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IN VITRO BIOSYNTHESIS OF CYCLOOXYGENASE METABOLITES IN OVALBUMIN-SENSITIZED AND CONTROL LUNGS OF GUINEA PIGS*

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Abstract—Cyclooxygenase metabolism may regulate mediator release in anaphylaxis. The *in vitro* biosynthesis of cyclooxygenase metabolites was examined, using isolated microsomal membranes from ovalbumin-sensitized and control guinea pig lungs, under conditions in which the substrate concentration ([^{3}H]arachidonic acid) was varied (5–168 μM). With microsomes from sensitized lungs, there was stimulation of cyclooxygenase activity at substrate concentrations from 20 to 100 μM, with the major increases in activity occurring in the biosynthesis of thromboxane B_2 and prostaglandin D_2. At substrate concentrations greater than 100 μM, the biosynthesis of thromboxane B_2 and prostaglandin D_2 decreased in microsomes of sensitized lungs to the level of production found in control-lung microsomes. In contrast, prostacyclin production was significantly higher in the control-lung microsomes at the higher substrate concentrations. The ratio of prostacyclin to thromboxane production was lower in sensitized-lung microsomes at all substrate concentrations. No changes in the biosynthesis of prostaglandins $F_{2\alpha}$ or E_2 were detected between the two groups. Kinetic analysis of the data demonstrated that the cyclooxygenases in control and sensitized-lung microsomes exhibited different apparent K_m and V_{max} values. When the enzymes from both the control and the sensitized-lung microsomes were assayed in the presence of indomethacin (1–10 μM), a cyclooxygenase inhibitor, thromboxane synthesis was preferentially inhibited. The microsomal enzymes from control and sensitized lungs showed similar responses to the drug. At the indomethacin concentrations used, no significant inhibition of prostacyclin or prostaglandin production occurred. The results of these *in vitro* experiments support the hypothesis that net changes in the biosynthesis of cyclooxygenase metabolites may modulate the anaphylactic response of the lungs in sensitized animals.

The lung serves as a major site for the biosynthesis [1, 2] and inactivation [1, 3] of arachidonic acid metabolites. The fluctuations of these metabolites, particularly the cyclooxygenase products and the leukotrienes, may play a critical role in inflammation [4, 5], in the immediate hypersensitivity response [6], and in numerous pulmonary diseases such as bronchial asthma, pulmonary edema, and pulmonary distress syndrome [3, 6–9].

The release and oxygenation of arachidonic acid from membranes generate leukotrienes [10] and hydroxy acids via the enzyme, lipoxygenase [11], and endoperoxides via the microsomal enzyme, cyclooxygenase [12]. Endoperoxides can be further metabolized in one of three pathways to the stable prostaglandins (PGE_2, $PGF_{2\alpha}$, and PGD_2), to thromboxane (TXA_2), and to prostacyclin (PGI_2) [1–3, 6, 10–12]. Numerous studies have demonstrated the release of prostaglandins from the lungs of guinea pigs [1, 13–15], rats, humans [6], and other laboratory animals [14, 16]. In the guinea pig lung, the major metabolite is thromboxane [17, 18]; however, prostacyclin [19] and other prostaglandins have also been detected [20–22]. The cyclooxygenase metabolites are active on airway smooth muscle, either by causing bronchoconstriction ($PGF_{2\alpha}$ and PGD_2) or bronchodilation (PGE_2), and have been implicated in platelet aggregation (TXA_2), vasodilation (PGI_2), and vasoconstriction ($PGF_{2\alpha}$, TXA_2, and PGE_2), [2, 23].

If sensitized guinea pig and human lungs are challenged with an antigen, there is an immunological release of mediators of inflammation. The primary mediators released are histamine and the leukotrienes [4, 5]. However, within minutes of primary mediator release, there is *de novo* synthesis of thromboxanes and prostaglandins [13, 17, 18, 24, 25]. It has been hypothesized that the generation of the cyclooxygenase metabolites represents a secondary event in inflammation which may serve a regulatory role by antagonizing and modifying further release of primary mediators [6, 24]. Investigations on isolated and perfused guinea pig lungs have suggested that the sensitized lungs have a greater capacity for prostaglandin and thromboxane production [18, 26–28]. However, these types of experiments fail to distinguish changes in the biosynthesis with changes in the metabolism and degradation of the cyclooxygenase metabolites. Furthermore, the availability of the substrate, arachidonic acid, is difficult to assess in intact tissue even though changes in the substrate concentrations can generate opposing responses in the isolated pulmonary vasculature [29].

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Thus, in light of the importance of the cyclooxygenase metabolites in immediate hypersensitivity reactions and in inflammation, we investigated *in vitro* changes in the biosynthesis of cyclooxygenase metabolites with various arachidonic acid concentrations in microsomes of sensitized and control lungs of guinea pigs. We used an isolated microsomal preparation. With this system, we were able to analyze changes solely in the biosynthesis of the metabolites, since the enzymes which degrade cyclooxygenase metabolites were removed.

