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Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical or mechanical stimulation of guinea-pig lungs

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Summary

1. Rabbit aorta contracting substance (RCS) and prostaglandins were released from guinea-pig isolated perfused lungs by gentle massage and also by infusion of Prosparol.

2. RCS and prostaglandins were also released by infusion into the pulmonary artery of bradykinin, arachidonic and dihomo-γ-linolenic acids or shock perfusate (containing RCS-releasing factor).

3. Arachidonic and dihomo-γ-linolenic acids caused a prolonged release of RCS and prostaglandins whereas bradykinin and shock perfusate gave a short-lasting output.

4. RCS and prostaglandins, together with histamine were released when superfused chopped lung tissue was agitated.

5. Challenge of sensitized guinea-pigs *in vivo* led to the release of an RCS-like substance into the carotid arterial blood.

6. Intravenous injection of bradykinin into guinea-pigs also released an RCS-like substance.

7. The release of RCS and prostaglandins was inhibited by aspirin or indomethacin in all experiments.

8. RCS contracted all vascular tissues investigated and also rat stomach strip.

9. The half-life of RCS was estimated as 1–2 minutes.

Introduction

Prostaglandins and rabbit aorta contracting substance (RCS) are released from guinea-pig isolated lungs by a variety of stimuli. As well as anaphylaxis, these include injection or infusion into the pulmonary artery of bradykinin, slow reacting substance released in anaphylaxis (SRS-A) or from egg yolk (SRS-C), arachidonic acid, or occasionally histamine (Piper & Vane, 1969a, b ; Piper & Vane, 1971 ; Vargaftig & Dao Hai, 1971). Mechanical agitation of chopped lungs (Palmer, Piper & Vane, 1970a) or spleen (Gryglewski & Vane, 1972) also releases prostaglandins and RCS. The present work amplifies and extends our previous results and in addition shows that a similar release of RCS can be evoked *in vivo*. Aspirin-like drugs, which block prostaglandin synthesis (Vane, 1971), block the release of prostaglandins and RCS both *in vitro* and *in vivo*. Some of this work has been presented to the British Pharmacological Society (Palmer, Piper & Vane, 1970 a, b).

Methods

Male guinea-pigs (200–300 g) were sensitized to ovalbumen by intraperitoneal (100 mg) and subcutaneous (100 mg) injection. Three weeks later the animals were killed. The lungs were removed, suspended in a chamber and perfused by a constant output pump at 5 ml/min via the pulmonary artery with Krebs bicarbonate solution at 37° C, pre-gassed with 95% oxygen and 5% CO_2. Lungs from unsensitized guinea-pigs were perfused in the same way. When all the blood had been washed from the lungs, the effluent from the pulmonary circulation was used to superfuse up to 6 isolated assay tissues (Vane, 1964 ; 1969 ; Piper & Vane, 1969a,b). These included rat colon, chick rectum and strips of cat or kitten terminal ileum, rabbit aorta, rat stomach strip, rat aorta, and longitudinal smooth muscle from guinea-pig ileum. All the tissues except one strip of cat or kitten terminal ileum were treated with a combination of antagonists infused to give final concentrations of mepyramine, hyoscine and phenoxybenzamine of 0·1 μg/ml, propranolol 2 μg/ml and methysergide of 0·2 μg/ml. These antagonists made the assay tissues more specific detectors of prostaglandins and RCS by eliminating the effects of histamine, acetylcholine, catecholamines and 5-hydroxytryptamine.

The assay tissues were arranged in two banks either in parallel or in series, with three tissues in each. When arranged in series the effluent from the lungs superfused first one bank and then the other, after re-gassing with 95% O_2/5% CO_2. Pressure in the pulmonary artery was measured from a side-arm on the pulmonary arterial cannula. Anaphylactic shock was induced in lungs from sensitized guinea-pigs by injection of ovalbumen (2–10 mg) into the pulmonary artery. Drugs or suspensions of particles were also injected or infused into the pulmonary artery.

For experiments with chopped lung tissue, the pulmonary artery of lungs from either sensitized or unsensitized guinea-pigs was cannulated and the lungs perfused with Krebs solution until all the blood was washed out. The lung parenchyma was chopped with scissors into pieces about 2 mm^3 and again washed in Krebs solution. The chopped tissue was placed in the barrel of a 10 ml plastic syringe and Krebs solution dripped through it at 5 ml/minute. The effluent then superfused the assay tissues (Piper & Vane, 1971).

For use in the blood-bathed organ technique (Vane, 1964 ; Piper, Collier & Vane, 1967), guinea-pigs (500–1,000 g) of either sex which had previously been sensitized to ovalbumen were anaesthetized with pentobarbitone sodium (60–80 mg/kg intraperitoneally). The trachea was cannulated and the guinea-pig artificially ventilated at 72 strokes/min and a constant volume of 5–9 ml/stroke. Airflow through the trachea was recorded by measuring differential pressure across a fine wire mesh in a tube attached to the tracheal cannula. The right carotid artery was cannulated to supply blood to the extracorporeal circulation via a constant output roller pump and blood pressure was recorded by a transducer connected to this cannula. In some experiments the left carotid artery was also cannulated and a fine cannula passed down until its tip was in the arch of the aorta. Assay tissues were superfused with blood at 2·5 ml/minute. An external jugular vein was cannulated for return of blood to the animal via a second channel in the roller pump. Drugs were given to the guinea-pig either intravenously by injection or infusion into the cannula in the jugular vein, or intra-arterially into the cannula in the arch of the aorta.

All changes in pressure and smooth muscle activity were detected by pressure transducers or Harvard 'smooth muscle' transducers and displayed on a six channel Watanabe pen recorder.

The Krebs solution was composed of the following in g/l (mM): NaCl, 6·9 (118); KCl, 0·35 (4·7); $CaCl_2$ $6H_2O$, 0·55 (2·5); KH_2PO_4, 0·16 (1·2); $MgSO_4$ $7H_2O$, 0·29 (1·17); glucose, 1·0 (5·6); $NaHCO_3$,2·1 (25·0).

Substances used were: acetylcholine chloride (British Drug Houses), acetylsalicylic acid (British Drug Houses), (−)-adrenaline bitartrate (British Drug Houses), Asp[1]-ileu[5]-angiotensin I (Schwarz, Batch number 700–725), Asp[1]-val[5]-angiotensin II (Ciba), arachidonic acid (Sigma), bradykinin (Parke Davis), dihomo-γ-linolenic acid (Upjohn), heparin (Boots), histamine acid phosphate (British Drug Houses), hyoscine hydrobromide (British Drug Houses), indomethacin (Merck, Sharpe & Dohme), linoleic acid (Hopkins & Williams), mepyramine maleate (May & Baker Ltd.), methysergide maleate (Sandoz), oleic acid (Hopkins & Williams), ovalbumen (British Drug Houses), sodium pentobarbitone (Abbot), phenoxybenzamine hydrochloride (I.C.I.), prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn).

