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MODULATION OF PULMONARY LEUKOTRIENE FORMATION AND PERFUSION PRESSURE BY BESTATIN, AN INHIBITOR OF LEUKOTRIENE A_4 HYDROLASE

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Abstract—We investigated the effects of bestatin, a prototype leukotriene A_4 (LTA_4) hydrolase inhibitor, on leukotriene (LT) formation and pulmonary artery perfusion pressure (P_{pa}) in isolated, perfused rat lungs. In lung parenchymal strips stimulated with a 10 μM concentration of the Ca^{2+} ionophore A23187, bestatin inhibited LTB_4 formation with an $IC_{50} = 10.4 \pm 3.0$ μM (mean ± SD, N = 4). It did not alter cysteinyl LT formation, confirming that it inhibited LTA_4 hydrolase selectively, without inhibiting phospholipase, 5-lipoxygenase, or LTC_4 synthase. In isolated, perfused lungs stimulated with 10 μM A23187, 300 μM bestatin inhibited LTB_4 release by 72.2 ± 10.6% (mean ± SEM, N = 6, $P < 0.01$) but had no significant effect on LTE_4 formation ($P > 0.5$). In these perfused lungs, bestatin did not alter the change in P_{pa} following stimulation with A23187. This effect is consistent with the insubstantial re-direction of LTA_4 toward formation of vasospactic cysteinyl LTs. Separate experiments used lungs from rats treated with lipopolysaccharide endotoxin *in vivo*, prior to isolation, perfusion, and stimulation with 5 μM formyl-methionyl-leucyl-phenylalanine, *in vitro*. In these inflamed lungs, 750 μM bestatin inhibited LTB_4 formation ($P < 0.05$) and increased LTE_4 formation ($P < 0.05$), compatible with selective inhibition of LTA_4 hydrolase. The re-direction of LTA_4 metabolism toward formation of cysteinyl LTs by inflamed, perfused lungs did not cause an increase in P_{pa}.

Key words: leukotriene B_4, leukotriene A_4 hydrolase; bestatin; selective inhibition; perfused lungs; rat

LTs† are lipid mediators of inflammatory disorders [1, 2]. Lungs exposed to immunological or chemical stimuli generate LTB_4, LTC_4, LTD_4 and LTE_4, via transformation of their common substrate, LTA_4 [3, 4]. LTA_4 hydrolase, the rate-limiting step in this biosynthetic pathway, is a pivotal determinant of LTA_4 disposition [5–9]; however, there is little emphasis on modulation of this enzyme as a tactic for alleviating inflammation. One factor accounting for disinterest in selective LTA_4 hydrolase inhibitors as therapeutic agents is a hypothetical concern that they might divert LTA_4 toward increased formation of cysteinyl LTs. This could have unpredictable or unfavorable consequences on pulmonary vascular tone. However, it is important to stress that this issue has never been examined experimentally. Our recent identification of bestatin as a prototype LTA_4 hydrolase inhibitor that does not affect 5-lipoxygenase, LTC_4 synthase or phospholipase A_2 [10] provides the opportunity to modulate LT biosynthesis, selectively, and to establish the corresponding effects of this modulation on P_{pa} in isolated lungs. Using experimental protocols to compare normal versus inflamed lungs, we asked: (i) How much does inhibition of pulmonary LTA_4 hydrolase *in vitro* divert LTA_4 metabolism away from LTB_4 formation and toward cysteinyl LT formation? and (ii) Does selective modulation of LTA_4 metabolism *in vitro* have a detrimental effect by increasing pulmonary perfusion pressure? Our results indicate that bestatin inhibits LTA_4 hydrolase selectively, reducing LTB_4 formation with small, and in most cases statistically insignificant, effects on cysteinyl LT formation. These changes in LT formation had no adverse effects on P_{pa} *in vitro*.

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† Abbreviations: LT, leukotriene; fmlp, formyl-methionyl-leucyl-phenylalanine; LPS, lipopolysaccharide; P_{pa}, pulmonary artery perfusion; Tx, thromboxane; and MPO, myeloperoxidase.