MATERIALS AND METHODS

Preparation of microsomes. Male albino guinea pigs (Hartley, 200–250 g) were actively sensitized with egg albumin [i.p., 10 mg in 1 ml of 0.9% (w/v) NaCl solution]. Control animals were not sensitized. After 28 days, all animals were killed by cervical dislocation and exsanguination. The lungs were removed and placed in chilled 0.05 M potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA. All subsequent procedures were done at 4°. Connective tissue was removed from the lungs. Then lungs from each group were pooled and homogenized in 3 vol. of cold buffer in a Virtis tissue homogenizer (2 × 30 sec intervals). The tissue was further homogenized with a Potter–Elvehjem homogenizer (2 × 30 sec). The homogenates were then centrifuged at 10,000 *g* for 10 min in a Beckman model L ultracentrifuge. The resultant pellet was discarded, and the supernatant fraction was spun at 100,000 *g* for 60 min. The pellet, designated the microsomal fraction, was resuspended in fresh phosphate buffer without EDTA and frozen at −70° until use. Protein was determined by the method of Lowry *et al.* [30] using bovine serum albumin as a standard.

Assay for cyclooxygenase activity. [^{3}H]Arachidonic acid was used as the substrate. Unlabeled arachidonic acid (stock 82.0 mM) was added to the labeled arachidonic acid to give a specific activity 10 μCi/μmole. The assay mixture for cyclooxygenase activity consisted of 2.5 mg microsomal protein, [^{3}H]arachidonic acid (in concentration from 5 to 168 μM), in a final volume of 1 ml of 0.1 M potassium phosphate buffer. The volume of arachidonic acid added to membrane did not exceed 80 μl. Indomethacin dissolved in ethanol was added to the incubation mixture as indicated in the figure legends. The reaction was initiated by the addition of the substrate. Incubations were for 5 min at 37°. The reactions were terminated by the addition of 2 mM citric acid (final pH 3.5). Nonenzymatic formation of products was measured after incubation of [^{3}H]arachidonic acid with microsomes which had been boiled for 5 min.

Determination of the cyclooxygenase metabolites. The reaction mixture was extracted first into petroleum ether (3 × volume) to remove the contaminating neutral lipids and the majority of the unreacted arachidonic acid [31]. Prostaglandins, thromboxane B_2 and prostacyclin were then extracted into diethyl ether (3 × 2 volume) [32]. The ether extracts were evaporated under a stream of nitrogen. The residue was resuspended in a final volume of approximately 50 μl of ether.

Samples were spotted on silica gel G paper chromatograms according to the method of Hamilton and Tobias [33]. The paper was then developed to a height of 16 cm in one of two solvents: solvent A consisted of 2,2,4-trimethylpentane–methyl ethyl ketone–acetic acid (100:41:1, by vol.); and solvent B consisted of chloroform–methanol–acetic acid (98:1:1, by vol.).

Quantitation of the cyclooxygenase products was via liquid scintillation counting. The chromatographic paper was cut into 1 cm squares, placed in Econofluor, and counted on a Hewlett Packard liquid scintillation counter. Specifically, $PGF_{2\alpha}$, PGE_2, TXB_2, PGD_2 and 6-keto-$PGF_{1\alpha}$ were quantitated. The products were identified by co-migration with authentic ^{3}Hstandards. The efficiency of counting for tritium was between 25 and 35%. Recovery of the label was between 65 and 80%. The R_f value for each metabolite in solvent A was as follows: 0.22, 0.34, 0.59, 0.59 and 0.84 for 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2, PGE_2 and PGD_2 respectively. In solvent B, the R_f values were: 0.19, 0.44, 0.44, 0.56 and 0.75 for $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2 and PGD_2 respectively. Arachidonic acid migrated to the solvent front in both solvent systems.

Statistics. Student's two tailed *t*-test was used to determine statistical significance. P values of less than 0.05 were considered significant.

Chemicals. The following chemicals, supplies and drugs were used: egg albumin five times recrystallized (Nutritional Biochemicals, Cleveland, OH); silica gel G chromatographic paper (Gelman Instrument Co., Ann Arbor, MI); indomethacin (Merck, Sharpe & Dohme, West Point, PA); arachidonic acid (NuCheck-Prep, Inc., Elysian, MN); [^{3}H]arachidonic acid (sp. act. 62.2 Ci/mmole), (New England Nuclear Corp., Boston, MA); [^{3}H]PGE_2, $PGF_{2\alpha}$, PGD_2 and 6-keto-$PGF_{1\alpha}$ (New England Nuclear Corp.); and Econofluor (New England Nuclear Corp.). Stock solutions of indomethacin were prepared in ethanol (concentration 1 mg/ml) and diluted to the required concentration with phosphate buffer upon use. Stock solutions of [^{3}H]arachidonic acid (2.1 mM) were dissolved in hexane and stored under nitrogen at −20° until the day of use.