Doses of adrenaline and histamine are expressed in terms of base. Aspirin was used as the sodium salt by mixing acetylsalicyclic acid with sodium bicarbonate in the ratio of 180:100 and dissolving in water. Indomethacin was dissolved by adding ethanol (0·4–0·8 ml) to 10–20 mg powder and then diluting to 1 mg/ml with Krebs solution. Solutions of aspirin and indomethacin were freshly made once or twice each day. Arachidonic acid was diluted with ethanol to a 10 mg/ml solution and further diluted with sodium carbonate solution (0·2 mg/ml) to a 1 mg/ml solution. Saline (0·9% w/v NaCl solution) was then used for further dilutions. Dihomo-γ-linolenic acid and prostaglandins were dissolved in the same way.

Particles used for infusion were 'Prosparol' which is a 50% v/v emulsion of arachis oil in water and has a 'particle' size of 1–2 μm, 'Bactolatex' which is a suspension of polystyrene spheres of 0·81 μm, diameter and Sephadex G10 of particle size 40–120 μm.

Results

In the following, contraction of the rabbit aorta in the presence of combined antagonists is taken to indicate RCS release. Where stated, additional characteristics of RCS (short half life and inhibition of release by aspirin-like drugs) were also obtained. Similarly, simultaneous contractions of the rat stomach strip, chick rectum and rat colon in the presence of combined antagonists indicated release of a prostaglandin-like substance (prostaglandin-LS). Where stated this was shown to be a prostaglandin by extraction and thin-layer chromatography.

Effect of rabbit aorta contracting substance on other tissues

Two banks of assay tissues were superfused in parallel with the effluent from sensitized lungs. The sensitivity of the recorders was adjusted so that challenge of the lungs induced similar contractions in each bank. A coil of silicone rubber tubing was then introduced between the lungs and one bank of tissues so that the lung effluent to that bank was delayed by 3–5 minutes. Another injection of ovalbumen was then made, either to the same lungs or to fresh ones and the

contractions of the assay tissues in both banks were compared. RCS decayed in the delay circuit, as shown by the lack of response of the rabbit aorta. The change in contraction size of the other assay tissues showed that RCS had little effect on the chick rectum or rat colon but did contribute to the contractions of the rat stomach strip.

Half life of rabbit aorta contracting substance

The above experiments showed that the rabbit aorta contracting activity of RCS was lost in less than 4 minutes. To time the loss of activity more accurately, two banks of assay tissues were superfused in parallel. A delay circuit of 0·75–2 min could be introduced between the lungs and one of the banks. The other bank was superfused with lung effluent, either undiluted or diluted by addition of Krebs solution to one-third or two-thirds the original concentration. In this way, the decay of RCS at various incubation times of 0·75–2 min could be assayed against the contractions caused by the diluted concentrations. RCS decayed to half concentration in 1–2 min (18 experiments).

Other vascular tissues

Spirally cut strips of arteries and veins from several species were tested for their sensitivity to RCS. All were contracted when RCS was released from sensitized lungs; the tissues included (with number of experiments in brackets), rabbit pulmonary (16), femoral (3), coeliac (2) and carotid (1) arteries, cat aorta (1), pulmonary (1), carotid (1), renal (1) and femoral (1) arteries, rat aorta (5) and guinea-pig aorta (2). Venous strips included rabbit inferior vena cava (2), pulmonary (2) and portal veins (4) and cat inferior vena cava (1) and portal vein (1). Cat, guinea-pig and rat vascular tissues were less sensitive than rabbit tissues, but further quantitation was not possible because of the lack of a standard preparation of RCS.

Stimuli which release rabbit aorta contracting substance

Bradykinin The observation that infusions of bradykinin into lungs from unsensitized guinea-pigs causes a release of RCS (Piper & Vane, 1969a) has been confirmed. In 8 experiments infusion of bradykinin (1–5 μg/ml) into guinea-pig lungs caused a short-lasting release of RCS and prostaglandin-LS, even when the infusion of bradykinin was continued for 10 min (Figure 1). The release of RCS was much smaller than that produced from unsensitized lungs by infusing perfusate collected during anaphylactic shock ('shock perfusate' which contains RCS releasing factor (RCS-RF); Piper & Vane, 1969a). Repeated infusions of bradykinin released similar amounts of RCS and prostaglandin-LS. In nine experiments, the assay tissues were continuously treated with indomethacin (1 μg/ml) to prevent possible intramural generation of prostaglandins. Similar results were obtained with bradykinin infusions (5 μg/ml) which released both RCS and prostaglandin-like activity. At its peak, the prostaglandin-like activity was equivalent to 1–5 ng/ml, assayed as E_2.

In three experiments, after release of RCS and prostaglandin-LS by bradykinin (1 μg/ml) had been demonstrated, aspirin (5 μg/ml) infused through the lungs abolished further release of RCS and prostaglandin-LS by bradykinin. Similar

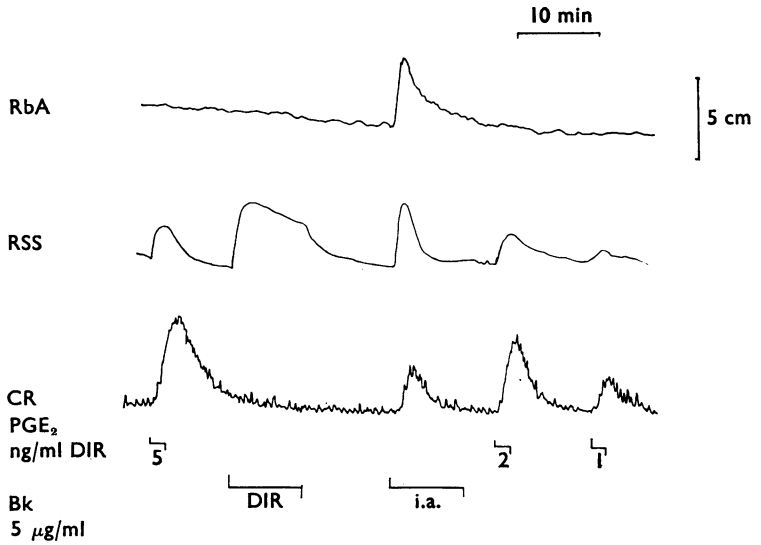

FIG. 1. Release of rabbit aorta contracting substance (RCS) and prostaglandins from guinea-pig lungs by bradykinin. A strip of rabbit aorta (RbA), a rat stomach strip (RSS) and a chick rectum (CR) were superfused with the effluent from guinea-pig isolated perfused lungs to which combined antagonists had been added. Prostaglandin E_2 (PGE_2) 5 ng/ml infused directly (DIR) to the tissues contracted RSS and CR. Bradykinin (Bk) 5 ng/ml DIR for 10 min caused a contraction of RSS but did not contract RbA or CR. A similar infusion into the pulmonary artery (i.a.) caused a short-lasting contraction of RbA, RSS and CR, showing the release of RCS and prostaglandins. Contractions of CR (which is not sensitive to RCS) showed that the prostaglandins released were equivalent to 1–2 ng/ml prostaglandin E_2 DIR. Time 10 min ; vertical scale 5 cm.

