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The release of spasmogenic substances from human chopped lung tissue and its inhibition

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Summary

1. Human lung tissue, passively sensitized with reaginic antibodies, released prostaglandins E_1, E_2 and $F_{2\alpha}$ in addition to histamine and slow reacting substance (SRS-A), when exposed to the appropriate antigen. No rabbit aorta contracting substance (RCS) was detected.

2. Experiments with rats and guinea-pigs showed that the release of RCS is not confined to anaphylactic reactions mediated by non-reaginic antibodies but may be a feature of anaphylaxis in guinea-pigs alone.

3. Human lung tissue gently agitated with a blunt nylon rod liberated an E-type prostaglandin and RCS in addition to histamine and SRS-A.

4. Human isolated bronchial muscle was contracted by RCS.

5. Disodium cromoglycate antagonized the release of prostaglandins during anaphylaxis but not during agitation of human lung tissue, whereas indomethacin blocked the release of prostaglandins during agitation and anaphylaxis.

6. The release of an E-type prostaglandin during anaphylaxis in human lung tissue, which inhibits the further release of histamine could be another example of the regulatory role of prostaglandins in body functions.

Introduction

In addition to the then known mediators of anaphylaxis, Piper & Vane (1969a) demonstrated the release of prostaglandins and rabbit aorta contracting substance (RCS) from isolated perfused lungs of guinea-pigs sensitized to ovalbumen, when these were challenged by intra-arterial injection of antigen. These substances were also released when chopped guinea-pig lung tissue was gently agitated (Palmer, Piper & Vane, 1970).

The anaphylactic reaction in ovalbumen sensitized guinea-pigs is a result of the interaction between non-reaginic antibodies of the IgG class and the challenging antigen (Ovary, Benacerraf & Bloch, 1963). Human isolated lung tissue can be passively sensitized with IgE antibody by incubation in sera from asthmatic patients which contain a relatively high concentration of reaginic antibody. Histamine and slow reacting substance (SRS-A) are known to be released when tissue sensitized in this way is subsequently challenged with the corresponding antigen (Sheard, Killingback & Blair, 1967) and both are bronchoconstrictor in man (Brocklehurst,

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1956). Prostaglandin $F_{2\alpha}$ is also broncho-constrictor in man (Sweatman & Collier, 1968) whereas prostaglandins E_1 and E_2 are bronchodilator (Cuthbert, 1969; Smith & Cuthbert, 1972; Herxheimer & Roetscher, 1971). RCS may be a precursor of prostaglandins (Gryglewski & Vane, 1971) but its effect on human bronchial muscle is not known. The following experiments were carried out to determine whether prostaglandins and RCS are released from human lung in anaphylaxis involving reaginic antibodies; to determine the nature of the prostaglandin(s) which might be released and to assess the possible significance of the release in the aetiology of asthma. The results obtained in the initial experiments prompted further investigations concerning the release of prostaglandins and RCS from human lung tissue by agitation and from lung tissue of other animal species, where the anaphylactic reaction was also mediated by reaginic antibodies. The abilities of disodium cromoglycate and indomethacin to antagonize the release of spasmogens from challenged, passively sensitized, human lung tissue and from agitated human lung tissue were compared.

Methods

Experiments with human lung

The following experiments were carried out on 26 samples of human lung. Macroscopically normal samples were obtained at pneumonectomy and immediately after excision, were placed in cold Tyrode solution (20° C) which had previously been gassed with 5% CO_2 and 95% O_2. The lung parenchyma was chopped with scissors into pieces of approximately 2 mm^3. Bronchial tissue was not chopped and where possible the bronchioles were dissected out, cut spirally and used as assay tissues. About 5 g of chopped lung tissue was washed in large volumes (about 2 l. in all) of Tyrode solution until the washing fluid was clear and incubated overnight at room temperature in 40 ml diluted serum from patients sensitive to *Dermatophagoides farinae*. Two different sera were used for sensitization and these were diluted either 1 in 4 or 1 in 8 with Tyrode solution according to their titre of reaginic antibodies. Chopped and washed lung tissue was also incubated overnight in Tyrode solution or in serum previously heated to 56° C for 4 h to destroy reaginic antibodies, to provide non-sensitized control tissue.

After passive sensitization, the lung tissue was washed and divided into aliquots of approximately 420 mg wet weight, as needed. A single aliquot of lung was spread out on nylon mesh which was stretched over a polythene funnel of 6 cm maximum diameter (Fig. 1). Krebs solution gassed with 5% CO_2 and 95% O_2 and warmed to 37° C was superfused at 5 ml/min over the chopped lung tissue. The funnel was placed above a series of 6 assay tissues arranged in two banks of three. The effluent from the lung superfused first one bank and then the other. The assay tissues included cat or kitten terminal ileum to detect histamine, the longitudinal smooth muscle layer from guinea-pig ileum (Rang, 1964) and spiral strips of human bronchus to detect SRS-A, rabbit aorta to detect RCS, rat stomach strip, rat colon and chick rectum to detect prostaglandins. All tissues except cat terminal ileum were treated with a combination of antagonists (referred to as combined antagonists) to eliminate the actions of histamine, catecholamines, 5-hydroxytryptamine and acetylcholine (Piper & Vane, 1969a). In most experiments, the combination of antagonists was hyoscine hydrobromide (0·1 μg/ml), methysergide bimaleate (0·2 μg/ml), mepyramine maleate (0·1 μg/ml), propranolol hydrochloride (2 μg/ml)

