid derived from six experiments.

The bronchial segments were washed and 10 min later, [Sar^9,$Met(O_2)^{11}$]substance P was added. Release of prostanoids was determined by collecting a sample of the organ bath fluid just before, 5 and 10 min after the addition of [Sar^9,$Met(O_2)^{11}$]substance P (10^{-7} M). Basal release of prostanoids, expressed as pg/mg wet tissue, was defined as the release of prostanoids during 10 min before the contractile challenge.

Thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$ were assayed using specific competitive enzyme immunoassay commercial kits (Spibio, Massy, France) and according to the method of Pradelles et al. (1985). The minimal detectable concentrations of thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$ were 13 and 24 pg/ml, respectively. Cross-reactivities of anti-thromboxane B_2 antibodies with 2,3-dinor thromboxane B_2 and other prostanoids were 8.2 and less than 1%, respectively. Cross-reactivities of anti-6 keto-prostaglandin $F_{1\alpha}$ antibodies were 8.7 and 2.1% with 2,3-dinor 6 keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$, respectively, and less than 1% with other prostanoids (manufacturer's specifications).

2.4. Morphological study

At the end of the experiments, bronchial rings were fixed (24 h, in 10% formyl saline) and 5 mm transverse sections of bronchi were obtained from paraffin blocks. The integrity of the epithelium and other structures in these sections was assessed by light microscopy.

2.5. Statistical analysis

All values in the text and in the figures are expressed as mean ± standard error of the mean (S.E.M.). Statistical differences were determined using variance analysis (ANOVA) and Student's *t*-test (two-tailed, for paired or unpaired data). *P*-values lower than 0.05 were considered to be significant.

2.6. Drugs

The drugs used were: U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) and indomethacin (Sigma, St. Louis, MO), [Sar^9,$Met(O_2)^{11}$]substance P and recombinant human interleukin-1β (Bachem, Bubendorf, Switzerland), GR 32191 ((1*R*-(1α(*Z*),2β,3β,5α))-(+)-7-(5-(((1,1′-biphenyl)-4-yl)-methoxy)-3-hydroxy-2-(1-piperidinyl) cyclopentyl)-4-heptenoic acid, hydrochloride) (kind gift of Dr. Coleman, Glaxo, Greenford, UK), CGP 28238 (6-(2,4-difluorophenoxy)-5-methyl-sulfonylamino-1-indanone) (kind gift of Dr. Anderson, Ciba-Geigy, Basel, Switzerland), acetylcholine (PCH, Paris, France), SR 140333 ((*S*)-1-(2[3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo [2.2.2]octane,chloride) synthesized at Sanofi Research Centre (Sanofi Recherche, Montpellier, France). Interleukin-1β was dissolved in distilled water at a concentration of 7.5 10^{-8} M and kept in small aliquots at −80°C until used. A fresh aliquot was used for each experiment. All drugs were dissolved in distilled water and then diluted in Krebs solution, except for indomethacin, which was dissolved in ethanol then diluted in Krebs solution. The final amount of ethanol (0.03%) did not alter acetylcholine reactivity.

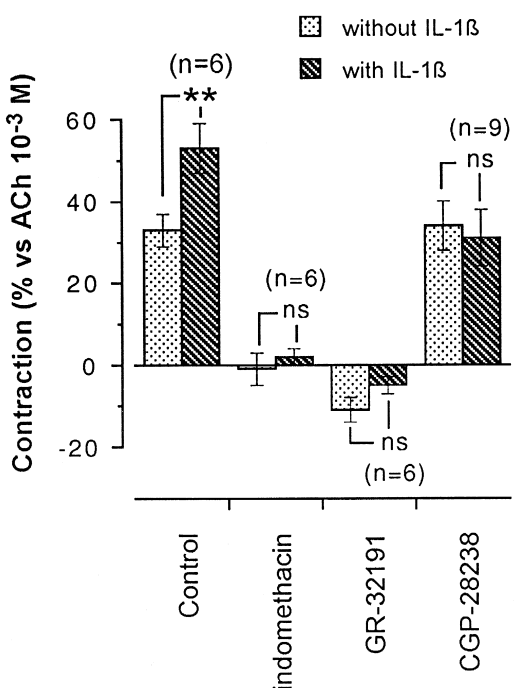

Fig. 4. Effect of indomethacin (10^{-6} M), the prostanoid TP-receptor antagonist GR 32191 (10^{-6} M) and the cyclooxygenase-2 specific inhibitor CGP 28238 (10^{-6} M), on [Sar^9,$Met(O_2)^{11}$]substance P (10^{-7} M)-induced contraction of isolated human small bronchi in the absence or presence of interleukin-1β (10 ng/ml, 15 h, 21°C) pre-treatment. Contractions are expressed as percentage of the effect induced by acetylcholine 10^{-3} M added at the end of the experiments. Results are reported as mean ± S.E.M. of six to nine experiments. Significant differences from studies without or after pre-treatment with interleukin-1β are shown by: $^{**}P < 0.01$.