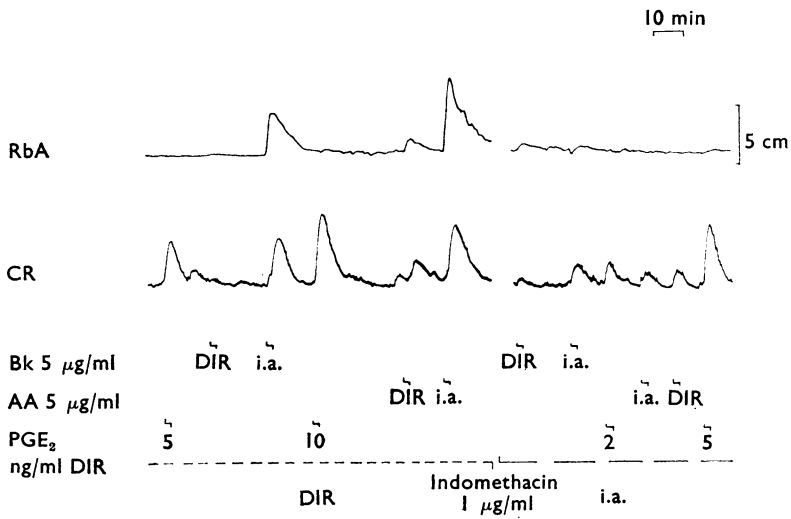

FIG. 2. Release of rabbit aorta contracting substance (RCS) and prostaglandins from guinea-pig lungs by bradykinin and arachidonic acid and its antagonism by indomethacin. The lungs and assay tissues (rabbit aorta (RbA) and chick rectum (CR)) were arranged as in Fig. 1. In the first panel, RbA and CR were superfused with indomethacin 1 μg/ml DIR. Bradykinin (Bk 5 μg/ml DIR) did not contract RbA or CR. Bradykinin (5 μg/ml i.a.) caused contraction of RbA and CR, showing release of RCS and of prostaglandins (equivalent to prostaglandin E_2 at 5 ng/ml.). Arachidonic acid (AA, 5 μg/ml DIR) caused small contractions of RbA and CR but when given i.a., much larger contractions of RbA and CR were induced, showing the release of RCS and prostaglandins (equivalent to prostaglandin E_2 at 5–10 ng/ml). In the second panel, indomethacin (1 μg/ml) was infused i.a. Contractions of RbA induced by bradykinin or arachidonic acid given i.a. were abolished, showing that the release of RCS had been inhibited and the amount of prostaglandins reduced to the equivalent of prostaglandin E_2 at 1–2 ng/ml. Time 10 min ; vertical scale 5 cm.

results were obtained in nine experiments with indomethacin (1 μg/ml ; Figure 2).

Histamine In two out of three experiments infusion of histamine (1 μg/ml) into lungs from unsensitized guinea-pigs led to a small release of RCS and of prostaglandin-LS.

Shock perfusate—rabbit aorta contracting substance-releasing factor The release of RCS and prostaglandin-LS from unsensitized lungs by infusion of shock perfusate containing RCS-RF has been shown many times (Piper & Vane,

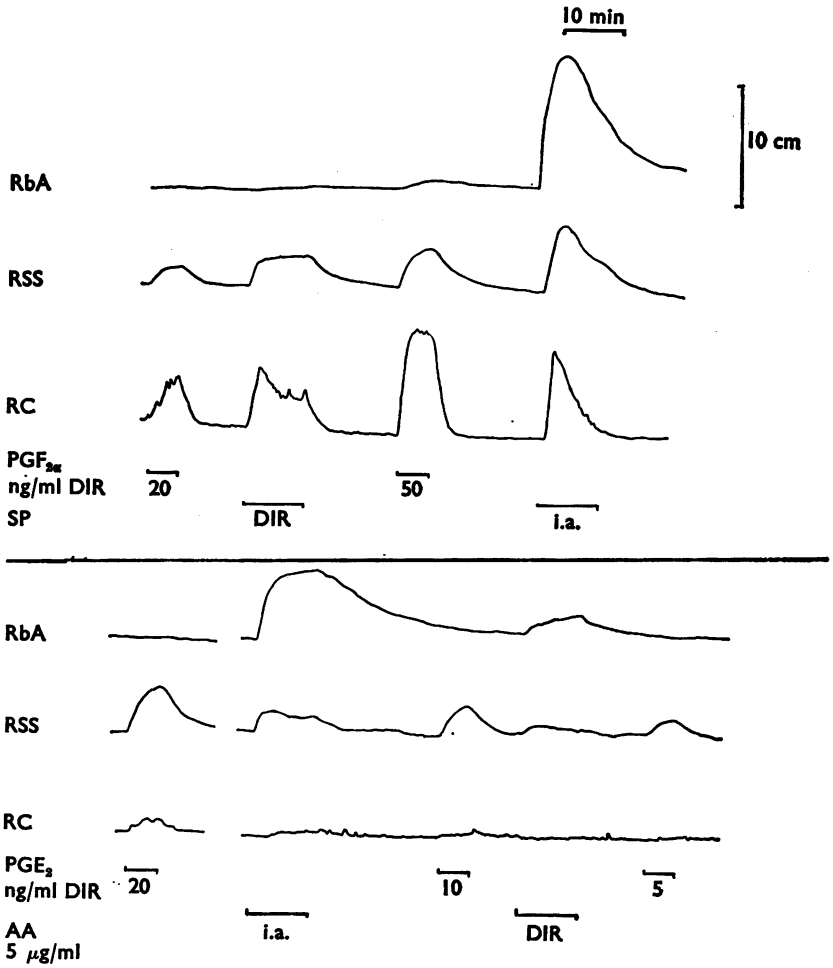

FIG. 3. Comparison of release of rabbit aorta contracting substance (RCS) and prostaglandins by shock perfusate and arachidonic acid. The lungs and assay tissues were arranged as in Fig. 1, but rat colon (RC) replaced chick rectum. In the upper panel, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ 20–50 ng/ml DIR) contracted rat stomach strip (RSS) and RC. An infusion of shock perfusate (SP) for 10 min DIR caused a maintained contraction of RSS and a partly sustained contraction of RC. When shock perfusate was infused i.a. for 10 min it caused a release of RCS and prostaglandins but this was not maintained for the duration of the infusion. In the lower panel infusions of prostaglandin E_2 (PGE_2 15–20 ng/ml DIR) contracted RSS and sometimes RC. When arachidonic acid (AA 5 μg/ml) was infused i.a. for 10 min it caused a release of RCS and a contraction of rabbit aorta (RbA) which did not decline until the infusion of arachidonic acid had stopped. There was also a maintained contraction of RSS and a small but maintained contraction of RC, showing the release of prostaglandins. When the same dose of arachidonic acid was infused DIR it gave much smaller contractions of RbA and RSS. Time 10 min ; vertical scale 10 cm.

