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RELEASE OF PROSTAGLANDINS AND HISTAMINE FROM SENSITIZED AND ANAPHYLACTIC GUINEA PIG LUNGS—CHANGES IN CYCLIC AMP LEVELS

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Abstract—Healthy control, antigen-sensitized and anaphylactic guinea pig lungs were perfused with Tyrode's solution, and immuno reactive prostaglandins E and $F_{2\alpha}$ (PGE, $PGF_{2\alpha}$), 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$ (metabolite) and histamine measured in the outflows. Histamine and cyclic AMP were also determined in the lung tissue. Significantly more PGE, $PGF_{2\alpha}$, metabolite and histamine were spontaneously released from sensitized lungs than control lungs. Anaphylaxis further increased the release of these compounds and also raised the tissue levels of cyclic AMP. The largest amounts of PGE and $PGF_{2\alpha}$ in the perfusate were found during min 2 and of histamine during min 1 after antigen administration. Pretreatment of the sensitized lungs with indomethacin, di-isopropylfluorophosphate (DFP) and epinephrine blocked the anaphylactic release of mediators. Indomethacin and DFP also reduced the cyclic AMP levels, while epinephrine pretreatment only marginally affected the rise in cyclic AMP induced by anaphylaxis. When given after the antigen, epinephrine had a minimal effect on the release of prostaglandins (PGs) and histamine, but enhanced the effect of anaphylaxis on cyclic AMP. The data indicate that sensitization with antigen may change the biological properties of the organism, as evidenced by the increased outflow of PGs and histamine. The differentiation of the effects of blockers points to an intricate relationship between the release of PGs and histamine and the tissue levels of cyclic AMP in sensitized and anaphylactic lungs.

Nearly half a century ago histamine was identified as a "mediator" released during the anaphylactic reaction [1, 2]. The histological localization of histamine and the conditions necessary for its synthesis, storage and participation in the antigen–antibody response have since been extensively investigated [3–5]. On the other hand, the discovery that sensitized, challenged lungs also release prostaglandins (PGs) is of relatively recent origin [6]. This finding was confirmed and extended by discovering 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$, the main lung metabolites in anaphylactic lung perfusates and in urines from anaphylactic guinea pigs [7, 8]. As far as sensitized, non-challenged lungs are concerned, the release of PGs was either not investigated previously or not detected when bioassay was used. At this time there are many indications that PGs may have a significant role in the respiratory and non-respiratory lung function. Thus, activities of PG synthetase and the metabolizing enzymes are high in the lung [9, 10]; PGs affect the cyclic nucleotide system in the lung tissue and isolated bronchi [11–13], and PGs influence the tone of airways [14–20].

Adenylate cyclase is hyper-reactive to epinephrine in homogenates from antigen-sensitized guinea pig lungs as compared to control guinea pig lungs. Furthermore, accumulation of cyclic AMP in response to both epinephrine and histamine is diminished in the whole perfused lungs from sensitized guinea pigs [21–23]. In anaphylaxis, elevated levels of cyclic AMP have been reported [24] and the regulatory role of cyclic AMP in the release of mediators has been amply demonstrated [25–27]. Epinephrine raises the cyclic AMP levels in lung and inhibits the antigen–antibody reaction [28–31]. Consequently, in the present study, the release of PGs and histamine from lung, the changes in the cyclic AMP levels in the lung tissue, and the effects of epinephrine on these were investigated.

Histamine, slow reacting substance of anaphylaxis, eosinophil chemotactic factor of anaphylaxis and platelet-activating factor are regarded as "primary" mediators of the antigen–antibody reaction, and it has been hypothesized that the release of PGs in anaphylaxis is dependent upon these mediators [27]. While the outflow of PGs can be increased by exogenous histamine [7, 32, 33], the converse relationship might also be true [11], and the conditions of mutual regulation have not been worked out. Therefore, we have investigated the effects of indomethacin, a known prostaglandin synthetase inhibitor [34], and di-isopropylfluorophosphate (DFP), an enzymatic blocker of histamine liberation [35, 36], on the release of histamine and PGs, and on the tissue levels of cyclic AMP in control, antigen-sensitized and anaphylactic guinea pig lungs. Since the antigen–antibody release of histamine is an energy-dependent process, the effect of 2-deoxyglucose (2-DG), a metabolic blocker [37], on the same variables was also studied.