MATERIALS AND METHODS

Materials. Bestatin [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl-L-leucine], amastatin [(2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoyl-L-valyl-L-valyl-L-aspartic acid], γ-glutamyl transferase, fmlp, (Sigma); Ca^{2+} ionophore A23187 (Calbiochem); LTB_4, LTC_4, LTE_4, TxB_2 standards and acetylcholinesterase tracers for enzyme immunoassay (Cayman Chemical Co.); pentobarbital Na^+ salt (Fort Dodge Laboratories Inc., Fort Dodge, IA); LPS endotoxin *Salmonella enteritidis* (Difco, Detroit, MI); and male Sprague–Dawley rats were used.

Modulation of leukotriene formation by lung parenchyma strips in vitro. Male Sprague–Dawley rats (300 ± 50 g) anesthetized with pentobarbital Na^+ salt (60 mg/kg) were ventilated with $O_2/CO_2/N_2$ (21%/5%/74%) via a tracheal cannula, using a Harvard animal respirator at 60 strokes/min, tidal volume = 2 mL. The lungs were removed, and a

steel cannula was inserted through the right ventricle into the pulmonary artery. Initially, the lung was suspended in a humidification chamber and perfused for 10 min at 0.03 mL·min^{-1}·g^{-1} rat weight with a physiological salt solution to remove most erythrocytes, neutrophils and platelets [11]. Parenchyma strips (150–350 mg, 3 × 3 × 10 mm) dissected from the lung lobe of male Sprague–Dawley rats were immersed in 2.0 mL of physiological salt solution, pH 7.4 (119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4 \cdot 7H_20$, 17 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 5.5 mM glucose, and 1.6 mM $CaCl_2$) containing 4% (w/v) inert Ficoll carbohydrate (mol. wt = 70,000) with 0–300 μM bestatin. The tissue was incubated at 37° for 10 min prior to stimulation with 10 μM Ca^{2+} ionophore A23187. Samples (200 μL) were withdrawn at intervals from 0 to 30 min for quantitation of LTB_4, LTC_4 and LTE_4. Results were expressed as nanograms of LT/milligram of lung (wet weight). A similar experiment was performed with 0–300 μM amastatin, an inhibitor of leucine aminopeptidase M and arginine aminopeptidase, but not LTA_4 hydrolase/aminopeptidase [10, 12].

Modulation of leukotriene formation and pulmonary perfusion pressure in normal lungs stimulated with A23187: Perfusion with bestatin in vitro. Lungs were isolated and perfused as described above for 10 min at 0.03 mL·min^{-1}·g^{-1} rat weight with a physiological salt solution to remove most erythrocytes, neutrophils and platelets [11]. Fresh perfusion buffer (50 mL), with or without 300 μM bestatin, was added to the reservoir and recirculated for 10 min to assure adequate drug distribution and to determine whether bestatin altered the baseline perfusion pressure. The lung was stimulated by adding 10 μM Ca^{2+} ionophore A23187 to the perfusion reservoir. Samples (2.0 mL = 40 sec) were removed via the left ventricle outflow from the lung at 1, 3, and 5 min after stimulation. P_{pa} was recorded continuously with a Statham pressure transducer. After 10 min, the remaining perfusate was collected and the lung was removed, homogenized immediately in 5.0 mL of ice-cold methanol, and centrifuged at 500 *g* for 10 min. The methanol contains any LTs that were retained within the lung and not released into the perfusate [13]. LTB_4, LTC_4, LTE_4 and TxB_2 in the perfusate and the homogenate were quantified by immunoassay [14, 15]. Data plotted as nanograms LT versus time represent the amount of LT released by the lung into the 2.0-mL fraction during the 40-sec collection interval, beginning at the time specified. Data plotted as bar graphs represent the cumulative release of LT in the total perfusion buffer.

Modulation of leukotriene formation and pulmonary perfusion pressure in lungs from endotoxin-treated rats: Perfusion with bestatin and fmlp in vitro. Rats were injected with LPS endotoxin (1 mg/kg, i.p.). After 1 hr, the lungs were isolated; perfused for 10 min by recirculating buffer containing 0, 300 or 750 μM bestatin; and then stimulated with 5 μM fmlp, an agonist that increases P_{pa} by approximately 10 mm of Hg under these conditions [11]. LTB_4, LTE_4 and TxB_2 were determined in samples (2.0 mL) collected from the left ventricle cannula at 1, 3, 5 and 10 min after stimulation. Total LT and TxB_2 in the perfusate and lung homogenate were determined as described above. P_{pa} was recorded continuously.

