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Piceatannol, a Syk-selective tyrosine kinase inhibitor, attenuated antigen challenge of guinea pig airways in vitro

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Abstract

Activation of nontransmembrane protein tyrosine kinases, such as Lyn and Syk, has been shown to be the earliest detectable signaling response to Fc receptor (FcεRI) cross-linking on mast cells leading to mast cell degranulation. The present study examined the effects of piceatannol (3,4,3,5′-tetrahydroxy-*trans*-stilbene,10–100 μM), a Syk-selective tyrosine kinase inhibitor, on ovalbumin-induced anaphylactic contraction of isolated guinea pig bronchi and release of histamine and peptidoleukotrienes from chopped lung preparations. Pretreatment with piceatannol slightly suppressed ovalbumin-induced peak anaphylactic bronchial contraction but markedly ($P<0.05$) facilitated relaxation of the anaphylactically contracted bronchi. Piceatannol did not inhibit direct histamine-, leukotriene D_4- or KCl-induced bronchial contraction, nor revert an existing anaphylactic bronchial contraction. Piceatannol, at 30 μM and above, significantly ($P<0.05$) prevented ovalbumin-induced release of both histamine and peptidoleukotrienes from lung fragments. Piceatannol did not inhibit exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments. Our data show for the first time that inhibition of Syk tyrosine kinase can attenuate anaphylactic bronchial contraction in vitro, probably via inhibition of mast cell degranulation.

Keywords: Bronchus; Ovalbumin; Histamine; Leukotriene D_4; Schultz–Dale reaction; Arachidonic acid

1. Introduction

Mast cell has been implicated to play a crucial role in asthma because mast cell degranulation induced by the cross-linking of high-affinity Fc receptor (FcεRI) releases a wide array of inflammatory mediators such as histamine, leukotrienes and cytokines, which can initiate, coordinate, and sustain the allergic inflammatory responses (Busse and Lemanske, 2001). Cumulative evidence obtained from rat basophilic leukemia cell line (RBL-2H3) and bone marrow-derived mouse mast cells show that activation of src-related kinase Lyn and 72-kDa Syk tyrosine kinase is the earliest detectable signaling response to FcεRI cross-linking (Kinet, 1999). This is followed by downstream signaling events such as activation of phospholipase Cγ, phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), and increase in inositol 1,4,5-trisphosphate and intracellular Ca^{2+} levels, which eventually leads to mast cell degranulation (Reischl et al., 1999). Broad-spectrum protein tyrosine kinase inhibitors (i.e., genistein, lavendustin A and tyrphostin 47) have been shown to block antigen-induced histamine release from isolated mast cells (Kawakami et al., 1992). In addition, piceatannol and ER-27319, two Syk-selective inhibitors, have also been shown to prevent mast cell degranulation in cell culture (Oliver et al., 1994; Moriya et al., 1997; Lavens-Phillips et al., 1998). Because mast cell degranulation is the hallmark of immediate-type hypersensitivity reaction, which is also the major mechanism for a variety of allergic diseases such as bronchial asthma, it is imperative to examine the effects of protein tyrosine kinase inhibitors on an in vitro model of allergic asthma.

The Schultz–Dale reaction (Chand and Eyre, 1978) has been extensively used to examine mast cell degranulation and anaphylactic contraction of airway tissues such as trachea, bronchi, and lung parenchymal strips in vitro (Jonsson and Dahlen, 1994; Roquet et al., 1997). It is believed that histamine and peptidoleukotrienes are the two major mast cell-derived mediators responsible for the anaphylactic contraction of isolated airways from both human and guinea pig. In guinea pigs, both immunoglobulin E (IgE) and IgG are able to sensitize mast cells to specific