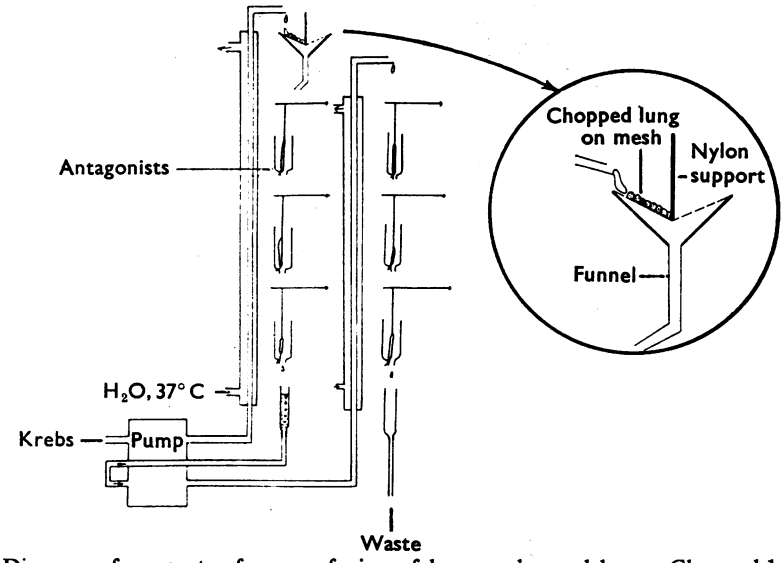

FIG. 1. Diagram of apparatus for superfusion of human chopped lung. Chopped lung tissue was spread on nylon mesh stretched over a polythene funnel of 6 cm diameter (see inset). A roller pump dripped Krebs solution (gassed with 95% oxygen, 5% carbon dioxide and heated to 37° C) at 5 ml/min over the chopped lung. The effluent from the funnel superfused six assay tissues which were arranged in two banks. After superfusing the first bank, the effluent was collected in a reservoir, regassed, rewarmed and pumped over the second bank of assay tissues. Combined antagonists were superfused over some or all of the assay tissues.

and phenoxybenzamine hydrochloride (0·1 μg/ml), but in some experiments propranolol and phenoxybenzamine were omitted because they depressed the sensitivity of guinea-pig ileum smooth muscle. The antigen used to challenge the sensitized lung tissue was a freeze-dried, partially purified extract of *D. farinae*. Changes in smooth muscle tension were recorded by Harvard smooth muscle transducers and records displayed on a Watanabe six channel pen recorder.

Experiments with rat lung

Two methods were used to stimulate the production of reaginic antibodies in rats.

(a) *Infection with* Nippostrongylus braziliensis

Female Sprague Dawley rats (100–150 g) were infected subcutaneously with about 5,000 larvae of *N. braziliensis*. Four to six weeks later the animals were killed. At this time there is a high titre of reaginic antibodies in the serum of the infected rats and the target cells of the lung tissue are sensitized with cell bound antibody (Ogilvie, 1964). The lungs were excised, finely chopped with a McIlwain chopper, washed free of blood and stored in Tyrode or Krebs solution until used. The antigen used to challenge the actively sensitized lung tissue was a homogenate of *Nippostrongylus braziliensis* larvae partially purified by centrifugation and Sephadex G 50 column separation. The antigen-containing fractions eluted from the column were freeze-dried and stored for use.

(b) *Immunization with ovalbumen and* Bordetella pertussis

Rats of either sex, 100–150 g, were sensitized by injection of ovalbumen 5 mg/kg intramuscularly and *B. pertussis* vaccine (Wellcome, 4×10^{10} organisms/ml) 0·5 ml

intravenously (Mota, 1964; Sheard & Blair, 1970). Eight to 12 days later the rats were killed, their lungs perfused by the method described for guinea-pig lungs (Piper & Vane, 1969a) and challenged by injection of 2 mg of ovalbumen into the pulmonary artery.

Experiments with guinea-pig lung

Reaginic antibody formation was promoted in Hartley guinea-pigs (200 g) by oral infection with about 500 larvae of *Trichinella spiralis*. Animals were bled by heart puncture five weeks after infection, when both reaginic and non-reaginic antibodies are present in the blood (Catty, 1970).

For passive sensitization, the serum obtained from infected animals was divided into two portions. One of these was heated at 56° C for 4 h to destroy the heat labile reagins. These two samples of serum were used to sensitize normal guinea-pigs by injection of 2 ml/kg intravenously. After an interval of eight days from passive sensitization, non-reaginic antibodies no longer remain in the tissues of the animals, whilst reaginic antibodes are still fixed to the target cells of the lung and skin (Catty, 1970). Animals passively sensitized with heated serum therefore had no exogenous antibodies remaining after 8 days and served as controls. At this time, the animals were killed, their lungs excised and prepared in the same way as rat lung. Lungs from non-sensitized guinea-pigs were also used as controls. The antigen used to challenge the *Trichinella*-sensitized animals was prepared from a sonicated suspension of *Trichinella* infective larvae (Catty, 1970).

