Peptidoleukotriene (PLT) Release and Absorption from the Airways of the Isolated Perfused Guinea Pig Lung Following Chemical and Antigenic Challenge

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Purpose. To study the release and absorption of peptidoleukotrienes (PLTs) from the airways of the guinea pig lung following calcium ionophore A23187 (CI), benzalkonium chloride (BAC), ethylene diamine tetra-acetic acid (EDTA) or ovalbumin (OA) challenge.

Methods. PLT C4/D4/E4 were quantified in the perfusate of the isolated perfused guinea pig lung (IPGPL) following intratracheal administration of CI, BAC, EDTA or OA in different doses. The formation and airway-to-perfusate transfer kinetics of PLTs were analyzed by fitting mean data for cumulative PLT in perfusate vs. time to an A → B → C first-order release and transfer model, with dose-dependent transfer rate constants.

Results. CI induced apparent first order release of PLTs with a $t\frac{1}{2} \simeq$ 1.2 minutes. The amount of PLT released was CI dose-dependent, as was the airway-to-perfusate transfer rate constant. These reached maxima of 0.254 μg and 0.0557 $min.^{-1}$, respectively, around a CI dose of 100 μg. In OA-sensitized IPGPL preparations, OA induced a similar dose-dependent release of PLTs, although the rates of PLT release were much greater and more variable than those seen with CI. In OA sensitized IPGPL preparations, at an OA dose of 1000 μg, the maximum amount of PLT released was 0.289 μg and the maximal airway-to-perfusate transfer rate constant was 0.0229 min^{-1}. BAC and EDTA failed to induce quantifiable PLT release from the airways.

Conclusions. Rapid release of the inflammatory mediators, PLT C4/D4/E4, could be induced in the unsensitized IPGPL by CI, and in the sensitized IPGPL by OA. Transfer into perfusate occurred in both cases with dose-dependent $t\frac{1}{2}$ ranging from 12.4 through 57.8 minutes.

KEY WORDS: peptidoleukotrienes; isolated lung; calcium ionophore A23187; ovalbumin; SRS-A; pulmonary kinetics.

INTRODUCTION

Peptidoleukotrienes C4, D4 and E4 (previously known as slow reacting substance of anaphylaxis, SRS-A) are inflammatory mediators with potent bronchoconstricting properties, orders of magnitude larger than histamine (1). Although they are known to be present in the airways of asthmatic patients (2–5), the kinetics of their formation, release and clearance from lung following a variety of airway challenges have not been studied. PLT release in the airways of hyper-responsive humans is known to occur in response to antigen (6) and exercize (7), but not methacholine—induced bronchoconstriction (6). Relationships between the quantity of PLTs released in airways and both the late phase of asthma (8) and chronic inflammatory lung damage are often assumed, even though the magnitude of bronchoconstriction in allergen—challenged asthmatics fails to correlate with urinary PLTE4 excretion (6,7). Furthermore, a report that PLT clearance from lung is reduced following antigenic asthma provocation (9) seems illogical, given that epithelial permeability to small molecules is usually increased by disease (10,11). Because of the importance of these mediators in asthma, and our need to improve the analysis of drug action in the airways, we have extended our studies of drug disposition in the lung (12–15) to include the characterization of PLT release and disposition in the isolated perfused guinea pig lung (IPGPL), following either chemical or antigenic challenge of the airways. Antigen sensitized and unsensitized guinea pig lung tissues have become the benchmark rodent model for asthma (16). This paper describes the release and clearance kinetics of PLTs from IPGPL as functions of the airway dose of calcium ionophore A23187 (CI, chemical challenge; unsensitized lungs) and ovalbumin (OA, antigen challenge; sensitized lungs). The model's response to the common preservative, benzalkonium chloride (BAC), and the chelating agent, ethylenediaminetetraacetic acid (EDTA), both of which have been used in nebulizer solutions and implicated in asthma attacks (17), was also explored.