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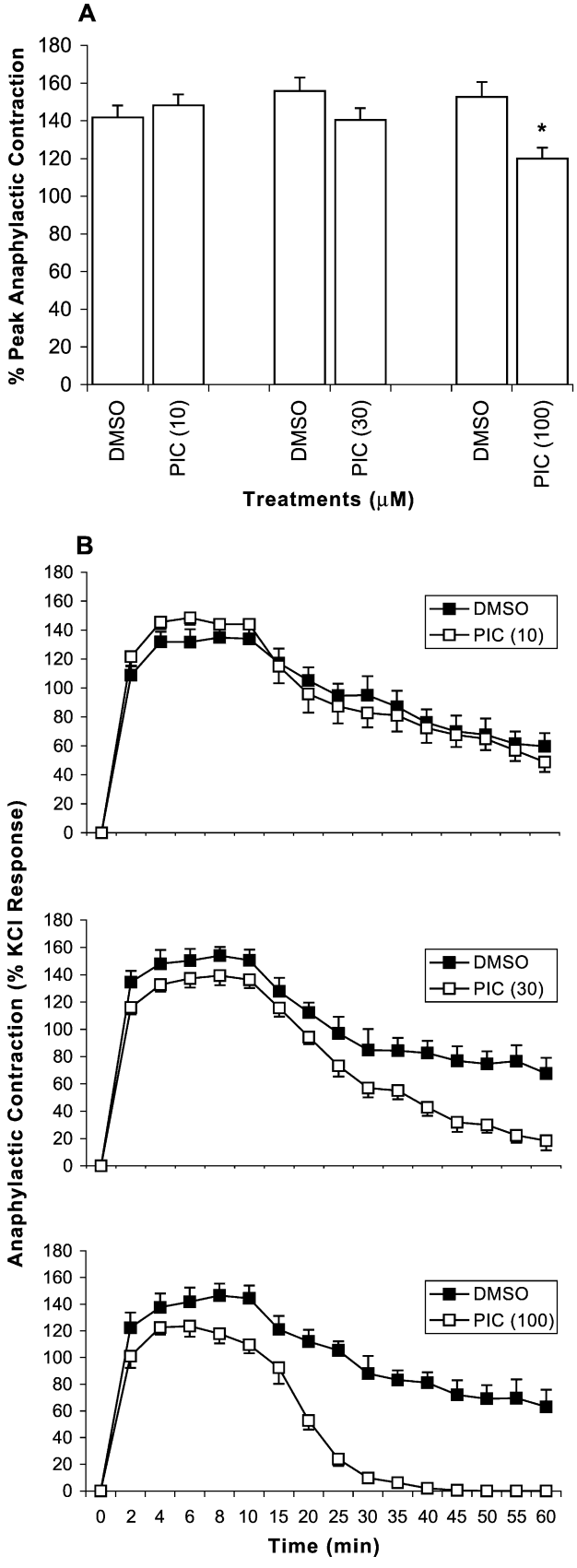

antigen (Ro et al., 1991) and cross-linking of their corresponding FcεRI and FcγR leads to mast cell degranulation. FcγR (e.g., FcγRIII) and FcεRI are structurally and functionally related, and both belong to a family of multi-subunit antigen receptors (Alber et al., 1992). Engagement of these cell surface receptors utilizes the same tyrosine kinase-signaling cascade for successful signal propagation and cellular activation (Sanchez-Majorada and Rosales, 1998).

Our previous studies showed that genistein and tyrphostin 47, two broad-spectrum tyrosine kinase inhibitors, significantly inhibited the Schultz–Dale reaction in guinea pig airways in vitro (Wong et al., 1997; Tsang and Wong, 2000). In the present study, we examined the effects of piceatannol, a Syk-selective inhibitor, on antigen-induced anaphylactic contraction of the bronchi and release of histamine and peptidoleukotrienes from chopped lung preparations. Our findings show that piceatannol only slightly reduced the peak ovalbumin-induced bronchial contraction; however, the anaphylactic contraction relaxed rapidly in the presence of piceatannol in a concentration-dependent manner. The rapid relaxation of anaphylactic contraction is likely mediated by the marked inhibition of ovalbumin-induced release of histamine and peptidoleukotrienes from mast cells in the airways by piceatannol.

2. Materials and methods

2.1. Materials

The following drugs and chemicals were used in this study: piceatannol, ovalbumin, salbutamol, histamine, indomethacin, L-cysteine, arachidonic acid (Sigma, St. Louis, MO, USA), rabbit IgG fraction to chicken egg albumin (Organon Teknicka, Durham, NC, USA), leukotriene D_4 (Cayman Chemical, Ann Arbor, MI, USA), histamine enzyme-linked immuno-sorbent assay (ELISA) kit (Immuno-Biological Laboratories, Hamburg, Germany), and leukotriene $C_4/D_4/E_4$ enzyme-immunoassay (EIA) kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

2.2. Sensitization procedures

Male Hartley guinea pigs (Interfauna, UK) weighing 350–450 g were passively sensitized by a single intraperitoneal injection of 1 mg/kg rabbit IgG antibody against ovalbumin (Tsang and Wong, 2000). The animals were sacrificed 2 days after injection.