Recovery of prostaglandin-like material

Perfusate from the lung tissue was collected after superfusion over the assay tissues, acidified to pH 2–3 and extracted twice with equal volumes of ethyl acetate. The ethyl acetate phase was evaporated to dryness under vacuum and the residue resuspended in 0·2–0·5 ml ethanol. Half of the extract was diluted with Krebs solution and the content of prostaglandin-like material estimated by bioassay. The remainder of the extract was subjected to thin-layer chromatography by the AI and AII solvent systems of Gréen & Samuelsson (1964). After running the plates in the solvent systems, the amounts of prostaglandin-like material were estimated by bioassay (Piper & Vane, 1969b).

Drugs used

Disodium cromoglycate (Fisons Pharmaceuticals Ltd.), histamine acid phosphate (May & Baker Ltd.), hyoscine hydrobromide (British Drug Houses Ltd.), indomethacin (Merck, Sharp & Dohme Ltd.), mepyramine maleate (May & Baker Ltd.), methysergide bimaleate (Sandoz Products Ltd.), phenoxybenzamine hydrochloride (Smith, Kline & French Ltd.), (±)-propranolol hydrochloride (Imperial Chemical Industries Ltd.), prostaglandin E_1, E_2 and $F_{2\alpha}$ (Upjohn Company).

Results

Release of prostaglandins

Histamine and SRS-A were released in all experiments with human lung whether the tissue was stimulated by antigen challenge or agitation (Figs. 2 and 3). Contractions of rat stomach strip, rat colon and chick rectum also occurred with either

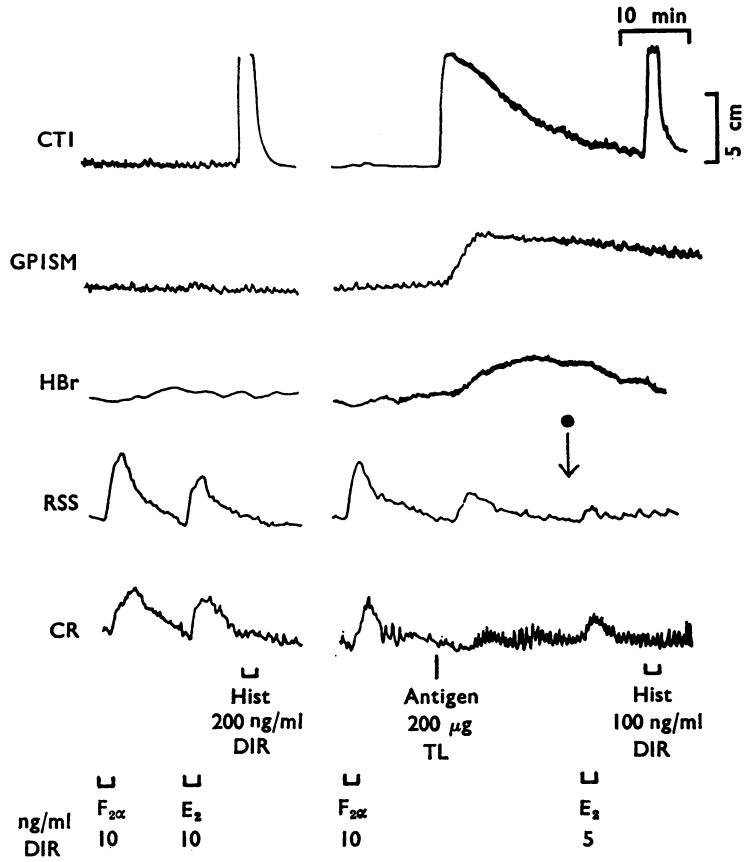

FIG. 2. Challenge of human passively sensitized lung tissue. The effluent from human passively sensitized lung tissue was superfused over a strip of cat terminal ileum (CTI), a strip of smooth muscle from guinea-pig ileum (GPISM), a spiral strip of human bronchiole (HBr), a rat stomach strip (RSS) and a chick rectum (CR). All tissues except CTI were blocked with mepyramine, hyoscine and methysergide. Calibrating doses of prostaglandin $F_{2\alpha}$, prostaglandin E_2 and histamine (Hist), were given directly (DIR) to all assay tissues except at ↓ which indicates that prostaglandin E_2 5 ng/ml was given only to RSS and CR. TL indicates through lung tissue.

stimulus indicating a release of prostaglandin-like material (Figs. 2, 3, 4, 5). The contractions could not be exactly matched by infusions of either prostaglandin E_2 or $F_{2\alpha}$ alone which suggested that a mixture of prostaglandins was released. The mean peak concentrations of histamine and prostaglandins (expressed as prostaglandin E_2 equivalents) released by antigen challenge or agitation in up to 14 separate experiments, are shown in Table 1. The amount of antigen used to challenge and the period of agitation were selected in order to match the quantities of histamine released as far as possible. Results were calculated by bracketing assay of the effluent from 420 mg of lung tissue, on the cat terminal ileum for histamine and rat stomach strip for prostaglandin E_2. The mean peak concentrations of histamine released by the two stimuli were not significantly different as calculated by Student's t test, but the mean peak concentration of prostaglandin released by agitation was significantly greater than that released by antigen challenge ($P<0{\cdot}01$).