RESULTS

Characteristics of cyclooxygenease activity in guinea pig lung microsomes. When cyclooxygenase activity was assayed in the microsomal fraction of sensitized and control lungs, metabolites from the three major pathways of cyclooxygenase were synthesized. Specifically, the metabolites were: prostaglandins (PG): $F_{2\alpha}$ and D_2; 6-keto-$PGF_{1\alpha}$, the stable breakdown product of prostacyclin (PGI_2); and thromboxane (TX) B_2, the stable metabolite of TXA_2. As seen in Fig. 1, when the microsomal fraction was incubated in the presence of 20 μM arachidonic acid, the major metabolite synthesized was TXB_2. Significant levels of $PGF_{2\alpha}$, PGD_2 and 6-keto-$PGF_{1\alpha}$ were also synthesized. Identification of the specific metabolites was determined by co-migration of the products with authentic standards. Nonenzymatic metabolism, as determined from a

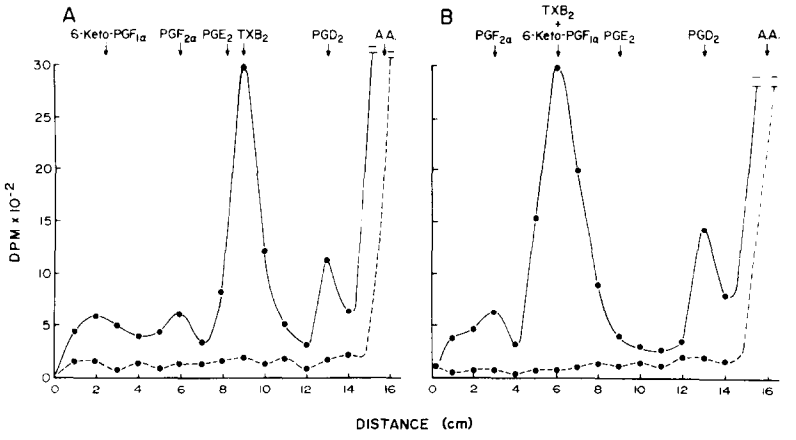

Fig. 1. Thin-layer paper chromatographic elution profile of cyclooxygenase production from guinea pig lung microsomes. Microsomal protein (2.5 mg) was incubated with 20 μM [^{3}H]arachidonic acid for 5 min at 37°. Key: (●——●) enzymatic production; (●- - -●) nonenzymztic production. (A) Solvent system A: 2,2,4-trimethylpentane–methyl ethyl ketone–acetic acid (100:41:1, by vol.). (B) Solvent system B; chloroform–methanol–acetic acid (98:1:1, by vol.).

boiled microsomal preparation, was significantly less than the enzymatic synthesis (Fig. 1, A and B). In perfused guinea pig lung preparations, Mathé *et al.* [13, 28] detected the release of PGE_2 from sensitized lung tissue. However, in our isolated microsomal preparation, no detectable levels of PGE_2 were synthesized (Fig. 1B). At 20 μM arachidonic acid, TXB_2 constituted approximately 60% of the total metabolites produced, while $PGF_{2\alpha}$, PGD_2, and 6-keto-$PGF_{1\alpha}$ constituted 10, 20, and 10% of the total respectively.

To further characterize the enzyme reaction, the microsomal fraction was incubated for various lengths of time at saturating substrate concentration (100 μM arachidonic acid) (Fig. 2). Under these conditions, the enzyme reaction was linear up to 5 min

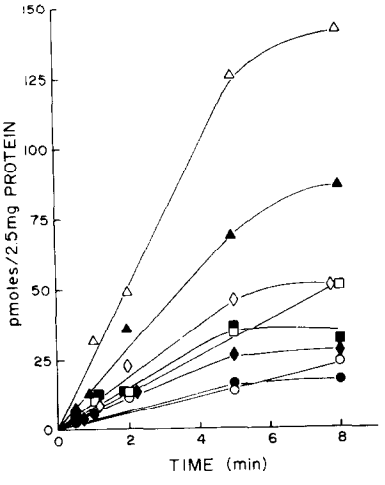

Fig. 2. Time course of cyclooxygenase metabolic production. Microsomal enzyme (2.5 mg protein) was incubated in the presence of 100 μM arachidonic acid. Values are the means of two to three experiments. Key: TXB_2 (▲—control, △—sensitized); 6-keto-$PGF_{1\alpha}$ (●—control, ○—sensitized); $PGF_{2\alpha}$ (■—control, □—sensitized); and PGD_2 (◆—control, ◇—sensitized).

in both the sensitized and control preparations for all cyclooxygenase metabolites measured. After 5 min of incubation, the rates of synthesis of TXB_2 and PGD_2 were 1.8 and 1.5 times greater, respectively, in the sensitized tissue than in the control. However, the synthetic rates for $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were identical in both groups.