3. Results

3.1. Influence of interleukin-1β pre-treatment on [Sar^9,Met(O$_2$)^{11}]substance P-, acetylcholine- and the thromboxane mimetic U-46619-induced contraction of isolated human bronchi

On isolated human small bronchi (diameter < 1 mm), [Sar^9,Met(O$_2$)^{11}]substance P (10^{-7} M) induced a transient contraction (Fig. 1). Incubation of the bronchi in Krebs–Henseleit solution at room temperature (21°C) for 15 h did not alter the responsiveness to [Sar^9, Met(O$_2$)^{11}]substance P. A pre-treatment with interleukin-1β (10 ng/ml, 15 h, 21°C) potentiated [Sar^9,Met(O$_2$)^{11}]substance P-induced contractions (Fig. 2, Table 1). Interleukin-1β pre-treatment induced an increased response to [Sar^9,Met(O$_2$)^{11}]substance P 10^{-8} and 10^{-7} M of 205 ± 37 mg ($n = 9$, $P < 0.001$) and 280 ± 90 mg ($n = 14$, $P < 0.01$), respectively. In contrast, under similar conditions, interleukin-1β pre-treatment did not modify airway basal tone, acetylcholine- or U-46619-induced contractions (Fig. 3a,b).

3.2. Effect of indomethacin, GR 32191 and CGP 28238 on [Sar^9,Met(O$_2$)^{11}]substance P-induced contraction of isolated human bronchi, in the absence of, or after a pre-treatment with interleukin-1β

Indomethacin (10^{-6} M) (a non-specific cyclooxygenase inhibitor) and GR 32191 (10^{-6} M) (a specific antagonist of the prostanoid TP-receptor) abolished the [Sar^9,Met(O$_2$)^{11}]substance P-induced contraction studied without or after a pre-treatment with interleukin-1β (Fig. 4). In contrast, the cyclooxygenase-2 inhibitor CGP 28238 (10^{-6} M) did not modify the [Sar^9,Met(O$_2$)^{11}]substance P-induced