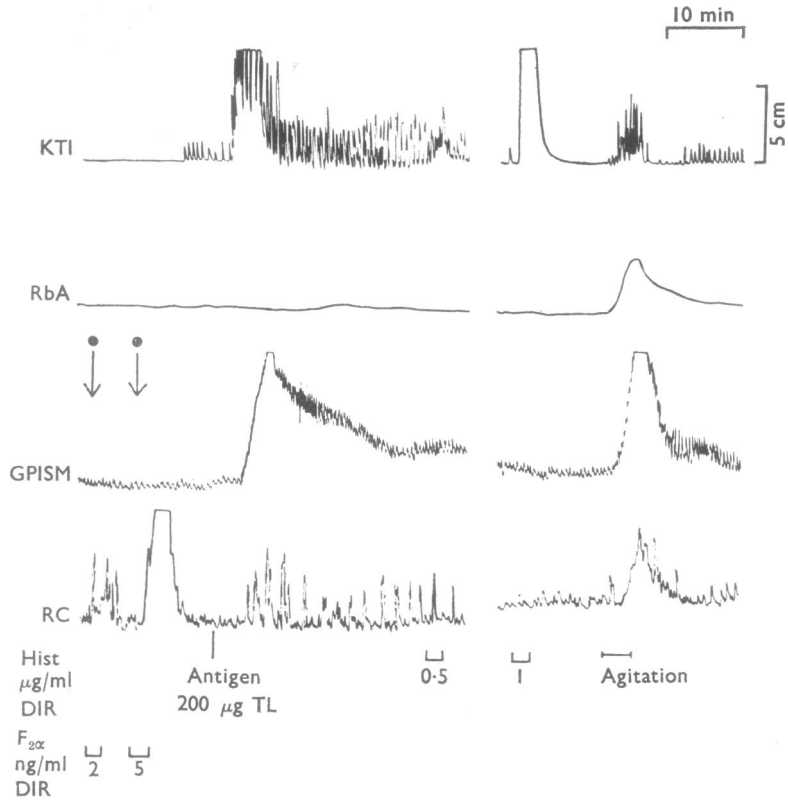

FIG. 3. Challenge and agitation of passively sensitized human lung. The effluent from passively sensitized human lung tissue was superfused over a strip of kitten terminal ileum (KTI), a spiral strip of aorta from a male rabbit (RbA), a strip of smooth muscle from guinea-pig ileum (GPISM) and a rat colon (RC). Tissues were treated with antagonists as in Figure 2. Calibrating doses of prostaglandin $F_{2\alpha}$ and histamine were given directly to the assay tissues. ⇩ Indicates doses given only to GPSIM and RC.

TABLE 1. *Comparison of the amounts of prostaglandin-like material and histamine released by antigen challenge or agitation of sensitized human lung tissue*

Stimulus	No. of experiments	Mean peak concentrations released ± S.E. of mean Histamine (ng/ml)	Prostaglandin (as E_2 ng/ml)
Antigen challenge (200 μg)	14	184·6±49·3	5·8±1·0
Agitation (4 min)	7	146·0±36·0	24·1±4·3

In 7 further experiments, the prostaglandin content of the effluents from antigen-challenged or agitated human lung were calculated after extraction and thin layer chromatography (Table 2). The quantity of E-type prostaglandin released by agitation was considerably greater than that released by antigen challenge, thus confirming the results obtained by bracketing assay. No F-type prostaglandin was detected in perfusate from either challenged or agitated lung when volumes of 100 ml or less were extracted. A small amount of prostaglandin $F_{2\alpha}$ was recovered after extraction of a larger volume of perfusate from challenged lung and thin layer chromatography with the AII solvent system of Gréen & Samuelsson. No prostaglandins were detected after extraction and thin layer chromatography of Krebs solution which had passed through unchallenged sensitized lung tissue. In all experiments where perfusate from lung tissue was extracted and subjected to thin

TABLE 2. *Comparison of the amounts of prostaglandin (PG) released by antigen challenge or agitation of sensitized human lung after extraction and thin layer chromatography (TLC) of the perfusate*

Stimulus	No. of experiments	Lung tissue (mg)	Volume of perfusate (ml)	Solvent	Original conc. of PGs in perfusate calculated from TLC (ng/ml)
Challenge (200 μg)	3	1,680	80	AI	2 E-type
	1	5,460	275	AII	2–4 E_1
					2 E_2
					2 $F_{2\alpha}$
Agitation (4 min)	3	1,680	100	AI	20–30 E-type

layer chromatography, when the prostaglandin activity was estimated by bioassay, there was also some substance present which contracted the chick rectum and was not prostaglandin E_1, E_2 or $F_{2\alpha}$. This may have been a phospholipid as it tended to run fairly low on the plate (van Dorp, 1971).