Fig. 1. (A) Effects of piceatannol (PIC) on ovalbumin-induced peak anaphylactic contraction of guinea pig bronchi. Bronchial rings were incubated with the indicated concentrations of piceatannol or same volume of DMSO for 30 min before 1 μg/ml ovalbumin challenge. Each point represents the mean ± S.E.M. of four to eight experiments. * Significant difference from DMSO controls, $P < 0.05$. (B) Time course of ovalbumin-induced anaphylactic bronchial contraction in the presence and absence of piceatannol. Each point represents the mean ± S.E.M. of four experiments.

2.3. Preparation of bronchial rings

Guinea pigs were sacrificed by CO_2 asphyxiation and subsequent decapitation. Lung lobes were isolated for studies on the release of histamine and peptidoleukotrienes (see below). Bronchial rings (~ 3 mm in length) were suspended isometrically under an optimum resting load of 2 g in organ baths containing 5 ml of Krebs–bicarbonate solution aerated with 95% O_2 and 5% CO_2 at 37 °C and of the following composition (mM): NaCl, 118.2; KCl, 4.6; $NaHCO_3$, 24.8; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2 and dextrose, 10.0. Contractile responses were monitored using force–displacement transducers (Grass FT-03) coupled to a MacLab/8 data-recording system (ADInstruments, Castle Hill, Australia).

2.4. Contractile studies

Bronchial rings were contracted to 60 mM KCl. The contraction was defined as the maximum tissue response, to which all subsequent anaphylactic contractions were compared. For antigen challenge studies, ring preparations were pre-incubated with indomethacin (4 μM) for 30 min before addition of ovalbumin (1 μg/ml). Indomethacin has been shown to reduce the production of relaxant prostanoids (e.g. prostaglandin E_2 and prostacyclin) capable of modulating the anaphylactic contraction (Ro et al., 1991; Abela and Daniel, 1994). To evaluate the role of Syk tyrosine kinase in mediating anaphylactic bronchial contraction, piceatannol or dimethyl sulfoxide (DMSO) was pre-incubated with bronchial rings 30 min before ovalbumin challenge. To examine the potential direct smooth muscle relaxant effects of piceatannol, we studied bronchial contraction induced by histamine, leukotriene D_4, or KCl in the presence and absence of the inhibitor. In the leukotriene D_4-induced bronchial contraction study, 4 μM indomethacin and 5 mM L-cysteine were pre-incubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanoids (e.g. prostaglandin E_2 and prostacyclin) were able to mask leukotriene D_4-induced canine bronchial contraction and that the addition of indomethacin restored the leukotriene D_4 effect (Abela and Daniel, 1994). L-cysteine is an inhibitor of an aminopeptidase that converts leukotriene D_4 to a less potent leukotriene E_4. Adding L-cysteine has been shown to enhance the smooth muscle contractile response to LTD_4 (Abela and Daniel, 1994). To further explore the potential direct smooth muscle relaxant effects, piceatannol was added to the bronchi after they reached their peak anaphylactic contractions. Salbutamol, a β_2-adrenoceptor agonist, was used as a positive control for airway smooth muscle relaxation.

2.5. Release of mediators from chopped lung preparations

Lung lobes obtained from guinea pigs were cut into approximately 1-mm^3 pieces using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY, USA). Duplicate aliquots of 200 mg lung fragments were weighed and placed in plastic scintillation vials containing 2 ml of oxygenated Krebs solution containing 4 μM indomethacin and 5 mM L-cysteine. Lung samples were then incubated in a shaker bath at 37 °C for 45 min before they were challenged with ovalbumin for 10 min (Tsang and Wong, 2000). To determine the mast cell-stabilizing effect, piceatannol was pre-incubated with the lung preparation for 30 min before ovalbumin challenge. To determine if piceatannol has any direct inhibitory effects on the de novo synthesis of pepti-