MATERIALS AND METHODS

Animals

The research adhered to the NIH Principles of Laboratory Animal Care. Hartley guinea pigs weighing 250–300 g (CI, BAC and EDTA experiments) or 100–150 g (OA experiments) were obtained from Charles River (Wilmington, MA). In the CI, BAC and EDTA experiments, the lungs from naive guinea pigs were used immediately. In the OA experiments, the guinea pigs were allowed to acclimate to their surroundings for at least 7 days before antigenic sensitization. The sensitization procedure involved intraperitoneal (ip) injections of 100 μg OA + 100 mg alum in 1 ml of Normal Saline for Injection USP on day 0 followed, on day 3, by half the initial dose, ip. Sensitized animals were used on days 21–28 when their weights were 296–384 g.

Chemicals

Bovine serum albumin (BSA, first fraction by heat shock), ovalbumin (OA), benzalkonium chloride (BAC) and calcium ionophore A23187 (CI) were obtained from Sigma Chemical (St. Louis, MO). Ethylene diamine tetra-acetic acid (EDTA) was obtained from JT Baker (Phillipsburg, NJ). Other chemicals were obtained from Fisher Scientific (Raleigh, NC).

Isolated Perfused Guinea Pig Lung (IPGPL)

The IPGPL preparation was established and dosed intratracheally (coarse sprays administered in 0.1 ml aqueous solution) as before (18). To permit PLT analysis in perfusate by enzyme immunoassay (EIA), Krebs-Hensleit buffer contained 0.1% (w/v) BSA (instead of the 4% described previously; 18). This

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was pumped at 15 ml/min. and 37°C, through the pulmonary vasculature, into collection vessels, prior to assay for PLTs. With only 0.1% BSA, the lungs began to show edema at t > 90 min. following isolation. Thus, experimental duration was limited to ≤80 min [we have shown that edema onset coincides most accurately with precipitate, discontinuous increases in epithelial permeability and airway-to-perfusate transfer of small solutes (12). However, small increases in permeability, as shown here for PLTs, can be induced and detected in this preparation, before edema becomes evident (15)].

PLT Release Induced By Airway Challenge

Following equilibration of the IPGPL preparation in an artificial thorax for 15 minutes, nominal 0.1 ml volumes of CI, OA, BAC or EDTA in aqueous solution were administered intratracheally, at time t = 0, as coarse aerosol sprays as described previously (18). Nominal doses of CI and OA ranged from 0.5–100 μg and 1–1000 μg, respectively. Each dose was tested in 4 separate IPGPL preparations (n = 4), with the exception of OA 1 μg where n = 2. Administered doses (amount reaching the airways) were 89.4 ± 5% of their nominal values, determined as described previously (18). The doses of EDTA and BAC were 0.1 and 0.25 μg, respectively (n = 2 in each case). Eluting perfusate was sampled for 60 min. following administration, stored at 0°C and assayed for PLTs, immediately following completion of each IPGPL preparation, by enzyme immunoassay (EIA; PerSeptive Biosystems, Cambridge, MA; this multi-well assay provided concentration of PLT [C4 + D4 + E4] by replicate spectrophotometric determination of dinitrophenol, liberated from its phosphate ester by PLT-bound alkaline phosphatase, not previously displaced by analytes). The assay was validated by spiking lung perfusate with standard PLT stock solutions (Cayman Chemical, Ann Arbor, MI) and shown to have a precision of RSD <18% (n = 3) throughout the range of 50–500 pg/ml. Sample dilution was performed in fresh perfusate, when necessary, to ensure that concentrations fell into this range and assays were performed in triplicate. Results for each experimental group were expressed as mean ± SEM cumulative amount of PLTs transferred into perfusate *vs.* time.

Data Analysis and Modeling

Based on the biexponential appearance of cumulative amount of PLT in perfusate (or vasculature, PLT_v) *vs.* time, t, profiles (Fig. 1) resulting from CI administration to the airways, Scheme I

$$PLT_p(t) \xrightarrow{k_{01}} PLT_a(t) \xrightarrow{k_{10}} PLT_v(t)$$

Scheme I

was proposed to describe PLT release into, and transfer from, airway to the perfusate, following challenge. The model assumed that there was a maximal amount of PLT precursor, $(PLT_p)_0$ that could be liberated from cells into the airway (airway side of the epithelium; PLT_a), and that the apparent first-order rate constants, k_{01} and k_{10}, described challenge-induced formation or release and airway-to-perfusate transfer, respectively. In all cases, PLT release was assumed to be faster than airway-to-perfusate transfer which was assumed to be rate-limiting (k_{01} > k_{10}). Two approaches were used to fit the data following CI challenge. Both employed nonlinear, least mean square regression analysis (Scientist, MicroMath Scientific Software, Salt Lake City, UT).