Attempts were made to increase the amounts of prostaglandin released by varying the antigen concentration used to challenge the lung from 50 to 500 μg, by using larger amounts of tissue (up to 700 mg), by doubling the perfusion rate (to decrease contact time with the lung tissue and so reduce possible enzymic degradation of prostaglandin) and by varying the serum used to sensitize the lung. These measures had no effect on prostaglandin release, although the quantities of histamine and SRS-A released into the perfusate were modified.

Release of rabbit aorta contracting substance

There was no contraction of rabbit aorta in any experiment where human lung was challenged, although the rabbit aorta had previously been shown to be sensitive to guinea-pig RCS. In contrast, when aliquots of the same human lung tissue were agitated, the rabbit aorta contracted on each occasion, indicating the release of RCS (Figs. 3 and 5). If the lung tissue was very gently agitated so that the amount of prostaglandin released was equivalent to that released in challenge (2–5 ng/ml prostaglandin E_2) the rabbit aorta still showed a definite contraction. To check that this RCS had the same characteristics as that described by Piper & Vane (1969a), the perfusate was collected and kept at room temperature until the tissues had relaxed (12 minutes). When the perfusate was recirculated over the same assay tissues, the rabbit aorta did not contract, although the contractions of the remaining assay tissues were not changed.

TABLE 3. *Comparison of the release of histamine, slow reacting substance (SRS-A), prostaglandin and rabbit aorta contracting substance (RCS) from lung tissue of rat, guinea-pig and man sensitized with reaginic antibodies when challenged or agitated*

	Rat lung*		Guinea-pig lung† Challenged, passively sensitized 8 days previously with			Human lung	
	Challenged actively sensitized	Agitated	Unheated serum	Heated serum	Agitated	Challenged passively sensitized	Agitated
Antibody	Reaginic		Reaginic	None		Reaginic	
Histamine	+	+	+	−	+	+	+
SRS-A	+	+	+	−	+	+	+
RCS	−	+	+	−	+	−	+
Prostaglandin	+	+	+	−	+	+	+

* Rats were actively sensitized to *N. braziliensis* or to ovalbumen and *B. pertussis*. † Guinea-pigs were passively sensitized to *T. spiralis*. +, Indicates release; −, indicates no detectable release.

Since RCS was not released when human lung passively sensitized with reaginic antibodies was challenged, it seemed possible that the release of RCS might only occur when the antibodies involved in anaphylaxis were non-reaginic. To clarify this, experiments were carried out with rats sensitized to ovalbumen and *B. pertussis* and to *N. braziliensis* and guinea-pigs sensitized to *T. spiralis*. The results of these qualitative experiments are shown in Table 3. Histamine, SRS-A and prostaglandins were released from challenged lung tissue of all three species when sensitized with reaginic antibodies but RCS was released only from guinea-pig lung. Lung tissue of all three species released RCS on agitation.

Effect of rabbit aorta contracting substance on human bronchial muscle

Agitation of aliquots of human lung tissue released smaller amounts of RCS than challenged guinea-pig lungs. Sensitized guinea-pig lungs were therefore used as the source of RCS in the following experiments. The assay tissues used were rabbit aorta, human bronchus, rat stomach strip, rat colon and chick rectum (2 experiments). These tissues were superfused with the effluent from isolated perfused lungs from guinea-pigs sensitized to ovalbumen. When the lungs were challenged all the assay tissues contracted. After superfusing the tissues, the perfusate from the lungs was collected for 6 min starting 30 s after injection of ovalbumen, and kept at room temperature until the tissues had relaxed (30–45 minutes). When it was reapplied to the tissues (in place of Krebs solution) rabbit aorta no longer contracted showing that RCS had disappeared. The contraction of human bronchus was reduced but chick rectum and rat colon contracted to the same extent and rat stomach strip to almost the same extent showing that prostaglandins were still present. To avoid the possibility of dilution during the collection of perfusate containing RCS, a similar experiment was carried out in which the effluent from guinea-pig sensitized lungs was divided to superfuse simultaneously two banks of three assay tissues, rabbit aorta, human bronchus and chick rectum. When the lungs were challenged, assay tissues in both banks contracted. A new pair of lungs was set up and a length of tubing, introducing a delay of 5 min, was placed between the lungs and one bank of tissues. When the lungs were challenged, the tissues in the first bank contracted as before. In the bank with the delay between the lungs and assay tissues, the contraction of chick rectum was unchanged, that of the rabbit aorta was very much reduced and the contraction of human bronchus was also diminished. These experiments showed that RCS contributed to the contraction of human bronchial muscle.

