**Effects of Tyrosine Kinase Inhibitors on Antigen Challenge of Guinea Pig Lung In Vitro**

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**ABSTRACT**

The present study was conducted to examine the effects of two protein tyrosine kinase inhibitors, genistein and tyrphostin 47, on an in vitro model of allergic asthma. Guinea pigs were sensitized with purified IgG raised against ovalbumin (OA). Isolated sensitized bronchial rings contracted in response to OA in a concentration-dependent manner, maximum contraction being achieved at 1 μg/ml. Genistein and tyrphostin 47 concentration-dependently (10–100 μM) inhibited OA-induced anaphylactic contraction of the bronchi, as well as release of histamine and peptide leukotrienes from chopped lung preparations. Genistein, but not tyrphostin 47, significantly suppressed bronchial contraction to leukotriene D₄ at 50 μM and to histamine at 100 μM. Daidzein, an inactive congener of genistein, did not alter OA-induced anaphylactic contraction. However, it slightly reduced bronchial contraction to leukotriene D₄ and the OA-stimulated release of peptide leukotrienes. The inhibitory effects were significantly weaker than those of genistein. Taken together, our results show that tyrphostin 47 inhibited anaphylactic contraction mainly by preventing mast cell degranulation, whereas genistein exerted inhibitory effects partly by blocking mast cell degranulation and partly by attenuating leukotriene D₄-induced bronchial contraction. These findings suggest that protein tyrosine kinase inhibitors have a therapeutic potential as mast cell stabilizers in the treatment of allergic diseases such as bronchial asthma.

Antigen-stimulated mast cell degranulation is known to be associated with increased intracellular Ca²⁺ concentration and protein kinase C activity (Beaven and Metzger, 1993; Ozawa et al., 1993). Recently, cumulating evidence obtained from rat basophilic mast cell line (RBL-2H3) and bone marrow-derived mast cells showed that activation of non-transmembrane PTKs is the earliest detectable signaling response to FcεRI cross-linking. This is followed by downstream signaling events such as activation of PLCγ (Li et al., 1992; Jouvin et al., 1994) and mitogen-activated protein kinase (Fukamachi et al., 1993), increase in inositol 1,4,5-trisphosphate and intracellular Ca²⁺ levels and enhanced protein kinase C activity, and it eventually leads to mast cell degranulation (Beaven and Metzger, 1993; Scharenberg and Kinet, 1994). Specific tyrosine kinases such as src-related kinase Lyn (Eiseman and Bolen, 1992), 72-kDa Syk (Hutchcroft et al., 1992; Benhamou et al., 1993), 94-kDa Fgr (Penhallow et al., 1995) and 77-kDa Btk (Kawakami et al., 1994) have been shown to be activated rapidly after FcεRI aggregation. Inhibitors of PTK have been shown to block antigen-induced activation of PTK, related downstream signaling events (e.g., inositol 1,4,5-trisphosphate production) and histamine release from mast cells (Kawakami et al., 1992; Lavens et al., 1992; Oliver et al., 1994). 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 logical to examine the effects of PTK inhibitors on an in vitro model of allergic asthma.

The Schultz-Dale reaction (Schultz, 1910; Dale, 1913; Chand and Eyre, 1978) has been used extensively to study anaphylactic contraction of airway tissue preparations such as trachea, bronchi and lung parenchymal strips. Among a wide array of mast cell-derived inflammatory mediators, such as thromboxane A₂ and platelet-activating factor, peptide leukotrienes and histamine have been shown to be the major mediators responsible for the anaphylactic contraction of the airways (Ro et al., 1991; Bjorck and Dahlen, 1993; Jonsson and Dahlen, 1994). A combination of histamine (H₁) receptor antagonist and peptide leukotriene receptor antagonist has been shown to block substantially the anaphylactic contraction of airway tissue preparations (e.g., bronchi and lung parenchymal strips) from both human and guinea pig (Regal, 1985; Bjorck and Dahlen, 1993; Jonsson and Dahlen, 1994). In guinea pigs, both IgE and IgG are able to sensitize mast cells to specific antigen (Regal, 1985; Undem et al.,