MATERIALS AND METHODS

Animal preparation. Male guinea pigs weighing 350–450 g were injected with 100 mg ovalbumin in 1 ml of 0.9% saline (50 mg administered intraperitoneally and 50 mg subcutaneously) on day 1. A boos-

ter dose of 50 mg ovalbumin was injected intraperitoneally on day 3. Control animals were similarly injected with the vehicle only. Four to six weeks later the animals were stunned and exsanguinated, the thoracic cavity was opened, and catheters were inserted *in situ* via the right ventricle into the pulmonary artery and via the left atrium into the pulmonary vein. The lungs were perfused with Tyrode's solution (37° bubbled with 5% CO_2 in O_2) at a rate of 5 ml/min using a Harvard pulsatile pump, model 1405. A bubbletrap was used in order to prevent air emboli. A side-arm from the inflowing catheter was connected to a Statham pressure transducer, P23 DC, and the pressure in the pulmonary artery continuously recorded on a Grass polygraph. As in our other experiments [7, 38], only animals with a basal perfusion pressure below 20 mm Hg were used.

The experiments were carried out according to the following general schedule. After a 15-min rinse period, the perfusates were continuously collected for an appropriate period of time. All drugs and the vehicle (0.9% NaCl) were infused directly into the pulmonary artery using a Harvard 940 syringe pump. Immediately after the procedure, lung was frozen in liquid nitrogen and kept at −70° until analyzed for cyclic AMP and histamine. The different perfusate samples were also kept at −70° until analyzed for PGs and histamine as described below.

In the first series of experiments, infusion of indomethacin (10 μM), DFP (5 mM), 2-DG (15 mM, no glucose added to the Tyrode's solution) or 0.9% NaCl at a rate of 0.5 ml/min was started 5 min before the antigen challenge. Anaphylactic reaction was elicited by a 30-sec infusion of 50 mg ovalbumin in 1 ml of 0.9% NaCl; in sensitized control animals 1 ml of 0.9% NaCl was infused. Perfusates were collected for 4 min after the end of ovalbumin or NaCl administration.

In experiments with epinephrine, anaphylaxis was provoked by 100 mg ovalbumin infused over a period of 60 sec. Continuous infusion of epinephrine (1 or 10 μg/ml) was started 60 sec before, or immediately after, the end of ovalbumin administration. Perfusates for assay of histamine and PGs were collected from 60 sec before until 4 min after ovalbumin infusion.

Prostaglandins. As reported elsewhere [7, 33], immunoreactive prostaglandins E_2/E_1, $F_{2\alpha}/F_{1\alpha}$, and 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$ were determined by the radioimmunoassay procedure developed by Levine *et al.* [39, 40]. Appropriate buffer controls without antiserum were run with every assay. The sample binding was calculated as per cent inhibition (total binding representing 0 per cent), and the immunoreactive PG content obtained by referring to the standard curve. Each sample was assayed in duplicate and the means were calculated. The rabbit antiserum used for determination of E-type prostaglandins was not sufficiently specific to distinguish between PGE_1 and PGE_2; thus 50 per cent inhibition was obtained with either 0.13 ng PGE_1 or 0.15 ng PGE_2. Consequently, although it is probable that PGE_2 and not PGE_1 is predominantly released from the lung, no attempt was made to distinguish between the two PGs, and the notation PGE is used. In contrast, the specificity of the $PGF_{2\alpha}$ antibody allowed for a reasonable differentiation between $PGF_{2\alpha}$ and $PGF_{1\alpha}$; thirty times more $PGF_{1\alpha}$ than $PGF_{2\alpha}$ was needed to achieve 50 per cent inhibition. Moreover, using a $PGF_{1\alpha}$ antiserum on a few samples, no $PGF_{1\alpha}$ was detected. Therefore, the notation $PGF_{2\alpha}$ is used. As far as anti-15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$, 50 per cent inhibition was achieved with 0.3 ng 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and 20 ng 15-keto-13,14-dihydro-PGE_2. In view of equal or higher concentrations of $PGF_{2\alpha}$ than PGE in lung outflows and the relatively small cross-reactivity (0.3 ng vs 20 ng), it appears that the bulk of metabolites measured was of the F-type. Nevertheless, since the determinations were not carried out before and after alkali treatment, the immunoreactive metabolites are denoted as 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$.

Histamine. Histamine in tissues and perfusates was determined according to the fluorometric method of Håkansson and Rönnberg [41] and is based on the extraction of histamine and its reaction with *o*-phthaldialdehyde (OPT) to produce a fluorescent condensation product. Fluorescence readings were made on a Perkin–Elmer MPF-2A set at 350/440 nm and converted to histamine units from the standard curve. In order to decrease variability due to different content of histamine/g of lung tissue, all results were expressed as per cent of histamine released, that is, histamine in perfusate/histamine in lung and perfusate.