Eicosanoid immunoassay. Typically, samples were diluted from 1:20 to 1:1000 with assay buffer (0.1 M phosphate buffer, pH 7.4, containing 1 mg BSA/mL, 1 mM EDTA, 400 mM NaCl and 0.1% NaN_3). LTB_4 was determined by immunoassay with an LTB_4-acetylcholinesterase tracer [14, 16]. A separate portion of the sample (100 μL) was diluted with 0.05 M Tris, pH 8.5 (100 μL) containing 1.2 mM $MgCl_2$, γ-glutamyl transferase and dipeptidase (1 U/mL), and this was incubated for 12 hr at 25° to convert LTC_4 and LTD_4 into LTE_4, a single species that reflects total cysteinyl leukotriene formation. LTE_4 was determined by enzyme immunoassay. When specified, LTC_4 was also determined by immunoassay, prior to its conversion to LTE_4. Control samples, containing known amounts of LTC_4, verify that the enzymatic transformation of LTC_4 and LTD_4 is quantitative. TxB_2 was determined by immunoassay with a TxB_2-acetylcholinesterase tracer substituting for [^{3}H]TxB_2 [17].

MPO assay. MPO activity was determined spectrophotometrically [18]. Lungs were homogenized in 7.5 mL of 0.05 M phosphate buffer, pH 6.0, containing 0.5% (w/v) hexadecyltrimethylammonium bromide. Homogenates were centrifuged at 40,000 *g* for 10 min at 4°. The supernatant was retained, and the pellet was resuspended, frozen at −70°, thawed and homogenized again. This procedure was repeated five times. A portion (0.1 mL) of the combined supernatant fractions, containing MPO, was mixed with 2.9 mL of 0.05 M phosphate buffer, pH 6.0, containing 0.0005% (w/v) hydrogen peroxide and 0.17 mg/mL orthodianisidine dihydrochloride. The absorbance at 450 nm was recorded for 5 min with a Titertek Multiscan MCC/340 reader (Flow Laboratories).

Statistics. Data were evaluated by unpaired, two-sided *t*-tests or, for multiple comparisons, by analysis of variance. Values reported are the means ± SEM for N = 4–8 experiments. The concentration of bestatin for half-maximal inhibition (IC_{50}) of LTB_4 formation in Fig. 1 was estimated from curve fitting the data to a sigmoidal concentration–response curve using the program GraphPad®.

RESULTS

Modulation of leukotriene formation by rat lung parenchyma strips in vitro. LTB_4 formation by rat lung parenchyma approached a maximum 10 min after stimulation with 10 μM A23187 (Fig. 1, upper panel). Bestatin inhibited LTB_4 formation in a concentration-dependent manner with an IC_{50} = 10.4 ± 3.0 μM (mean ± SD, N = 4) (Fig. 1, lower panel). There were no significant changes in LTC_4 and LTE_4 formation, consistent with selective inhibition of LTA_4 hydrolase (Fig. 2). The slight increase in LTC_4 and LTE_4 did not equal the decrease in LTB_4, indicating that LTA_4 was not diverted quantitatively toward the LTC_4 synthase metabolic pathway. Amastatin and bestatin each inhibit leucine aminopeptidase and aminopeptidase M. If the effects of bestatin on LTB_4 formation were indirect and attributable to its inhibition of these