1969a). In five experiments the shock perfusate was collected directly from the lungs (and was therefore uncontaminated with combined antagonists). This perfusate was then infused into lungs from unsensitized guinea-pigs and also directly to the assay tissues. The contraction of cat terminal ileum was smaller when the shock perfusate was infused into the lungs than when given directly to the assay tissues, showing that the histamine content of shock perfusate did not increase when passed through the lungs.

Infusion of shock perfusate for 5–10 min released an initial burst of RCS which diminished whilst the infusion was maintained (Fig. 3, upper panel). In three experiments sensitized guinea-pigs were given aspirin (10 mg/kg) intravenously 15–30 min before being killed. The lungs were removed and perfused in the usual way. When the lungs were challenged, histamine (0·5–2 μg/ml) and prostaglandin-LS equivalent to (5–10 ng/ml) prostaglandin E_2 were released but there was no release of RCS. The amount of prostaglandin usually released in anaphylaxis was 10–100 ng/ml in terms of E_2 (Piper & Vane, 1969b). The perfusate from the lungs was collected for 6 min after challenge. This perfusate was then infused into perfused lungs from unsensitized guinea-pigs when it released RCS and prostaglandin-LS (equivalent to 5–20 ng/ml E_2). The release of histamine could not be estimated because the perfusate contained combined antagonists. These experiments show that whereas aspirin blocked the release of RCS during anaphylaxis it did not antagonize the release of RCS-RF.

Arachidonic acid Injections of arachidonic acid of 2–50 μg or infusions of 5 μg/ml were made into nine lungs from sensitized and fourteen lungs from unsensitized guinea-pigs. When given directly to the assay tissues arachidonic acid sometimes caused contractions of rabbit aorta, rat stomach strip and rat colon. When the same doses of arachidonic acid were given through the lungs, there were much larger contractions of rabbit aorta but smaller contractions of rat colon, showing that the substance contracting rat colon had been removed by the lungs. The direct effects of arachidonic acid on the assay tissues for prostaglandins often precluded accurate estimation of prostaglandin release. The archidonic acid used was stated by the manufacturers to be 99% pure so that smooth muscle activity could have been due to an impurity or to peroxides of arachidonic acid which are very easily formed. However, with some samples of arachidonic acid, these direct effects were attenuated by treating the assay tissues with indomethacin 1 μg/ml). Under these conditions, it was clear that arachidonic acid released from the lungs not only RCS but also prostaglandin-like activity.

Effluent from the lungs infused with arachidonic acid was collected whilst the assay tissues were contracting. When the assay tissues had returned to baseline (15–35 min) the collected perfusate was re-applied to them. It no longer had rabbit aorta-contracting activity, showing that the RCS was unstable. Similar amounts of RCS were released from sensitized and unsensitized lungs. The fact that arachidonic acid had less activity on the rat colon when infused into the lungs than when applied directly, suggests that arachidonic acid is removed by the lungs, as are prostaglandins (Ferreira & Vane, 1967 ; Piper, Vane & Wyllie, 1970). Infusion of arachidonic acid did not alter the perfusion pressure (3 experiments).

The release of RCS by infusion of arachidonic acid (6 experiments) was maintained throughout the infusion (Fig. 3, lower panel). This contrasts with the release

of RCS by bradykinin, histamine or shock perfusate when the release occurred only at the start of the infusion (Figs. 1 and 3, upper panel). In one experiment arachidonic acid infused into the lungs caused a small contraction of the kitten ileum, suggesting slight histamine release. However, this was not seen in other experiments.

Aspirin (5 μg/ml ; 3 experiments) or indomethacin (1 μg/ml ; 6 experiments) given through the lungs blocked the release of RCS and prostaglandin-LS by arachidonic acid (Figure 2).

Dihomo-γ-linolenic acid

Dihomo-γ-linolenic acid (5–10 μg/ml) behaved like arachidonic acid and released RCS and prostaglandin-LS (5–10 ng/ml in terms of prostaglandin E_1) from unsensitized guinea-pig lungs (7 experiments). The release of RCS and prostaglandin-LS lasted as long as the infusion of dihomo-γ-linolenic acid and was abolished by aspirin (5 μg/ml).

Other fatty acids

Oleic and linoleic acids are not precursors of prostaglandins. At concentrations of 5–10 μg/ml these acids neither contracted the assay tissues when applied directly, nor released smooth muscle contracting substances from unsensitized perfused lungs.

Angiotensins

Rat aorta contracts strongly to an initial dose of angiotensin but rapidly becomes tachyphylactic to repeated doses of 0·05–5 μg/ml (Türker, Yamamota, Bumpus & Khairallah, 1971). However it retains sensitivity to RCS and so provides a suitable assay tissue to detect RCS release in the presence of angiotensin II. Infusions of angiotensin II (0·05–5 μg/ml) through unsensitized guinea-pig lungs did not cause contraction of rat aorta made tachyphylactic to angiotensin II, although RCS produced from the same lungs by shock perfusate did contract the rat aorta. Angiotensin I (50 ng/ml) infused through unsensitized lungs did not release RCS, although the contraction of the rat colon showed that there had been conversion to angiotensin II.

Infusion of particles

Particles infused into dog spleen (Gilmore, Vane & Wyllie, 1969) or lungs from rats or guinea-pigs (Lindsey & Wyllie, 1970) evoke a release of prostaglandins which behave like prostaglandin E_2. Injection of particles into guinea-pig lungs also causes a release of RCS and prostaglandin-LS (Piper & Vane, 1971).

Injections of Prosparol, Bactolatex and Sephadex G10 (particle diameter 0·8–120 μm) into the pulmonary artery of sensitized guinea-pigs caused a rise in pulmonary arterial pressure (4–64 mmHg) and a release of RCS and prostaglandin-LS (2–50 ng/ml assayed as prostaglandin E_2). Only in three out of ten experiments was a small release of histamine (10–20 ng/ml) detected.

In lungs from sensitized animals peak release of mediators by injection of particles was less than the amounts released by anaphylaxis (histamine 0·5–2

μg/ml ; prostaglandin 10–100 ng/ml). In lungs from unsensitized guinea-pigs, release of RCS and prostaglandin-LS by injection of particles was much more difficult to demonstrate. In 28 out of 32 trials in 10 lungs, injection of particles into unsensitized guinea-pig lungs failed to release RCS or prostaglandin-LS and in only two trials was there a small release of RCS ; in these and two other trials there were small contractions of one assay tissue, either rat stomach strip or chick rectum, equivalent to prostaglandin E_2 at less than 10 ng/ml. In one trial histamine (50 ng/ml) was released.