Cyclic AMP. The tissue was homogenized in 0.4 N perchloric acid containing tracer amounts of [8-^{14}C]cyclic AMP and centrifuged at 5000 *g* for 20 min. An aliquot of the supernatant was neutralized with 3 M Tris base and the cyclic nucleotide separated by a modification of the method of Mao and Guidotti [42]. The lyophilized eluate was reconstituted in sodium acetate buffer (0.05 M, pH 6.2) and the concentrations of cyclic AMP were measured by radioimmunoassay according to the method of Steiner *et al.* [43] using the commercially available kit (Schwarz/Mann, Orangeburg, N.Y.). The results were corrected for recovery, which varied from 62 to 80 per cent. The data were also corrected for the amount of [8-^{14}C]cyclic AMP added. The purity of the cyclic AMP was checked by determination of the nucleotide in aliquots incubated with phosphodiesterase.

Drugs. Epinephrine bitartrate, indomethacin, 2-deoxyglucose and ovalbumin were obtained from Sigma Chemical Co., and DFP from Aldrich Chemical Co. Other reagents and solvents were analytical grade from ordinary commercial sources.

RESULTS

The basal outflows of PGE, $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and histamine from control, sensitized and anaphylactic guinea pig lungs are presented in Fig. 1. Small amounts of PGs and histamine were found in the perfusates from control lungs. The amounts of $PGF_{2\alpha}$, the metabolite and histamine were significantly larger (P values <0.01) in the outflows from the sensitized than from the control lungs. Since the increase in $PGF_{2\alpha}$ was larger than that of PGE, the ratio of PGE to $PGF_{2\alpha}$ decreased from 2.05 ± 0.07 in control to 0.55 ± 0.18 in sensitized animals. Anaphylaxis increased PGE, $PGF_{2\alpha}$, the metabolite and histamine in the perfusates (P values

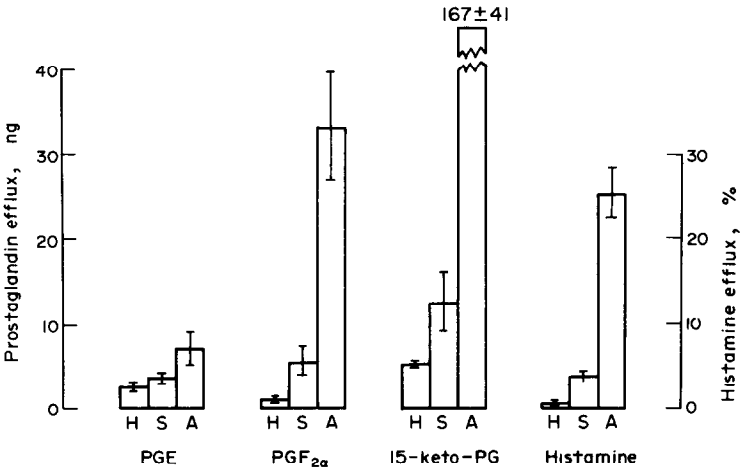

Fig. 1. Efflux of PGE, $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$ (15-keto-PG) and histamine from healthy control (H), sensitized (S) and anaphylactic (A) guinea pig lungs. Lungs from control and ovalbumin-sensitized guinea pigs were perfused with Tyrode's solution via the pulmonary artery. Ovalbumin, 50 mg in 1ml of 0.9% NaCl, or 0.9% NaCl was infused for 30 sec and perfusates were collected for an additional 4 min. The results are expressed as means ± S. E., prostaglandins as ng released and histamine as per cent released. N = eight animals/group. Since ovalbumin had no effect in control animals, only results from control + NaCl, sensitized + NaCl and sensitized + ovalbumin are shown.

<0.01). The ratio of PGE to $PGF_{2\alpha}$ further decreased to 0.20 ± 0.05.

The effects of indomethacin, DFP and 2-DG on cyclic AMP levels in the lung tissue and release of PGs and histamine from sensitized and anaphylactic lungs are shown in Table 1. Anaphylaxis caused an approximately 4-fold elevation in cyclic AMP levels. As in other experiments, the release of PGs and histamine was increased during the antigen–antibody reaction. Indomethacin did not affect the cyclic AMP levels in the sensitized lungs, but it significantly diminished the rise in cyclic AMP in the anaphylactic lungs ($P < 0.01$). The outflows of PGE, $PGF_{2\alpha}$ and the metabolite from both sensitized and anaphylactic lungs were decreased (P values < 0.01). The release of histamine was not changed in sensitized but was reduced in anaphylactic lungs ($P < 0.01$).