**ABBREVIATIONS**: OA, ovalbumin; LTD₄, leukotriene D₄; HNMT, histamine N-methyltransferase; PTK, protein tyrosine kinase; FcεRI, high affinity IgE-binding Fc receptor; FcγR, IgG-binding Fc receptor; [³H]-r-MHm, tritiated N-γ-methylhistamine; PLCγ, phospholipase Cγ.
1985; Ro et al., 1991; Moore and Dannenberg, 1993), and cross-linking of their corresponding FcεRI and FcγRII leads to mast cell degranulation. It has been shown that IgG-sensitized guinea pig airway tissues released more histamine and peptidoleukotrienes (Undem et al., 1985; Ro et al., 1991) and that their anaphylactic contractions were more sensitive to inhibition by combined H1-receptor and peptidoleukotriene receptor antagonists, results that closely resemble the IgE-dependent anaphylactic responses in human (Regal, 1985; Bjorck and Dahlen, 1993). FcγRII (e.g., FcγRIIa) and FcγRII leads to mast cell degranulation and functional activation, and both belong to a family of multisubunit antigen receptors (Alber et al., 1992; Bolen, 1995). It has been shown that engagement of these cell surface receptors activates PTKs as the initial events for successful signal propagation (Bolen, 1995).

In this study, we passively sensitized guinea pigs with IgG raised against OA and studied the effects of two PTK inhibitors, genistein (Akiyama et al., 1987) and tyrphostin 47 (Gazit et al., 1989; Levitzki and Gazit, 1995), on antigen-induced anaphylactic contraction of the bronchi and release of histamine and peptidoleukotrienes from chopped lung preparations. Our findings show that genistein and tyrphostin 47 substantially inhibited both antigen-induced release of mediators and anaphylactic contraction. Tyrphostin 47 acted mainly by preventing mast cell degranulation, whereas genistein exerted inhibitory effects partly by blocking mast cell degranulation and partly by attenuating LTD4-induced bronchial contraction. Taken together, these data suggest that PTK inhibitors have a therapeutic potential as mast cell stabilizers in the treatment of allergic diseases such as bronchial asthma.

Materials and Methods

Drugs. The following drugs and chemicals were used in this study: OA (grade V), histamine dihydrochloride, indomethacin, tyrphostin 47 (RG50864), L-cysteine, bovine serum albumin (Sigma Chemical Co., St. Louis, MO), tolune, isoamyl alcohol, boric acid, potassium phosphate, dimethyl sulfoxide (DMSO) (Merck, Durmas-tadt, Germany), rabbit IgG fraction to chicken egg albumin (OA) (Organon Teknica Corp., Durham, NC), genistein and daidzein (Research Biochemicals International, Natick, MA), LTD4 (Cayman Chemical Co., Ann Arbor, MI), tritiated S-adenosyl-L-[methyl-3H]methionine (60–85 Ci/mmol), leukotriene C4/D4/E4 radioimmunoassay kit and biodegradable liquid scintillant (Amersham Life Science, Buckinghamshire, U.K.) and HNMT (New England Nuclear, Boston, MA). Rabbit anti-OA IgG antibody was stored in sterile H2O, rabbit IgG fraction to chicken egg albumin (OA) (Berkshire, U.K.) and HNMT. Briefly, a total incubation volume of 60 μl of reaction reagent in 12-mm tissue culture wells was preincubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanooids (e.g., PGE2 and PGD2) capable of modulating the anaphylactic contraction (Abela and Daniel, 1994; Bertrand et al., 1991; Ro et al., 1991). To determine maximum antigen-induced contraction, bronchial rings were exposed to increasing concentrations of OA (0.001–10 μg/mL). To evaluate the role of PTK in mediating bronchial smooth muscle anaphylactic contraction, PTK inhibitors such as genistein, tyrphostin 47 and daidzein, a structural analog of genistein devoid of tyrosine kinase inhibitory activity (Akiyama et al., 1987), were preincubated with bronchial rings 30 min before addition of OA. The effects of PTK inhibitors were compared to those of their corresponding control ring preparations in the absence of inhibitor. To confirm that the inhibitory effects of PTK inhibitors on anaphylactic contraction are mediated by mast cell stabilization, we examined the effects of genistein and tyrphostin 47 on histamine- or LTD4-induced bronchial contraction. In the LTD4-induced bronchial ring contraction study, 4 μM indomethacin and 5 mM L-cysteine were preincubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanooids (e.g., PGE2 and PGD2) were able to mask LTD4-induced canine bronchial contraction and that the addition of indomethacin restored the LTD4 effect (Abela and Daniel, 1994). L-cysteine is an inhibitor of an aminopeptidase that converts LTD4 to less potent LTE4. Adding L-cysteine has been shown to enhance the smooth muscle contractile response to LTD4 (Abela and Daniel, 1994).

