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In vitro-induced human airway hyperresponsiveness to bradykinin

M. Molimard*+, E. Naline+, E. Boichot‡, P. Devillier#, V. Lagente‡, B. Bégaud*, C. Advenier+

In vitro-*induced human airway hyperresponsiveness to bradykinin. M. Molimard, E. Naline, E. Boichot, P. Devillier, V. Lagente, B. Bégaud, C. Advenier. ©ERS Journals Ltd 1998.*

ABSTRACT: Lipopolysaccharide (LPS) and interleukin (IL)-1β have been reported to induce airway hyperresponsiveness in several animal models. This study investigated the effect of LPS or IL-1β on bradykinin-induced human isolated bronchi contraction.

LPS (100 ng·mL^{-1} for 3–6 h) and IL-1β (3×10^{-10} and 3×10^{-9} M for 20 min to 3 h) time-dependently potentiated bradykinin-induced contraction. This contraction was abolished, as in control experiments, by indomethacin (10^{-6} M) or by the thromboxane (Tx) receptor antagonist GR 32191 but not by the cyclo-oxygenase-2 inhibitor, CGP28238. In contrast, the Tx mimetic U46619-induced contraction of human bronchi was not enhanced by IL-1β pretreatment. In the presence of GR 32191 (10^{-6} M), bradykinin induced a prostanoid dependent relaxation that was not significantly modified by IL-1β pretreatment. Determination of prostanoids in the organ bath fluid showed that bradykinin induced TxB_2, the stable metabolite of TxA_2, and 6-keto prostaglandin $F_{1\alpha}$, the stable metabolite of PGI_2, release. Only TxA_2 release was potentiated by IL-1β.

Taken together our results suggest that interleukin-1β (1–3 h)-induced potentiation of the effect of bradykinin is linked to an increased activity of thromboxane synthase and, in turn, to increased thromboxane synthesis.

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*Dépt de Pharmacologie, Université Victor Segalen Bordeaux II, Bordeaux, France. +Laboratoire de Pharmacologie, Faculté de Médecine Paris Ouest, Paris, France. ‡Laboratoire de Pharmacodynamic et de Pharmacologie Moléculaire, INSERM U 456, Faculté des Sciences pharmaceutiques, Rennes, France. #Laboratoire de Pharmacologie, CHU de Reims, Reims, France.

Correspondence: M. Molimard
Dépt de Pharmacologie
CHU Pellegrin-Carreire
33076 Bordeaux cedex
France
Fax: 33 556245889

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Airway inflammation is one of the main features of asthma. It has been well established that people with respiratory infections may experience increased bronchial reactivity and impaired bronchial airflow [1, 2]. Inhalation of endotoxin or lipopolysaccharide (LPS), a component of the outer cell wall of Gram-negative bacteria, has been reported to induce airway hyperresponsiveness in both normal [3] and asthmatic subjects [4]. The pathophysiological mechanisms underlying these changes after administration of LPS to the airways are not fully understood. Effects of LPS are likely to be indirect, through the activation of various inflammatory cells [5] which release the different endogenous inflammatory mediators and cytokines responsible for the host response. Among them, interleukin (IL)-1β is notable [6–8] and has been described as inducing airway hyperresponsiveness. Indeed, intratracheal administration of IL-1β has been shown to induce airway hyperresponsiveness to bradykinin in rats [9].

Bradykinin has been implicated in the pathophysiology of asthma [10]. Inhaled bradykinin is a potent bronchoconstrictor in asthmatic patients, but is almost ineffective in normal subjects [11]. This feature may explain a key mechanism in the occurrence of airway hyperresponsiveness, which is a pathological characteristic of asthma.

It has been established that bradykinin-induced human isolated bronchi contraction is linked to bradykinin B_2 receptor stimulation and subsequent prostanoid release [12–14]. The purpose of this study was to determine whether LPS and thereafter IL-1β induce hyperresponsiveness to bradykinin on human bronchial tissue *in vitro* and, if so, to analyse the mechanism of this hyperresponsiveness.