Antagonism of release of spasmogens

Disodium cromoglycate

Disodium cromoglycate (0·3–20 μg/ml) was perfused through human chopped lung for 5–10 min before and during challenge of the lung (9 experiments). Concentrations of 3–20 μg/ml reduced the amounts of histamine, SRS-A and prostaglandin released during challenge (Fig. 4). When these concentrations of disodium cromoglycate were perfused directly over the assay tissues, they did not alter the sensitivity of the tissues to the substances released during challenge. Histamine and SRS-A release were inhibited in proportion to the dose of disodium cromoglycate applied but the release of prostaglandin was reduced only when that of histamine and

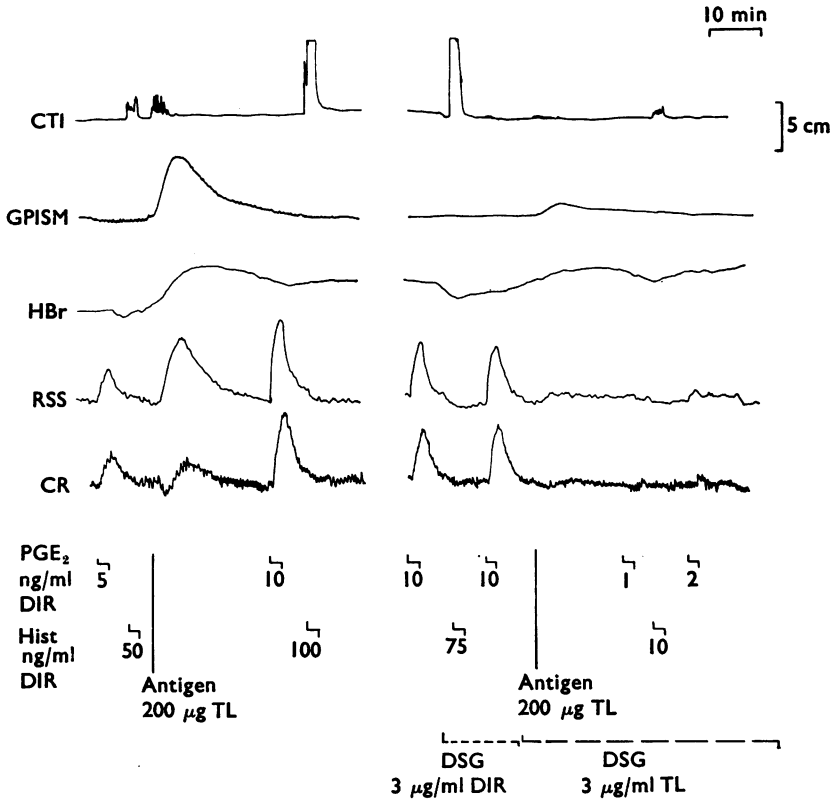

FIG. 4. Effect of disodium cromoglycate (DSG) on release of spasmogens during challenge of passively sensitized human lung. The effluent from passively sensitized lung tissue was superfused over 5 assay tissues as in Figure 2. In the first panel when an aliquot of lung tissue was challenged all the assay tissues contracted showing the release of histamine, SRS-A and prostaglandin-like material. In the second panel, calibrating doses of prostaglandin E_2 and histamine were given while disodium cromoglycate, 3 μg/ml, was perfusing the assay tissues. The same concentration of disodium cromoglycate was then perfused through a fresh aliquot of lung tissue for 5 min before and during challenge. The responses of all the assay tissues were very much reduced.

SRS-A had been almost completely suppressed. When disodium cromoglycate (10 μg/ml) was infused through human lung tissue it had no effect on the release of spasmogens by agitation (3 experiments).

Indomethacin

Sensitized human lung tissue was kept in a solution of indomethacin (1 μg/ml) for one hour before aliquots (420 mg) were removed and challenged, still in the presence of indomethacin. Lung tissue kept in Krebs solution for a similar period and challenged in the absence of indomethacin served as a control. The effluents from the control and treated lung tissue were collected for 4·5 min beginning 30 s after antigen was injected into the Krebs solution perfusing the lung. The prostaglandin content of both perfusates was assayed on the rat stomach strip, chick rectum and rat colon as prostaglandin E_2 equivalents. The remainder of the perfusate, collected during challenge of 14 separate aliquots of indomethacin treated and control lung tissue, was extracted for prostaglandins and the extract subjected to thin layer chromatography by the AII solvent system.

Immediate bioassay of the perfusate from the challenged control tissue showed that it contained approximately 5 ng/ml prostaglandin E_2 equivalents, that from indomethacin-treated lung contained no detectable quantities of prostaglandins. Assay of samples scraped from the chromatography plate showed that the original perfusate from the control tissue had contained 2–4 ng/ml prostaglandin E_1, 2 ng/ml prostaglandin E_2 and 2 ng/ml prostaglandin $F_{2\alpha}$, whereas that from the indomethacin-treated lung had contained only 0·5 ng/ml prostaglandin E_1, 0·5 ng/ml prostaglandin E_2 and approximately 1 ng/ml prostaglandin $F_{2\alpha}$. These concentrations of prostaglandins were too small to have been detected in the immediate bioassay. Histamine and SRS-A were not assayed.