In three experiments aspirin (5 μg/ml) blocked the release of RCS by Prosparol (Figure 4). Aspirin did not antagonize the rise in pulmonary arterial pressure when particles were injected.

Mechanical stimulation of perfused lungs

Gentle massage of the external surface of isolated perfused guinea-pig lungs released histamine, RCS and prostaglandin-LS (Piper & Vane, 1971).

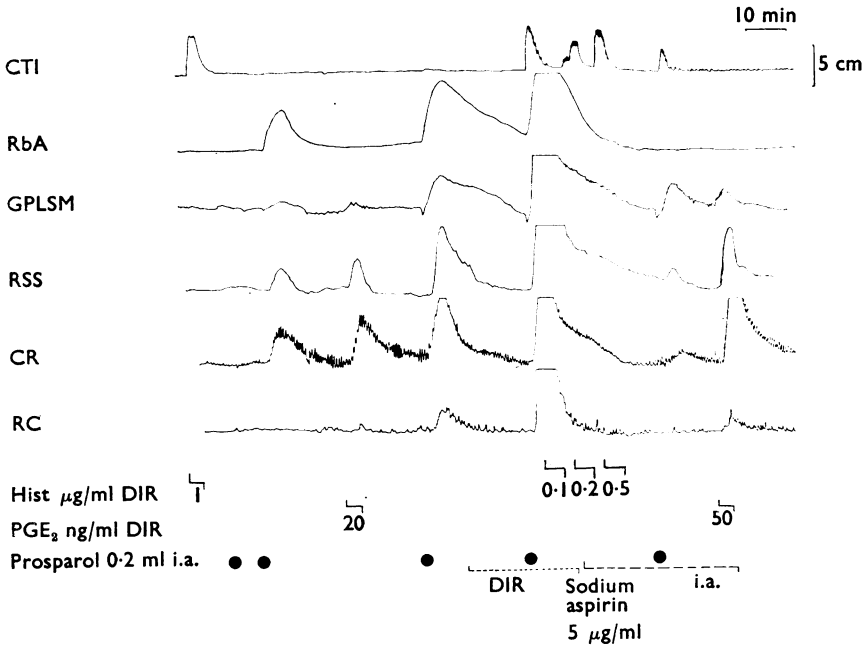

FIG. 4. Release of rabbit aorta contracting substance (RCS), prostaglandins and histamine by Prosparol. The antagonism by aspirin of the release of RCS. The effluent from sensitized guinea-pig isolated perfused lungs was superfused over a strip of cat terminal ileum (CTI), rabbit aorta (RbA), a strip of smooth muscle from guinea-pig ileum (GPLSM), rat stomach strip (RSS), chick rectum (CR) and rat colon (RC). All tissues except CTI were blocked with combined antagonists. An infusion of histamine (Hist) 1 μg/ml given DIR contracted CTI only. The first injection of Prosparol (0·2 ml i.a., ●) given slowly over 30 s had no effect but a second injection caused release of RCS and prostaglandins, as shown by contractions of RbA, RSS and CR and a small contraction of GPLSM. An infusion of prostaglandin E_2 (PGE_2) (20 ng/ml DIR) caused contractions of GPLSM, RSS and CR which almost matched the contractions caused by Prosparol given i.a. A third i.a. injection of Prosparol caused an even larger release of RCS and prostaglandins. In other experiments, similar amounts of Prosparol given DIR had no effect on the assay tissues. Sodium aspirin (5 μg/ml) was infused continuously DIR before and during i.a. injection of Prosparol, which caused an even larger release of RCS and prostaglandins and also released more than 0·5 μg/ml of histamine. The aspirin was then infused through the lungs (i.a.) before and during an injection of Prosparol. Aspirin abolished the release of RCS by Prosparol and reduced the amounts of histamine and prostaglandins released. A final dose of prostaglandin E_2 (50 ng/ml) was given DIR. Time 10 min ; vertical scale 5 cm.

In nine experiments, aspirin (5–10 μg/ml) was infused into the isolated lungs after massage had demonstrated release of histamine, prostaglandin-LS and RCS. Further massage during aspirin infusion released histamine as before, but in four of the experiments the release of similar amounts of RCS and prostaglandin-LS was reduced or abolished. The lack of effect of aspirin in the other experiments may have been due to inadequate perfusion of parts of the lung with Krebs solution containing aspirin. For this reason guinea-pigs were given an intravenous dose of aspirin (10–20 mg/kg). Thirty to sixty minutes later the lungs were removed and perfused in the usual way. When these aspirin-treated lungs were massaged, histamine was released but there was no release of RCS (in four out of five experiments). The contractions of the assay tissues which detected prostaglandins were also less than usual (Figure 5).

Release of rabbit aorta contracting substance and other mediators from chopped lungs

When superfused chopped lung from sensitized guinea-pigs was challenged by injecting ovalbumen into the Krebs solution, histamine, SRS-A, RCS and prostaglandin-LS were released. The same mediators were released when chopped lung tissue from sensitized or unsensitized guinea-pigs was stirred manually at a rate of about 1/s for 5–6 min (35 experiments, Piper & Vane, 1971). Stirring the

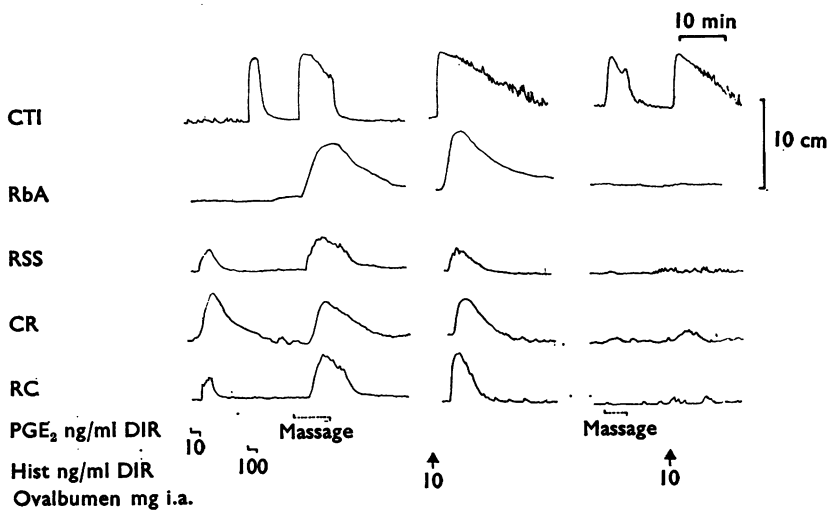

FIG. 5. Release of histamine, rabbit aorta contracting substance (RCS) and prostaglandins from lungs by gentle massage and anaphylaxis. Antagonism by aspirin of release of RCS and prostaglandins. The effluent from isolated perfused lungs taken from a guinea-pig sensitized to ovalbumen was superfused over cat terminal ileum (CTI), rabbit aorta (RbA), rat stomach strip (RSS), chick rectum (CR) and rat colon (RC). All tissues except CTI were blocked with combined antagonists. An infusion of prostaglandin E_2 (10 ng/ml DIR) contracted only RSS, CR and RC. An infusion of histamine (100 ng/ml DIR) contracted the CTI showing that histamine, RCS and prostaglandins were released. In the second panel, effluent from a fresh pair of sensitized lungs superfused the tissues. When the lungs were challenged by injection of ovalbumen (10 mg i.a.) all the tissues contracted showing the release of histamine, RCS and prostaglandins. In the third panel, the effluent from lungs taken from a sensitized guinea-pig which had been given sodium aspirin (10 mg/kg i.v.) 30 min before killing was superfused over the tissues. When these lungs were massaged and challenged, RbA did not contract and the contractions of RSS, CR and RC were very much reduced showing that aspirin blocked the release of RCS and prostaglandins. The contractions of CTI were practically unchanged. Time 10 min ; vertical scale 10 cm.