The specific response of lung to antigen challenge may be dependent on the amount of arachidonic acid released from the membrane [13, 29]. Thus, cyclooxygenase activities of lung microsomes from sensitized and control guinea pigs were assayed with various substrate concentrations of 5–168 μM arachidonic acid. As the substrate concentration was increased, the percentage of the total products represented by 6-keto-$PGF_{1\alpha}$ increased with a concomitant decrease in TXB_2 synthesis (Fig. 3A). In the microsomal fractions from both the control and sensitized lungs, TXB_2 represented up to 70% of the total cyclooxygenase metabolites at 10 μM arachidonic acid, but it progressively decreased to approximately 40% of the total between 10 and 168 μM arachidonic acid. As TXB_2 decreased, the percentage of the total metabolites represented by 6-keto-$PGF_{1\alpha}$ increased from 15 to 40%. For the prostaglandins, there was an initial increase in the percentage of the total represented by $PGF_{2\alpha}$ and PGD_2 (Fig. 3B). However, at saturating substrate concentrations, PGD_2 and $PGF_{2\alpha}$ remained constant at 25 and 15% of the total respectively.

Cyclooxygenase synthesis in the microsomal fractions from control and sensitized guinea pig lungs. Sensitized guinea pig lung microsomal fraction had a greater capacity for the synthesis of cyclooxygenase metabolites than that from the control lungs (Fig. 4). However, this increase in synthesis was not due to changes in the lung wet weight or in the microsomal protein content. Neither the lung wet weight (3.16 ± 0.09 g/lung for control, N = 23, versus 3.04 ± 0.12 g/lung for the sensitized, N = 24) nor the microsomal protein content (16.68 ± 1.48 mg/lung for the control versus 14.57 ± 1.90 mg/lung for the sensitized lungs) was significantly altered in the two

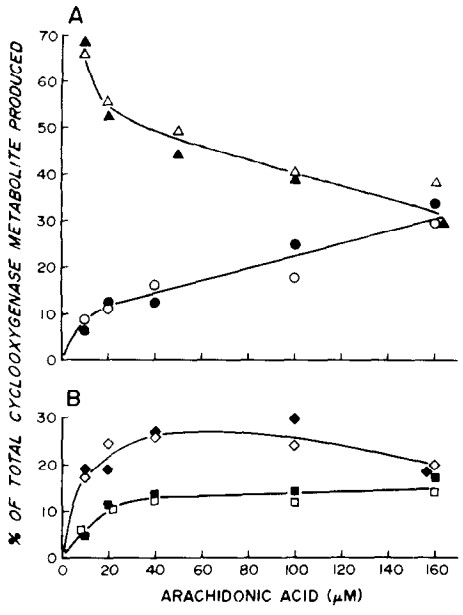

Fig. 3. Distribution of cyclooxygenase metabolite production with various substrate concentrations. Each point is the mean of three to eight experiments and is expressed as the percentage of total metabolites produced. (A) TXB_2 (▲—control, △—sensitized); 6-keto-$PGF_{1\alpha}$ (●—control, ○—sensitized). (B) PGD_2 (◆—control, ◇—sensitized); $PGF_{2\alpha}$ (■—control, □—sensitized).

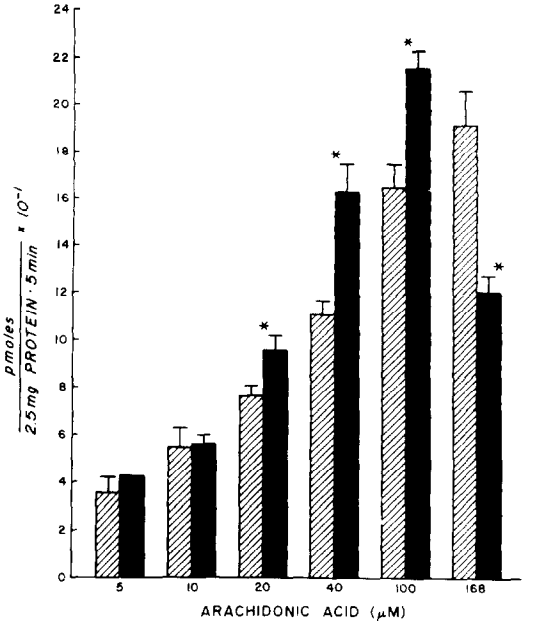

Fig. 4. Total cyclooxygenase products in ovalbumin-sensitized and control guinea pig lung microsomes. Cyclooxygenase activity in microsomal fraction was assayed for TXB_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGD_2 and PGE_2 biosynthesis. Microsomal protein (2.5 mg) was incubated in the presence of [^{3}H]arachidonic acid for 5 min at 37°. Key: hatched bars, control; solid bars, sensitized biosynthesis. Each value is the mean ± S.E. from three to eight experiments. An asterisk (*) indicates that the value was significantly different from the corresponding control value ($P < 0.05$, Student's t-test).