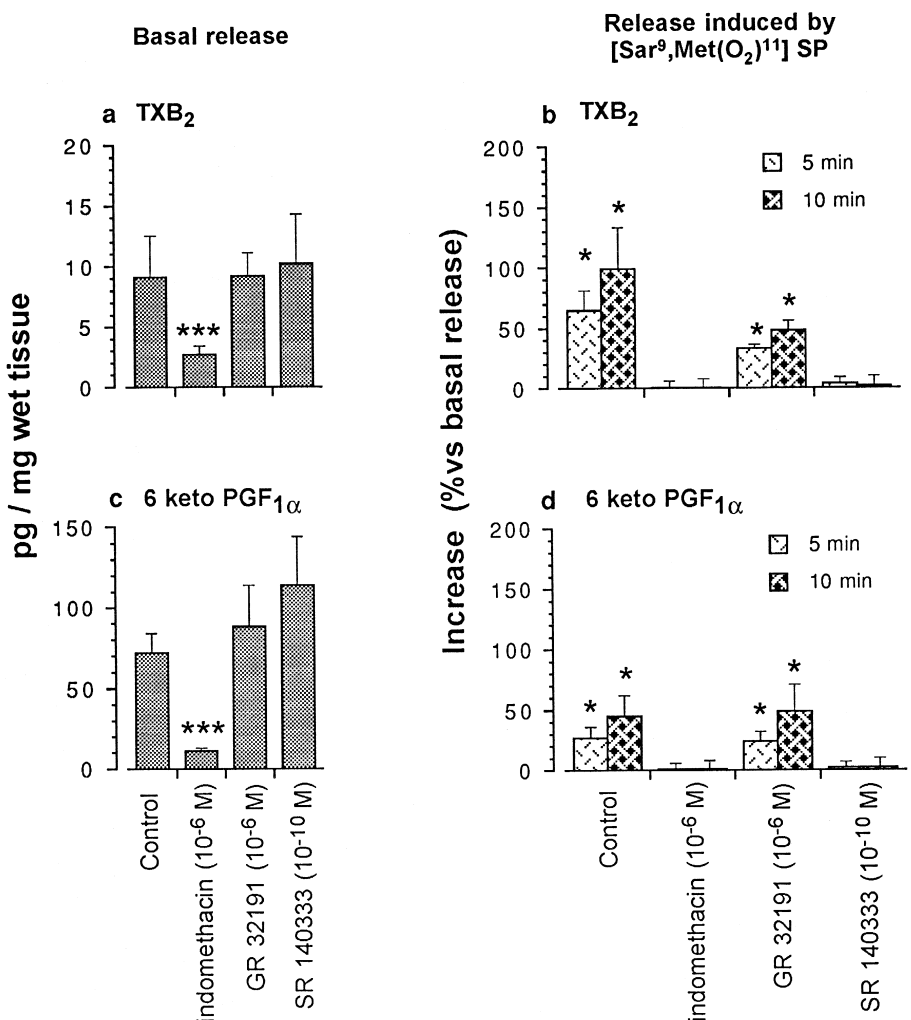

Fig. 5. Effect of indomethacin (10^{-6} M), the prostanoid TP-receptor antagonist GR 32191 (10^{-6} M) and the tachykinin NK$_1$ receptor antagonist SR 140333 (10^{-10} M) on thromboxane B$_2$ release (a,b) and 6 keto-prostaglandin F$_{1\alpha}$ release (c,d), before (basal release) (a,c) or after 5 or 10 min stimulation with [Sar^9,Met(O$_2$)^{11}]substance P (10^{-7} M) (b,d). Results are reported as mean ± S.E.M. of six to seven experiments. Significant differences from basal tone are shown by: $^*P < 0.05$; $^{***}P < 0.001$.

response but abolished the increase of response induced by interleukin-1β pre-treatment (Fig. 4).

3.3. Effect of [Sar^9,Met(O$_2$)^{11}]substance P on thromboxane B$_2$ and 6 keto-prostaglandin F$_{1\alpha}$ release and its modifications by drugs

Fig. 5 represents the isolated human bronchi release of the metabolites of thromboxane A_2 and prostaglandin I_2, which are thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$, respectively, at basal tone during 10 min (basal release) (Fig. 5a,c). The non-specific cyclooxygenase inhibitor, indomethacin, significantly decreased the release of thromboxane B_2 (2.7 ± 0.7 vs. 9.1 ± 3.4 pg/mg wet tissue in control, $n = 6$, $P < 0.001$) and 6 keto-prostaglandin $F_{1\alpha}$ (11 ± 2 vs. 72 ± 12 pg/mg wet tissue in control, $n = 7$, $P < 0.001$). The prostanoid TP-receptor antagonist GR 32191 (10^{-6} M) and the tachykinin NK_1 receptor antagonist SR 140333 (10^{-10} M) did not modify basal values of these metabolites (Fig. 5a,c).

[Sar^9,Met(O$_2$)^{11}]substance P significantly increased production of the metabolites thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$, 5 and 10 min after its addition to the bath in control group. Pre-treatment with indomethacin or SR 140333 prevented the increase of these metabolites. In contrast, pre-treatment with GR 32191 did not prevent such production (Fig. 5b,d).

3.4. Influence of interleukin-1β on thromboxane B$_2$ and 6 keto-prostaglandin F$_{1\alpha}$ release induced by [Sar^9,Met(O$_2$)^{11}]substance P

Basal release of thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$ determined before addition of [Sar^9,Met(O$_2$)^{11}]substance P were not modified by interleukin-1β pre-treatment (10 ng/ml, 15 h, 21°C) (Fig. 6a,c). With regard to basal state, [Sar^9,Met(O$_2$)^{11}]substance P-induced release of thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$ were significantly increased by interleukin-1β pre-treatment (Fig. 6b,d).