DFP did not change the cyclic AMP levels in the sensitized lungs. In the anaphylactic lungs, the levels were markedly reduced ($P < 0.01$). The amounts of PGE, $PGF_{2\alpha}$ and the metabolite in the outflows were diminished both in the sensitized (P values < 0.05)

Table 1. Effect of enzyme and metabolic blockers on tissue levels of cyclic AMP and release of PGE, $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}/E_2$ (15-keto-PG) and histamine from sensitized and anaphylactic guinea pig lungs*

	Control	IND	DFP	2-DG
Sensitized				
Cyclic AMP	1.6 ± 0.2	1.1 ± 0.3	1.3 ± 0.2	1.4 ± 0.1
PGE	3.4 ± 0.8	1.0 ± 0.3	1.2 ± 0.2	1.5 ± 0.4
$PGF_{2\alpha}$	5.5 ± 1.7	1.7 ± 0.2	1.4 ± 0.3	1.9 ± 0.3
15-Keto-PG	12.5 ± 3.5	7.0 ± 3.5	6.1 ± 1.5	7.9 ± 3.3
Histamine	3.8 ± 0.3	3.0 ± 0.4	1.5 ± 0.2	1.4 ± 0.2
Anaphylactic				
Cyclic AMP	6.7 ± 1.1†	3.2 ± 0.6‡	3.0 ± 0.5‡	3.5 ± 0.6
PGE	7,1 ± 2.1†	2.3 ± 0.7‡	3.8 ± 1.3‡	12.8 ± 2.7
$PGF_{2\alpha}$	33.3 ± 2.6†	5.0 ± 3.4‡	13.7 ± 3.1‡	25.7 ± 4.2
15-Keto-PG	167.2 ± 41.8†	9.8 ± 4.7‡	5.8 ± 1.2‡	96.7 ± 50.8
Histamine	25.1 ± 3.2†	11.7 ± 2.2‡	2.7 ± 0.4‡	20.0 ± 3.4

* Lungs from ovalbumin-sensitized guinea pigs were perfused with Tyrode's solution via the pulmonary artery. Ovalbumin, 50 mg in 1 ml of 0.9% NaCl, or 1 ml 0.9% NaCl, was infused for 30 sec and the perfusates were collected for an additional 4 min. Infusion of indomethacin (IND, 10 μM) di-isopropylfluorophosphate (DFP, 5 mM), or 2-doexyglucose (2-DG, 15 mM) was started 5 min before administration of ovalbumin or saline and continued until the end of the experiment. Results are expressed as mean ± S. E., cyclic AMP as nmoles/g of lung wet weight, prostaglandins as ng released and histamine as per cent released. N = eight and five lungs/group, for control and treated lung preparations respectively. Statistical differences of most interest, from sensitized controls and anaphylactic controls, are indicated in the subsequent footnotes. For complete statistical analysis, see the text.

† Sensitized control, P values < 0.01.

‡ Anaphylactic control, P values < 0.01.

Table 2. Effect of epinephrine on tissue levels of cyclic AMP and release of PGE, $PGF_{2\alpha}$ and histamine from sensitized and anaphylactic guinea pig lungs*

Treatment	Cyclic AMP	PGE	$PGF_{2\alpha}$	Histamine
I NaCl	1.8 ± 0.2	3.5 ± 0.9	4.7 ± 1.0	3.7 ± 0.8
II Ovalbumin	8.1 ± 1.3	8.5 ± 2.1	14.3 ± 3.4	30.0 ± 6.1
III Epinephrine, 5 min				
Epi 1 + NaCl	2.3 ± 0.4	3.5 ± 0.3	4.1 ± 0.6	1.1 ± 0.2†
Epi 10 + NaCl	6.4 ± 1.2†	2.6 ± 0.4	4.2 ± 0.2	1.6 ± 0.3†
Epi 1 + ovalbumin	11.1 ± 2.0	4.8 ± 1.1‡	5.9 ± 0.7‡	14.9 ± 2.3‡
Epi 10 + ovalbumin	9.2 ± 3.2	3.9 ± 0.4‡	6.4 ± 1.5‡	17.6 ± 4.0‡
IV Epinephrine, 3 min				
NaCl + Epi 1	2.1 ± 0.3	3.7 ± 0.4	4.3 ± 0.9	4.4 ± 0.2
NaCl + Epi 10	5.1 ± 0.8†	3.1 ± 0.8	4.5 ± 0.8	3.9 ± 1.3
Ovalbumin + Epi 1	16.7 ± 1.9‡	8.4 ± 2.1	16.7 ± 5.3	39.0 ± 4.9
Ovalbumin + Epi 10	14.5 ± 1.8‡	4.9 ± 0.9	11.4 ± 2.4	27.9 ± 6.3

* Lungs from ovalbumin-sensitized guinea pigs were perfused with Tyrode's solution via the pulmonary artery. Perfusates were collected for 5 min and 0.9% NaCl (I) or 100 mg ovalbumin in 0.9% NaCl (II) was infused during min 2 of collection. Epinephrine (1 or 10 μg/ml) was infused during the entire 5-min period (III) or only the last 3 min (IV). The results are expressed as means ± S. E., cyclic AMP as nmoles/g of lung wet weight, prostaglandins as ng released and histamine as per cent released. N = five animals/group. Statistical differences of most interest, from sensitized controls (I, NaCl) and anaphylactic controls (II, ovalbumin) are indicated in the subsequent footnotes. For complete statistical analysis, see the text.