Materials and methods

Human bronchial tissue preparation

Bronchial tissues were removed from 22 patients (mean age 63 yrs, range 49–79 yrs) with lung cancer at the time of the surgical procedure. All were previous smokers. None were asthmatic. Just after resection, segments of bronchi with an inner diameter of 0.5–1 mm were taken from as far away as possible from the malignancy. They were placed in oxygenated Krebs–Henseleit solution (NaCl 119 mM, KCl 5.4 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 0.6 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 11.7 mM) and stored overnight at 4°C. After removal of adhering fat and connective tissues, four to eight rings of the same bronchus were prepared. Each set of bronchial rings was suspended under an initial tension of 1.5 g in a 5 mL organ bath containing Krebs–Henseleit solution, bubbled with 95% O_2/5% CO_2 and maintained at 37°C. The tissue was allowed to equilibrate over 1 h, during which time the Krebs–Henseleit solution was changed every 15 min. Changes in

force of contraction were measured isometrically with UF1 strain gauges and amplifiers, and displayed on an IOS-Moise 3 recorder (Dei Lierre, Mitry Mory, France).

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

Protocols

Concentration–response curves for bradykinin (10^{-9} to 10^{-6} M) or for the thromboxane mimetic U-46619 (10^{-9} to 10^{-6} M) were recorded by applying increasing concentrations of drugs in logarithmic increments.

Incubation with LPS 100 ng·mL^{-1} was initiated 3 or 6 h before the addition of bradykinin. Incubation with IL-1β (3×10^{-10} or 3×10^{-9} M) was began 20 min, 1 h or 3 h before bradykinin addition. IL-1β concentrations were chosen in agreement with the work of HAKONARSON *et al.* [15]. Pretreatment with the thromboxane prostanoid receptor an-tagonist GR 32191 10^{-6} M for 1 h and with indomethacin 10^{-6} M for 1 h was performed according to our previous studies [12, 14] and precontraction of bronchi with acet-ylcholine (ACh) 10^{-5} M was performed to study bradykinin-induced relaxation in the presence of GR 32191. A concentration of the cyclo-oxygenase (COX)-2 inhibitor CGP 28238 of 10^{-6} M was chosen for a maximal inhibition of COX-2 without inhibition of COX-1 according to KLEIN *et al.* [16] and was added 1 h before cumulative concentration–response curves to bradykinin were obtained. Pretreatment with the bradykinin B_2 receptor antagonist Hoe 140 at 10^{-6} M was performed 15 min before the concentration–response curves to bradykinin were obtained, according to our previous studies [12, 14, 17].

Contractile responses were expressed from baseline as a percentage of contraction induced by ACh (1 mM) added at the end of the experiments. Relaxant responses were expressed from the precontraction level as a percentage of the maximal relaxation induced by theophylline (3 mM) added at the end of the experiments.

Measurement of prostanoid release

Prostanold release by the airway preparation was measured by determination of prostaglandin (PG) E_2, 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2, and thromboxane B_2 (TxB_2), the stable metabolite of TxA_2, in the organ bath fluid derived from six experiments. Baseline release was determined by collecting the organ bath fluid after 90 min. Bradykinin-induced prostanoid release was measured in the organ bath fluid after the concentration–response curve to bradykinin had been obtained (which took 30 min), after 60 min pretreatment with or without IL-1β (3×10^{-10} M).

PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 were assayed according to the method of PRADELLES *et al.* [18] by using specific enzyme-linked immunosorbent assay (ELISA) commercial kits (Stallergènes, Fresnes, France). The minimal detectable concentrations of TxB_2, 6-keto $PGF_{1\alpha}$ and PGE_2 were 10, 100 and 26 pg·mL^{-1}, respectively. The cross-reactivities of TxB_2 with TxB_1, dinor TxB_2 and other prostaglandins were 17%, 11% and <1%, respectively. The cross-reactivities of PGE_2 with 15-keto PGE_2, PGE_1, $PGF_{2\alpha}$ and other prostaglandins were 5%, 6.2%, 5% and <1%, respectively. The cross-reactivities of 6-keto $PGF_{1\alpha}$. were 12% with dinor 6-keto $PGF_{1\alpha}$, 8% with $PGF_{1\alpha}$, 1.5% with 6-keto $PGF_{2\alpha}$, <0.7% with PGE_2, <0.2% with TxB_2 and <0.1% with PGD_2 (manufacturer's specifications)

Statistical analysis

All values in the text and figures are expressed as mean ±SEM. Statistical differences were determined using analysis of variance (ANOVA) and Student's t-test for paired or unpaired data. A p-value <0.05 was considered to be statistically significant.