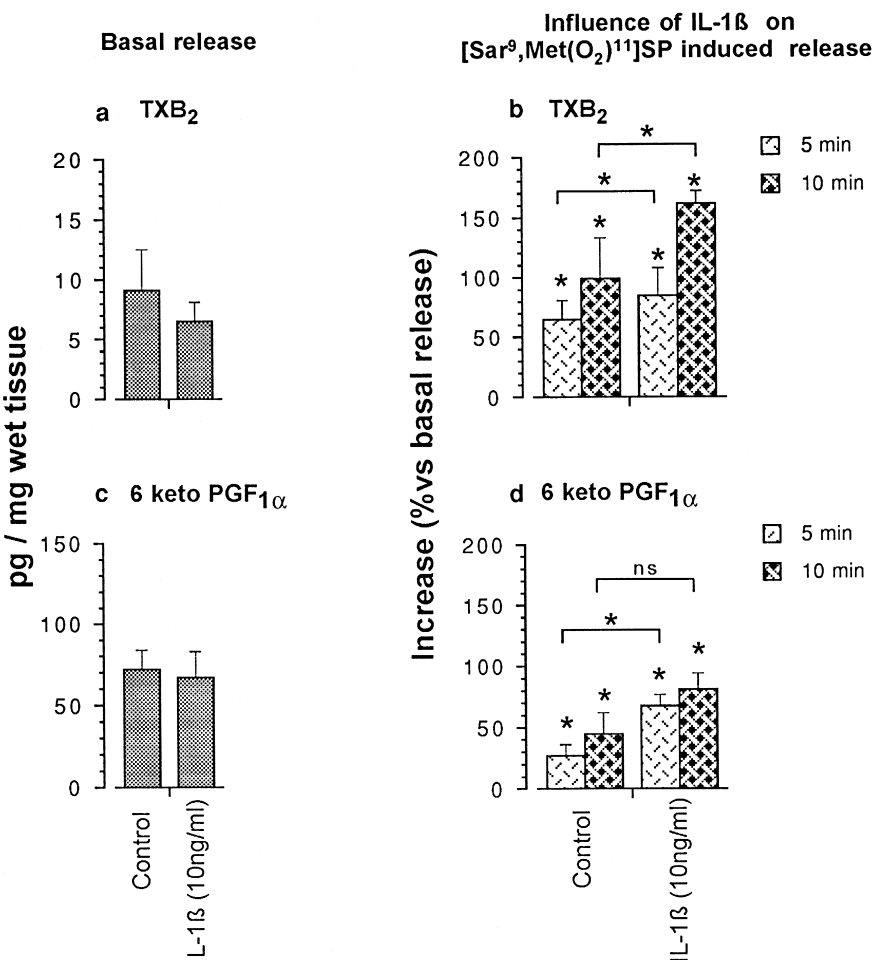

Fig. 6. Effect of a prolonged incubation with or without IL-1β (10 ng/ml, 15 h, 21°C) on thromboxane B_2 release (a,b) and 6 keto-prostaglandin $F_{1\alpha}$ release (c,d), before (basal release) (a,c) or after 5 or 10 min stimulation with [Sar^9,Met(O$_2$)^{11}]substance P (10^{-7} M) (b,d). Results are reported as mean ± S.E.M. of six to seven experiments. Significant differences from basal tone are shown by: $^*P < 0.05$.

4. Discussion

Substance P, a neuropeptide of the tachykinin family is localized in the lung in the sensory unmyelinated C-fibers which innervate all compartments of the airway wall, from the trachea down to the bronchiole and is, along with neurokinins A and B and calcitonin gene related peptide (CGRP), a transmitter of the excitatory non-adrenergic non-cholinergic (NANC) system (Lembeck and Holzer, 1970; Lundberg and Saria, 1987; Ellis and Undem, 1994; Fischer et al., 1996; Lundberg, 1996). The stimulation by different stimuli of chemosensitive C-fiber afferents in airways leads to a local release of tachykinins that is responsible for several biological effects in the bronchopulmonary system including bronchospasm, increase in vascular permeability from postcapillary venules, stimulation of glandular secretion, facilitation of cholinergic neurotransmission and recruitment and activation of some types of inflammatory cells (Maggi et al., 1993; Ellis and Undem, 1994; Lundberg, 1996; Advenier et al., 1997; Kraneveld et al., 1997). The effects of tachykinins are mediated via three types of receptors denoted tachykinin 1–3 (NK_1, NK_2 and NK_3) which have the highest affinity for substance P, neurokinin A and neurokinin B, respectively (Regoli et al., 1994). Several papers suggest that tachykinin content in sensory nerves and/or tissues may be increased in inflammatory processes. Indeed, after allergen exposure in sensitized guinea-pigs, there is increased preprotachykinin mRNA concentrations in nodose ganglions (Fischer et al., 1996). Similarly, lipopolysaccharide induces preprotachykinin gene-I mRNA up-regulation in rat alveolar macrophages (Killingsworth et al., 1997), in dendritic cells derived from bone marrow cultures of rats (Germonpré et al., 1998), and in the human monocyte cell line U937 (Germonpré et al., 1997). Asthmatic lung tissue may contain more substance P than that of normal subjects as reflected by the increase in substance P-like immunoreactivity detected in the bronchoalveolar lavage fluid (Niebber et al., 1992) and in the sputum, as well as in macrophages obtained from the sputum (Tomaki et al., 1995; Germonpré et al., 1997). Finally, in tissue obtained at autopsy or after lobectomy or bronchoscopy, both the number and the length of substance P-immunoreactive nerve fibers were increased in airways of asthmatics compared to normal subjects (Ollerenshaw et al., 1991; Howarth et al., 1995).