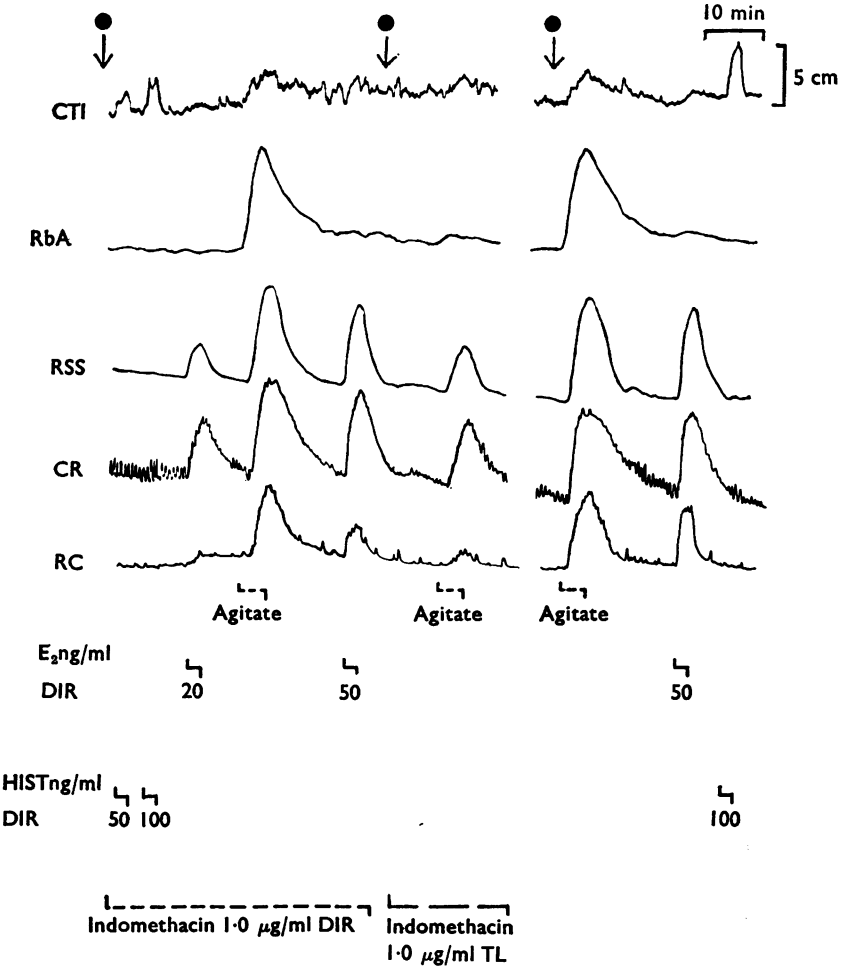

FIG. 5. Release of histamine, rabbit aorta contracting substance (RCS) and prostaglandins from human lung by agitation. The effluent from human chopped lung was superfused over a strip of cat terminal ileum (CTI), a spiral strip of aorta from a rabbit (RbA), a rat stomach strip (RSS), a chick rectum (CR) and a rat colon (RC). Tissues were treated as in Figure 2. ↓ Indicates a fresh aliquot of lung tissue. Indomethacin (1 μg/ml) was superfused (DIR) and calibrating doses of histamine and prostaglandin E_2 given. The lung tissue was gently agitated for 4 minutes. All the assay tissues contracted showing the release of histamine, RCS and prostaglandin-like material. Indomethacin 1 μg/ml was then infused through a fresh aliquot of lung tissue (TL). On agitation, there was no contraction of RbA and contractions of CTI, RSS, RC and CR were reduced. After indomethacin had been washed out of the system, agitation of the next aliquot of lung tissue again caused release of histamine, RCS and prostaglandin-like material.

TABLE 4. *Effects of disodium cromoglycate and indomethacin on the release of histamine, slow reacting substance (SRS-A), prostaglandin and rabbit aorta contracting substance (RCS) from human lung tissue, when agitated or challenged with antigen*

	Disodium cromoglycate (10 μg/ml)		Indomethacin (1 μg/ml)	
	Challenge	Agitation	Challenge	Agitation
Histamine	−	+	Not assayed	±
SRS-A	−	+	Not assayed	+
Prostaglandin	−	+	−	−
RCS	Absent	+	Absent	−

−, ±, and + indicate complete, partial or no inhibition respectively.

When indomethacin (1 μg/ml) was infused through the chopped lung tissue for 5 min before and during agitation (3 experiments) the release of RCS was either completely abolished or severely reduced (Fig. 5). As estimated by the contractions of the assay tissues, the output of prostaglandin was reduced from the equivalent of >>50 ng/ml to approximately 30 ng/ml prostaglandin E_2 and the peak release of histamine was reduced from approximately 75 ng/ml to 50 ng/ml (mean of 3 experiments). The same concentration of indomethacin superfused over the assay tissues had no effect on the responses to the mediators released during agitation. The comparative effects of disodium cromoglycate and indomethacin on challenged and agitated human lung are summarized in Table 4.

Discussion

Prostaglandins were detected in the effluent from challenged human lung tissue sensitized with reaginic antibodies in all experiments carried out (14). E-type prostaglandins were predominant although small amounts of prostaglandin $F_{2\alpha}$ were also present.

