Impaired cAMP production in human airway smooth muscle cells by bradykinin: role of cyclooxygenase products

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Pang, Linhua, Elaine Holland, and Alan J. Knox. Impaired cAMP production in human airway smooth muscle cells by bradykinin: role of cyclooxygenase products. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19):L322–L329, 1998.—Interleukin (IL)-1β impairs human airway smooth muscle (ASM) cell cAMP responses to isoproterenol (Iso). We investigated if bradykinin (BK) could cause a similar effect and the role of cyclooxygenase (COX) products in this event, since we have recently reported that BK, like IL-1β, also causes COX-2 induction and prostanoid release in human ASM cells. BK pretreatment significantly attenuated Iso-induced cAMP generation in a time- and concentration-dependent manner. cAMP generation by prostaglandin (PG) E2 but not by forskolin was also impaired. The COX inhibitor indomethacin completely prevented the impairment, whereas the selective COX-2 inhibitors NS-398 and nimesulide, protein synthesis inhibitors cycloheximide and actinomycin D, and steroid dexamethasone were all partially effective. The impairment was mimicked by the B2 agonist [Tyr(Me)8]BK, the Ca2+ ionophore A-23187, and PGE2 and prevented by the B1 antagonist HOE-140, but anti-IL-1β serum was ineffective. The results indicate that BK impairs human ASM cell responses to Iso, and the effect is largely mediated by B2 receptor-related COX product release via both COX isozymes and is independent of IL-1β.

Airway inflammation; prostaglandin E2; asthma; cyclooxygenase induction; isoproterenol; adenosine 3′,5′-cyclic monophosphate

BRADYKININ (BK) is a naturally occurring inflammatory peptide generated by the cleavage of kininogen. Asthmatic patients have elevated kinin concentrations in plasma and in nasal and bronchoalveolar lavage fluid after allergen challenge (5, 7). BK elicits many features of bronchial asthma such as bronchoconstriction (17), microvascular leakage (27), and recruitment of inflammatory cells (28), and inhaled BK is a potent bronchoconstrictor in asthmatic patients (26). BK may therefore play a role in the pathogenesis of bronchial asthma.

Enhanced cytokine production, inflammation, and impaired responsiveness of airway smooth muscle (ASM) to adrenoceptor agonists are characteristic features in asthma. Increasing evidence suggests that proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF-α) play critical roles in the development of inflammatory responses in the airway and in regulating ASM responsiveness to adrenoceptor agonists. IL-1β causes α-adrenergic hyporesponsiveness in human ASM cells (29) and guinea pig tracheas (34); TNF-α also causes similar impairment in guinea pig tracheas (34) and canine ASM cells (10). Although it has been suggested that there is uncoupling of ASM β-receptors from adenyl cyclase, the precise mechanism(s) underlying this hyporesponsiveness has not been fully explored, and there are no reports on whether BK can cause the same hyporesponsiveness in ASM cells.

Cyclooxygenase [COX; prostaglandin (PG) endoperoxide synthase, EC 1.14.99.1] is the rate-limiting enzyme for the conversion of arachidonic acid (AA) to prostanoids and exists in two isoforms, the constitutive COX-1 and the inducible COX-2, which can be switched on by cytokines and inflammatory mediators (3). Accumulating evidence suggests that the induction and regulation of COX-2 may be key elements in the pathophysiological process of a number of inflammatory disorders. Enhanced expression of COX-2 in asthmatic airways has recently been reported (30), suggesting that COX-2-derived products may play an essential role in the inflammatory processes present in asthmatic airways. We and others have shown that IL-1β (23, 33) or a mixture of cytokines (6) induces COX-2 expression in cultured human ASM cells and that the induction is accompanied by a marked increase in PGE2 and PGI2 production (23). BK has been shown to stimulate AA release from cultured ASM cells via the rise in cytosolic free Ca2+ and the activation of the 85-kDa cytosolic phospholipase A2 (24, 32). We have recently reported that BK, like IL-1β, causes the induction of COX-2 and the release of prostanoids from human ASM cells, and the effect is mediated by the activation of the B2 receptors rather than the B1 receptors (24). Because PGE2 and PGI2 are both coupled to adenylyl cyclase and increase intracellular cAMP, chronically elevated levels of PGE2 and PGI2 would be expected to cause heterologous desensitization of adenylyl cyclase. We postulated that BK may also cause impaired cAMP generation in response to α-adrenoceptor agonists in BK-treated human ASM cells and BK-induced COX-2 expression, and prostanoid release may be responsible for the impairment.