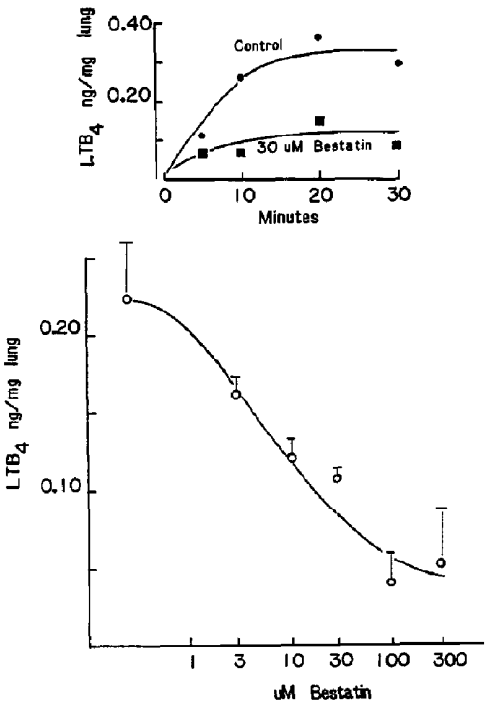

Fig. 1. Effect of bestatin on LTB_4 formation by rat lung parenchyma strips stimulated with 10 μM Ca^{2+} ionophore A23187. Upper panel = LTB_4 formation versus time. Values represent nanograms of LTB_4 per milligram of lung (wet weight). Lower panel = bestatin concentration versus LTB_4 formation (mean ± SD, N = 4). The IC_{50} (10.4 ± 3.0 μM) for bestatin was estimated from the sigmoidal concentration–response curve using the program GraphPad®.

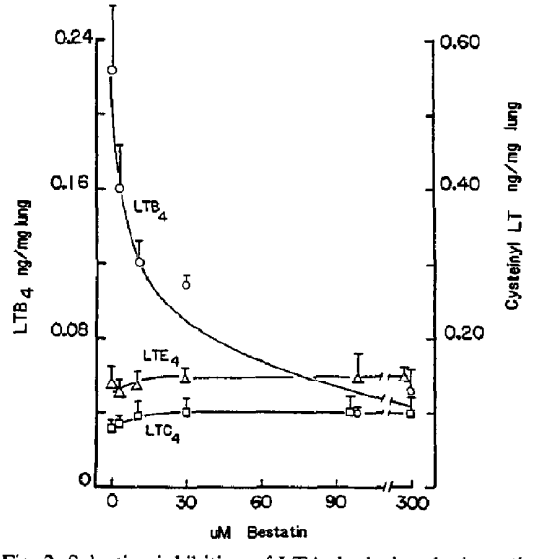

Fig. 2. Selective inhibition of LTA_4 hydrolase by bestatin: Formation of LTB_4, LTC_4 and LTE_4 (mean ± SD, N = 4) by rat lung parenchymal strips after stimulation with 10 μM A23187 in the presence of bestatin.

other enzymes, amastatin would have a similar effect. This was not the case; amastatin did not inhibit LTB_4 formation, consistent with previous results [10]. Lung strips incubated with 3, 10, 30, 100 and 300 μM amastatin produced 0.079 ± 0.009 (mean ± SEM, N = 6), 0.072 ± 0.011 (N = 4), 0.065 ± 0.006 (N = 6), 0.094 ± 0.009 (N = 4), and 0.071 ± 0.005 (N = 6) ng LTB_4/mg lung tissue, respectively. These values were indistinguishable (P = 0.2 to 0.8) from the control value (0.089 ± 0.015 ng LTB_4/mg tissue).

Modulation of leukotriene formation and pulmonary perfusion pressure in normal lungs stimulated with A23187: Perfusion with bestatin in vitro. We examined the effect of bestatin on isolated, perfused lungs stimulated with 10 μM A23187. This protocol emphasizes the contributions of non-inflammatory, parenchymal cells to LT biosynthesis and pulmonary pressure because normal perfused rat lungs do not sequester appreciable amounts of neutrophils and platelets in their capillaries [11]. The increase in P_{pa} induced by A23187 originates primarily from LT formation, not TxA_2 or other cyclooxygenase metabolites [19].