† Sensitized control, P values < 0.01.

‡ Anaphylactic control, P values < 0.01.

and anaphylactic (P values < 0.01) animals. The liberation of histamine was inhibited in a similar manner.

2-DG had no effect on the cyclic AMP levels in the sensitized lungs. In anaphylactic lungs, the elevation of cyclic AMP levels was decreased (P < 0.05). In the sensitized animals, 2-DG diminished the release of PGE, $PGF_{2\alpha}$ and histamine (P values < 0.05). However, in anaphylaxis, the changes in PG and histamine did not reach the conventional levels of significance.

The effects of epinephrine and anaphylaxis on cyclic AMP levels and the release of PGs and histamine are shown in Table 2. The anaphylactic reaction (ovalbumin infusion), as compared to the sensitized non-challenged control state (NaCl infusion), elevated the cyclic AMP levels and also increased the outflows of PGE, $PGF_{2\alpha}$ and histamine (P values < 0.01).

Infusion of epinephrine, 1 or 10 μg/ml, over a period of 5 min in sensitized non-challenged lungs suppressed (P < 0.05) the spontaneous release of histamine without influencing that of the PGs. The higher epinephrine concentration also increased the tissue levels of cyclic AMP. In anaphylactic lungs (ovalbumin was given 1 min after the start of epinephrine infusion), both epinephrine concentrations reduced (P values < 0.05 and 0.01) the release of histamine and PGs but had no clear-cut effect on cyclic AMP accumulation.

Infusion of epinephrine, 1 or 10 μg/ml, over a period of 3 min had no effect on the release of PGs and histamine in sensitized non-challenged lungs. However, the higher epinephrine concentration raised the cyclic AMP levels (P < 0.01). In anaphylactic lungs (epinephrine administration was started immediately after the end of ovalbumin injection), the lower concentration of epinephrine had a potentiating action on the cyclic AMP accumulation (P < 0.05). The release of PGs and histamine, however, was not significantly modified. The higher epinephrine concentration had an additive effect on the cyclic AMP rise caused by the anaphylactic reaction. The mean release of mediators was decreased, reaching significance (P < 0.05) for PGE only. In conclusion, the effects of epinephrine were dose dependent. They also differed when given before or after ovalbumin. A dissociation between the action on cyclic AMP and the release of mediators was also found. In the sensitized non-challenged lungs, only the higher epinephrine concentration, regardless of the duration of administration, elevated the cyclic AMP levels. The outflow of PGs was not influenced. In anaphylactic lungs, both the lower and higher epinephrine concentrations raised the cyclic AMP levels when administered after the ovalbumin challenge. In contrast, the release of PGE, $PGF_{2\alpha}$ and histamine was inhibited only by preinfused epinephrine.

The inhibitory effect of epinephrine on the anaphylactic release of PGs was abolished by preinfusion of propranolol in the final concentration of 1 μg/ml.

A correlation matrix for the data presented in Table 2 was done according to Snedecor and Cochran [44]. The sensitized and anaphylactic groups were analyzed separately. In the sensitized lungs, the outflow of histamine was negatively correlated to that of PGE (P < 0.05), whereas the outflows of PGE and $PGF_{2\alpha}$ gave a positive correlation (P < 0.01). In anaphylactic lungs, there was a positive correlation between the release of histamine and PGE (P < 0.01), histamine and $PGF_{2\alpha}$ (P < 0.001), and PGE and $PGF_{2\alpha}$ (P < 0.001). In these lungs, a positive correlation between cyclic AMP levels and the release of both $PGF_{2\alpha}$ (P < 0.05) and histamine (P = 0.06) was also found. The correlation between cyclic AMP and PGE was not significant.