The present study was therefore aimed to investigate if BK impaired cAMP production in human ASM cells in response to isoproterenol (Iso) and if so to determine the mechanisms responsible for the impaired response. We paid particular attention to whether COX-2 isoenzyme induction and COX products were involved. The nonselective COX inhibitor indomethacin (Indo), the selective COX-2 inhibitors N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide (NS-398) and nimesulide (Nim), the protein synthesis inhibitors cycloheximide and actinomycin D, and the anti-inflammatory steroid dexamethasone were used to assess the role of COX-2 induction and COX products in the process. We also characterized the receptors involved by comparing the effect of BK with that of the selective B1 and B2 receptor.
agonists and by using the selective B₁ and B₂ receptor antagonists to prevent the effect of BK. In addition, the ability of Ca²⁺ ionophore A-23187, which has a similar effect as BK in causing cytosolic free Ca²⁺ increase and AA release, and the exogenously applied COX product PGE₂ to mimic the impaired responses by BK was also investigated. Because BK has been shown to stimulate IL-1 release from isolated lung strips (22), we used rabbit anti-IL-1β (human) antiserum to examine if the effect of BK was dependent on IL-1β release.

MATERIALS AND METHODS

Cell culture. Human tracheas were obtained from two individuals postmortem (one male aged 44 and one female aged 52, with no evidence of airway diseases) within 12 h of death. Primary cultures of human ASM cells were prepared from explants of ASM according to methods previously reported (16, 23, 24). Cells at passages 3–4 were used for all experiments. We have previously shown that the cells grown in this manner depict the immunohistochemical and light-microscopic characteristics of typical ASM cells (23).

Experiment protocol. The cells were cultured to confluence in 10% fetal calf serum (Seralab, Crawley Down, Sussex, UK)–Dulbecco’s modified Eagle’s medium (Sigma, Poole, Dorset, UK) in humidified 5% CO₂-95% air at 37°C in 24-well culture plates and growth arrested in serum-deprived medium for 24 h before experiments. Immediately before each experiment, fresh serum-free medium containing BK (Sigma) was added. In the time-course experiments, the cells were incubated with BK (10 µM) for 1–24 h, whereas in the concentration-response experiments the cells were incubated for 24 h with 0.01–10 µM BK. In most experiments thereafter, the cells were incubated with 10 µM BK for 24 h. At the indicated times, the culture media were harvested and stored at −20°C until the radiomunoassay of PGE₂ content (23) as a representative of COX products. The anti-PGE₂ antiserum (Sigma) had negligible cross-reactivity in our hands (23). To test the inhibition by various drugs on the effect of BK, Indo, cycloheximide, actinomycin D, dexamethasone, the B₁ receptor antagonist des-Arg⁸[Leu⁹]BK, the rabbit anti-human IL-1β antiserum (Sigma), NS-398, and Nim (Cayman Chemical, Ann Arbor, MI) and the B₂ receptor antagonist d-Arg⁹Hyp³Thi⁵Dtc⁶Oic⁷BK (HOE-140, kind gift from Dr. R. N. Zalhin and Dr. B. A. Scholken, Hoechst Aktiengesellschaft, Frankfurt, Germany) were added 30 min before the addition of BK. Experiments with the selective BK B₁ receptor agonist des-Arg⁸BK, the B₂ receptor agonist [Tyr(Me)⁸]BK, Ca²⁺ ionophore A-23187, and exogenous PGE₂ (all from Sigma) were conducted in the same way as BK. BK and its receptor agonists and antagonists and the anti-human IL-1β antiserum were dissolved in serum-free medium, and all other agents were dissolved in dimethyl sulfoxide (Sigma; final concentration 1.0% vol/vol), except PGE₂, which was dissolved in ethanol (Sigma; final concentration 1.0% vol/vol). In all of the studies, a group of control cells was incubated with the vehicles used to dissolve the agents applied in the experimental cells for the same period of time.