Release of mediators from chopped lung preparations. Lung lobes obtained from sensitized guinea pigs were cut into approximately 1-mm3 pieces using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY). Fragmented lung preparations were washed thoroughly with oxygenated Krebs-NaHCO3 buffer before incubation. Duplicate aliquots of 200-mg lung fragments were preincubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanooids (e.g., PGE2 and PGD2) capable of modulating the anaphylactic contraction (Abela and Daniel, 1994; Bertrand et al., 1991; Ro et al., 1991). To determine maximum antigen-induced contraction, bronchial rings were exposed to increasing concentrations of OA (0.001–10 μg/mL). To evaluate the role of PTK in mediating bronchial smooth muscle anaphylactic contraction, PTK inhibitors such as genistein, tyrphostin 47 and daidzein, a structural analog of genistein devoid of tyrosine kinase inhibitory activity (Akiyama et al., 1987), were preincubated with bronchial rings 30 min before addition of OA. The effects of PTK inhibitors were compared to those of their corresponding control ring preparations in the absence of inhibitor. To confirm that the inhibitory effects of PTK inhibitors on anaphylactic contraction are mediated by mast cell stabilization, we examined the effects of genistein and tyrphostin 47 on histamine- or LTD4-induced bronchial contraction. In the LTD4-induced bronchial ring contraction study, 4 μM indomethacin and 5 mM L-cysteine were preincubated with the tissue for 30 min. It has been reported that very minute amounts of relaxant prostanooids (e.g., PGE2 and PGD2) were able to mask LTD4-induced canine bronchial contraction and that the addition of indomethacin restored the LTD4 effect (Abela and Daniel, 1994). L-cysteine is an inhibitor of an aminopeptidase that converts LTD4 to less potent LTE4. Adding L-cysteine has been shown to enhance the smooth muscle contractile response to LTD4 (Abela and Daniel, 1994).

Histamine radioenzymatic assay. Histamine release from lung samples in response to OA was determined via a radioenzymatic assay as described by Henry et al. (1991), utilizing highly purified HNMT. Briefly, a total incubation volume of 60 μl was prepared by sequential addition of 10 μl of biological samples (or H2O for the blank), 25 μl of H2O (or H2O containing 500 pg of histamine as internal standard) and 25 μl of reaction reagent in 12 × 75-mm polypropylene culture tubes. Reaction reagent contained 21 μl of 0.4 M potassium phosphate/0.1% bovine serum albumin, pH 7.8, 2 μl of HNMT, and 2 μl of tritiated S-adenosylmethionine (80 Ci/mmol). After a 1-hr incubation in a shaker bath at 2°C, the enzymatic reaction was terminated by the addition of 75 μl of 2.5 M potassium

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borate, pH 11; 4 ml of toluene-isoamyl alcohol (3:1, v/v) was then added to each tube. After centrifugation for 3 min, 3.8 ml of the organic phase, which contained tritiated N-\( \tau \)-methylhistamine ([\( ^3\)H]-\( \tau \)-M) formed by the HNMT reaction, was transferred to another set of tubes containing 500 \( \mu \)l of 0.5 M HCl for back extraction of the [\( ^3\)H]-\( \tau \)-M into the aqueous phase. Tubes were centrifuged for 3 min, and the organic phase was aspirated and discarded. The aqueous phase was mixed with 1.25 ml of toluene-isoamyl alcohol. After centrifugation and removal of the organic phase, 300 \( \mu \)l of the acidified aqueous phase was transferred to scintillation vials containing 8 ml of biodegradable counting scintillant. Radioactivity was quantitated by liquid scintillation spectrometry (Beckman LS 3801, Beckman Instruments, Inc., Fullerton, CA).

**Leukotrienes radioimmunoassay.** The release of peptidoleukotrienes from chopped lung preparations in response to OA was quantitated by radioimmunoassay (Amersham Life Science, Buckinghamshire, U.K.). Briefly, total incubation volumes of 400 \( \mu \)l were prepared by sequential addition of 100 \( \mu \)l of biological samples or LTC\(_4\) standard, 100 \( \mu \)l of [\( ^3\)H]-LTC\(_4\), 100 \( \mu \)l of peptidoleukotriene-specific antiserum (cross-reactivity for LTC\(_4\)/D\(_4\)/E\(_4\) = 100%/100%/41%), and 100 \( \mu \)l of assay buffer (pH 7.4) in polypropylene tubes. Antigen-antibody competition reaction was allowed to take place overnight at 2\(^\circ\)C to 8\(^\circ\)C. Dextran-coated charcoal suspension (250 ml) was then added to each reaction tube to adsorb any unbound leukotrienes. After centrifugation, the supernatant was transferred to scintillation vials containing 10 ml of scintillant. Radioactivity was measured using liquid scintillation spectrometry. Samples were assayed in duplicate.