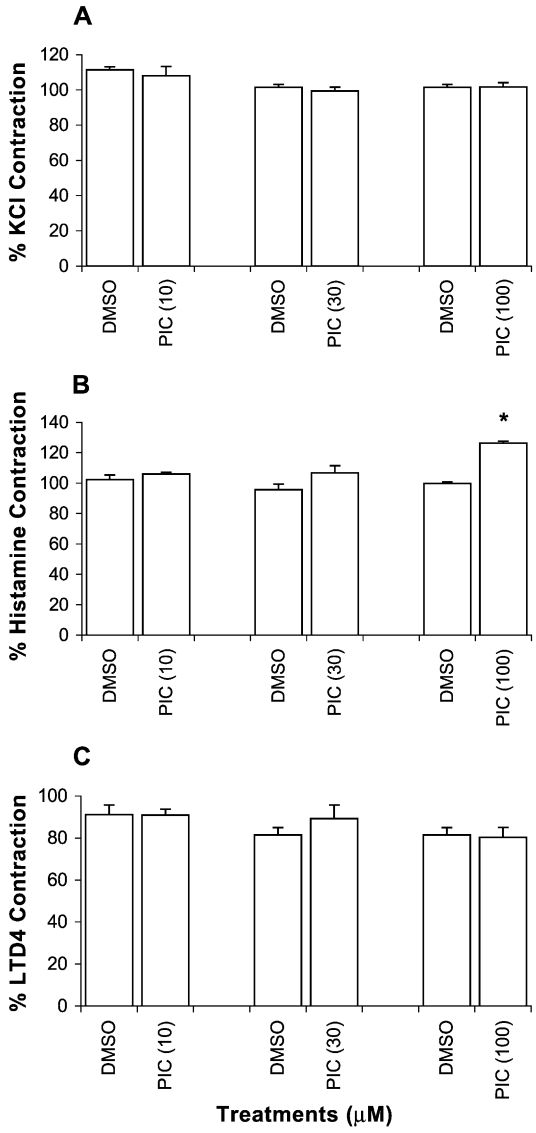

Fig. 2. Effects of piceatannol (PIC) on (A) 60 mM KCl-, (B) 30 μM histamine-, or (C) 0.1 μM leukotriene D_4-induced bronchial contraction. A DMSO control was carried out in parallel with each piceatannol concentration. Each point represents the mean ± S.E.M. of four to six experiments. * Significant difference from DMSO control, $P < 0.05$. (D) Reversal effects of piceatannol on existing ovalbumin (OA)-induced bronchial contraction. DMSO and salbutamol (1 μM, SAL) were used in parallel with piceatannol as negative and positive controls, respectively.

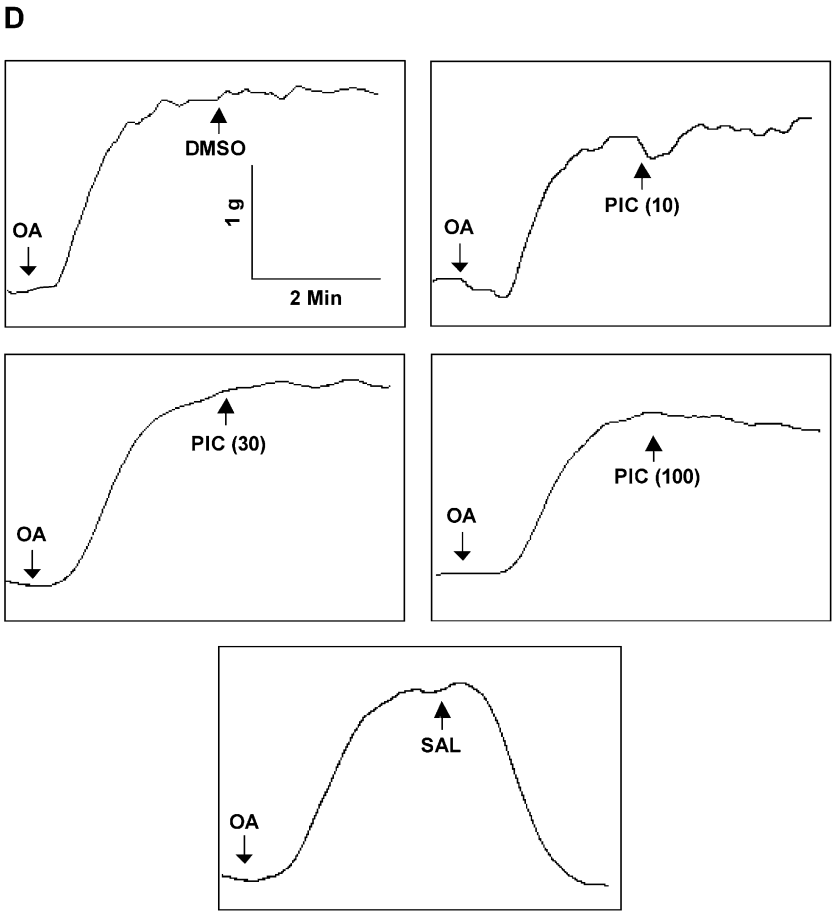

Fig. 2 (*continued*).