CAMP assay. After the incubation with BK, A-23187, or PGE₂ and the removal of culture media, the cells were washed three times with PBS and incubated in 0.5 ml of fresh medium with 1.0 mM IBMX (Sigma) to prevent cAMP degradation. The cAMP production reaction was initiated with the addition of ISO (Calbiochem-Novabiochem, La Jolla, CA) and was terminated 10 min later with 0.1 ml ice-cold trichloroacetic acid (Sigma), which was then removed by amine-freon (Sigma) extraction (18), and cAMP content in the extract was determined by a protein binding assay (12). The protein kinase A-dependent cAMP and cAMP used in the assay were from Sigma; [8-3H]cAMP (specific activity 962 GBq/mmol) was from Amersham Life Science (Little Chalfont, Bucks, UK). The cAMP production in response to forskolin and PGE₂ (Sigma) was conducted in the same way as ISO.

Statistical analysis. Data are expressed as means ± SE from n determinations. Statistical analysis was performed by using the statistical software from SPSS (31). One-way analysis of variance and/or unpaired two-tailed t-test was used to determine the significant differences between the means. The results were adjusted for multiple testing by using Bonferroni’s correction. P values of <0.05 were accepted as statistically significant.

RESULTS

Effect of BK on cAMP formation in response to ISO, PGE₂, and forskolin. The capacity of human ASM cells to synthesize cAMP in unstimulated conditions and after BK pretreatment was examined first. As shown in Fig. 1, basal cAMP levels were low in both control cells and cells pretreated with BK (10 µM for 24 h). ISO caused a concentration-dependent increase in cAMP synthesis. BK markedly reduced cAMP formation in response to 1.0 and 10 µM ISO (P < 0.01 and P < 0.001, respectively). Forskolin, a direct adenyl cyclase activator, and PGE₂ also caused a concentration-dependent increase in cAMP generation, and BK significantly attenuated cAMP formation in response to 0.001 and 0.01 µM PGE₂ (P < 0.01 and P < 0.001, respectively.

The toxicity of all the chemicals used in this study and their vehicles dimethyl sulfoxide and ethanol (final concentration 1.0% vol/vol; Sigma) to human ASM cells was determined by thiazolyl blue [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterrazolium bromide; MTT; Sigma] assay (23, 24). After 24-h incubation with the chemicals, 20 µl of 5 mg/ml MTT were added to the culture medium in 96-well plates and incubated for 1 h at 37°C. After the medium was removed, 200 µl of DMSO were added to solubilize the blue-colored tetrazolium, the plates were shaken for 5 min, and values for the optical density at 550 nm were read in a microplate reader. Viability was set as 100% in control cells.

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Fig. 2. Concentration response of prostaglandin (PG) E2 and forskolin on cAMP generation from control human ASM cells and cells pretreated with BK. After being pretreated with or without BK (10 µM) for 24 h, cells were washed with PBS and further incubated in fresh medium with 1.0 mM IBMX plus various concentrations of either PGE2 (A) or forskolin (B) for 10 min. Each point is the mean ± SE of 8 determinations from 2 independent experiments. **P < 0.01 and ***P < 0.001 compared with control cells.

Fig. 2A). However, unlike Iso and PGE2, there was no significant change in cAMP between the control cells and cells pretreated with BK (10 µM for 24 h) in response to forskolin (Fig. 2B). Human ASM cells pretreated with BK (10 µM) showed a time-dependent decrease in cAMP formation in response to Iso in the time-course experiments (Fig. 3A), whereas cAMP formation in the control cells over the time course remained unchanged, with cAMP concentration around 12.5 pmol/well. The desensitization was significant from after 8 h of incubation (P < 0.001) and peaked after 24 h (P < 0.001). Treatment of the cells with various concentrations of BK for 24 h also produced a concentration-dependent desensitization response of cAMP production (Fig. 3B). The effect was significant from 0.1 µM (P < 0.001) and reached a maximum at 10 µM (P < 0.001).