groups. Increased cyclooxygenase activity in the sensitized-lung microsomal fraction was observed at arachidonic acid concentrations of 20 to 100 μM (Fig. 4). No significant differences in the enzyme activities were detected at substrate concentrations below 20 μM. Maximum stimulation (50%) of synthesis in the microsomes from sensitized lungs occurred at 40 μM arachidonic acid. At 168 μM arachidonic acid, there was a significant decrease in the cyclooxygenase activity in the sensitized-lung microsomes while the control-lung microsomes displayed continual synthesis.

To assess the specific differences in biosynthesis of each cyclooxygenase metabolite by the microsomal fractions of the control and sensitized lungs, microsomal enzyme activity was assayed in the presence of various arachidonic acid concentrations. The profile of each metabolite versus substrate concentration is shown in Fig. 5. Major differences in TXB_2, PGD_2 and 6-keto-$PGF_{1\alpha}$ production were detected. TXB_2 synthesis was significantly higher in the sensitized-lung microsomes at substrate concentrations between 20 and 100 μM (Fig. 5C). However, when arachidonic acid concentrations exceeded 100 μM, there was an inhibition of TXB_2 synthesis in the sensitized-lung microsomes. In contrast, for 6-keto-$PGF_{1\alpha}$, no significant differences in biosynthesis were detected except at arachidonic acid concentrations greater than 100 μM (Fig. 5A). Whereas the enzymatic activity of the sensitized guinea pig microsomes reached maximum production by 100 μM, the control-lung microsomes were capable of continued synthesis of the prostacyclin metabolite.

Enzymatic activity of sensitized-lung microsomes had a greater capacity for the synthesis of TXB_2 than the enzymeatic activity of the control microsomes (Table 1), but a decreased substrate affinity (Fig. 6A). The response of the sensitized-lung microsomal enzyme activity to PGI_2 production was reversed. There was greater substrate affinity (Fig. 6B) but decreased synthetic capacity (Table 1) for 6-keto-$PGF_{1\alpha}$ in the sensitized-lung microsomal fraction.

Prostaglandin production was also altered in the sensitized-lung microsomes. Stimulation of PGD_2 synthesis occurred at substrate concentrations between 20 and 100 μM. The profile of PGD_2 production was parallel to TXB_2 production for microsomes from the sensitized and control lungs (Fig. 5D). Control enzymatic activity reached maximum synthesis by 100 μM. There was also a decrease in PGD_2 synthesis in the sensitized-lung microsomes when the arachidonic acid concentration exceeded 100 μM. The apparent enzymatic rate was higher in the sensitized-lung microsomal fraction, although there was no significant difference in substrate affinity between the control and sensitized enzyme (Table 1). No significant differences in $PGF_{2\alpha}$ production were detected (Fig. 5B). The apparent kinetic constants were not different between the two groups (Table 1). No detectable levels of PGE_2 were found in the isolated enzyme preparations.

Effect of indomethacin on cyclooxygenase activity. Indomethacin is a nonsteroidal anti-inflammatory drug which inhibits cyclooxygenase activity [34]. We

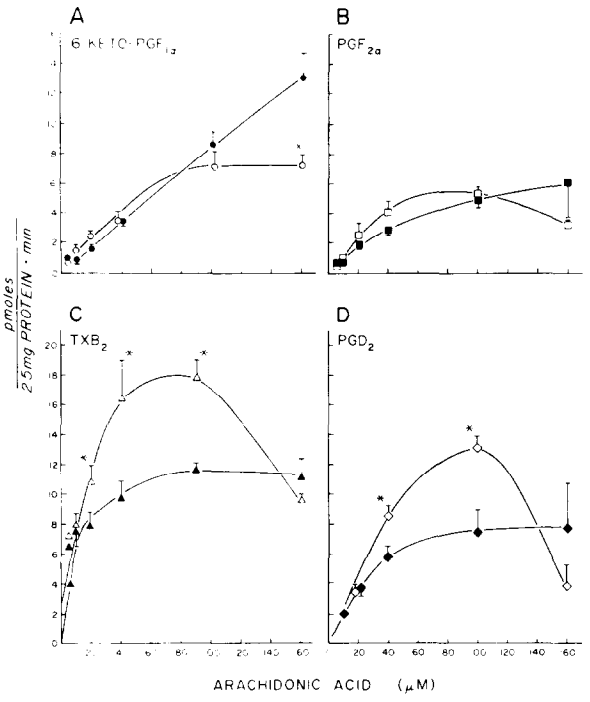

Fig. 5. Effects of substrate concentration on cyclooxygenase metabolite production. Microsomal protein (2.5 mg) from control or sensitized lungs was incubated in [^{3}H]arachidonic acid for 5 min at 37°. Each value is the mean ± S.E. from three to eight experiments. Metabolites measured were: (A) 6-keto-$PGF_{1\alpha}$ (●—control, ○—sensitized); (B) $PGF_{2\alpha}$ (■—control, □—sensitized); (C) TXB_2 (▲—control, △—sensitized); and (D) PGD_2 (◆—control, ◇—sensitized). An asterisk (*) denotes that the value was significantly different from control value ($P < 0.01$, Student's t-test).