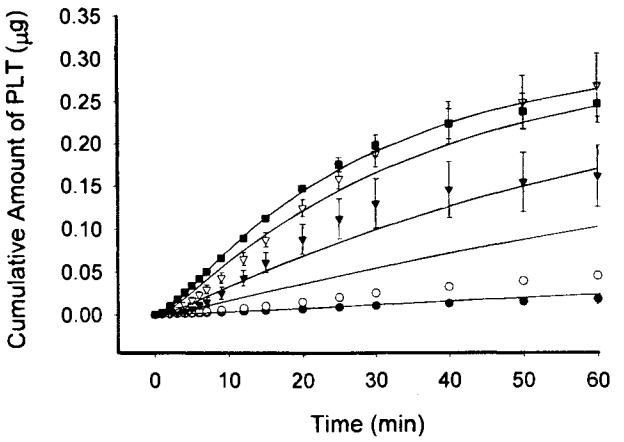

Fig. 1. Cumulative PLT_v *vs.* time induced by calcium ionophore A23187. Each point is the mean of n = 4 ± SEM following CI doses of ■ 100 μg, ∇ 10 μg, ▼ 5 μg, O 2.5 μg, ● 0.5 μg. The solid lines are the best fits predicted by simultaneous data fitting across doses of CI to eq. 1–4 with $(PLT_p)_0$ = 0.3 μg, k_{01} = 0.544 min^{-1} and k_{10} values are the best estimates according to eq. 1 (Fig. 2). By the best across dose fit, Dsat = 12.6 μg and $(k_{10})max$ = 0.0358 min^{-1}. The model selection criteria for this fit was 3.30 with 3 degrees of freedom; r^2 = 0.977.

Simultaneous Curve Fitting Across Doses of CI

All of the data was used simultaneously to estimate dose-dependency parameters for k_{10}, and a single value for k_{01}. In this approach, $(PLT_p)_0$ was assumed to remain constant at 0.30 μg, a value for PLT_v which was approached at 60 minutes but never exceeded. Values for k_{10} were assumed to escalate with dose according to equation 1

$$\text{for } D \le Dsat \quad k_{10} = (k_{10})max \; D / Dsat$$

$$\text{for } D > Dsat \quad k_{10} = (k_{10})max \qquad (1)$$

where D refers to the dose of calcium ionophore A23187 administered to the airways and Dsat is the dose at which k_{10} attains a maximum value (k_{10}) max (e.g., Fig. 2). Cumulative mean data for PLT_v *vs.* t (Scheme I), resulting from each challenge dose, were fitted using unweighted nonlinear least mean square regression across doses, to equations 2 through 4

$$dPLT_p/dt = k_{10}\, PLT_a \qquad (2)$$

$$\text{where } dPLT_a/dt = k_{01}\, PLT_p - k_{10}\, PLT_a \qquad (3)$$

$$\text{and } dPLT_v/dt = -k_{01}\, PLT_p \qquad (4)$$

With initial conditions:

$$PLT_{p(t=0)} = (PLT_p)_0$$

$$PLT_{a(t=0)} = 0$$

$$PLT_{v(t=0)} = 0$$

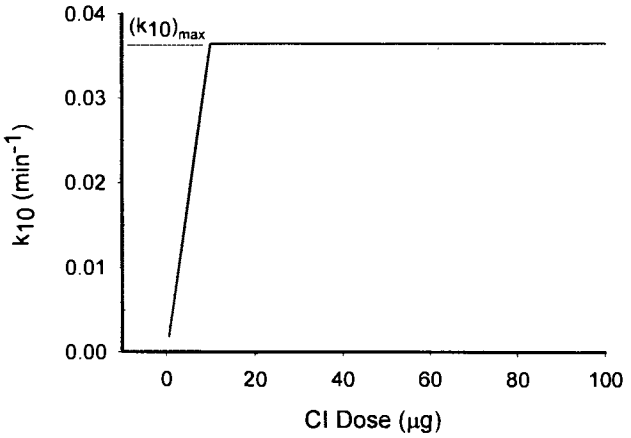

Fig. 2. Predicted profile for k_{10}, as a function of CI dose (eq. 1), following the simultaneous across dose data fitting shown in Fig. 1.

