In vitro-induced human airway hyperresponsiveness to bradykinin

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


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 (3×10⁻⁹ and 3×10⁻⁸ 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 TxB2, the stable metabolite of TxA2, and 6-keto-prostaglandin F1α, the stable metabolite of PGI2, release. Only TxA2 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.


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. Effective 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 broncho-constrictor 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 B₁ 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, CaCl₂ 2.5 mM, KH₂PO₄ 0.6 mM, MgSO₄ 1.2 mM, NaHCO₃ 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% O₂/5% CO₂ 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 begun 20 min, 1 h or 3 h before bradykinin addition. IL-1β concentrations were chosen in agreement with the work of Hérion et al. [15]. Pretreatment with the thromboxane prostaglandin 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 acetylcholine (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 TxB2, the stable metabolite of TxA2, in the organ bath (fig. 2b). 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 10^{-6} 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.

Measurement of prostanoid release

Prostanoid release by the airway preparation was measured by determination of prostaglandin (PG) E2, 6-keto PGFα1, the stable metabolite of PGH2, and thromboxane B2 (TxB2), the stable metabolite of TxA2, in the organ bath fluid derived from six experiments. Baseline release was determined by collecting the organ bath fluid after 90 min. Bradykinin-induced prostaglandin 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). PGE2, 6-keto PGFα1, and TxB2 were assayed according to the method of Paysages et al. [18] by using specific enzyme-linked immunosorbent assay (ELISA) commercial kits (Stallergènes, Fresnes, France). The minimal detectable concentrations of TxB2, 6-keto PGFα1 and PGE2 were 10, 100 and 26 pg·mL^{-1}, respectively. The cross-reactivities of PGE2 with 15-keto PGE2, PGEα1, PGFα2, and other prostaglandins were 5%, 6.2%, 5% and <1%, respectively. The cross-reactivities of 6-keto PGFα1 were 12% with dinor 6-keto PGFα1, 8% with PGFα2, 1.5% with 6-keto PGFα2, <0.7% with PGFα2, <0.2% with TxB2 and <0.1% with PGD2 (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_{2α}), indomethacin, LPS from Escherichia coli serotype 0111:B4 (Sigma, St Louis, MO, USA), recombinant human interleukin-1β (Bachem, Bubendorf, Switzerland), Hoe140 (o-Arg[Hypr5, Thr1,Tic5,Oic8]bradykinin) (gift from J. Winicki, Hoechst, Puteaux, France), GR 32191 ([(1R,3S,7S)-2,3,5,6]-7-(5-(((1,1′-biphenyl)-4-yl)-methoxy-3-hydroxy-2-(1-piperidinylcyclopentyl)-4-heptanoic 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^{-6} 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).
Involvement of bradykinin B₂ receptor in bradykinin-induced contraction following interleukin-1β pretreatment

The bradykinin B₂ receptor antagonist Hoe140 (10⁻⁶ M), at a concentration known to abolish the effect of bradykinin on bradykinin B₂ receptors but not on bradykinin B₁ 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. 1](image-url)  – 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.

![Fig. 2](image-url)  – 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; ▲: 3 h); d) 1-h incubation with IL-1β (●: control without IL-1β; ○: IL-1β 0.3 nM; ▲: 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.
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 TxA₂ 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 TxB₂ and 6-keto PGF₁α (stable metabolite of PGI₂) but not PGE₂ in the organ bath fluid of human isolated bronchi.

**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 B₁ and B₂ [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 B₂ receptor stimulation. The B₁ type receptor has, however, attracted interest because of its apparent
upregulation following some types of tissue inflammation and, consequently, the view that B₂ receptors dominate kinin pharmacology may be tempered by the fact that inflamed tissue may exhibit an enhanced response to kinins through B₁ receptor upregulation [21]. Indeed, upregulation of response to the B₁ receptor agonist des-Arg⁹-bradykinin and de novo synthesis of B₁ receptor was demonstrated after prolonged incubation of tissues in Krebs solution [22, 23] and was increased by LPS [24] or IL-1β [25, 26]. B₁ receptor upregulation within only 1 h is unlikely. The increased response to bradykinin observed in this study was mediated by B₂ receptors, since the bradykinin B₂ antagonist Hoe140 completely suppressed the contracting effect of bradykinin after IL-1β pretreatment. However, these results do not exclude that B₁ receptor upregulation may be induced by a more prolonged incubation.

Upregulation of the number or affinity of bradykinin B₂ 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 B₂ receptors. More recently, TSURUGOSHI et al. [27, 28] showed that bradykinin B₂ receptors mediate airway hyperresponsiveness to bradykinin induced 24 h after intratracheal administration of IL-1β but that B₂ 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 B₂ 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 B₂ 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 B₂ receptor upregulation.

It has previously been demonstrated that bradykinin-induced human bronchi contraction was linked to bradykinin B₂ 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 A₂ 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 PGI₂ and PGE₂ 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. 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.

**Fig. 6.** – Concentrations in the organ bath fluid of a) thromboxane (TxB₂), the stable metabolite of TxA₂; b) 6-keto prostaglandin (PG)F₁α, the stable metabolite of PGI₂; and c) PGE₂ 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 Husmann et al. [13], this study found that PGI₂ release is greater than PGE₂, and TXA₂ 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 that suggest that IL-1β 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 A₂ release. Moreover, the lack of potentiation of bradykinin-induced PGI₂ release by IL-1β 3×10⁻¹⁰ M confirms that the potentiation of the thromboxane A₂ 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, lipopoly saccharide and interleukin-1β may rapidly induce human airway hyperresponsiveness to bradykinin. This interleukin-1β induced hyperresponsiveness to bradykinin is linked to bradykinin B₂ receptor stimulation and increased thromboxane B₂ synthesis, due more to increased thromboxane synthase activity than to increased cyclooxygenase 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.

References