Drugs

The drugs used were: bradykinin, U-46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$), indomethacin, LPS from *Escherichia coli* serotype 0111:B4 (Sigma, St Louis, MO, USA), recombinant human interleukin-1β (Bachem, Bubendorf, Switzerland), Hoe140 (D-Arg^0[Hyp^3, Thi^5,D-Tic^7,Oic^8]bradykinin) (gift from J. Winicki, Hoechst, Puteaux, France), 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) (gift from R.A. Coleman, Glaxo, Greenford, UK), CGP 28238 (gift from G.P. Anderson, Ciba-Geigy, Basel, Switzerland), and acetylcholine (PCH, Paris, France); theophylline sodium anisate was used as a proprietary injectable solution (Theophylline Bruneau®, Paris, France). IL-1β was dissolved in distilled water at a concentration of 10^{-9} M and stored 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 ACh reactivity.

Results

Effect of lipopolysaccharide and interleukin-1β pretreatment on bradykinin-induced contraction of human bronchi

Bradykinin induced a contraction of isolated human small bronchi which reached its maximum within 6 min after each addition (figs. 1 and 2). Concentration–response curves for bradykinin were not significantly modified after incubation of human bronchi in Krebs solution for 1–6 h (n=6) (fig. 2a).

An LPS 100 ng·mL^{-1} pretreatment for 1–3 h had no significant effect on either isolated airway basal tone or the response to acetylcholine 1 mM (table 1), but time dependently potentiated bradykinin-induced contraction (n=6) (fig. 2b).

Similarly to LPS, IL-1β (3×10^{-10} M) had no significant effect on either airway basal tone or contractions induced by 1 mM acetylcholine (table 1), but time-dependently potentiated bradykinin-induced contractions (n=6) (fig. 2c). No difference in the potentiating effect of bradykinin concentration–response curves was observed between the two doses of IL-1β (3×10^{-10} and 3×10^{-9} M) incubated for 1 h (n=6) (fig. 2d).

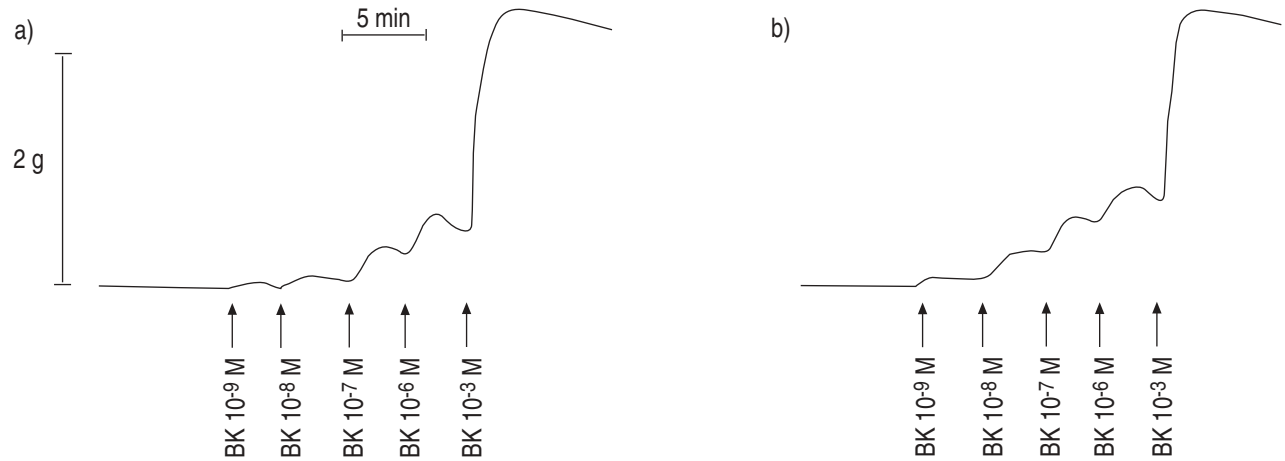

Fig. 1. – Example of the original tracing: contraction induced by increased concentrations of bradykinin (BK, 10^{-9} to 10^{-6} M), a) without or b) with 1-h pretreatment with interleukin-1β (3×10^{-10} M). ACh: acetylcholine.