**Data analysis.** All data are presented as mean ± S.E.M. Statistical differences in contractile responses to OA challenge, histamine or LTD\(_4\), and in the release of mediators in response to OA, in the presence and absence of inhibitors were analyzed using ANOVA followed by Student-Newman-Keuls test (Armitage and Berry, 1987). The critical level for significance was set at \( P < .05 \).

**Results**

**Antigen challenge.** Sensitized guinea pig bronchial rings contracted in response to 60 mM KCl with an active force of 1.45 ± 0.10 g (\( n = 19 \)). It also produced graded contractile responses to increasing concentrations of OA (fig. 1A). The sensitized bronchial rings did not contract to irrelevant protein such as bovine serum albumin. The threshold concentration of OA to induce anaphylactic contraction was 0.01 \( \mu \)g/ml, and maximum response was achieved at 1 \( \mu \)g/ml. The concentration that caused 50% maximum contraction was 0.05 \( \mu \)g/ml (fig. 1B). For all subsequent antigen-challenge studies, OA at a concentration of 1 \( \mu \)g/ml was used to induce maximum anaphylactic bronchial smooth muscle contraction (1.83 ± 0.10 g, \( n = 19 \)).

**Effects of PTK inhibitors on Schultz-Dale reaction.** To determine the effects of PTK inhibitors on the Schultz-Dale reaction, the inhibitors were preincubated with the ring preparations for 30 min before OA challenge. Genistein concentration-dependently inhibited OA-induced bronchial contraction by 15%, 60%, 73% and 99% at 10 \( \mu \)M, 30 \( \mu \)M, 50 \( \mu \)M and 100 \( \mu \)M, respectively (fig. 2A). In contrast, 50 \( \mu \)M diadzein, a structural analog of genistein devoid of tyrosine kinase inhibition activity, did not affect OA-induced bronchial contraction. On the other hand, tyrphostin 47 significantly inhibited anaphylactic contraction by 25%, 31%, 73% and 77% at 10 \( \mu \)M, 30 \( \mu \)M, 50 \( \mu \)M and 100 \( \mu \)M, respectively (fig. 2B).

**Effects of PTK inhibitors on mediator-induced bronchial contraction.** To determine whether the inhibition of anaphylactic contraction is mediated by blocking the release of mast cell-derived mediators or by attenuating bronchial smooth muscle contraction, we evaluated the PTK inhibitors in histamine- or LTD\(_4\)-induced bronchial ring contraction. At 50 \( \mu \)M, a concentration that significantly blocked the OA-induced bronchial contraction, neither genistein nor tyrphostin 47 attenuated bronchial contraction (1.45 ± 0.07 g, \( n = 26 \)) induced by 30 \( \mu \)M histamine (fig. 3A). At 100 \( \mu \)M, genistein but not tyrphostin 47 substantially suppressed histamine-induced bronchial contraction by 88%. Daidzein (100 \( \mu \)M) did not exhibit any inhibitory effect on histamine-induced bronchial contraction. Bronchial contraction induced by 0.1 \( \mu \)M LTD\(_4\) (1.45 ± 0.06 g, \( n = 14 \)) was significantly reduced by genistein (75%), but not by tyrphostin 47, at a concentration of 50 \( \mu \)M. Daidzein (50 \( \mu \)M) also inhibited LTD\(_4\)-induced contractile response by 44%. Nevertheless, the inhibitory effect of daidzein was significantly (\( P < .05 \)) less than that of genistein (fig. 3B).
Effects of PTK inhibitors on the release of mediators. Chopped lung preparations released low levels of histamine (75.5 ± 21.0 ng/g tissue, n = 13) and peptidoleukotrienes (1.2 ± 0.1 ng/g tissue, n = 8) spontaneously. Upon 1 μg/ml OA challenge, the release of histamine and that of peptidoleukotrienes from lung fragments were significantly increased by 24-fold (1809.0 ± 135.2 ng/g tissue, n = 13) and 60-fold (71.6 ± 5.7 ng/g tissue, n = 8), respectively. Genistein concentration-dependently inhibited antigen-induced histamine release by 32%, 54% and 67% at 10 μM, 50 μM and 100 μM, respectively (fig. 4A). Likewise, it substantially reduced OA-induced release of peptidoleukotrienes by 19%, 58% and 74% at 10 μM, 50 μM and 100 μM, respectively (fig. 4B). In contrast, daidzein (50 μM), failed to block histamine release but significantly reduced the release of peptidoleukotrienes from lung fragments by 29%. Nevertheless, the inhibitory effect of daidzein on the release of peptidoleukotrienes was significantly (P < .05) weaker than that of genistein. On the other hand, tyrphostin 47 at concentrations of 10 μM, 50 μM and 100 μM significantly reduced histamine release from lung fragments by 22%, 41% and 48%, respectively, and markedly blocked the release of peptidoleukotrienes by 40%, 92% and 98%, respectively (fig. 4). The inhibitory effect of tyrphostin 47 on the release of leukotrienes was substantially more pronounced than that of genistein.
Itzki and Gazit, 1995). Their relative potencies against non-transmembrane PTKs (e.g., Jak, Fer, Syk, Btk, and Lyn) remain to be determined.