chopped lung up to four times at 20–30 min intervals released similar amounts of RCS, prostaglandin-LS and histamine each time. The amount of prostaglandin-LS (assayed at E_2) released was 10–80 ng/ml and that of histamine 0·5–2 μg/ml. Prostaglandin E_2 and $F_{2\alpha}$ were identified in the effluent from stirred lung tissue of guinea-pigs by extraction and thin-layer chromatography in the AII system of Gréen & Samuelsson (1964).

A similar release of histamine, RCS and prostaglandin-LS occurred on stirring chopped lung tissue from rabbits (3 experiments), rats (3 experiments) and kitten (1 experiment). When the effluent was re-tested on the assay tissues the RCS had disappeared, although histamine and prostaglandin-LS were still present. This effluent also contained RCS-RF, for when it was used to perfuse lungs from unsensitized guinea-pigs, a further release of RCS occurred.

Release of histamine, RCS and prostaglandin-LS was demonstrated by stirring chopped lung from guinea-pigs, then indomethacin (0·01–1 μg/ml) or sodium aspirin (0·1–5 μg/ml) was infused through the lung choppings. When they were stirred again, the release of RCS and of prostaglandins was reduced or abolished (Fig. 6) but that of histamine was relatively unaffected. When indomethacin was washed out of the chopped lung tissue, the amounts of RCS and prostaglandin-LS released by subsequent stirring gradually increased towards the control levels (Figure 6).

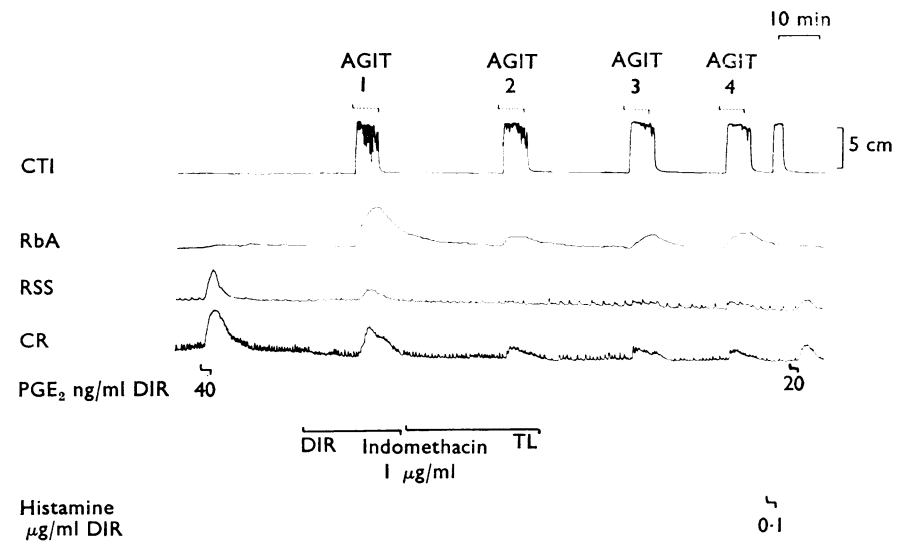

FIG. 6. Release of histamine, rabbit aorta contracting substance (RCS) and prostaglandins by agitation of guinea-pig chopped lung tissue. The effluent from guinea-pig chopped lung tissue was superfused over cat terminal ileum (CTI), rabbit aorta (RbA), rat stomach strip (RSS) and chick rectum (CR). All tissues except CTI were blocked with combined antagonists. Prostaglandin E_2 (40 and 20 ng/ml DIR) contracted RSS and CR only. Indomethacin (1 μg/ml) was infused DIR before and during agitation of the lung tissue for 4 min (AGIT 1). All the assay tissues contracted, showing the release of histamine, RCS and prostaglandins. The infusion of indomethacin was made through the lung tissue (TL) before and during further agitation of the lung tissue (AGIT 2). The contraction of CTI was unchanged showing that the release of histamine was unaffected, but the contractions of RbA, RSS and CR were reduced, showing that the release of RCS and prostaglandins had been inhibited. The infusion of indomethacin was stopped and the drug allowed to wash out of the lung tissue. With further agitation (AGIT 3 and 4) the amount of RCS released began to increase. The amount of prostaglandins released did not recover appreciably. An infusion of histamine (0·1 μg/ml DIR) contracted CTI and showed that approximately this amount of histamine was released by agitation of the lung tissue. Time 10 min ; vertical scale 5 cm.

Release of active substances in vivo.

Guinea-pigs sensitized to ovalbumen were prepared for use in the blood-bathed organ technique. Three assay tissues were used—cat terminal ileum and two strips of rabbit aorta, one of which had been treated with phenoxybenzamine (1 μg/ml for 1 hour) to block the effects of histamine and the α-effects of catecholamines.

In eight experiments when ovalbumen (0·1–5 mg/kg) was given intravenously, anaphylactic shock occurred, as shown by reduction in airflow and a rise in arterial blood pressure. Piper *et al.* (1967) showed that catecholamines were released by anaphylactic shock or bradykinin injection in guinea-pigs and such a release would account for the contraction of the untreated rabbit aortic strip. There was also a