doleukotrienes such as inhibition of 5-lipoxygenase activity, exogenous arachidonic acid at a final concentration of 70 μM was added to the lung fragments alone or together with ovalbumin challenge for 10 min in the presence and absence of piceatannol (Kumlin and Dahlen, 1990; Tsang et al., 1998). Diffusates were then collected and stored at − 70 °C until assay.

2.6. Quantitation of mediators

Histamine release from lung samples in response to ovalbumin was determined using an ELISA kit according to the manufacturer's instructions. The release of peptidoleukotrienes from chopped lung preparations in response to ovalbumin or combined ovalbumin and arachidonic acid was measured using an EIA according to the manufacturer's instructions. Optical density was determined using a microplate reader (Tecan, Austria) at 450 nm. Samples were assayed in duplicate.

2.7. Data analysis

All data are presented as mean±S.E.M. Statistical differences in contractile responses to ovalbumin challenge, histamine, leukotriene D_4 or KCl, and in the release of mediators in response to ovalbumin, in the presence and absence of piceatannol, were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. The critical level for significance was set at $P<0.05$.

3. Results

3.1. Effects of piceatannol on anaphylactic contraction

Sensitized guinea pig bronchial rings contracted in response to 60 mM KCl with an active force of contraction that amounted to 1.6±0.1 g ($n=19$ rings from 10 guinea pigs). To determine the role of Syk kinase in the Schultz–Dale reaction, piceatannol (10–100 μM) was pre-incubated with the ring preparations for 30 min before ovalbumin challenge. The concentrations of piceatannol were chosen based on reports from cell culture studies (Oliver et al., 1994; Lavens-Phillips et al., 1998; Law et al., 1999; Raeder et al., 1999). Ovalbumin at 1 μg/ml was able to induce a strong and sustaining anaphylactic bronchial contraction reaching 2.1 ± 0.1 g ($n=12$ rings from 6 guinea pigs). Piceatannol (100 μM) produced only a minor, but statisti-

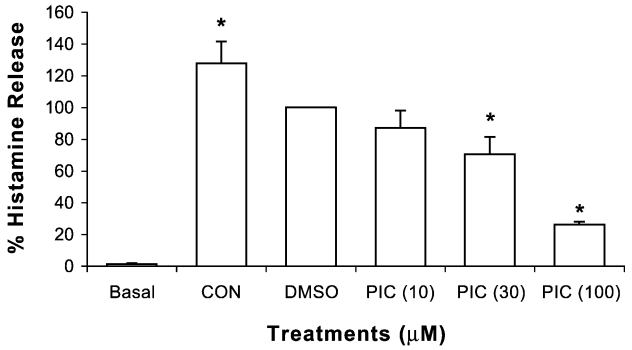

Fig. 3. Effects of piceatannol (PIC) on the release of histamine from ovalbumin-stimulated guinea pig chopped lung preparations. Various concentrations of piceatannol were incubated with the lung preparations in the presence of 4 μM indomethacin and 5 mM L-cysteine for 30 min before ovalbumin challenge. Each point represents the mean ± S.E.M. of four experiments. * Significant difference from DMSO control, $P < 0.05$.

cally significant ($P < 0.05$) reduction in ovalbumin-induced peak anaphylactic contraction of the guinea pig bronchi (Fig. 1A). In contrast, the anaphylactically contracted bronchi relaxed at a substantially greater extent in the presence of piceatannol as compared to the DMSO controls (Fig. 1B). In the first 30 min, ovalbumin-induced bronchial contraction relaxed by 60% and 90% in the presence of 30 and 100 μM piceatannol, respectively. Complete relaxation was achieved in 40 min with 100 μM piceatannol and 90% relaxation was reached in 60 min with 30 μM piceatannol. In contrast, substantial anaphylactic contraction was still maintained in the control bronchial preparations after 60 min.