In addition to an increased release of substance P, an increased effect of substance P may be observed in asthma and airway inflammation. Increased tachykinin receptor synthesis and/or expression revealed by an increase in plasma leakage evoked by substance P has also been reported in rats in a model of chronic inflammatory disease produced by *Mycoplasma pulmonis* (McDonald et al., 1991; Baluk et al., 1995; McDonald, 1995). In human tissues, Ben-Jebria et al. (1993), have shown that passive sensitization of isolated human bronchi enhances the functional response to neurokinin A and substance P. An increase in mRNA transcription for tachykinin NK_1 receptors has also been reported in lung tissue from asthmatics (Adcock et al., 1993) and in rabbit airway smooth muscle following treatment with human atopic asthmatic serum (Hakonarson et al., 1998).

Among mediators involved in airway inflammation in asthma, there is increasing evidence that a variety of cytokines, in particular, interleukin-1β, play an important role. In rats, intra-tracheal administration of interleukin-1β has been shown to induce airway hyperresponsiveness to bradykinin (Tsukagoshi et al., 1994). This cytokine has also been shown to induce a potentiation of bradykinin's effects on the human isolated bronchus through thromboxane synthase potentiation and subsequently increased thromboxane A_2 release (Molimard et al., 1998). Recently, Ahluwalia et al. (1998) have shown that in tachykinin NK_1 receptor knockout mice, interleukin-1β-induced neutrophil accumulation was significantly attenuated by approximately 50%.

Our results show that interleukin-1β pre-treatment is able to induce human small bronchi hyperresponsiveness to the tachykinin NK_1 receptor specific agonist [Sar^9,$Met(O_2)^{11}$]substance P. As tachykinin NK_1 agonist-induced contraction of human bronchi is entirely mediated by prostanoids since it is abolished by indomethacin (Naline et al., 1996), the mechanisms of the hyperresponsiveness we observed could be due to: (1) an increase in tachykinin NK_1 receptor number, (2) an increase in prostanoid synthesis, (3) an increase in prostanoid contractile effect, (4) a non-specific effect of [Sar^9,$Met(O_2)^{11}$]substance P. The latter may be ruled out by the persistence of abolition of [Sar^9,$Met(O_2)^{11}$]substance P-induced contraction by the specific tachykinin NK_1 receptor antagonist SR 140333 in the presence of interleukin-1β pre-treatment. Thromboxane receptor up-regulation may be ruled out by the lack of potentiation of the thromboxane mimetic U-46619 induced contraction by interleukin-1β pre-treatment. An increase in tachykinin NK_1 receptor number is unlikely the predominant mechanism of the hyperresponsiveness we observed as interleukin-1β induced potentiation of [Sar^9,$Met(O_2)^{11}$]substance P contractile effect is abolished by the specific cyclooxygenase-2 inhibitor, CGP 28238 (Klein et al., 1994). Therefore, an increased prostanoid synthesis through cyclooxygenase-2 induction seems the most likely mechanism of the effect we observed. Prostanoid release assay in the organ bath confirmed our functional study. Thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$, the stable metabolites of thromboxane A_2 and prostaglandin I_2, respectively, release is increased by [Sar^9,$Met(O_2)^{11}$]substance P and their release is further increased by interleukin-1β pre-treatment. Interestingly, as mentioned in Section 1, interleukin-1β (Pang and Knox, 1997; Vigano et al., 1997; Pang et al., 1998) or a mixture of cytokines (Belvisi et al., 1997) have been reported to induce cyclooxygenase-2 expression in cultured human airway smooth

muscle cells. This induction was accompanied by a marked increase in prostaglandins E_2 and I_2 production (Pang and Knox, 1997).

In conclusion, our results show that, in vitro, interleukin-1β pre-treatment may induce human airway hyperresponsiveness to the tachykinin NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]substance P. This interleukin-1β-induced hyperresponsiveness appears to be linked to tachykinin NK_1 receptor stimulation and cyclooxygenase-2 induction.

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