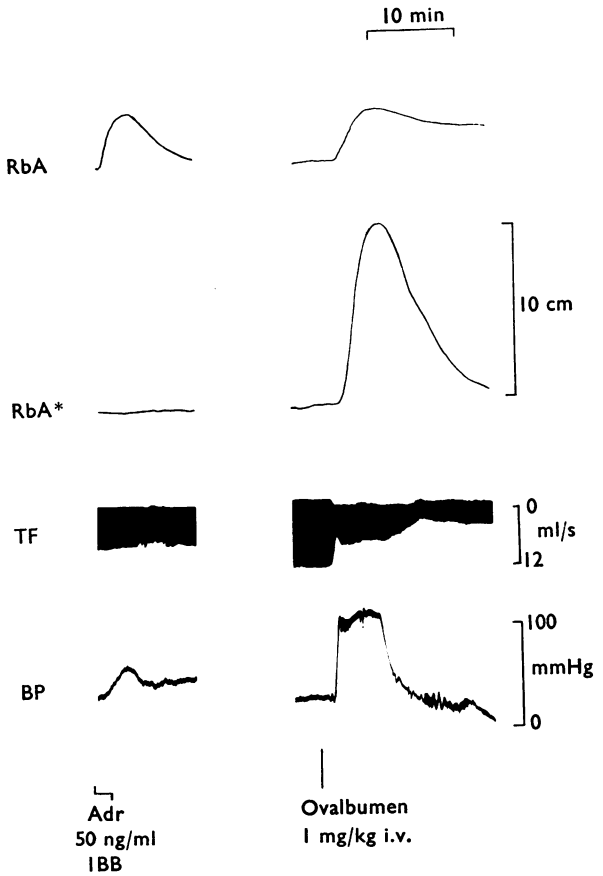

FIG. 7. Release of an RCS-like substance during anaphylaxis *in vivo*. Two rabbit aortic strips (RbA) were superfused with carotid blood from a male guinea-pig (680 g) previously sensitized to ovalbumen. The flow of air through the trachea (TF) in ml/s (inflation downwards) and carotid blood pressure (BP) in mmHg are also shown. RbA* had previously been soaked in phenoxybenzamine (1 μg/ml) for 1 hour. In the first panel, adrenaline (Adr) (50 ng/ml) was infused into the blood bathing the RbA (IBB) and caused a contraction of the untreated RbA but not of the blocked RbA*. The adrenaline then entered the circulation and caused a rise of BP. In the second panel the guinea-pig was challenged by i.v. injection of ovalbumen 1 mg/kg. There was a rapid increase in BP, reduction in TF and contractions of both RbAs. Since RbA* did not contract to adrenaline, it detected the release of an RCS-like substance. Time 10 min ; vertical scale 10 cm, ml/s and mm Hg.

contraction of the phenoxybenzamine-treated rabbit aorta which showed the release of an RCS-like substance into the arterial blood (Figure 7). The onset of (2–5 min) and time to maximum (5 min) release of catecholamines and RCS were similar, but the output of catecholamines (15–30 min) lasted longer than that of RCS (8–10 minutes). In one experiment, after the guinea-pig had been shocked and a substance like RCS had been detected in the extracorporeal circulation, the lungs were removed from the animal and perfused with Krebs solution via the pulmonary artery. The effluent from the lungs was superfused over another rabbit aortic strip which was blocked with 'combined antagonists' and the lungs challenged with ovalbumen. The rabbit aorta contracted showing the release of RCS.

Six guinea-pigs were given sufficient bradykinin (1–8 μg) intravenously to cause marked reduction in airflow. There was a transitory fall of blood pressure followed by a rise, and a release of catecholamines, as previously shown by Piper *et al.* (1967). There was also a release of an RCS-like material into the arterial blood (Figure 8). In two of these animals, bradykinin (8 μg) was given through a cannula, the tip of which was in the arch of the aorta. There was a prolonged fall in blood pressure and release of catecholamines (Piper, 1969) but no effect on tracheal flow and no release of RCS (Figure 8).

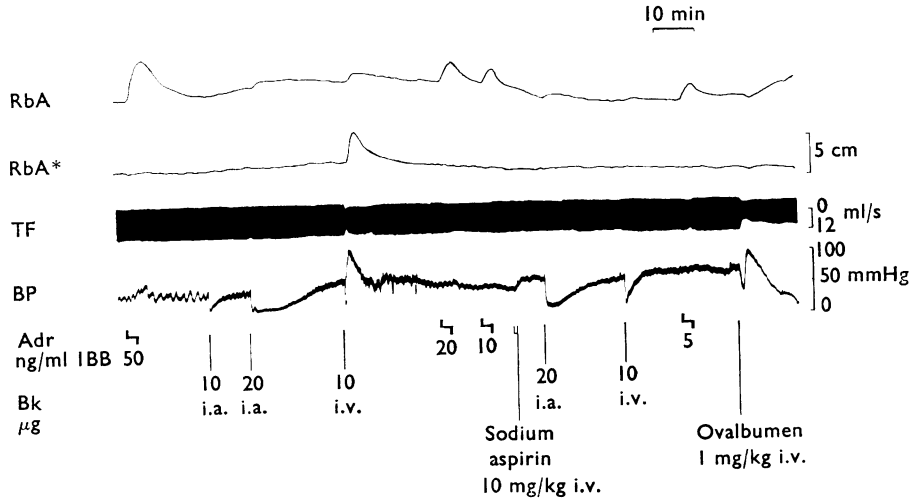

FIG. 8. The release of an RCS-like substance by intravenous injection of bradykinin and its antagonism by aspirin. Tracings as in Fig. 7. Adrenaline (Adr 50 ng/ml) was infused into the blood bathing (IBB) the rabbit aortae (RbA) and contracted the untreated RbA; it then entered the circulation and caused a rise in BP. Injection of bradykinin (Bk 10 μg) given into the arch of the aorta (i.a.) caused a fall in BP. Bradykinin 20 μg i.a. caused a more prolonged fall in BP, small reduction in TF and small contraction of untreated RbA. Bradykinin 10 μg given i.v. caused a transitory fall in BP followed by a rise, a reduction in TF and contractions of RbA and RbA*. Since RbA* did not respond to adrenaline its contraction indicated the release of an RCS-like substance into the carotid blood. Calibrating doses of adrenaline (20 and 10 ng/ml IBB) contracted RbA but not RbA*. Sodium aspirin 10 mg/kg was injected i.v. This caused a maintained increase in BP and relaxation of RbA. Bradykinin (20 μg i.a.) caused a fall of BP which was of shorter duration than before aspirin. Bradykinin 10 μg i.v. caused a fall of BP which lasted longer than before aspirin but no secondary rise in BP and no reduction in TF or contraction of RbA* Adrenaline (5 ng/ml IBB) contracted RbA. When the guinea-pig was challenged by i.v. injection of ovalbumen (1 mg/kg) anaphylaxis occurred and there was an immediate fall in BP followed by a secondary rise and then progressive fall, a reduction of TF and slow contraction of RbA but not RbA* (see Fig. 4). Aspirin therefore antagonized the release of the RCS-like substance by BK given i.v. and by anaphylaxis. Time 10 min; vertical scales 5 cm, ml/s and mmHg.

In one guinea-pig, histamine (1–10 μg i.v.) caused reduction in airflow of similar magnitude to that caused by bradykinin but there was no release of RCS. However bradykinin and histamine may have had different effects on lung function which resulted in approximately the same change in airflow. Aspirin (8 mg/kg) was given intravenously in four guinea-pigs. This dose blocked the reduction in airflow produced by intravenous bradykinin (Collier & Shorley, 1960) and the release of the RCS-like substance. Although aspirin did not block the reduction in airflow or the release of catecholamines which occurred during anaphylaxis, it completely antagonized the release of RCS-like material (Figure 8).