Bestatin inhibited LTB_4 formation by perfused lungs stimulated with A23187 (Fig. 3, lower panel). Control lungs released 68.7 ± 21.8 ng LTB_4/40 sec; lungs treated with 300 μM bestatin released 22.8 ± 6.6 ng LTB_4/40 sec (P < 0.05, N = 6) at 5 min after stimulation (Fig. 3, lower panel—left). LTB_4 in the total perfusate declined by 72.2% from the control: (641 ± 123 ng LTB_4 to 178 ± 68 ng LTB_4 released by lungs perfused with 300 μM bestatin, *in vitro*, P < 0.01, N = 6) (Fig. 3, lower panel—right). Bestatin had no statistically significant effect on cysteinyl LT formation, verifying that it inhibited LTA_4 hydrolase selectively. Control lungs released 6.6 ± 2.7 ng LTC_4/40 sec; lungs perfused with 300 μM bestatin released 10.9 ± 4.7 ng LTC_4/40 sec at 5 min after addition of A23187 (P = 0.51, N = 6) (Fig. 3, middle panel—left). Control lungs released 16.7 ± 3.2 ng LTE_4/40 sec; lungs perfused with 300 μM bestatin released 23.1 ± 4.4 ng LTE_4/40 sec at 5 min after addition of A23187 (P = 0.28, N = 6) (Fig. 3, upper panel—left). Total cysteinyl LTs in the control perfusate, 295 ± 88 ng LTE_4, were indistinguishable from 355 ± 77 ng LTE_4 in the perfusate from bestatin-treated lungs (P = 0.62, N = 6) (Fig. 3, upper panel—right). LTC_4 in the control perfusate, 151 ± 51 ng, was indistinguishable from 298 ± 133 ng LTC_4 in the perfusate from bestatin-treated lungs (P = 0.32, N = 6) (Fig. 3, middle panel—right). The data from lung homogenates were similar. A 300 μM concentration of bestatin inhibited LTB_4 formation by 69.5 ± 9.5% (P < 0.01), while LTE_4 formation, at 150 ± 29% of control (P = 0.23), and LTC_4 formation, at 148 ± 32% of control (P = 0.26), were unchanged. TxB_2 formation by perfused lungs stimulated with A23187 was <3 ng/mL, consistent with previous observations [19].

Treatment with 300 μM bestatin did not augment the change in P_{pa} following stimulation with A23187 (Fig. 4). The left panel of Fig. 4 depicts the P_{pa} versus time profile for three separate control lungs; the right panel depicts data from three lungs perfused with 300 μM bestatin for 10 min, prior to stimulation

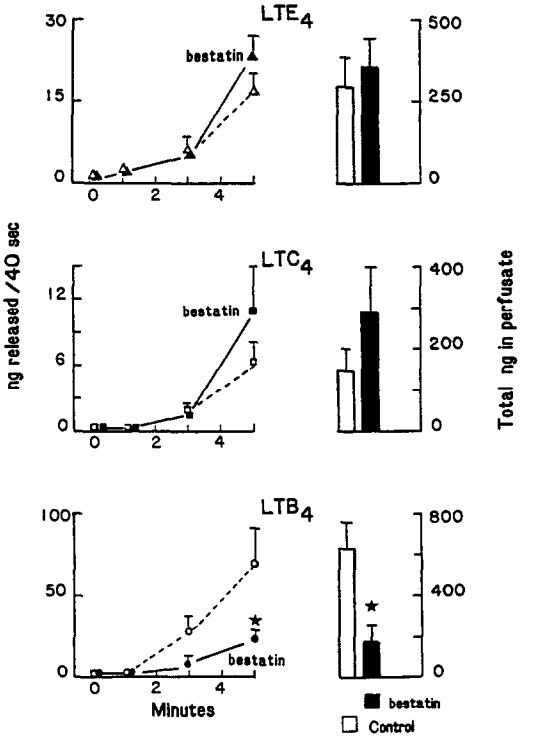

Fig. 3. Effect of bestatin, *in vitro*, on leukotriene formation by isolated, perfused rat lungs stimulated with 10 μM A23187. The left-hand panels depict the release of LTB_4 (lower), LTC_4 (middle) and LTE_4 (upper) versus time after the addition of 10 μM A23187 to lungs perfused with buffer (open symbols) or 300 μM bestatin (filled symbols). Data points represent nanograms of LT (mean ± SEM, N = 6) released by the lung during a 40-sec collection interval (2.0 mL perfusate collected) beginning at the time point specified. The right-hand panels show the amount of LTB_4, LTC_4 and LTE_4 in the total perfusate after 10 min. The total nanograms of LT released represent the average concentration (ng/mL) in the total perfusate x perfusate volume. Key: (*) $P < 0.05$.