Bromchial rings from guinea pigs passively sensitized with purified IgG raised against OA contracted in response to OA, but not to bovine serum albumin, in a concentration-dependent manner (fig. 1). This observation is consistent with our previous study using guinea pig lung parenchymal strips (Wong et al., 1992a) and with other reports using guinea pig trachea (Undem et al., 1985; Bertrand et al., 1991; Ro et al., 1991), which showed that IgG is able to sensitize guinea pig mast cells to specific antigen. Both IgG-binding FcεRI (e.g., FcεRIII) and IgE-binding FcεRI are expressed on the surface of human and murine mast cells (Ravetch and Kinet, 1991; Alber et al., 1992). IgG-mediated aggregation of FcεRIII has been shown to stimulate the release of arachidonic acid metabolites (Alber et al., 1992) and serotonin (Daeron et al., 1992) from murine mast cells. In an effect analogous to that of FcεRII, cross-linking of two or more FcεRs triggers non-transmembrane PTK activities as the initial signaling events, followed by increases in intracellular Ca²⁺ level and protein kinase C activity, which in turn leads to exocytosis of mast cell-derived inflammatory mediators (Alber et al., 1992; Beaven and Metzger, 1993).

OA-induced anaphylactic contraction of the bronchi was significantly inhibited by either genistein or tyrphostin 47 in a concentration-dependent manner (fig. 2). At 50 μM, both genistein and tyrphostin 47 markedly suppressed bronchial anaphylactic contraction by at least 70%. In contrast, 50 μM daidzein, an analog of genistein that has no inhibitory activity for PTK (Akiyama et al., 1987), failed to alter the anaphylactic contraction. On the other hand, the OA-induced release of histamine and peptidoleukotrienes from chopped lung preparations was also concentration-dependently reduced by genistein and tyrphostin 47 (fig. 4). Lavens et al. (1992) previously showed that genistein inhibited anti-IgE-induced histamine release from human lung mast cells. These findings suggest that PTK is involved in the Schultz-Dale reaction and that the inhibitors of PTK interrupted the early signaling pathway of FcεR cross-linking in the mast cells (Alber et al., 1992; Bolen, 1995) and, therefore, attenuated the anaphylactic contraction by preventing the release of mast cell-derived inflammatory mediators such as histamine and peptidoleukotrienes.

In order to ascribe the inhibitory effects of genistein and tyrphostin 47 on anaphylactic contraction solely to mast cell stabilization, we must first determine whether the PTK inhibitors themselves have any effects on the bronchial smooth muscle contraction. Our current understanding of the biological activities of PTK has been derived mainly from studies of growth factor-associated responses such as mitogenesis, differentiation and proliferation of immune cells (Bolen, 1995; Levitzki and Gazit, 1995). Lately, evidence has been accumulating that protein tyrosine phosphorylation also participates in the regulation of smooth muscle contraction (Di Salvo et al., 1994; Hollenberg, 1994; Semenchuk and Di Salvo, 1995; Jin et al., 1996). In addition to playing a central role in the EGF-induced gastric smooth muscle contraction (Hollenberg, 1994), PTK mediates vascular smooth muscle contraction induced by serotonin or phenylephrine (Semenchuk and Di Salvo, 1995; Watts et al., 1996) and gastric longitudinal smooth muscle contraction induced by angiotensin-II (Yang...
et al., 1993). These findings indicate that PTK is involved in the signal transduction of neurotransmitters known to act through G protein-coupled receptors. Inhibitors of PTK such as genistein and tyrphostins have been shown to inhibit smooth muscle contraction to carbachol, norepinephrine, phenylephrine, serotonin and angiotensin-II (Di Salvo et al., 1993; Yang et al., 1993; Abebe and Agrawal, 1995; Watts et al., 1996).