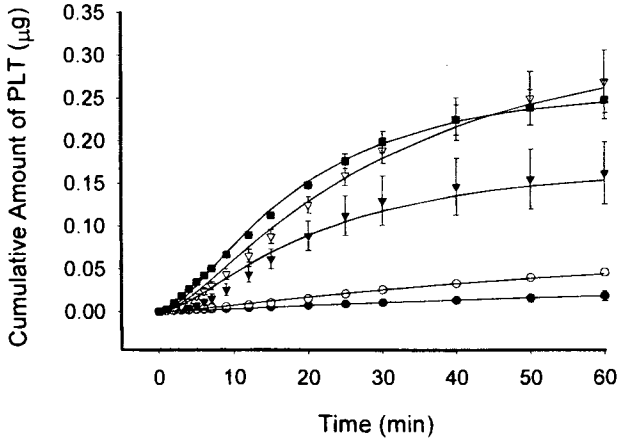

Fig. 3. Cumulative PLT_v *vs.* time induced by calcium ionophore A23187. Each point is the mean of n = 4 ± SEM following CI doses ■ 100 μg, ∇ 10 μg, ▼ 5 μg, O 2.5 μg, • 0.5 μg. The solid lines represent the best fits predicted by the model for single challenge doses when $(PLT_p)_0$ and k_{10} were varied and k_{01} was fixed at 0.292 min^{-1}. The best estimates for $(PLT_p)_0$ and k_{10} are shown in Table I.

The values for k_{10}, Dsat and (k_{10})max were allowed to float according to eq. 1 resulting in dose-dependent k_{10} values. Initial estimates for k_{01}, k_{10}, (k_{10})max and Dsat were derived by fitting mean (single dose) PLT_v *vs.* t profiles to eqs. 2 through 4 with k_{01} and k_{10} allowed to float. An "across dose mean value" was then determined for k_{01}, after which Eq. 1 was used to fit k_{10} *vs.* D, with Dsat and (k_{10}) max allowed to float. Following this (knowing the dependence of k_{10} upon CI dose, but floating the value of k_{01}) the mean data were refitted simultaneously across doses to eqs. 2, 3 and 4 to determine the best overall fit and estimate for k_{01}.

Curve Fitting for Individual Challenge Doses

In the second procedure, mean PLT_v *vs.* t data, for individual doses of CI were used to determine best estimates of $(PLT_p)_0$ and k_{10}, both of which were allowed to float with dose. In this approach, the PLT release rate constant, k_{01} = 0.292 min^{-1}, was held constant at the "across dose mean value" determined previously. Values for $(PLT_p)_0$ were held constant with dose and assigned equal to best estimates of $(PLT_v)_\infty$, following curve fitting of mean PLT_v *vs.* t data (t ≥ 20 min), for each challenge dose, to eq. 5

$$(PLT_v)_\infty - PLT_v = (PLT_v)_\infty e^{-k_{10}(t-t_{lag})} \quad (5)$$

Mean PLT_v *vs.* t profiles (0–60 min) were fitted to eqs. 2 through 4 to determine a best estimate for k_{10} at each challenge dose. Data for PLT_v *vs.* t following OA challenge of sensitized IPGPL preparations was also treated according to this approach. The model fits were validated using goodness-of-fit parameters such as MSC and r^2, review of SD of final parameter estimates and visual inspection of the residuals.