assayed the effect of this drug on cyclooxygenase activity in the isolated microsomal fractions of sensitized and control lungs. Indomethacin (concentration 1–10 μM) preferentially inhibited TXB_2 production in both the control and the sensitized-lung microsomes (Fig. 7). The apparent IC_{50} varied from 2.8 to 4.7 μM as the substrate concentration increased from 20 to 100 μM. There were no significant differences in the responses to the drug by microsomal enzymes from sensitized and control animals.

In contrast to the inhibition of TXB_2 synthesis by indomethacin, the synthesis of prostacyclin and prostaglandins was not markedly affected at the concentrations of the drug used (Table 2). Although there was an apparent increase in 6-keto-$PGF_{1\alpha}$ production in microsomes from sensitized lungs, this increase was not significantly different from the initial value (without indomethacin). Indomethacin did not alter 6-keto-$PGF_{1\alpha}$ production in microsomes from the control lungs. At concentrations of indomethacin which inhibited TXB_2 production by 50%, $PGF_{2\alpha}$ synthesis was only inhibited by 30% (Table 2). The results suggest that more than one cyclooxygenase enzyme complex may be present in the lung [35]. The microsomal enzyme activities of control and sensitized lungs did not differ in their responses to indomethacin (Table 2).

DISCUSSION

The changes in biosynthesis and degradation of cyclooxygenase metabolites by sensitized-lung microsomes suggest that these metabolites may regulate, in part, the immunological release of mediators of inflammation [4–6]. Cyclooxygenase metabolites

Table 1. Apparent enzymatic kinetic constants for cyclooxygenase of sensitized- and control-lung microsomes

Product	≃ K_m (μM) Control	Sensitized	≃ V_{max}* (pmoles/min · mg protein) Control	Sensitized
TXB_2	6	20	11.76 ± 0.27	20.83 ± 1.05
$PGF_{2\alpha}$	40	25	6.08 ± 2.60	5.25 ± 0.58
PGD_2	24	26	7.95 ± 2.84	13.27 ± 0.71
6-keto-$PGF_{1\alpha}$	500	53	46.71 ± 2.37	7.29 ± 0.84

* Values are means ± S.E.

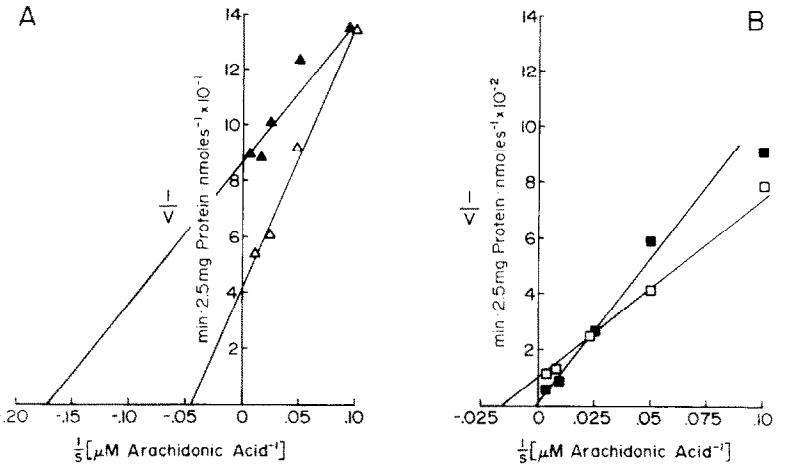

Fig. 6. Lineweaver–Burk plots for TXB_2 and 6-keto-$PGF_{1\alpha}$ biosynthesis. (A) TXB_2 production: $\simeq K_m$ value for control (▲) was 5.69 μM, linear regression analysis revealed correlation coefficient (r) = 0.915; $\simeq K_m$ value for the sensitized (△) was 20.8 μM, r = 0.993. (B) 6-keto-$PGF_{1\alpha}$ production: $\simeq K_m$ value for the control (■) was 500 μM, r = 0.997; $\simeq K_m$ value for sensitized (□) was 53 μM, r = 0.947. Each point is the mean of three to eight experiments.