Discussion

In 1969, we identified the release of RCS, RCS–RF, prostaglandin E_2 and $F_{2\alpha}$ from guinea-pig lung during anaphylactic shock (Piper & Vane, 1969a, b). At that time, we knew that RCS was unstable and that its release was antagonized by release of histamine. It is probable that release of both RCS and prostaglandins (Vane, 1971; Smith & Willis, 1971; Ferreira, Moncada & Vane, 1971). Our present results further establish the link between RCS and prostaglandin release, as well as defining more clearly the properties of RCS.

In all the situations we have studied, RCS and prostaglandin release from lung tissue go hand in hand and can sometimes occur without an accompanying release of histamine. It is probable that release of both RCS and prostaglandins represents fresh synthesis (Piper & Vane, 1971) not only by lung tissue, but also by other tissues, such as chopped spleen (Gryglewski & Vane, 1972). When this is considered, along with the fact that prostaglandin precursors such as arachidonic acid and dihomo-γ-linolenic acid lead to a sustained release of RCS when infused into isolated lungs, it seems possible that RCS is a product formed during prostaglandin biosynthesis. Alternatively, it could be a substance formed by the prostaglandin synthetase system but not converted to prostaglandins. An unstable RCS was formed when arachidonic acid was incubated with a crude cell-free prostaglandin synthetase from dog spleen (Gryglewski & Vane, 1971; 1972) suggesting that RCS was an intermediate in prostaglandin synthesis, perhaps the cyclic endoperoxide postulated as an unstable intermediate by Nugteren, Beerthuis & van Dorp (1967) and Samuelsson, Granström & Hamberg (1967). Our results are consistent with this hypothesis and further show that the half-life of RCS in the lung effluent is between 1–2 minutes.

By comparing the effects of lung perfusate before and after degeneration of RCS, we have also assessed the effects of RCS on the tissues we normally use to assay prostaglandins. The rat stomach strip is contracted by RCS, a conclusion supported by our initial work (Piper & Vane, 1969a) in which we found that the amount of contractor activity measured on stomach strips immediately after lung anaphylaxis could not be matched by the activity remaining after extraction of the effluent for prostaglandins. The rat colon and chick rectum are not contracted by RCS, so they should be more suitable assay tissues for prostaglandins when RCS is likely to be present. In the light of this finding, it is interesting to note that in our first paper on RCS (Piper & Vane, 1969b) there was evidence for antagonism by aspirin not only of the release of RCS but also of the release of prostaglandins (see, for instance, Fig 7, Piper & Vane, 1969b).

All vascular tissues tested, including strips of arteries and veins from several species, were contracted by RCS. Whether it is vaso-dilator or vaso-constrictor *in vivo*, RCS is strongly vaso-active, so that local generation of RCS and prostaglandins in blood vessel walls may contribute to the local control of vascular resistance.

Whether RCS is broncho-active is still not completely clear, although Piper & Walker (1973) found that it contracted strips of human bronchi. Both RCS and prostaglandins are released by bradykinin ; aspirin, which prevents their release, also antagonizes the bradykinin-induced reduction in airflow in the guinea-pig (Collier & Shorley, 1960). Thus, it has been argued (Piper & Vane, 1969a ; Collier, 1969 ; Collier, 1971 ; Vargaftig & Dao Hai, 1972) that a local release of RCS and/or prostaglandin $F_{2\alpha}$ accounts for the reduction in airflow induced by bradykinin. Our present results accord with this hypothesis. However, the demonstration that the release of RCS by bradykinin is short-lived suggests further experiments which may distinguish between a direct and indirect action of bradykinin.

Shock perfusate (which contains RCS-RF) also induced a transient release of RCS and prostaglandins, in contrast to the release induced by arachidonic acid or dihomo-γ-linolenic acid, which lasted for the duration of the infusion. This result shows that RCS-RF is unlikely to be a prostaglandin precursor. Since aspirin did not inhibit release of RCS-RF, it is also unlikely to be a product of prostaglandin synthetase. Thus, the mechanism by which RCS-RF (and bradykinin) releases RCS and prostaglandins seems different from that by which the prostaglandin precursors cause release. Vargaftig & Dao Hai (1972) came to a similar conclusion, for they found that mepacrine, a phospholipase-A inhibitor, selectively prevented the release of RCS by bradykinin without affecting the release induced by arachidonic acid. This suggested that bradykinin may be activating phospholipase-A, leading to release of prostaglandin precursor. Activation of phospholipase-A by bradykinin or other chemical stimuli may be related to the broncho-constrictor or vaso-constrictor activity of the substances. However, Alabaster (1971) could find no correlation between the rise in perfusion pressure and the release of spasmogens for perfused lungs challenged by tryptamine.

Distortion of the cell membrane may lead to RCS and prostaglandin biosynthesis and release (Piper & Vane, 1971). Prostaglandins are synthesized *de novo* at the time of release, as shown by release of larger amounts than can be extracted from the tissue (see Piper & Vane, 1971) and the abolition of release by inhibitors of prostaglandin synthesis, such as aspirin. This contrasts with histamine release, which takes place from pre-formed stores in mast cells. Thus, prostaglandins may be released from many different cell types of mechanical or chemical challenge, whereas histamine depends on the involvement of the mast cell.

Release of spasmogens by infusion of particles was much more readily obtained from sensitized than from unsensitized lungs. This suggests that sensitization to a specific antibody in some way makes the lungs more sensitive to non-specific stimuli. Such an increased susceptibility to release of mediators has not been reported, although Fink & Schlaueter (1969) found monkeys were more sensitive to metacholine challenge (as an aerosol) after passive sensitization. Similarly, Mathé, Hedqvist, Holmgren & Svanborg (1973) found that asthmatic patients were 10,000 fold more sensitive to the broncho-constrictor effects of prostaglandin $F_{2\alpha}$ than were normals.

The increase in perfusion pressure when particles were infused into the pulmonary artery must have been induced, at least partially, by an increase in resistance to perfusion, associated with mechanical blockage of the blood vessels. Such a blockage would lead to cell damage and consequent release of RCS and prostaglandins; it is uncertain, however, whether the vaso-activity of the released substances contributes to or attenuates the rise in perfusion pressure.

Prostaglandins, infused into the pulmonary artery, are inactivated by the lungs (Ferreira & Vane, 1967; Piper *et al.*, 1970) and our present results show that some of the smooth-muscle stimulating activity of arachidonic acid is also reduced on passage through the pulmonary circulation. Aspirin in the doses used did not affect this inactivation. Since it is likely that the spasmogenic activity of arachidonic acid is due to peroxide formation in the solution (Dakhil & Vogt, 1962) it seems probable that the lungs can inactivate fatty acid peroxides.

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