Effect of various inhibitors on BK-induced changes in cAMP formation. The effect of the nonselective COX inhibitor Indo, selective COX-2 inhibitors NS-398 and Nim, protein synthesis inhibitors cycloheximide and actinomycin D, and the steroid dexamethasone was assessed on BK-induced desensitization in cAMP production in response to Iso. As shown in Fig. 4, Iso (10 µM) caused a marked increase in cAMP production; the response was strongly desensitized by BK pretreatment (10 µM, 24 h, P < 0.001), and this desensitization was significantly attenuated by Indo, NS-398, cycloheximide, actinomycin D, dexamethasone (P < 0.001), and Nim (P < 0.01). However, when the cells were incubated with the inhibitors themselves, no significant change in Iso-induced cAMP production was observed (data not shown), suggesting that COX products after BK pretreatment, including those from the inducible COX-2 isoform, are involved in BK-induced desensitization of human ASM cell responses to Iso.

Effect of the selective BK receptor agonists on cAMP formation in response to Iso. To characterize the BK receptor(s) involved in BK-induced desensitization of the cell responses to Iso, we examined the effect of the selective B1 receptor agonist des-Arg9-BK and the selective B2 receptor agonist [Tyr(Me)8]BK on the cAMP production.
production of human ASM cells in response to Iso. [Tyr(Me)³]BK mimicked the effect of BK by reducing the cAMP production in a similar concentration-dependent manner as that of BK, significant from 0.1 µM (P < 0.001), and the maximum effect was observed at 10 µM (P < 0.001; Fig. 5). In contrast, pretreatment of the cells with the B₁ receptor agonist des-Arg⁹-BK had no effect on cAMP formation compared with the control cells (Fig. 5). The results suggest that the B₂ receptor is responsible for mediating BK-induced impairment of human ASM cell responses to Iso.

Effect of the selective BK-receptor antagonists on BK-induced changes in cAMP formation. Pretreatment of human ASM cells with the selective B₂ receptor agonist des-Arg⁹-BK or the selective B₂ receptor antagonist HOE-140 (1–100 µM) strongly antagonized BK-induced attenuation in cAMP production in a concentration-dependent fashion and abolished the effect of BK at 100 µM (Fig. 6), whereas pretreatment with the B₁ receptor antagonist des-Arg⁹, [Leu⁶]BK over the same concentration range (1–100 µM) did not show any significant effect (Fig. 6). The data therefore provide further evidence that B₂ receptors are largely responsible for mediating BK-induced impairment of human ASM cell responses to Iso.

Effect of the anti-human IL-1β serum on BK-induced impaired changes in cAMP formation. Because reports have shown that BK stimulates the release of IL-1β (22) and IL-1β causes impaired responses of human ASM cells to Iso (29), we examined if BK-induced impaired responses to Iso were dependent on IL-1β by using the anti-human IL-1β antiserum to block any effect of IL-1β during the 24-h incubation with BK. cAMP production by control cells in response to Iso (10 µM, 10 min) was 11.0 ± 0.69 pmol/well. With the pretreatment of BK (10 µM, 24 h), the cAMP production was reduced to 1.64 ± 0.62 pmol/well. Coincubation of the cells with BK and a series of dilutions of the antiserum (1:500, 1:250, 1:125, and 1:62.5) did not significantly affect the impaired cAMP production induced by BK, with the cAMP production ranging from 1.5 to 1.76 pmol/well. However, the same range of dilution of the antiserum completely abolished the impaired cAMP production induced by IL-1β (data not shown), indicating that BK-induced impairment of human ASM cell responses to Iso is independent of IL-1β.