The time course of the appearance of PGs in the outflows from anaphylactic lungs and its relation to that of histamine as well as the influence of epinephrine on these events are presented in Figs. 2 and 3. The largest amount of PGE was found 1 min after the infusion of ovalbumin (Fig. 2). Preinfusion of

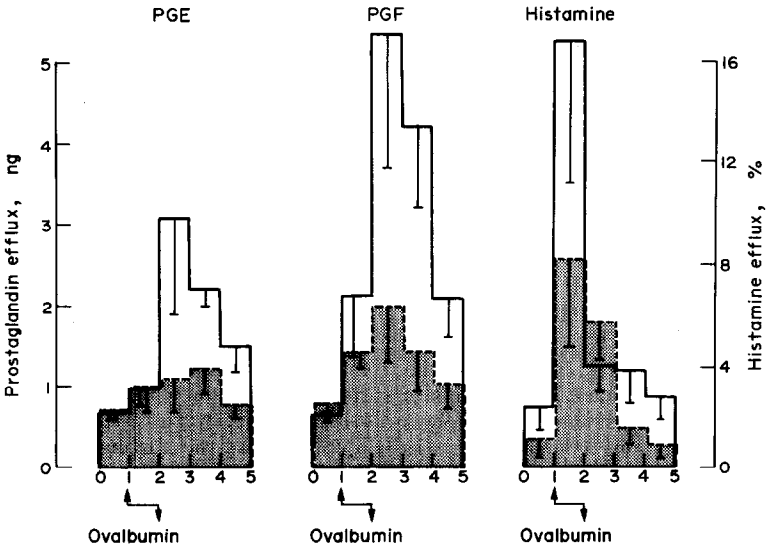

Fig. 2. Spontaneous and ovalbumin-elicited release of PGE, $PGF_{2\alpha}$ (PGF) and histamine from sensitized guinea pig lungs. Lungs from ovalbumin-sensitized guinea pigs were perfused with Tyrode's solution via the pulmonary artery. Ovalbumin, 100 mg in 1 ml of 0.9% NaCl, was infused for 1 min. Shaded area represent experiments in which epinephrine, 10 μg/ml, was infused from 1 min before ovalbumin until the end of perfusate collection. The results are expressed as means ± S. E., prostaglandins as ng released and histamine as per cent released. N = five animals/group.

epinephrine, 10 μg/ml, did not change the basal outflow of PGE but effectively suppressed the anaphylactic release of PGE ($P < 0.01$). The highest concentration of $PGF_{2\alpha}$ in the outflows was also reached 1 min after ovalbumin challenge. Preinfusion of epinephrine raised the mean baseline $PGF_{2\alpha}$ concentration, but the differences were not significant. The anaphylactic outflow of $PGF_{2\alpha}$ was inhibited by epinephrine ($P < 0.01$). The highest concentrations of histamine were found in the perfusate concurrent with the infusion of ovalbumin. Epinephrine antagonized both the spontaneous and anaphylactic release of histamine ($P < 0.01$ for the anaphylactic release). As shown in Fig. 3, the maximal outflows of $PGF_{2\alpha}$ and PGE were simultaneous and occurred 1 min after the maximal outflow of histamine. More $PGF_{2\alpha}$ than PGE was found in the total perfusates, as the concentrations were higher and the duration of elevated release was somewhat longer. Maximal mean per cent increase elicited by antigen challenge was approximately 700, 800 and 400 for histamine, $PGF_{2\alpha}$ and PGE respectively.

DISCUSSION

In previous investigations, either no PGs and histamine [45], or only traces of PGs in lung perfusates from healthy animals have been reported [6, 7]. In this study, using an improved assay, PGs in outflows could be quantified. Significantly more PGs were

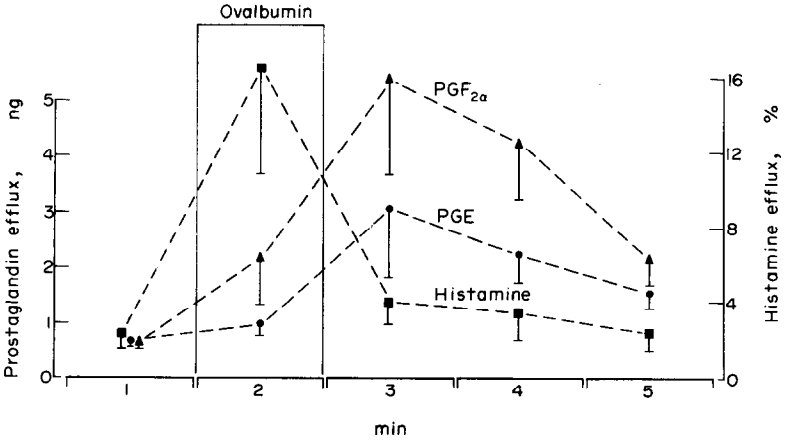

Fig. 3. Time course of histamine and prostaglandin appearance in guinea pig lung perfusates during anaphylactic reaction. Lungs from ovalbumin-sensitized guinea pigs were perfused with Tyrode's solution via the pulmonary artery. Ovalbumin, 100 mg in 1 ml of 0.9% NaCl, was infused for 1 min. The results are expressed as means ± S. E., prostaglandins as ng released and histamine as per cent released. N = five animals/group.