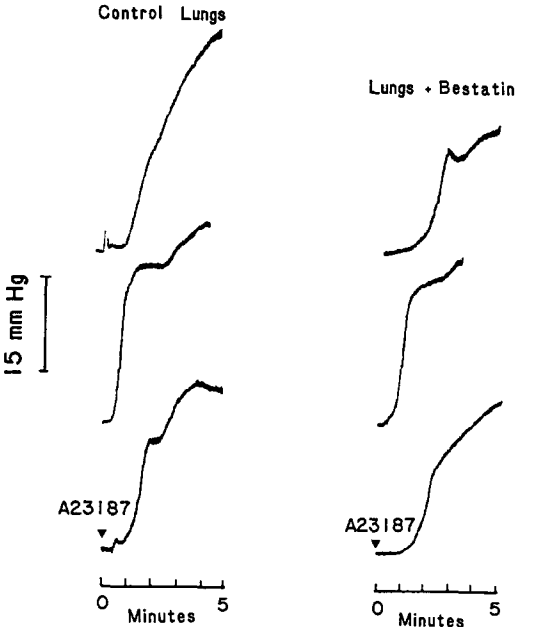

Fig. 4. Effect of bestatin on the perfusion pressure in lungs stimulated with 10 μM A23187. Tracings on the left depict P_{pa} versus time for three control lungs stimulated with 10 μM A23187 at 0 min. Tracings on the right depict data for three lungs perfused for 10 min with 300 μM bestatin prior to stimulation with 10 μM A23187.

with 10 μM A23187. The change in maximal P_{pa} was indistinguishable between the groups ($P = 0.09$). P_{pa} increased by 32.3 ± 2.8 mm Hg (mean ± SEM, N = 3) in the control lungs, and by 25.2 ± 1.3 (mean ± SEM, N = 3) in lungs perfused with 300 μM bestatin.

Modulation of leukotriene formation and pulmonary perfusion pressure in lungs from endotoxin-treated rats: perfusion with bestatin and fmlp in vitro. We examined the effect of bestatin, perfused *in vitro*, in lungs containing inflammatory cells. Rats exposed to LPS, *in vivo*, sequester leukocytes and platelets in their lung capillaries [11, 20, 21]. When these lungs are isolated, perfused and stimulated with fmlp, *in vitro*, the neutrophils contribute prominently to LTB_4 formation; the platelets contribute to TxB_2 formation. MPO activity reflects the leukocyte content of the lung [18]. We verified that the lungs from rats treated with LPS *in vivo* contained 1425 ± 144 U MPO, after perfusion. This was significantly different ($P < 0.01$) from the perfused lungs of normal rats, which contained only 39 ± 3 U MPO.

At 5 min after addition of fmlp, control lungs released 76.9 ± 10.3 ng LTB_4/40 sec (N = 8). Lungs treated with 300 μM bestatin released 64.5 ± 11.1 ng LTB_4/40 sec ($P = 0.40$, N = 7) and lungs treated with 750 μM bestatin released 39.1 ± 4.8 ng LTB_4/40 sec ($P < 0.05$, N = 5), (Fig. 5, lower panel—left). Total LTB_4 formation (perfusate plus homogenate) was 1224 ± 148 ng (mean ± SEM, N = 8) by the control lung, 1082 ± 199 ng (mean ± SEM, N = 7, $P = 0.56$) by lungs treated with 300 μM bestatin, and 615 ± 108 ng LTB_4 (mean ± SEM, N = 5, $P < 0.05$) by lungs treated with 750 μM bestatin. The latter value was statistically different from the control (Fig. 5, lower panel—right). In the same samples, LTE_4 levels increased, consistent with bestatin inhibiting LTA_4 hydrolase and diverting unconsumed LTA_4 toward cysteinyl LT formation. Control lungs produced 302 ± 57 ng LTE_4; lungs perfused with 300 μM bestatin produced 559 ± 51 ng LTE_4 ($P < 0.05$); lungs perfused with 750 μM bestatin produced 557 ± 128 ng LTE_4 ($P < 0.05$) (Fig. 5, middle panel—right). Bestatin did not alter TxB_2 formation by inflamed lungs stimulated with 5 μM fmlp (Fig. 5, upper panel—left). Control lungs produced 1456 ± 204 ng TxB_2; lungs perfused with 300 μM bestatin produced 1601 ± 287 ($P = 0.66$, N = 7); and lungs perfused with 750 μM bestatin produced 859 ± 169 ($P = 0.11$, N = 5).