At a concentration (50 μM) that significantly inhibited OA-induced anaphylactic contraction and mediator release, neither genistein nor tyrphostin 47 altered histamine-induced bronchial contraction. However, as the concentration was increased to 100 μM, genistein, but not tyrphostin 47, abolished bronchial contraction induced by histamine. As expected, 100 μM daidzein had no effect on histamine-mediated contraction (fig. 3A). The inhibitory effect of genistein might be related to the substantial inactivation of certain PTKs that participate in the signal transduction of histamine-induced bronchial contraction. It has been shown in human umbilical vein endothelial cells that histamine induced a delayed tyrosine phosphorylation of the 42/44-kDa mitogen-activated protein kinase, which suggests that PTK is a mediator in the histamine signal transduction cascade (Fleming et al., 1995). However, it remains to be determined whether tyrosine phosphorylation of the same or other substrates occurs in histamine-treated bronchial smooth muscle cells. Alternatively, genistein at a concentration of 100 μM might have exhibited some nonspecific activities against histamine-induced bronchial contraction. Wijetunge et al. (1992) showed that 100 μM genistein, but not daidzein, inhibited voltage-operated calcium channel currents in vascular smooth muscle. However, we still do not know whether the inhibition of calcium channel currents is a direct blockade by genistein or a result of PTK inactivation.

On the other hand, LTD₄-induced bronchial smooth muscle contraction was significantly inhibited by genistein, but not by tyrphostin 47, at 50 μM concentration. Daidzein (50 μM) also attenuated the LTD₄-mediated contraction, but its inhibitory effect was significantly weaker than that of genistein (fig. 3B). The effect of genistein on LTD₄-induced contraction is unlikely to be associated with nonspecific activity on other serine/threonine kinases. It has been shown that genistein scarcely inhibited Ca²⁺-dependent protein kinase, protein kinase C, phosphorylase kinase and phosphodiesterase, even at concentrations above 300 μM (Akiyama et al., 1987). Another possible explanation for genistein’s inhibitory effect is blockade of Ca²⁺ channel currents in the bronchial smooth muscle. This does not appear to be the case, however, because 50 μM genistein failed to block the histamine-induced bronchial contraction. Thus it is likely that genistein attenuated LTD₄-induced contraction by mitigating the PTK activities stimulated by LTD₄ receptor activation. It has been demonstrated in human epithelial cells that LTD₄ induced tyrosine phosphorylation of PLCγ and of two other associated protein substrates (Groonsoos et al., 1995). Activation of PLCγ produces inositol 1,4,5-triphosphate and diacylglycerol, resulting in increased intracellular Ca²⁺ concentration and protein kinase C activity, which ultimately regulate smooth muscle contractility (Hollenberg, 1994).

The inhibitory effects of daidzein on LTD₄-induced bronchial contraction were unexpected, given the reported inactivity of this compound on EGF receptor kinase and pp60⁷-src (Akiyama et al., 1987). In addition, 50 μM daidzein reduced the release of peptide leukotrienes from lung fragments. Nevertheless, the inhibitory effects of daidzein are significantly weaker than those of genistein (figs. 3 and 4). Recently, daidzein has been shown to attenuate serotonin-, norepinephrine- or KCl-induced vascular smooth muscle contraction (Toma et al., 1995; Watts et al., 1996). These recent findings, together with our results, suggest that daidzein might possess some activity against PTK (Toma et al., 1995; Watts et al., 1996). Alternatively, the inhibitory effects of both genistein and daidzein on LTD₄-induced bronchial contraction and peptide leukotrienes release from lung fragments might be due to certain unidentified nonselective effects of the drugs.

In summary, genistein and tyrphostin 47 substantially inhibited the Schultz-Dale reaction in the guinea pig airways. Genistein exhibited its modulatory effects partly by mast cell stabilization and partly by reduction of smooth muscle contraction in response to LTD₄. Tyrphostin 47 acted primarily as a mast cell stabilizer without having any significant effect on smooth muscle contraction. Because mast cells play a pivotal role in initiating allergic disorders such as bronchial asthma (Wasserman, 1994), our findings suggest that PTK inhibitors may have therapeutic potential as mast cell stabilizers in the treatment of asthma.

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