Involvement of bradykinin B_2 receptor in bradykinin-induced contraction following interleukin-1β pretreatment

The bradykinin B_2 receptor antagonist Hoe140 (10^{-6} M), at a concentration known to abolish the effect of bradykinin effect on bradykinin B_2 receptors but not on bradykinin B_1 receptors [12, 19], totally abolished bradykinin-induced contraction studied without or after pretreatment with IL-1β (3×10^{-10} M) for 1 h (n=4) (data not shown).

Effect of cyclo-oxygenases inhibition on interleukin 1β-induced bradykinin hyperresponsiveness

The specific COX-2 inhibitor CGP 28238 at submaximal concentration (10^{-6} M) failed to inhibit the potentiation of the effects of bradykinin induced by IL-1β (1 h) (n=6) (fig. 3a). In contrast, the nonspecific COX inhibitor indomethacin at 10^{-6} M, a concentration known to abolish the effect of bradykinin [12, 14], abolished bradykinin-induced

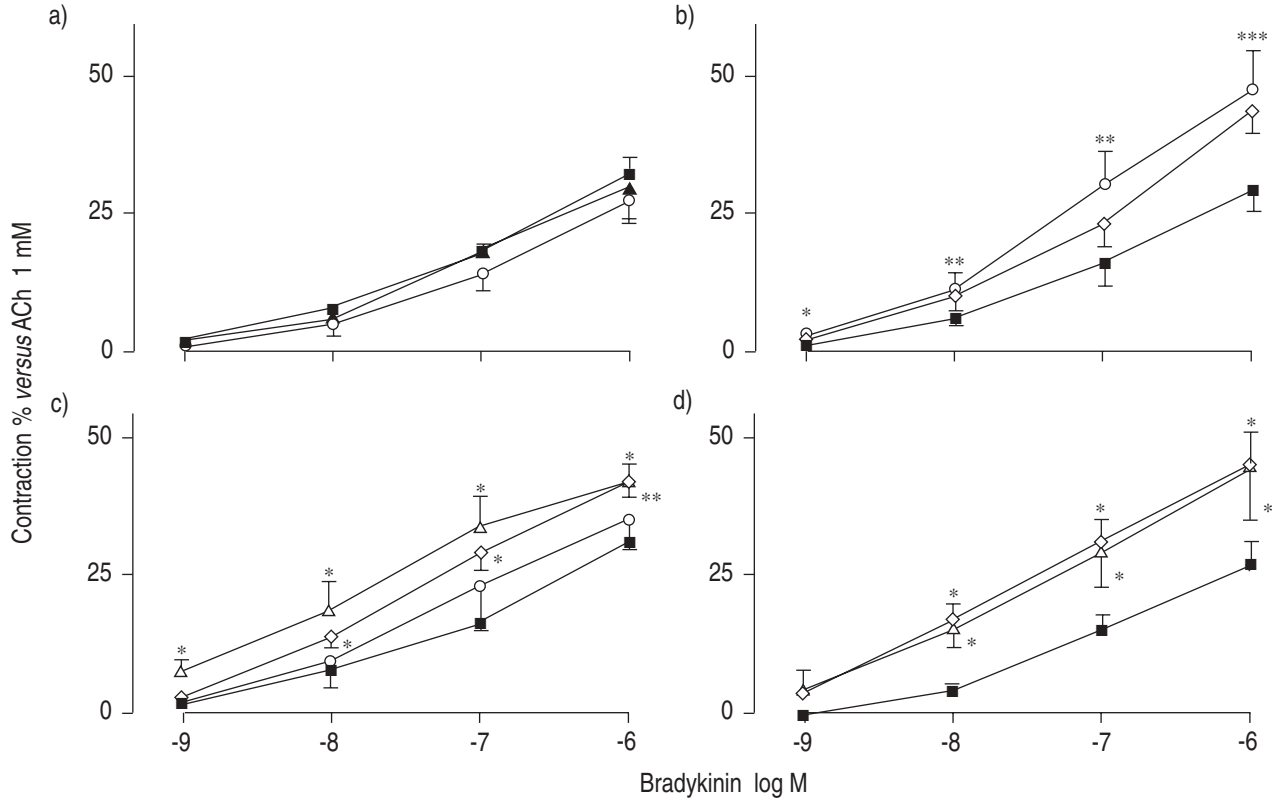

Fig. 2. – Concentration–response curves in human isolated small bronchi induced by bradykinin (10^{-9} to 10^{-6} M) after a) prolonged incubation in Krebs (■: control; ○: 3 h; ▲: 6 h); b) prolonged incubation with lipopolysaccharide (LPS) 100 ng·mL^{-1} (■: control; ◇: 3 h; ○: 6 h); c) prolonged incubation with interleukin-1β (IL-1β, 3×10^{-10} M) (■: control without IL-1β; ○: 20 min; ◇: 1 h; ý: 3 h); d) 1-h incubation with IL-1β (■: control without IL-1β; ◇: IL-1β 0.3 nM; ý: IL-1β 3 nM). ACh: acetylcholine. Results are reported as mean±SEM for six to 10 experiments. Significant differences from control are shown as: *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Table 1. – Effect of interleukin-1β (IL-1β, 3×10^{-10} M) and lipopolysaccharide (LPS, 100 ng·mL^{-1}) on baseline tone and on variation of tension induced by acetylcholine (ACh, 1 mM) in human isolated bronchi (n=6–22)

Treatment	Baseline mg	Variation in tension induced by 1 mM ACh mg
Control (n=22)	1992±259	2482±196
LPS 100 ng·mL^{-1}, 1 h (n=6)	1920±358	2542±320