Figure 6 depicts the pressure (P_{pa}) versus time

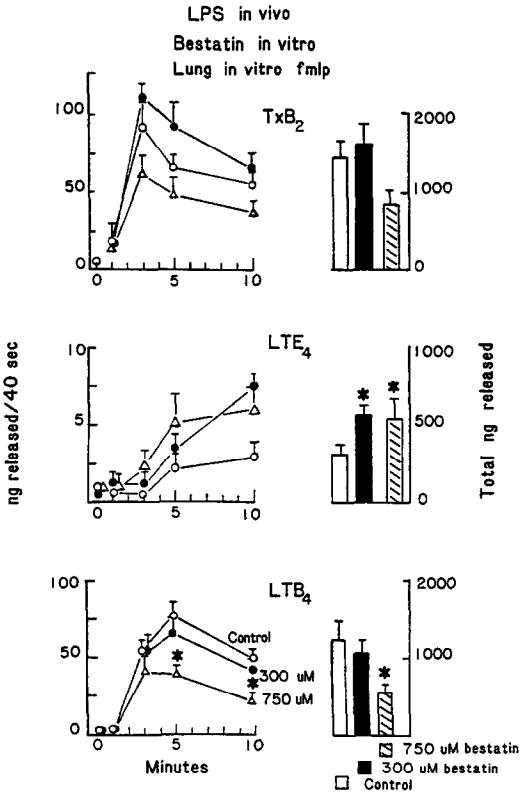

Fig. 5. Effect of bestatin, *in vitro*, on LTB_4, LTE_4 and TxB_2 formation by perfused lungs from rats treated with LPS *in vivo*. Lungs were isolated 1 hr after LPS injection, *in vivo* (1 mg/kg, i.p.), perfused, and then stimulated *in vitro* with 5 μM fmlp. The left-hand panels show the release of LTB_4 (lower), LTE_4 (middle) and TxB_2 (upper) as a function of time after the addition of fmlp. Control, N = 8 (○—○); 300 μM bestatin, N = 7, (●—●); or 750 μM bestatin, N = 5, (△—△). The right-hand panels show the total amount of LTB_4, LTE_4 and TxB_2 in the perfusate plus homogenate at 10 min. Values are means ± SEM. Key: (*) $P < 0.05$.

profile for lungs from animals treated with LPS *in vivo*, prior to isolation and perfusion of the lungs. The left panel shows data from six different control lungs, the middle panel shows data from six lungs perfused with 300 μM bestatin, and the right panel shows data from five lungs perfused with 750 μM bestatin, prior to stimulation with 5 μM fmlp. The change in maximal P_{pa} (mean ± SEM) was indistinguishable among the groups. P_{pa} increased by 5.8 ± 1.5 mm Hg in control lungs, by 3.9 ± 0.8 mm Hg in lungs perfused with 300 μM bestatin ($P = 0.28$), and by 5.2 ± 1.3 mm Hg in lungs perfused with 750 μM bestatin ($P = 0.75$). The time until the onset, and the duration of the P_{pa} response following stimulation with fmlp were indistinguishable between control and bestatin-treated lungs. Thus, the diversion of LTA_4 toward cysteinyl LT formation had no adverse effect on pulmonary pressure.

DISCUSSION

Bestatin, a prototype LTA_4 hydrolase inhibitor [10], can reduce LTB_4 formation by lung parenchyma tissue without shunting LTA_4 completely into the cysteinyl LT pathway. This has positive implications for the use of selective inhibitors of LTA_4 hydrolase as anti-inflammatory agents. Namely, adverse effects due to metabolic diversion of LTA_4 are not inevitable. Our results with isolated lungs from normal rats reinforce this conclusion. These lungs have negligible amounts of leukocytes and platelets adhering to their capillary vasculature after they are isolated and perfused [11]. The Ca^{2+} ionophore A23187 initiates a robust formation of LTs by this *in vitro* preparation, with an accompanying increase in pulmonary perfusion pressure [19, 22]. Cyclooxygenase-derived eicosanoids, such as TxA_2, do not contribute to this response. In this preparation, inhibition of LTB_4 formation occurred without a corresponding increase in cysteinyl LT formation or pulmonary perfusion pressure. Non-enzymatic hydration to inactive 5,12-dihydroxy-eicosatetraenoic acids and rapid deactivation of LTC_4 and LTD_4 could each contribute to this effect.