Effect of the Ca²⁺ ionophore A-23187 on cAMP formation in response to Iso. To further clarify the role of COX products in BK-induced desensitization of the cell responses to Iso, we examined if Ca²⁺ ionophore A-23187, which has a similar effect as BK in causing free Ca²⁺ increase and release of endogenous AA, could cause impaired cAMP production. A-23187 was found to cause a concentration-dependent generation of prostanoids (measured as PGE₂) after 24 h of incubation with human ASM cells, significant from 0.1 µM (P < 0.001, Fig. 7A), and the subsequent cAMP production in response to Iso (10 µM) was strongly...
reduced by A-23187 in a concentration-dependent manner, significant from 0.1 µM (P < 0.001; Fig. 7B). Coincubation of the cells with A-23187 (1.0 µM) and the COX inhibitor Indo (0.01–1.0 µM) concentration dependently abolished the A-23187-induced PGE2 release (Fig. 8A) as well as impairment of cAMP production (Fig. 8B), suggesting that it is the prostanoids produced after A-23187 treatment that mediate the impaired cAMP production.

Effect of exogenous PGE2 on cAMP formation in response to Iso and PGE2. Pretreatment of the cells with the exogenously applied COX product PGE2 also resulted in marked attenuation of cAMP production in response to Iso (10 µM) in a concentration-dependent fashion, significant from 0.01 µM (P < 0.001, Fig. 9A). When PGE2 was used as a cAMP stimulant, it caused a concentration-dependent cAMP generation in control cells, and PGE2 (1.0 µM) pretreatment of the cells for 24 h markedly reduced cAMP production in response to subsequent PGE2 stimulation, significant for all the subsequent PGE2 concentrations tested (P < 0.01 and P < 0.001) compared with the control cells (Fig. 9B). The results thus provide further evidence that the COX products can cause heterologous desensitization of human ASM cells to various receptor-lined cAMP stimulants and are mainly responsible for the impaired responses caused by BK.

Cell viability. Cell viability after 24 h of treatment with all the chemicals used in this study was consistently >95% compared with cells treated with the vehicles.

DISCUSSION

Increasing evidence suggests that β-adrenergic relaxant mechanisms may be dysfunctional in asthmatic airways (4). ASM relaxation to β-adrenoceptor agonists in vitro is impaired in airways taken from patients who...
died of asthma exacerbation (2, 13), in surgical lobectomy samples from patients with stable asthma (8), and in animal models of asthma (9). Reports have accumulated to support the hypothesis that cytokines, most notably IL-1β and to a lesser extent TNF-α, contribute to the impaired airway relaxation in asthma. IL-1β and TNF-α have been reported to cause β-adrenergic hyporesponsiveness in various human and animal tissues and cells (10, 29, 34). Recently, Hakonarson and associates (15) demonstrated that the altered responsiveness of atopic/asthmatic sensitized rabbit ASM was largely attributed to autologously induced expression and autocrine action of IL-1β. However, very little is known about the role of other proinflammatory mediators in the development of the β-adrenergic hyporesponsiveness in the airway. Because asthmatic patients have elevated levels of kinin concentrations in nasal and bronchoalveolar lavage fluid (5, 7) and BK exerts similar effects as IL-1β in human ASM cells such as causing COX-2 induction and prostanoid release (23, 24), we postulated that BK could also cause impaired responses of human ASM cells to the β-adrenoceptor agonist Isc.

The results in our present study demonstrated that pretreatment of human ASM cells with BK resulted in a time- and concentration-dependent impairment of the cell responses to Iso (Fig. 3). The impaired responses were completely reversed by the COX inhibitor Indo, which blocked the generation of COX products, and were partially prevented by the COX-2 selective inhibitors NS-398 and Nim, the protein synthesis inhibitors cycloheximide and actinomycin D, and the steroid dexamethasone (Fig. 4). We have recently reported the details of BK-induced PGE2 release and COX-2 induction in human ASM cells and the inhibition of the induction by some of the above inhibitors (24). In view of this, the Western blot results were not shown here. The fact that Indo produced a greater effect than the COX-2 selective inhibitors, the protein synthesis inhibitors, and dexamethasone suggests that prostanoids produced by phospholipase A2 activation and constitutive COX-1 also play a role in BK-induced impaired cAMP generation. We have previously shown that phospholipase A2 activation is responsible for the early phase of prostanoid production (measured as PGE2) in response to BK and that COX-2 is responsible for the later phase (24). Our findings therefore provide the first direct evidence that BK induces impaired responses of human ASM cells to β-adrenoceptor agonists, and COX products from both COX-2 induction and constitutive COX-1 contribute largely to this impairment. The relevance of these findings to human airway function could be made clear by further investigations of the effect of BK on the responsiveness of intact human airways.