3.2. Effects of piceatannol on mediator-induced bronchial contraction

To determine whether the rapid relaxation of the contracted bronchi produced by piceatannol is mediated by blocking the release of mast cell-derived mediators or by direct relaxant effects on the bronchial smooth muscle, we evaluated the effects of piceatannol on histamine-, leukotriene D_4-, or KCl-induced bronchial contraction. Piceatannol failed to prevent bronchial contraction induced by 30 μM histamine, 0.1 μM leukotriene D_4, or 60 mM KCl (Fig. 2A–C). Compared with salbutamol, piceatannol failed to revert the anaphylactically contracted bronchi to any significant extent when the inhibitor was added at the plateau phase of the contraction (Fig. 2D). These findings indicate that piceatannol-mediated rapid relaxation of anaphylactic contraction was not a result of any potential nonselective activities such as receptor antagonism or inhibition of voltage-dependent calcium channels. However, piceatannol (100 μM) significantly ($P < 0.05$) potentiated bronchial contraction induced by histamine, but not by leukotriene D_4 or KCl.

3.3. Effects of piceatannol on the release of mediators

There were low levels of histamine (22.0 ± 2.2 ng/g tissue, $n = 4$) and peptidoleukotrienes (50.8 ± 10.6 ng/g tissue, $n = 4$) released from the chopped lung preparations. Upon ovalbumin challenge of the lung fragments, the release of histamine increased by 60-fold (2908.5 ± 601.3 ng/g tissue, $n = 4$) and that of peptidoleukotrienes increased by 8-fold (396.4 ± 65.9 ng/g tissue, $n = 4$). At 30 and 100 μM, piceatannol significantly ($P < 0.05$) suppressed ovalbumin-induced histamine release from lung fragments by 30% and 74%, respectively (Fig. 3). At 30 μM, piceatannol markedly ($P < 0.05$) blocked peptidoleukotrienes release by 52% (Fig. 4A). The effect of 100 μM piceatannol on peptidoleukotrienes release was not examined because it was observed that, at this higher concentration, piceatannol interfered with the EIA. To determine if piceatannol possesses other activity such as inhibition of 5-lipooxygenase, we examined the effects of piceatannol (30 μM) on ovalbumin-induced peptidoleukotrienes release in the presence of 70 μM exogenous arachidonic acid (Fig. 3B). Arachidonic acid alone triggered a minor release of peptidoleukotrienes from lung fragments (23.6 ± 8.1%, $n = 4$) as

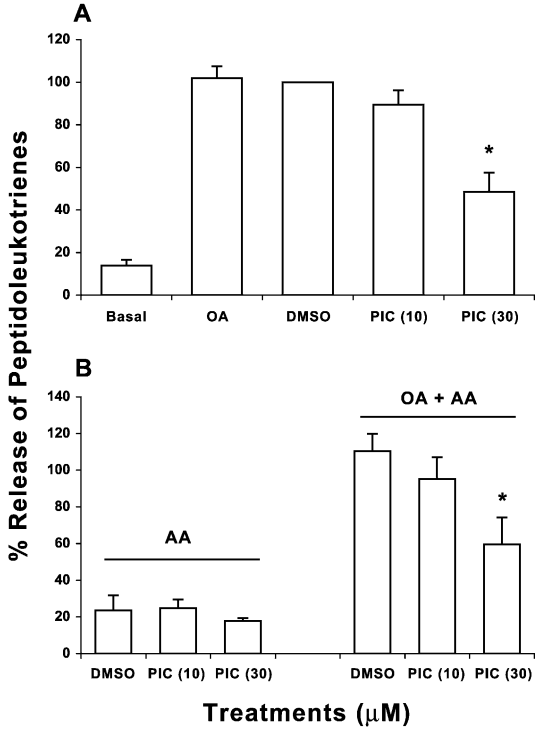

Fig. 4. Effects of piceatannol (PIC) on the release of peptidoleukotrienes in the absence (A) and presence (B) of 70 μM arachidonic acid (AA) from ovalbumin (OA)-stimulated guinea pig chopped lung preparations. Various concentrations of piceatannol were incubated with the lung preparations in the presence of 4 μM indomethacin and 5 mM L-cysteine for 30 min before ovalbumin or combined ovalbumin and arachidonic acid challenge. Each point represents the mean ± S.E.M. of three to four experiments. * Significant difference from DMSO control, $P < 0.05$.

compared to ovalbumin control (100%, $n=4$). Piceatannol did not block the arachidonic acid-induced release of peptidoleukotrienes, indicating that their inhibitory effects on ovalbumin-induced peptidoleukotrienes release were not mediated by inhibition of 5-lipooxygenase. When ovalbumin and arachidonic acid were added together to the lung fragments, there was an additional increase in peptidoleukotrienes release (110.5 ± 9.2, $n=4$). Piceatannol (30 μM) markedly ($P<0.05$) reduced the component of peptidoleukotrienes release induced by ovalbumin by 51%.