LPS 100 ng·mL^{-1}, 3 h (n=10)	2120±254	2470±212
IL-1β 3×10^{-10} M, 1 h (n=22)	2226±285	2705±268
IL-1β 3×10^{-10} M, 3 h (n=6)	2072±380	2598±363

Data presented as means±SEM.

contraction studied either without or after 1 h of IL-1β (3×10^{-10} M) pretreatment (fig. 3b).

Effect of interleukin-1β on the relaxant component of bradykinin effect observed after thromboxane receptor blockade

After pretreatment with the thromboxane prostanoid receptor antagonist GR 32191 (10^{-6} M), bradykinin induced a relaxation of human bronchi. This response was not modified by IL-1β (3×10^{-10} M) pretreatment (n=6) (fig. 4).

Effect of interleukin-1β on U46619-induced contraction of human bronchi

The TxA_2 mimetic U46619 contracted human isolated small bronchi but this contraction was not potentiated by 1 h of IL-1β (3×10^{-10} M) pretreatment (n=4) (fig. 5).

Effect of interleukin-1β 10^{-10} M on bradykinin-induced prostanoid release by human bronchi

Bradykinin (10^{-9} to 10^{-6} M) induced the accumulation of TxB_2 and 6-keto $PGF_{1\alpha}$ (stable metabolite of PGI_2) but not PGE_2 in the organ bath fluid of human isolated bronchi.

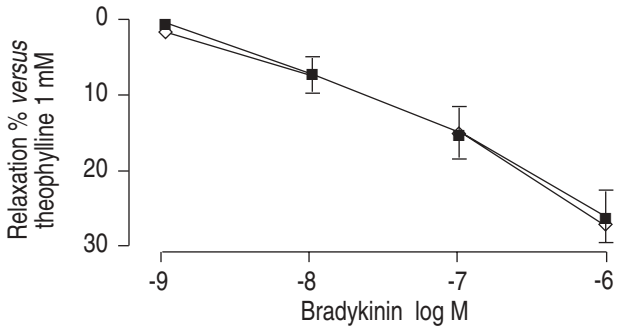

Fig. 4. – Relaxation of human isolated small bronchi induced by bradykinin (10^{-9} to 10^{-6} M) in the presence of the thromboxane receptor antagonist GR 32191 (10^{-6} M) in control experiments (■) or after 1-h incubation with interleukin-1β 3×10^{-10} M (◇). Results are reported as mean±SEM for six experiments.

Pretreatment for 1 h with IL-1β (3×10^{-10} M) increased bradykinin-induced TxB_2, but not 6-keto $PGF_{1\alpha}$ or PGE_2 accumulation in the organ bath fluid (n=6) (fig. 6).

Discussion

The present study demonstrates that LPS, a component of the outer cell wall of Gram-negative bacteria, and IL-1β time-dependently induced human airway hyperresponsiveness to bradykinin *in vitro*, whereas neither had any effect on ACh-induced contraction at the concentrations studied.