are continually synthesized *de novo* after antigen stimulation and release of primary mediators (histamine and leukotrienes) [13, 18, 25]. The cyclooxygenase products (prostaglandins, thromboxane, and prostacyclin) induce physiological changes in the bronchial and vascular tissue of the lung and may augment the anaphylactic responses initiated by histamine and leukotrienes [2, 23]. To assess biochemical changes in the synthesis of the cyclooxygenase metabolites, we analyzed the kinetics of microsomal cyclooxygenase activity from ovalbumin-sensitized and control lungs of guinea pigs. Others have shown that, after antigen challenge in perfused or isolated lungs of sensitized guinea pigs, there is an increase in the release of prostaglandins and thromboxanes [13–15]. However, one of the problems with these studies is the difficulty in distinguishing between the biochemical changes due to synthesis and the changes due to degradation of the cyclooxygenase metabolites. Using the isolated microsomal fraction, we demonstrated a marked increase in the synthesizing capacity of the sensitized guinea pig lung. Significant increases in TXB_2 and PGD_2 synthesis occurred, while no changes in $PGF_{2\alpha}$ or PGE_2 synthesis were detected. Furthermore, there were substrate-dependent differences in 6-keto-$PGF_{1\alpha}$ production between the control and sensitized lungs. These results are in agreement with the hypothesis that inducement of pathological conditions in guinea pigs modifies prostaglandin-synthesizing enzymes [36].

Additional biochemical changes in the lung enzymes may also occur with sensitization. Concurrent with the changes in the synthesizing enzymes, there may be decreased degradative enzyme activity. After exposure to environmental pollutants, Matsumura *et al.* [37] demonstrated increases in the anaphylactic responses in guinea pig lungs. The pollutants inhibited prostaglandin dehydrogenase activity which resulted in a net increase in anaphylactic responses in guinea pig lungs [38, 39]. It is not known if sensitized lungs have a decreased prostaglandin dehydrogenase activity; contradictory results have been found [27, 40]. Since sensitized guinea pigs have increased synthesizing ability, if the enzymes which degrade cyclooxygenase metabolites were also decreased, then the effects of the cyclo-

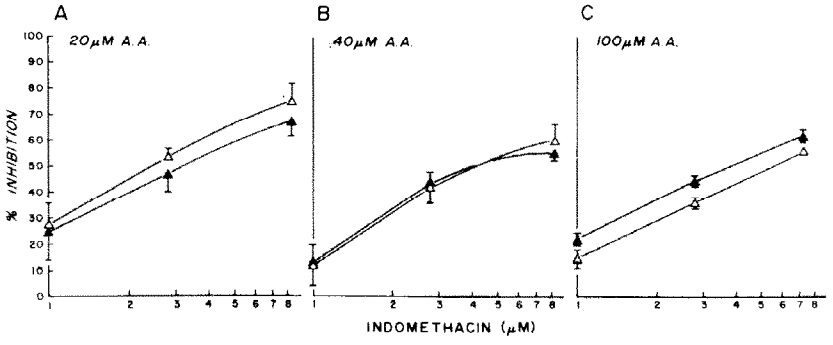

Fig. 7. Effect of indomethacin on TXB_2 production in microsomes from control and sensitized lungs. Indomethacin (1–10 μM) was preincubated for 1.5 min at 25° with microsomal protein (2.5 mg) and then incubated further in [^{3}H]arachidonic acid for 5 min at 37°. Key: (▲——▲) control, and (△——△) sensitized lungs; A.A. = arachidonic acid.

Table 2. Effect of indomethacin on cyclooxygenase metabolite production*

Product	Indomethacin (μM)	Control (pmoles/mg protein · min^{-1})	Control % Inhibition	Sensitized (pmoles/mg protein · min^{-1})	Sensitized % Inhibition
6-Keto-$PGF_{1\alpha}$	0	3.56 ± 0.28 (6)	0	3.53 ± 0.62 (6)	0
	1	3.76 ± 0.36 (3)	0	5.8 ± 1.01 (3)	0†
	3	3.87 ± 0.43 (8)	0	2.91 ± 0.39 (5)	18
	10	3.02 ± 0.41 (4)	15	5.68 ± 1.84 (4)	0†
$PGF_{2\alpha}$	0	2.92 ± 0.38 (7)	0	4.11 ± 0.72 (4)	0
	1	2.39 ± 0.25 (3)	18	3.98 ± 1.00 (2)	0
	3	2.30 ± 0.25 (6)	22	2.84 ± 0.58 (4)	31
	10	2.13 ± 0.41 (4)	28	2.89 ± 0.51 (4)	30
TXB_2	0	9.81 ± 0.95 (7)	0	16.41 ± 2.35 (7)	0
	1	8.57 ± 0.58 (4)	13	11.30 ± 1.93 (5)	32
	3	6.15 ± 0.68 (8)	33	8.08 ± 1.43 (8)	51
	10	5.05 ± 0.59 (4)	49	7.49 ± 1.49 (4)	55

* Microsomal protein (2.5 mg) was incubated in the presence of indomethacin for 5 min at 37° at a substrate concentration of 40 μM arachidonic acid. Each value is the mean ± S.E.; numbers in parentheses indicate the number of experiments.