4. Discussion

Cross-linking of FcεRI on mast cells triggers immediate activation of nontransmembrane protein tyrosine kinases, Lyn and Syk, resulting in mast cell degranulation. Activation of Lyn has been shown to be upstream of Syk (Kinet, 1999), and activation of Syk leads to propagation of multiple signaling pathways and manifestation of multiple biological responses including membrane ruffling, granule secretion and cytokine production (Reischl et al., 1999; Oliver et al., 2000). According to the concept of signaling networks perceived by Jordan et al. (2000), there are two classes of signal interconnections: junctions, which are signal integrators, and nodes, which split the signal and route them to multiple outputs. Syk can be considered an example of a node in FcεRI-triggered signaling networks in mast cells and a strategic signaling target for inhibition. Indeed, Syk-deficient mast cell line and basophils failed to degranulate upon FcεRI aggregation (Zhang et al., 1996; Kepley et al., 2000), and transfection of Syk into the mast cell line reconstituted FcεRI-mediated degranulation (Zhang et al., 1996). Inhibition of Syk by piceatannol, a widely reported Syk-selective inhibitor, has been shown to inhibit FcεRI-mediated histamine and serotonin release from isolated mast cells and basophils (Oliver et al., 1994; Kepley et al., 1998; Lavens-Phillips et al., 1998). Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) was originally isolated from the seeds of *Euphoribia lagascae* and is now readily available through organic synthesis (Lawrence and Niu, 1998). Piceatannol has been shown to potently inhibit the activity of Syk at concentrations that have little or no effect on Lyn, Fyn, cylcooxygenase (COX)-1 and COX-2 (Oliver et al., 1994; Kepley et al., 1998; Lavens-Phillips et al., 1998; Law et al., 1999; Raeder et al., 1999; Lee et al., 2001). Although piceatannol has been shown to inhibit focal adhesion kinase (FAK) activity in platelets (Law et al., 1999), this potential inhibitory effect does not affect the findings of the present study because FAK acts downstream of Syk. Inhibition of Syk kinase will ultimately inhibit FAK in a cascade manner.

Schultz–Dale reaction manifested in sensitized airways from both human and guinea pig has been shown to be mediated by two major mast cell-derived mediators, namely, histamine, a preformed biogenic amine, and peptidoleukotrienes, biosynthetic metabolites of arachidonic acid (Roquet et al., 1997). Histamine appears to be relatively more important as a mediator during the initial peak phase of anaphylactic contraction, whereas peptidoleukotrienes appear to have a dominant role in maintaining the plateau phase of the contraction (Jonsson and Dahlen, 1994). Our present findings show that 30 μM piceatannol significantly reduced histamine release by 30% and peptidoleukotrienes release by 52%, but failed to suppress the initial peak anaphylactical bronchial contraction. This observation can be explained by the fact that the dramatic increase in histamine release (60-folds) and in peptidoleukotrienes release (8-folds) induced by 1 μg/ml ovalbumin demands for a very substantial inhibition of mediator release in order to see suppression of the initial peak contraction. In addition, the concentration of histamine and peptidoleukotrienes in the microenvironment of the bronchial tissues might be higher than that measured in the supernatants. As the concentration of piceatannol was increased to 100 μM, the initial peak of anaphylactical contraction was significantly inhibited by 22% ($P<0.05$) (Fig. 1A) with a 74% inhibition of histamine release and presumably additional inhibition of peptidoleukotrienes release (Fig. 3). These observations indicate that (1) inhibition of Syk by piceatannol can block mast cell degranulation in airways in vitro, consistent with the findings observed in isolated mast cells (Oliver et al., 1994; Kepley et al., 1998; Lavens-Phillips et al., 1998), and (2) a substantial reduction in histamine release is required in order to significantly attenuate the peak anaphylactic bronchial contraction.