RESULTS

PLT Release After Calcium Ionophore Airway Challenge

Figs. 1 and 3 show the PLT_v *vs* time profiles resulting from IPGPL challenge with different doses of CI. The curves drawn through the data are the result of either simultaneous data fitting across doses [Fig.1; $(PLT_p)_0$ = 0.3 μg, k_{01} (best estimate) = 0.544 min^{-1}, k_{10} escalates with dose according to eq. 1] or single dose data fitting [Fig. 3; k_{01} = 0.292 min^{-1}, $(PLT_p)_0$ and k_{10} both allowed to escalate with dose]. Fig. 2 is a partner to the curve fit shown in Fig. 1, and illustrates the "best fit" theoretical dependence of k_{10}, assuming the validity of eq. 1.

Fits of the mean PLT_v *vs* t data shown in Figs. 1 and 3, according to Scheme I, which were unrestrained with respect to $(PLT_p)_0$, k_{01} and k_{10}, failed to provide unambiguous solutions for the estimated parameters. When $(PLT_p)_0$ was fixed, however, the data shown in Fig. 1 resulted in good estimates for k_{01} and k_{10}, even though, from these data alone, it was impossible to tell kinetically whether PLT release or airway-to-perfusate transfer was rate-determining (identical curves result from k_{01}/k_{10} < or > 1; 19). Because other hydrophilic molecules of the ≈500 Da size of the PLTs, are known to transfer from airways to vasculature with absorption half-lives in the range 6.3–26.5 minutes (10), and values for the larger half-life (of $0.693/k_{01}$ or $0.693/k_{10}$) fell consistently in this range, airway-to-perfusate transfer was assumed to be the rate-determining step in Scheme I. Because absorption through the pulmonary epithelium is known to be enhanced during inflammation (10), and the PLTs are inflammatory mediators, it was intuitive to assume that $(PLT_p)_0$ and k_{10} could escalate with dose, while k_{01} was only PLT formation and release-mechanism-dependent.

The solid curves and parameter estimates shown in Figs. 1 and 2 were the successful result of simultaneous data fitting across doses, provided that values for k_{10} were calculated using eq. 1. Despite numerous attempts using Emax and other models to describe the dose-dependency in k_{10}, (20), it was not possible to obtain unambiguous parameter estimates, by simultaneous across-dose fitting, without either fixing $(PLT_p)_0$, and allowing k_{10} to float, or vice-versa. As a result of these across-dose modeling experiments (e.g., Figs. 1 and 2), the better goodness-of-fit and more reasonable parameter estimates shown in Fig. 3 and Table I, were finally obtained by first estimating $(PLT_v)_\infty$ for each dose, assigning this value to $(PLT_p)_0$ in Scheme I, and then fitting the single dose data

Table I. Best Estimates of $(PLT_p)_0$ and k_{10} Following CI Challenge and the Curve Fitting Shown in Fig. 3

CI dose (μg)	$(PLT_p)_0$ (μg) [SD]	k_{10} (min^{-1}) [SD]	MSC[a]	Goodness of fit, r^2
0.5	0.0312 [0.00601]	0.0153 [0.000290]	4.97	0.997
2.5	0.0736 [0.00655]	0.0158 [0.000300]	5.11	0.997
5	0.163 [0.0245]	0.0482 [0.00293]	3.73	0.989
10	0.307 [0.0188]	0.0330 [0.000755]	5.26	0.998
100	0.254 [0.0120]	0.0557 [0.000789]	6.51	0.999

[a] MSC = model selection criteria with 2 degrees of freedom.