† Value was not significantly different from the initial value where indomethacin concentration equals 0 μM.

oxygenase products could potentiate the anaphypactic response.

In contrast to the work of other investigators [13, 18, 26–28], we saw no difference in $PGF_{2\alpha}$ production by microsomes of the control and sensitized lungs. Since the previous reports used perfused or isolated lungs, both synthetic and degradative enzymes were present. We have shown that there were no changes in the synthesis of $PGF_{2\alpha}$ by microsomes of sensitized lungs. However, if the activity of the degradative enzymes decreased, there could possibly be a net increase in $PGF_{2\alpha}$ present in the intact lungs.

The present experiments with microsomal enzyme activity of sensitized guinea pig lung also demonstrated increased production of PGD_2. PGD_2 has been shown to play a key role in acute inflammation [41, 42]. Anhut *et al.* [43] detected PGD_2 in the perfusate from guinea pig lungs after antigen challenge. Furthermore, PGD_2 is a more potent bronchoconstrictor than $PGF_{2\alpha}$ in the guinea pig [44, 45]. In the microsomal system, PGD_2 production paralleled the increases in TXB_2 production. Failure of other investigators to detect PGD_2 may have been due to the insensitivity of the biochemical techniques used or to the presence of enzymes *in situ* which convert PGD_2 to other prostaglandins [46]. Although nonenzymatic production of PGD_2 by a boiled microsomal preparation was considered in analyzing the data, chemical conversion of PGH_2 to PGD_2 may also occur.

The major *in vitro* differences in microsomal cyclooxygenase activity of sensitized and control lungs occurred in the synthesis of TXA_2 and PGI_2. Sensitized-lung microsomal fraction had a greater capacity for the synthesis of TXB_2 than the control-lung microsomal fraction. However, the control-lung microsomes produced more PGI_2 than the sensitized-lung microsomes. The balance between TXA_2 and PGI_2 is important in lung physiology [47]. TXA_2 causes vaso- and bronchoconstriction [44, 48] and platelet aggregation [49], while PGI_2 causes vasodilation [50], antagonizes bronchoconstriction [51], and prevents platelet aggregation [52]. Under normal physiological conditions, when hyperventilation occurs, PGI_2 is the major metabolite produced [53]. The ratio of PGI_2 to TXA_2 production increases. Increased PGI_2 production may serve as a protective mechanism against the damaging effects of TXA_2 [53]. However, we found that, with sensitization, the ratio of PGI_2 to TXB_2 production decreased in the lung microsomes. Thus, with the impaired PGI_2 production in the sensitized-lung microsomes, the effects of TXA_2 and PGD_2 may be augmented when anaphylaxis occurs. Our results suggest that there are biochemical differences in the substrate receptors and/or in the enzymes responsible for TXA_2 and PGI_2 production. An additional possibility for the changes in metabolite biosynthesis by the sensitized-lung microsomes is an alteration in the cell types present in the lung. It has been postulated that TXA_2 is produced by the parenchymal tissue [54, 55], while PGI_2 is synthesized in the endothelial tissue [50, 55]. Our results indicate that there was no difference in the total microsomal protein of the control and sensitized lungs, but they do not discriminate between the microsomal enzymes from the different cell types in the lungs.

Since the cyclooxygenase enzymes of microsomes from both control and sensitized lungs displayed altered kinetic properties, we investigated the possibility that the enzymes responded differently to cyclooxygenase inhibitors. Our results support previous findings that indomethacin competes with the substrate for binding sites on the enzyme [56]. However, there were no significant differences in the responses of the control and sensitized lung microsomes to the drug. At concentrations of 1–10 μM, indomethacin preferentially inhibited TXB_2 production. Interestingly, at these concentrations, no significant inhibition of $PGF_{2\alpha}$ or 6-keto-$PGF_{1\alpha}$ occurred in either the control or sensitized lung microsomes. Why this occurred is not known. Recently, Lysz and Needleman [35] demonstrated the presence of two forms of the cyclooxygenase in brain tissue. One form was predominantly involved

in $PGF_{2\alpha}$ production while the other was involved in PGE_2 production. The enzymes were differentially affected by indomethacin. Our results cannot eliminate the possibility of more than one cyclooxygenase having been present in the microsomal fraction isolated from the lung.

In summary, the present *in vitro* study demonstrated increased cyclooxygenase activity in sensitized guinea pig lung microsomes. The kinetic properties of the enzymatic activity were altered in the sensitized-lung microsomes. TXB_2 and PGD_2 syntheses were markedly increased while PGI_2 production appeared to be impaired. The balance between PGI_2 and TXA_2 production may affect the ability of the lung tissue to respond under pathological conditions.

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