Much effort has been made to understand the mechanism(s) by which cytokines cause the β-adrenergic hyporesponsiveness. In ASM, Iso binds to the β-adrenergic receptors that couple to the stimulatory G protein Gs, the α-subunit of which in turn activates the enzyme adenyl cyclase to generate cAMP (4). Increased cAMP activates protein kinase A and protein kinase G to cause relaxation of ASM (4). The observations that 1L-1β did not affect the ability of the direct adenyl cyclase activator forskolin to cause cAMP accumulation (14, 29) or smooth muscle relaxation (14) suggest that the decreased β-adrenergic responsiveness by IL-1β is not due to any change in the activity or expression of adenyl cyclase. The fact that a phosphodiesterase inhibitor, IBMX, was used together with Iso (29) also excludes the involvement of changes in phosphodiesterase activity in the effects of 1L-1β within the experimental design. The effects, therefore, are likely to be mediated upstream of the adenyl cyclase enzyme. Studies looking at whether the effect is at the level of G proteins have recently shown that, although IL-1β had no effect on the expression of the stimulatory G protein subunit Gsα (29), IL-1β-attenuated relaxation of tracheal smooth muscle to Iso was ablated by a muscarinic M2-receptor antagonist and was associated with en-
hanced induction of the inhibitory G protein subunits Ga1,2 and Ga1,3 (14). This suggests that the cytokine-induced impairment of airway responsiveness to β-adrenoceptor agonists is attributable to enhanced M2 receptor/G protein-coupled inhibition of adenylly cyclase.

PGs (mainly PGE2 and PG12) activate the PGEP2 and PGEP4 receptors, which are also coupled to Ga and adenylly cyclase (21), to increase cAMP production in human ASM cells. Thus PGs share a similar receptor-mediated signal transduction system with β-adrenoceptor agonists, and the functional responses to PGE2 receptor stimulation, like that to β-adrenoceptor stimulation, are downregulated by the activation of Ga protein (20). It is therefore reasonable to speculate that elevated levels of PGs could cause heterologous desensitization of adenylly cyclase. In fact, BK not only caused impaired responses to Iso but also caused impaired responses to PGE2 (Fig. 2A), and exogenously applied PGE2 also caused heterologous desensitization of cAMP production in response to both Iso and PGE2 (Fig. 9, A and B). BK caused impaired responses to lower concentrations of PGE2 (0.001–0.01 µM) but not to higher concentrations (0.1–1 µM). The most likely explanation for this is that the PGE2 concentration after 24 h of incubation with BK was ~0.01–0.02 µM, which would only be expected to impair cAMP accumulation to subsequent application of similar concentration of PGE2. However, when the cells were preincubated with 1 µM exogenously applied PGE2 for 24 h, impaired responses to subsequent stimulation with PGE2 at concentrations up to 1 µM were observed (Fig. 9B). Observations from our previous studies have demonstrated that IL-1β causes induction of COX-2 in human ASM cells and consequently results in a marked increase in prostanooid generation with PGE2 and PG12 as the major products (23) and that BK has a similar effect (24). BK attenuated the capacity of human ASM cells to form cAMP in response to Iso, and this impairment was prevented completely or partially by reagents that blocked either the activity of COX or the induction of COX-2 (Fig. 4). The Ca2+ ionophore A-23187, which has a similar effect as BK in causing cytosolic free Ca2+ increase and AA release, induced PGE2 release and mimicked the impaired responses by BK (Fig. 7, A and B), and when the PGE2 release was blocked by Indo, the impaired responses were also reversed (Fig. 8, A and B). The exogenously applied PGE2 also mimicked the impaired responses by BK (Fig. 9, A and B). These findings are in agreement with the report that pretreating human ASM cells with agents that induce cAMP formation resulted in a marked decrease in the capacity of the cells to produce cAMP after subsequent application of Iso (16). The fact that the anti-human IL-1β antiserum did not affect BK-induced impairment excludes the possibility that the effect of BK is mediated by the release of IL-1β. It is therefore likely that BK-induced impairment of responses of human ASM cells to β-adrenoceptor agonists is largely mediated by the large quantity of COX products involving both existing COX-1 and induced COX-2, possibly through the uncoupling of β-receptors from adenylly cyclase activation. The precise mechanism(s), however, remains to be further investigated.