The recent introduction of kinin analogues, acting as selective agonists or antagonists of kinin receptors, has allowed confirmation of the long-standing proposal of the existence of two types of bradykinin receptor, termed B_1 and B_2 [20]. The authors [12, 14] and others [13] have demonstrated that bradykinin-induced contraction of human bronchi is linked, under usual experimental conditions, to bradykinin B_2 receptor stimulation. The B_1 type receptor has, however, attracted interest because of its apparent

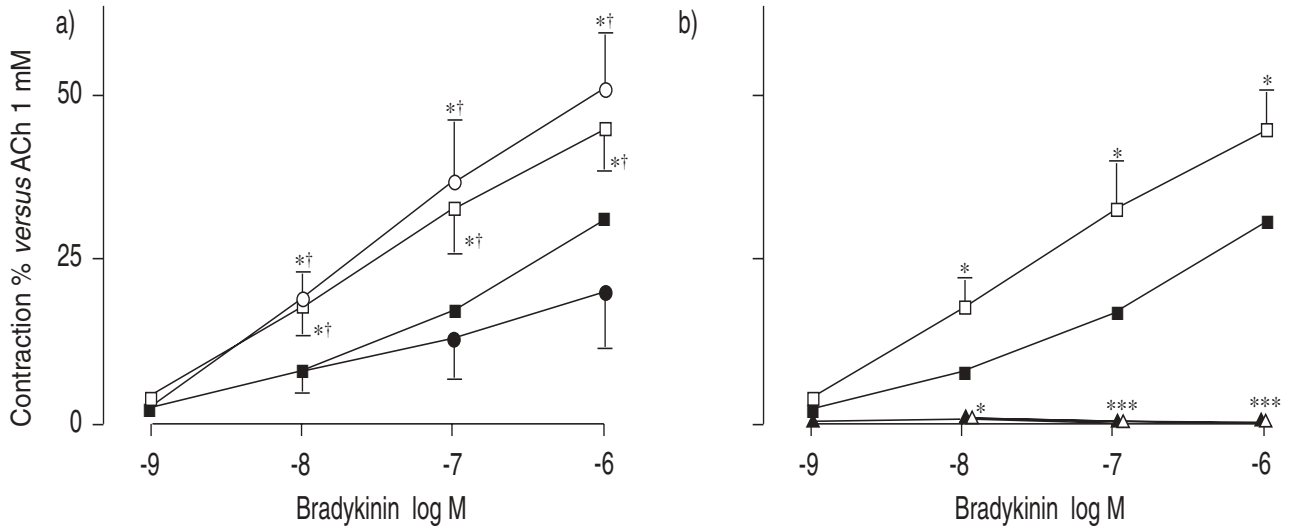

Fig. 3. – Effect of the cyclo-oxygenase-2 specific inhibitor CGP 28238 (10^{-6} M) (a) or indomethacin 10^{-6} M (b) on contraction of human isolated small bronchi induced by bradykinin (10^{-9} to 10^{-6} M) with or without interleukin-1β (IL-1β, 3×10^{-10} M) pretreatment. Controls without (■) or with IL-1β (❑); experiments performed after the pretreatment with CGP 28238 in the presence (○) or the absence (●) of IL-1β; experiments performed after pretreatment with indomethacin in the presence (ý) or absence (▲) of IL-1β. ACh: acetylcholine. Results are reported as mean±SEM for four or five experiments. *: $p<0.05$ *versus* control, ***: $p<0.001$ *versus* control; †: $p<0.05$ *versus* pretreatment with CGP 28238 in the absence of IL-1β.