found in the perfusates from sensitized than control lungs. Moreover, since $PGF_{2\alpha}$ was increased more than PGE, the ratio of PGE to $PGF_{2\alpha}$ decreased. Histamine was also significantly augmented in the same perfusates. Similar results were also obtained in experiments with guinea pig lung fragments incubated in Tyrode's solution (unpublished data). The levels of PGs were not measured in the lung tissue, since it is generally assumed that they are not stored in tissues but are synthesized *de novo*. However, the level of histamine, which is prestored, was determined in the lungs. No differences were found between the control and sensitized guinea pigs (means ± S. E.: 13.08 ± 2.71 and 14.2 ± 3.1 μg/g of lung wet weight. N = 18 lungs/group. Thus, the increased amounts of histamine found in the outflows were not simply a reflection of an increase in lung content. This was even more apparent when histamine data were expressed as per cent release, which is obtained by taking into account the lung histamine content. The results would seem to suggest an enhanced ability to synthesize and release PGs and an increased turnover of histamine in the sensitized lung. Bronchial hyperreactivity to histamine and to $PGF_{2\alpha}$ has been reported [18, 19, 46, 47]. In animals, an increased mortality to injected histamine and an altered pattern of cyclic nucleotide system response to the autonomic nervous system transmitters and autacoids after immunization with *Bordetella pertusis* or ovalbumin have been described [21–23, 48–50]. These investigations and the data reported here indicate that antigen sensitization may change the metabolic and enzymatic properties of an organism.

Anaphylaxis further increased the outflows of PGs and histamine, which agrees with other reports in the literature [6, 7, 45, 51]. It is of interest that the PGE to $PGF_{2\alpha}$ ratio declined in sensitized compared to control lungs, and again in anaphylactic compared to sensitized lungs, as there was a larger increase in $PGF_{2\alpha}$ than PGE. Whether this is due to a larger increase in synthesis of $PGF_{2\alpha}$ than PGE or conversion of PGE to $PGF_{2\alpha}$ is not known. Our preliminary data (unpublished) indicate a faster conversion of PGE to $PGF_{2\alpha}$ in anaphylactic and sensitized lungs as compared to control lungs.

Indomethacin abolished the increased outflows of PGs during anaphylaxis and also decreased the release of histamine. This is in agreement with the results obtained on human lung tissue [51], but not with the data from another study on guinea pig lung [45]. Intradermally injected PGE_1 and PGE_2 in man and rat appear to liberate histamine [52]. Prostaglandins can also release histamine from human and guinea pig lung preparations [11, 53]. Thus, the effects of indomethacin on histamine release could have been secondary either to its general membrane-stabilizing property, or to its inhibition of PG synthesis.

It has been demonstrated in several preparations that cyclic AMP analogs and substances raising the endogenous levels of cyclic AMP diminish the subsequent release of mediators. Conversely, low cyclic AMP levels enhance the release of mediators in anaphylaxis [4, 27, 29, 30]. In the present experiments, low cyclic AMP levels after pretreatment with indomethacin did not result in enhanced release of PGs and histamine, suggesting that the release was inhibited primarily and that the decrease in cyclic AMP change by anaphylaxis was at least in part secondary to this inhibition. Consistent with such an explanation is the finding that indomethacin, in the concentration used, had no effect on adenylate cyclase or phosphodiesterase activity in guinea pig lungs (data not shown). It should be kept in mind that the cyclic AMP levels were measured 4.5 min after the introduction of antigen challenge. Thus, the possible early changes in cyclic AMP, preceding or simultaneous with the major release of mediators, would not be detected. The results presented here reflect a state when most of the histamine and PGs had already been released and could have acted to change the tissue levels of cyclic AMP.

DFP blocks the anaphylactic release of histamine from guinea pig, rat and human tissues, possibly by inhibiting activation of a serine esterase [35, 36, 54]. In this experiment, DFP also markedly inhibited the release of PGE, $PGF_{2\alpha}$ and the metabolite in sensitized and anaphylactic lungs. While activation of serine esterase could be as important for the release of PGs as it is for histamine, other known effects of DFP, such as inhibition of a variety of enzymes, could constitute the mode of action. The inhibition of cyclic AMP rise during anaphylaxis by DFP indicates further that the cyclic AMP elevation several minutes after the beginning of the antigen–antibody reaction [24] may be predominantly due to the release of mediators.