In contrast, ovalbumin-induced bronchial contraction relaxed markedly faster in piceatannol-pretreated bronchi by 30% and 61% at 30 and 100 μM, respectively, based on the area under the curves for contractile responses over 60 min (Fig. 1B). This increased relaxation rate is not secondary to the reduced peak ovalbumin-induced bronchial contraction since at 30 μM piceatannol, the peak contraction was the same as that of the control (Fig. 1A). The rapid relaxation induced by piceatannol might be due to either potential smooth muscle relaxant effect or inhibition of secondary lipid mediator release (i.e., peptidoleukotrienes) upon antigen challenge. Our findings show that piceatannol neither inhibited direct histamine-, leukotriene D_4- or KCl-induced bronchial contraction, nor relaxed ovalbumin-induced bronchial contraction when the inhibitor was added at the plateau phase of the contraction (Fig. 2). These suggest that the rapid relaxation of ovalbumin-induced anaphylactic bronchial contraction by piceatannol is not mediated by histamine or leukotriene D_4 receptor antagonism, or by other non-specific smooth muscle relaxing activities such as inhibition of voltage-dependent and receptor-operated Ca^{2+} channels. Interestingly, we observed that 100 μM piceatannol slightly ($P<0.05$) potentiated histamine-induced bronchial contraction. Similar enhancing effect was also observed with PD098059, a specific MAPK kinase inhibitor, in our previous studies (Tsang et al., 1998;

Tsang and Wong, 2000). It has been reported that relaxant prostanoids such as prostaglandin E_2 and prostacyclin are constantly being released from the bronchial preparations (Ro et al., 1991; Abela and Daniel, 1994). Inhibition of MAPK kinase by PD098059 prevented the release of prostaglandin E_2 from human bronchial epithelial cells by inhibiting the activity of phospholipase A_2 (Newton et al., 2000). We speculate that piceatannol, by inhibiting Syk, attenuated the downstream MAPK activity and the release of prostaglandin E_2, leading to enhanced histamine-induced bronchial contraction observed in the present study.

On the other hand, 30 μM piceatannol markedly inhibited the release of peptidoleukotrienes from ovalbumin-challenged lung fragments by 52% (Fig. 4A). At this concentration, the anaphylactically contracted bronchi relaxed 30% faster than the DMSO control. Upon aggregation of FcεRI on mast cells, tyrosine phosphorylation of Syk leads to activation of MAPK signaling pathway (Zhang et al., 1997; Reischl et al., 1999). As mentioned above, MAPK can activate phospholipase A_2 to liberate arachidonic acid from the phospholipid membrane (Lin et al., 1993; Zhang et al., 1997). In turn, arachidonic acid is converted to peptidoleukotrienes by the action of 5-lipooxygenase (Lepley and Fitzpatrick, 1996). Therefore, inhibition of Syk is expected to result in a reduction in the biosynthesis of peptidoleukotrienes. Our results showed that piceatannol did not block exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments, suggesting that the inhibitor does not have direct effect on 5-liopoxygenase activity (Fig. 4B). Therefore, the substantial reduction in peptidoleukotrienes release by piceatannol via Syk inhibition is likely linked to the rapid relaxation of the ovalbumin-induced anaphylactic bronchial contraction. We anticipate more substantial reduction in ovalbumin-induced peptidoleukotrienes release from the lung fragments by 100 μM piceatannol, which was shown to facilitate relaxation of the anaphylactically contracted bronchi by 61% as compared to DMSO control (Fig. 1B). However, in the preliminary study, we found out that 100 μM piceatannol interfered with the EIA. Therefore, the effect of 100 μM piceatannol on the ovalbumin-induced release of peptidoleukotrienes from lung fragments was not examined.

Mast cell-derived mediators such as histamine and peptidoleukotrienes produce a plethora of pathological effects in the airways, including bronchoconstriction, plasma exudation, mucus secretion, recruitment and activation of inflammatory cells, and airway smooth muscle hyperplasia (Barnes et al., 1998; Busse and Lemanske, 2001). Our present study demonstrates that piceatannol produced substantial inhibition of histamine and peptidoleukotriene release from sensitized lung fragments and rapid relaxation of anaphylactic bronchial contraction in vitro. On the other hand, Syk kinase has been implicated as a pivotal upstream signaling molecule for the activation of various inflammatory cells including T cells, B cells and monocytes (Dustin and Chan, 2000; Latour and Veillette, 2001). Taken together, our findings indicate that inhibition of Syk kinase in inflammatory cells (e.g. mast cells) may have therapeutic potential in the treatment of allergic diseases such as asthma.

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