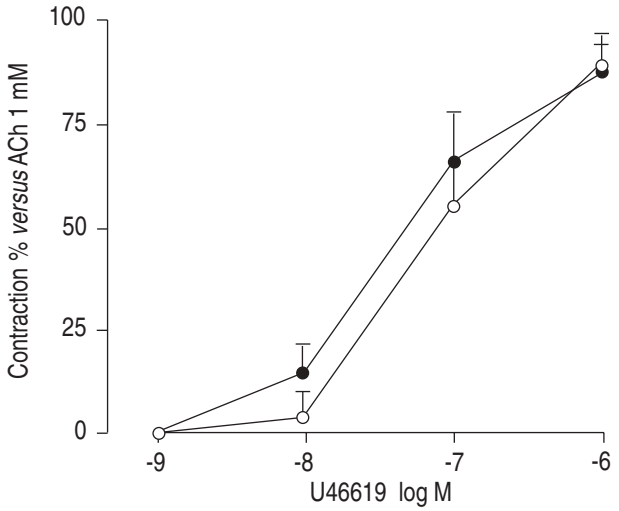

Fig. 5. – Contraction of human isolated small bronchi induced by the thromboxane mimetic U46619 (10^{-9} to 10^{-6} M) in control experiments (●) or after 1-h incubation with interleukin-1β 3×10^{-10} M (○). ACh: acetylcholine. Results are expressed as mean±SEM for four experiments.

upregulation following some types of tissue inflammation and, consequently, the view that B_2 receptors dominate kinin pharmacology may be tempered by the fact that inflamed tissue may exhibit an enhanced response to kinins through B_1 receptor upregulation [21]. Indeed, upregulation of response to the B_1 receptor agonist des-Arg^9-bradykinin and *de novo* synthesis of B_1 receptor was demonstrated after prolonged incubation of tissues in Krebs solution [22, 23] and was increased by LPS [24] or IL-1β [25, 26]. B_1 receptor upregulation within only 1 h is unlikely. The increased response to bradykinin observed in this study was mediated by B_2 receptors, since the bradykinin B_2 antagonist Hoe140 completely suppressed the contracting effect of bradykinin after IL-1β pretreatment. However, these results do not exclude that B_1 receptor upregulation may be induced by a more prolonged incubation.

Upregulation of the number or affinity of bradykinin B_2 receptors could be hypothesized to explain the potentiating effect of IL-1β. BATHON *et al.* [25] showed that 24 h incubation of synovial cells with IL-1β induces a two-fold increase in the number of bradykinin B_2 receptors. More recently, TSUKAGOSHI *et al.* [27, 28] showed that bradykinin B_2 receptors mediate airway hyperresponsiveness to bradykinin induced 24 h after intratracheal administration of IL-1β but that B_2 receptor upregulation is not involved in this increased response. In the present experiments, incubation with IL-1β was far shorter (1 h), so that the synthesis of new receptors seems unlikely. Further argument against bradykinin B_2 receptor upregulation is provided by the lack of potentiating effect of IL-1β on the relaxant component of the effect of bradykinin observed after thromboxane prostanoid receptor blockade by GR 32191 (10^{-6} M). Indeed, it has previously been demonstrated that the relaxant effect of bradykinin observed in these conditions is linked to bradykinin B_2 receptor stimulation [14]. One would therefore, have, to expect an IL-1β-induced potentiation of the relaxant effect of bradykinin in the case of bradykinin B_2 receptor upregulation.

It has previously been demonstrated that bradykinin-induced human bronchi contraction was linked to bradykinin B_2 receptor stimulation and subsequent prostanoid release [12]. In this paper, it was shown that the effect of bradykinin remains entirely linked to this release after IL-1β pretreatment, since indomethacin still abolishes bradykinin-induced contraction under these experimental conditions. Several mechanisms may be hypothesized to explain IL-1β-induced potentiation of the effect of bradykinin, including: 1) a rapid increase in phospholipase A_2 activity induced by IL-1β, as demonstrated in human synovial fibroblasts [29], or 2) increased COX activity or COX-2 induction, as shown in human pulmonary epithelial cells [30]. These mechanisms are unlikely to be involved in the present experiments, since one would expect an IL-1β- induced potentiation of the relaxant effect of bradykinin due to increased PGI_2 and PGE_2 release, conversely to the present functional observations and prostanoid measurements. In addition, the specific COX-2 inhibitor CGP28-238 [16], at concentrations tending to inhibit the effect of bradykinin, failed to inhibit the potentiating effect of IL-1β on bradykinin-induced contraction.