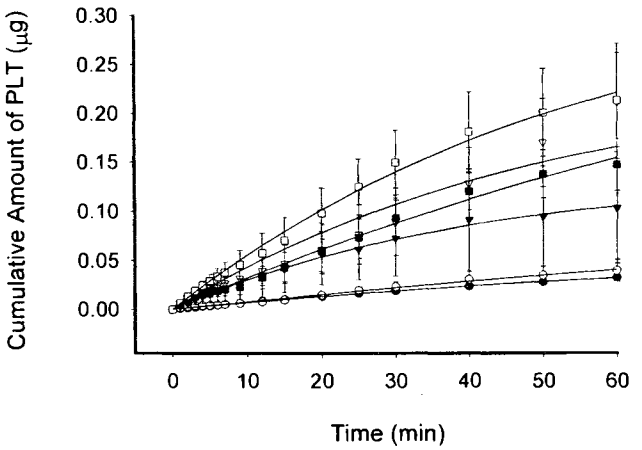

Fig. 4. Cumulative PLT_v *vs.* time from pre-sensitized IPGPL preparations, induced by ovalbumin. Each point is the mean of n = 4 (except for 1 μg where n = 2) ± SEM following OA doses of □ 1000 μg, ■ 100 μg, ∇ 50 μg, ▼ 10 μg, ○ 5 μg, ● 1 μg. The solid lines represent the values predicted by the model when $(PLT_p)_0$ and k_{10} were varied (best estimates are shown in Table II).

with k_{10} floating and k_{01} held constant. In this way, curve fitting showed that Scheme I was feasible to explain simultaneous PLT formation and airway-to-perfusate transfer in the IPGPL, following challenge with CI, and that PLT disposition could be modeled with minimal variance using the parameters shown in Table I. Overall, simultaneous fitting across doses (Fig. 1 and 2) showed that the data were consistent with absorptive clearance kinetics (k_{10}) increasing with increasing CI dose. Because that fitting approach had required a single fixed value for $(PLT_p)_0$, we sought independent confirmation by the second technique, in which values for $(PLT_p)_0$ were also allowed to increase with CI dose. Those single dose fits (Fig. 3, Table I) confirmed that Scheme I was consistent with the data with both k_{10} and $(PLT_p)_0$ increasing with CI dose. These observations are consistent with literature evidence for increasing epithelial permeability following airway challenge (10,11,15) and suggest that reported reductions in PLT clearance from lung following antigenic asthma provocation (9) were the result of those author's experimental methods and were not a true reflection of the way in which endogenously released PLTs are handled by the lung.

PLT Release After Ovalbumin Challenge

Fig. 4 shows the PLT_v *vs* time profiles resulting from pre-sensitized IPGPL airway challenge with different doses of ovalbumin. In the case of antigen challenge, there were several notable differences in the appearance of the profiles. In contrast to the curves for CI (Fig. 1), PLT appearance following OA was largely monoexponential in appearance, but with similar apparent rate constants for transfer into perfusate (k_{10}, Scheme I). The mean PLT_v *vs* time data at each dose showed much larger variance and, in many cases with individual preparations, there was evidence that further bolus doses (pulses) of PLT were released at times >0. These effects are illustrated in Fig. 5. The curves drawn through the data in Fig. 4 are the result of mean single dose data fitting according to eq. 5 with t_{lag} held = 0. In effect, this is a fit according to Scheme I with k_{01} approaching infinity. It ignores the pulsatile behavior seen in Fig. 5 which was not sufficiently reproducible to model. A comparison of the "best estimates" for $(PLT_p)_0$ and k_{10}, and their dependence upon dose, with those following CI delivery to the airways (compare Table I and II), showed similar overall values for $(PLT_p)_0$ and, as expected in the case of OA, increased variance in the parameter estimates along with a "flattened" dependence of k_{10} upon dose. Fig. 6a and b show a comparison of the $(PLT_p)_0$ *vs.* dose curves derived (a) from the curve fits shown in Fig. 4, alongside (b) the experimental values for $(PLT_v)_{60min}$. The dependence on dose for both $(PLT_p)_0$ and $(PLT_v)_{60min}$ and an apparent upper limit of both variables for the IPGPL preparation are shown clearly. There was no measurable PLT release induced by OA from the unsensitized IPGPL.

PLT Release After BAC and EDTA Challenge

There was no measurable PLT release induced, from the unsensitized IPGPL, by either BAC or EDTA. Two animals were tested for each entity and PLT concentrations