To extend our investigation, we used lungs from rats treated with LPS. Leukocytes and platelets accumulate in their capillary vasculature, *in vivo*, and remain there during the perfusion [11, 20, 21]. When these lungs are stimulated with fmlp, *in vitro*, this cellular infiltrate contributes to eicosanoid formation and the subsequent alteration of pulmonary vascular tone. This model is attractive because it uses stimuli that resemble those likely to occur in pulmonary inflammation; the change in perfusion pressure involves TxA_2 and LTs; and LT biosynthesis can originate from multiple cellular sources including neutrophils, platelets and lung parenchymal tissue. Stimulation with 5 μM fmlp may initiate LTB_4 formation directly and via transcellular biosynthesis from neutrophil-derived LTA_4 [23]. The effects of bestatin, *in vitro*, on LTB_4 levels were modest in the inflamed lung, implying that deleterious effects of neutrophil infiltration and activation, including their release of LTA_4 and LTB_4, may be difficult to control once the neutrophils assemble in cell clusters in the lung capillaries [11]. Bestatin inhibited LTA_4 hydrolase less effectively in inflamed lungs, compared with normal lungs. This is consistent with appreciable neutrophil accumulation in these lungs and with our data showing that bestatin inhibits LTA_4 hydrolase in intact neutrophils with a low potency [10]. The neutrophil influx also increases the LTA_4 hydrolase content in the lung, necessitating greater amounts of bestatin for inhibition. Neutrophil influx or lung damage might also have increased release of other peptidase enzymes that bind bestatin and lower its effective concentration. In contrast to the results from normal lungs, bestatin diverted sufficient LTA_4 into the cysteinyl LT pathway of inflamed lungs to increase LTE_4 levels. However, this biochemical change did not cause an increase in pulmonary perfusion pressure.

Bestatin acts *in vivo* as an immunomodulator [24–27], but its immunomodulatory mechanism is not correlated directly with inhibition of peptidase [28]. Our data raise the possibility that inhibition of LTA_4 hydrolase also contributes to these effects. Interest in the therapeutic potential of LTA_4 hydrolase

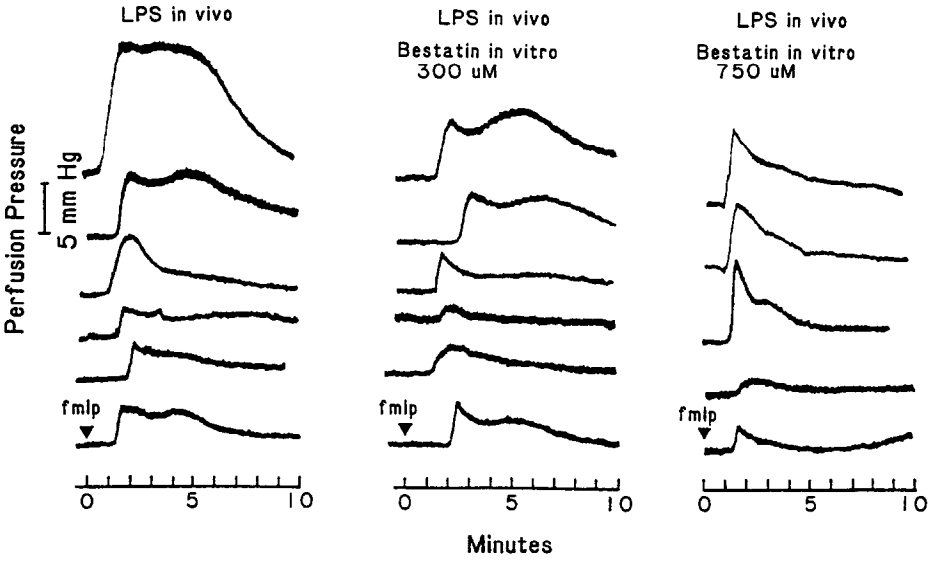

Fig. 6. Effect of bestatin on the perfusion pressure in inflamed lungs stimulated with fmlp. Tracings depict P_{pa} versus time for lungs from animals treated with LPS *in vivo*, prior to isolation and perfusion of the lungs. The left panel shows data from six separate control lungs, the middle panel shows data from six lungs perfused with 300 μM bestatin, and the right panel shows data from five separate lungs perfused with 750 μM bestatin, prior to stimulation with 5 μM fmlp. The change in maximal P_{pa} (mean ± SEM) was statistically indistinguishable among the groups.

inhibitors is emerging [10, 29–32]. Our experiments assessing the effects of bestatin *in vitro* show that this class of inhibitors may have potential value for treatment of inflammatory disorders mediated by LTB_4. Detrimental effects on pulmonary function may not necessarily limit their use.

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