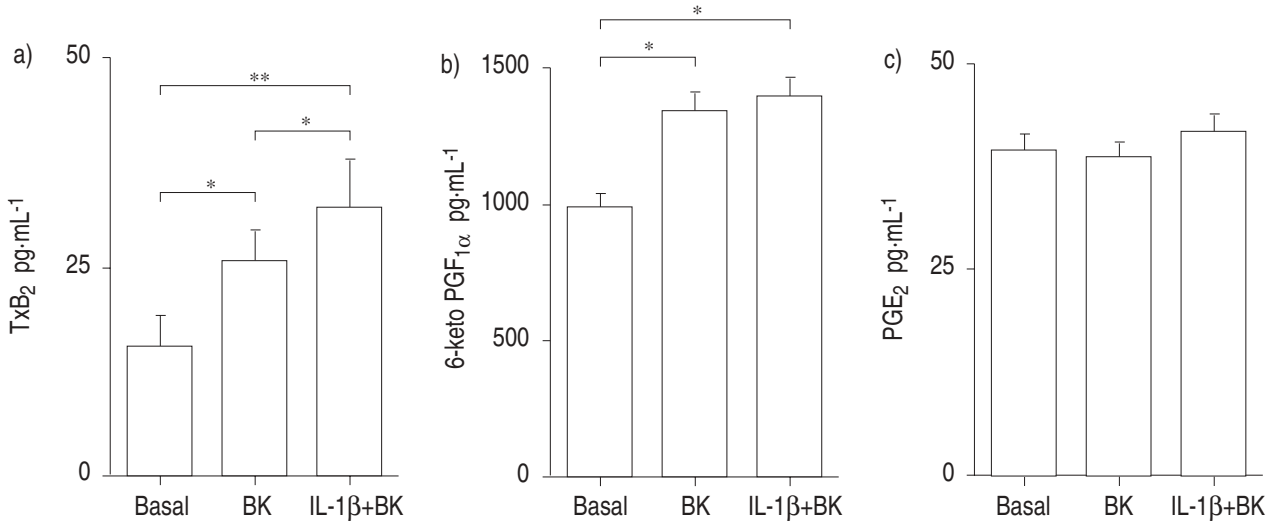

Fig. 6. – Concentrations in the organ bath fluid of a) thromboxane (Tx)B_2, the stable metabolite of TxA_2; b) 6-keto prostaglandin (PG)$F_{1\alpha}$, the stable metabolite of PGI_2; and c) PGE_2 without (basal) or after stimulation of human isolated bronchi with bradykinin (10^{-9} to 10^{-6} M) without interleukin-1β (IL-1β) (BK) or after 1-h IL-1β 3×10^{-10} M pretreatment (IL-1β+BK). Results are shown as mean±SEM for six experiments. *: $p<0.05$; **: $p<0.01$

This study has demonstrated that the potentiating effect of IL-1β is not linked to thromboxane receptor upregulation, since the effect of the thromboxane mimetic U46619 was not modified by IL-1β pretreatment. Taken together, these functional results suggest that IL-1β-induced short-term potentiation of the effect of bradykinin is linked to increased synthesis of constrictor prostanoids.

In agreement with HULSMANN *et al.* [13], this study found that PGI_2 release is greater than PGE_2 and TxA_2 release in human isolated small bronchi both under basal tone and after bradykinin pretreatment. The prostanoid release measurements performed in organ bath fluid confirm the functional results that suggest that IL-1β- induced short-term potentiation of the effect of bradykinin is linked to an increased constrictor prostanoid synthesis. Indeed, it was demonstrated that the potentiating effect of IL-1β (3×10^{-10} M) is linked to the potentiation of bradykinin-induced thromboxane A_2 release. Moreover, the lack of potentiation of bradykinin-induced PGI_2 release by IL-1β 3×10^{-10} M confirms that the potentiation of the thromboxane A_2 release observed in these experiments is not linked to COX induction and suggest a thromboxane synthase induction. A similar shift in favour of constrictor prostanoids in the balance of the dilator/constrictor prostanoids was recently described in response to angiotensin II after IL-1β pretreatment in rat aorta [31].

In conclusion, these results show that, *in vitro*, lipopolysaccharide and interleukin-1β may rapidly induce human airway hyperresponsiveness to bradykinin. This interleukin-1β induced hyperresponsiveness to bradykinin is linked to bradykinin B_2 receptor stimulation and increased throm-boxane synthesis, due more to increased thromboxane syn-thase activity than to increased cyclo-oxygenase activity. Further *in vitro* experiments are necessary to determine the effect of more prolonged incubation with lipopolysaccharide and interleukin-1β on the airway response to bradykinin.

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