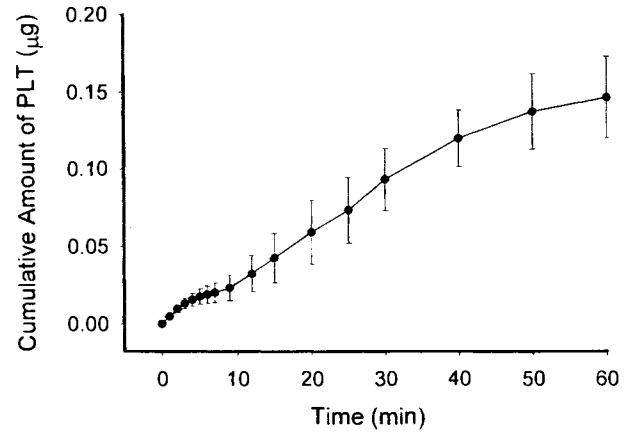

Fig. 5. Cumulative PLT_v *vs.* time following administration of 100 μg ovalbumin to illustrate the pulsatile release of PLTs. Each point is the mean of n = 4 ± SEM.

Table II. Final Parameter Estimates of $(PLT_p)_0$ and k_{10} Following OA Challenge and the Curve Fits Shown in Fig. 4

OA dose (μg)	$(PLT_p)_0$(μg) [SD]	k_{10} (min^{-1}) [SD]	MSC[a]	Goodness of fit, r^2
1	0.0619 [0.00454]	0.0120 [0.0011]	5.54	0.999
5	0.0904 [0.0202]	0.0096 [0.0027]	3.94	0.999
10	0.124 [0.00860]	0.0295 [0.0039]	3.05	0.999
50	0.230[b]	0.0217 [0.0030]		0.968
100	0.274 [0.0598]	0.0133 [0.0039]	3.19	0.998
1000	0.289 [0.0261]	0.0229 [0.0035]	3.37	0.999

[a] MSC = Model selection criteria with 2 degrees of freedom.
[b] $(PLT_p)_0$ was assigned this value in order to fit the data.

in the perfusate were consistently below the LOQ of the assay (50 pg/ml). Doses of BAC (0.25 μg) and EDTA (0.1 μg) were selected which corresponded to typical human doses which could be inhaled from commercially available nebulizer solutions. No attempt was made to scale these doses; rather an attempt was made to overdose the IPGPL and maximize possible PLT release. Experiments with these entities were terminated following observations of a consistent negative response (n = 2). In spite of reports of acute bronchoconstriction in humans and animals (17) following inhalation of formulations containing these pharmaceutical excipients, there was no evidence of their inducing PLT release in the IPGPL.

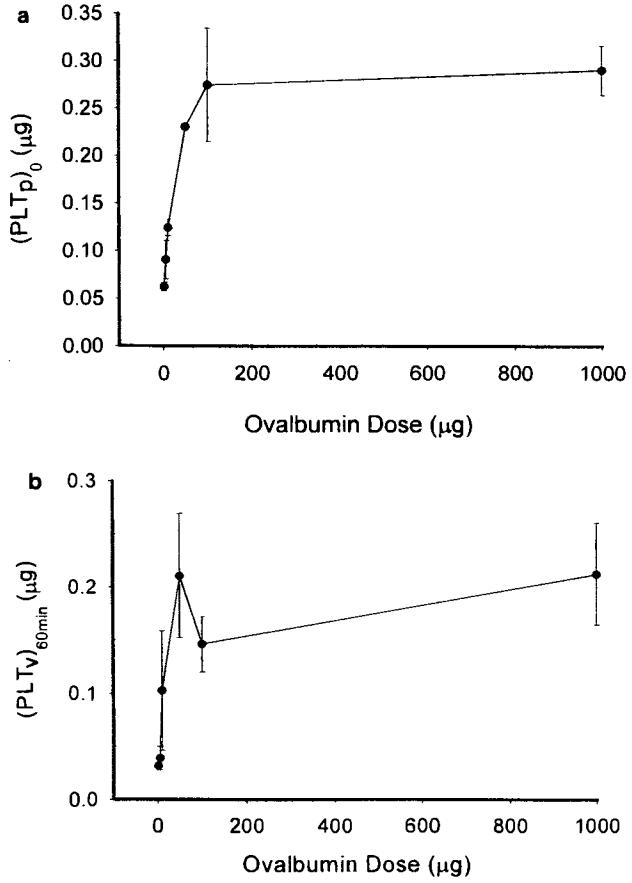

Fig. 6. (a) Apparent dependence of $(PLT_p)_0$ and $(PLT_v)_{60minutes}$ on dose of ovalbumin. Each point shows the value obtained for $(PLT_p)_0$ to produce the curves shown in Fig. 4 (Table II). (b) Each point shows the experimentally determined value of PLT_v at 60 minutes ± SEM.