Two BK receptor subtypes (B1 and B2) have been identified (11). B1 and B2 receptor-mediated responses can be distinguished pharmacologically on the basis of relative potencies of agonists or by the use of receptor-selective antagonists. In the present study, BK-induced impaired responses were mimicked by the selective B2 receptor agonist [Tyr(Me)2]BK but not by the selective B1 receptor agonist des-Arg9-BK (Fig. 5), and the effect of BK was strongly reversed and abolished by the selective B2 receptor antagonist HOE-140, whereas the selective B1 receptor antagonist des-Arg9,[Leu9]BK was ineffective (Fig. 6), suggesting that BK-induced impaired responses to Iso are mediated by the B2 receptors. Our results are in agreement with previous findings that B2 receptors are responsible for various effects of BK in airway tissues and cells (17, 24, 27).

Our present findings also provide fresh insights into the argument about whether the consequences of COX-2 induction and prostanoild production in human ASM would be detrimental or beneficial for airway functions in asthma. PGE2 is an important anti-inflammatory mediator that has bronchoprotective effects in the airways (25). It is possible therefore that the exaggerated PGE2 production as a result of COX-2 induction is part of a negative feedback mechanism that is exerting a braking effect on the inflammatory response. The induction of COX-2 itself may also shut the released AA away from the generation of the potent bronchoconstrictor of the lipoxygenase pathway toward the synthesis of bronchodilators of the COX pathway, such as PGE2 and PG12. However, PGE2 at higher concentrations also causes ASM contraction due to weak agonism at the thromboxane receptor (1, 19), and other products of COX, such as PGF2α, thromboxane A2, and PGD2, are potent proinflammatory modulators that cause bronchoconstriction via the activation of the thromboxane prostaglandin receptor (1, 19). In addition, we report here that COX-2 induction forms an important part in BK-induced impairment of human ASM cell responses to Iso, and we speculate that COX-2 induction may also be involved in IL-1β-induced impaired responses to Iso. COX-2 induction and the consequent prostanoild production in human ASM may therefore do more harm than good in respect to airway function in inflammatory airway diseases such as asthma.

In summary, this study examined the role of COX products in BK-induced impairment of human ASM cell cAMP responses to the β-adrenoceptor agonist Iso. Our results demonstrated that 1) pretreatment of the cells with BK resulted in a marked time- and concentration-dependent decrease in cAMP accumulation after subsequent application of Iso; 2) reagents that inhibited or blocked either the activity of COX or the induction of COX-2 also completely or partially prevented BK-induced impairment in cAMP formation; 3) BK-induced impaired responses were mimicked and abolished by the selective B2 receptor agonist and antagonist, respectively, but were not affected by the
anti-IL-1β antiserum; and 4) pretreatment with A-23187 and exogenously applied PGE₂ also caused impairment on cAMP accumulation of the cells in a similar pattern as that of BK. Collectively, these findings indicate that COX products (including those from COX-2 induction) are critical in the development of BK-induced impairment of human ASM cell responses to ISO, and this may be helpful in the understanding of the pathogenesis of asthma.

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