Anaphylactic release of histamine is blocked by metabolic inhibitors, e.g. 2-DG [37, 54, 55]. In this investigation, 2-DG had only a minor inhibitory effect on the liberation of histamine. The mean release of $PGF_{2\alpha}$ and the metabolite was not significantly decreased. These results are in agreement with a report [56] that the release of PGs from rabbit heart was not modified by 2-DG and lack of glucose. However, rat mast cells incubated without metabolic substrates can keep up their energy production by utilizing endogenous sources and thereby allowing for a maximal histamine release [57]. The absence of a 2-DG effect, therefore, is not *per se* a proof of independence from energy requirements. Further experiments are necessary to elucidate this issue.

Epinephrine markedly diminished the anaphylactic release of PGs, confirming our previous finding [7]. It is noteworthy that the mean inhibitions of PGE, $PGF_{2\alpha}$ and histamine in the outflow were quite similar [58, 62 and 54 per cent respectively]. The signs and symptoms of the antigen–antibody reaction in lungs and other organs are alleviated by epinephrine. These effects are presumably mediated via the beta-adrenergic receptors. The inhibitory effect of epinephrine on the release of PGs, which was also antagonized by propranolol, is probably mediated via the beta-adrenergic receptor as well. It has been claimed that bronchoconstriction or distortion of the cell membrane induces synthesis and release of PGs [58]. As epinephrine counteracts bronchoconstriction, this effect could at least partially account for the results obtained. Another property of epinephrine is inhibition of the antigen-induced release of histamine [3, 4, 28]. If release of PGs is secondary to that of histamine, as some data suggest [7, 32, 33 45], then

suppression of histamine release should also be followed by a decrease in PG release. The high correlations, with and without epinephrine treatment, between anaphylactic release of histamine and PGs are consistent with such a possibility. The mechanism of histamine inhibition by epinephrine is by elevation of cyclic AMP levels[29]. Other substances which raise cyclic AMP also inhibit the release of histamine [11, 30, 59]. Subsequently, dibutyryl cyclic AMP was found to decrease not only the release of histamine but also that of PGs from both whole perfused and sliced anaphylactic guinea pig lungs (data not shown). Thus, an additional possibility is that the same process may lead to the release of histamine and PGs and that the intracellular levels of cyclic AMP control this process.

Epinephrine given 60 sec after the start of anaphylactic challenge had no effect on the outflow of histamine and PGs. It is possible that the anaphylactic process had already been fully developed, and the largest proportion of histamine and PGs released before epinephrine could exert its effect. In contrast, the effect of anaphylaxis on cyclic AMP accumulation was enhanced by epinephrine. As previously suggested [24], the "late" rise of cyclic AMP during anaphylaxis could be predominantly due to the release of histamine and PGs. An additional increase in cyclic AMP by a low concentration of epinephrine, which did not affect the anaphylactic release of mediators and does not change the cyclic AMP levels in sensitized non-challenged lungs, requires an explanation. In sensitized as compared to control animals, adenylate cyclase in lung homogenates is hypersensitive to stimulation with epinephrine, while in whole perfused lungs the accumulation of cyclic AMP in response to the same stimulus is diminished [21, 22]. Furthermore, altered uptake and metabolism of catecholamines have been found in anaphylactic lung [38, 60]. On the basis of these data it is suggested that the bio-availability of epinephrine at the appropriate receptor sites may be increased as a consequence of decreased catecholamine metabolism or increased membrane permeability secondary to the antigen–antibody binding [61, 62]. Epinephrine thus made available would act on the hypersensitive adenylate cyclase to raise cyclic AMP levels.

Changes in the time course of the outflow of histamine, PGE and $PGF_{2\alpha}$ would seem to indicate that the release of histamine preceded that of PGE and $PGF_{2\alpha}$, the latter two appearing simultaneously. This is consistent with the notion that histamine is a "primary" mediator of the antigen–antibody reaction [27] and that PGs are the "secondary" mediators, possibly released by the primary mediators [7, 32, 33]. However, the converse may also be true: under some circumstances PGs may release histamine [11, 52,53, 63]. Another possibility is that the initial release is simultaneous, reflecting an identical underlying mechanism, but the time required to reach the pulmonary circulation and be washed out is different for histamine and PGs.

In conclusion, while previous investigation of mediators and cyclic nucleotides have dealt with the differences between the control and anaphylactic lung, one of the aims of this study was to compare the control and sensitized animals. The increased spontaneous outflow of PGs and histamine after antigen sensitization found in this study, taken together with other experimental work, supports the view that sensitization alters biological properties of the organism [22, 23], and indicates that the state of sensitization in itself is different from both the healthy state and anaphylaxis. Differences in cyclic AMP accumulation after epinephrine stimulation in sensitized and anaphylactic lungs, as presented here, and in control and sensitized guinea pig lungs [22] are consistent with such a hypothesis.

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