DISCUSSION

This work is based upon studies in an isolated perfused lung preparation in which only the pulmonary circulation is perfused (12). Dissolved solute delivery (CI and OA in these studies) has been optimized previously (18) and shown to be reproducible to the lobar regions. Thus, the model can best be thought of as being applicable to the analysis of inflammatory diseases of the small airways, most like those chronic phases of asthma in which serosal and cellular mediators of inflammation are implicated.

When PLT C4 is released from cells into hyper-responsive airways in response to challenge (6,7) it may be metabolized, inside and outside the lung,to D4 and E4 (21). In the present study, an EIA was employed which grouped all these PLTs together. Further metabolism, beyond E4, which is known to occur in vivo, mainly in blood and liver (21), was prevented by isolating the lung and replacing blood with perfusate. PLT assays in perfusate were performed immediately after each IPGPL experiment and were validated to show that further metabolism and/or degradation failed to occur in lung-passaged perfusate. Assuming that guinea-pig lung studies have relevance to human allergic small airway disease (16), this study has several implications. First we should emphasize the apparently similar dependence of initial PLT formation and release upon the dose of CI or OA (Table I, Fig. 6), the latter in sensitized animals; both indicated that about 0.3 μg represented maximal release, probably from mast cells (2–5). The form of the dose dependencies was as expected. However, PLT formation and release kinetics (k_{01}, Scheme 1) were slow and quantifiable, only in the case of ionophore challenge. When antigen was used, a rapid mast cell response was apparently induced, probably because OA cross links IgE receptors on the mast cell surface and this triggers PLT release remotely. On the other hand, CI delivery of extra intracellular calcium ions is probably a diffusion-mediated (slower) process. With both challenge agents however, released PLTs appeared to be able to stimulate their own absorption into the pulmonary vasculature. Reliable half-life estimates ranged between 45 and 12 minutes. These values (a) were consistent with what is known about the lung's epithelial permeability for like-sized solutes (10) (b) implied that it takes between 1 and 5 hours to clear the lung of released PLTs (c) were consistent with the airway-challenge-induced 0–6 hour urinary excretion of PLT E4, the major metabolite in vivo (8,9,21) and (d) were inconsistent with reported reductions in PLT clearance from lung following antigenic asthma provocation (9). Those latter results (9) may have been due to maldistribution of airway instilled PLTs following antigenic challenge and/or additional release of endogenous PLTs reducing the clearance of exogenous (instilled) material. Antigen challenge however, produced more variable and pulsatile PLT release (Fig. 5). We speculated that this phenomenon was due to the gradual redistribution of OA to reach fresh mast cells at different times, and cause them to release their PLTs in turn. This would explain some of the data

fitting difficulties in the case of OA, because the model assumed "bolus delivery" to the PLT source. From an in vitro pharmacodynamic viewpoint, it appeared that CI-induced PLT transfer to perfusate in this model may be a less variable indicator of small-airway antiinflammatory drug effects than antigen challenge in sensitized preparations. Unfortunately, the results of our kinetic modeling showed clearly that the mechanism(s) of PLT formation and release, following CI challenge, were very different from those seen when antigen was used.

CONCLUSIONS

Calcium ionophore A23187 induced a rapid, formation or apparent first-order release ($\simeq$ 0.292 min.$^{-1}$) of up to 0.289 μg PLTs from the airways. Transfer to the perfusate was also apparent first-order and dose-dependent for CI, ranging 0.0153 through 0.0557 min.$^{-1}$. Ovalbumin induced similar PLT release and passage into perfusate from presensitized IPGPL preparations. In this case, however, release was faster and less reproducible, possibly because of small differences in the lung regions which were exposed to antigen at times in these experiments. The excipients BAC and EDTA failed to elicit PLT release following intratracheal administration of human doses to the airways of the IPGPL.

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