The origin of the prostaglandins released into the effluent following antigen challenge is open to conjecture. They could be synthesized when the membranes of the sensitized mast cells are distorted by the exocytosis of histamine granules, following antigen–antibody combination and/or during contraction of smooth muscle within the tissue aliquot. Although macroscopically visible bronchioles were removed before the lung tissue was chopped, an appreciable amount of bronchiolar tissue must have remained and would be expected to contract in response to the histamine and SRS-A liberated in anaphylaxis. The bronchoconstrictor agents histamine and bradykinin cause prostaglandin release from perfused guinea-pig lungs (Palmer, Piper & Vane, in preparation). If it can be assumed that the amounts of histamine and SRS-A liberated from the aliquots of lung tissue in anaphylaxis are far in excess of the amounts which cause maximal contraction of the smooth muscle present, then release of these mediators would have to be strongly inhibited before smooth muscle contraction would be affected. Disodium cromoglycate inhibits the anaphylactic reaction, reducing the amount of histamine and SRS-A released in a concentration-dependent manner (Sheard & Blair, 1970). It did not affect the release of prostaglandins until the release of the former mediators was almost completely suppressed when contraction of smooth muscle in the tissue would also be reduced. Furthermore, if prostaglandin release is a reflection of the distortion of mast cell membranes following antigen–antibody combination then it would be expected that the quantity of prostaglandin formed would be dependent on the amount of antigen used to challenge. In contrast to histamine and SRS-A however, the quantity of prostaglandin released was not

related to the amount of antigen used. These results suggest that the prostaglandin release detected in these experiments was not a direct result of the anaphylactic reaction but probably related to the subsequent contraction of bronchial tissue.

Mild mechanical stimulation, including gentle massage and agitation of guinea-pig lung causes release of the same mediators as anaphylactic shock in this species i.e. histamine, SRS-A, prostaglandin and RCS (Palmer, *et al.*, 1970; Piper & Vane, 1971). Agitation of human lung tissue caused the release of these same mediators but no RCS was detected in the effluent from challenged lung. The human lung tissue was sensitized with reaginic antibodies, whereas the guinea-pig lung used by Piper & Vane was sensitized with antibodies of the non-reaginic class. This fact could have accounted for the failure of human lung tissue to release RCS on challenge but the experiments with guinea-pigs passively sensitized to *Trichinella* showed that RCS release is not limited to non-reaginic systems. It may be a feature of anaphylaxis in guinea-pigs alone but experiments in other species are needed to clarify this point. There is evidence that RCS may be an unstable intermediate in the synthesis of prostaglandin (Gryglewski & Vane, 1971) and the amount of RCS released could thus be proportional to the amount of prostaglandin formed. As only small amounts of prostaglandin were released on challenge the quantity of RCS formed might have been too small to have been detected. However, RCS was detected when human lung was agitated gently, to release prostaglandin equivalent to that released on antigen challenge. RCS may thus not be involved in the release of prostaglandins and other mediators of anaphylaxis from human lung tissue.

The quantities of histamine released in anaphylaxis or agitation can be varied by changing the strength of the stimuli. When the stimuli were adjusted to release equivalent amounts of histamine, the quantity of prostaglandin released in anaphylaxis was significantly smaller than that released on agitation. This can be attributed to the fact that histamine is contained only within the mast cells of primate lung tissue (Ishizaka, Ishizaka & Tomioka, 1972) and therefore comparable disruption of these cells by either the anaphylactic or mechanical stimulus resulted in equivalent amounts of this mediator being released. However, prostaglandins are synthesized wherever cell membranes are distorted (Piper & Vane, 1971). They are therefore, produced from all cells disturbed by mechanical agitation but only from the sensitized mast cells and/or smooth muscle of the bronchioles following antigen challenge.

The release of prostaglandins from challenged lung tissue could be reduced either by indomethacin, which inhibits prostaglandin synthesis (Vane, 1971), or by disodium cromoglycate which interferes with the anaphylactic reaction (Sheard & Blair, 1970). Disodium cromoglycate had no effect on the release of prostaglandin by agitation which indicates that it does not interfere with prostaglandin synthesis. Indomethacin, in contrast, inhibited the release of both prostaglandins and RCS during agitation and slightly reduced the peak concentration of histamine release.

Significance of prostaglandins and rabbit aorta contracting substance in sensitized lung in vivo *and* in vitro

When passively sensitized human lung is challenged with antigen *in vitro* the resulting anaphylactic reaction resembles the immediate hypersensitivity reaction which occurs in patients suffering from extrinsic allergic bronchial asthma (Sheard

& Blair, 1970), so that it is logical to assume that mediators released *in vitro* are also released in asthma. RCS appeared to contract human bronchial muscle but since its release was not detected during antigen challenge it is not likely to contribute to anaphylactic bronchospasm. It has been suggested that prostaglandins might act as regulatory substances to maintain homeostasis (Horton, 1969). This may apply in the allergic release of mediators because E-type prostaglandins are bronchodilators and thus alleviate the bronchoconstrictor effects of histamine and SRS-A. They also modify the anaphylactic release of mediators from leucocytes of allergic human subjects (Lichtenstein & Bourne, 1971), from human lung (Walker, 1973) and from rat peritoneal mast cells (Koopman, Orange & Austen, 1971). The release of prostaglandins in anaphylaxis may thus be another example of the regulatory role of prostaglandin in body functions.

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