SRS-A. Results were expressed in arbitrary units. Histamine was assayed either biologically or fluorimetrically. Prostaglandin content was estimated by radioimmunoassay. Prostaglandin synthetase inhibitors were infused intra-arterially for 15 min before and during challenge.

Indomethacin (0.5–10 μg/ml) increased the amount of SRS-A released by up to 3.4 times (25 experiments). Treatment with indomethacin (1 μg/ml) produced the maximum increase in SRS-A (from 153 ± 26 to 525 ± 69 units/lung) and also reduced the output of prostaglandins. Histamine levels were also increased. Sodium aspirin (1–10 μg/ml) similarly potentiated release of SRS-A; the maximum increase produced by aspirin (5 μg/ml) was from 138 ± 26 to 465 ± 62 units/lung. This dose of aspirin also increased the amount of histamine released from 4.43 ± 0.6 to 19.5 ± 2.9 μg/lung as estimated by bioassay and fluorimetric assay (15 experiments).

Sodium meclofenamate 0.1–1.0 μg/ml increased SRS-A output by up to 2.4 times (maximum increase at 0.5 μg/ml i.a.; 16 experiments) and there was an increased amount of histamine released.

A bell-shaped dose-response curve was seen with all three drugs. The decreasing effects with increasing doses of anti-inflammatory drugs may be due to inhibition of enzymes such as phosphodiesterase.

Piper & Vane (1969) showed that a sample of SRS-A released rabbit aorta contracting substance (RCS; now thought to be a mixture of prostaglandin endoperoxides and thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975)) from unsensitized guinea-pig lungs. In the present experiments, injection of 0.5 units (laboratory standard) of two newly prepared samples of SRS-A i.a. into lung from unsensitized guinea-pigs released RCS and prostaglandins into the effluent (6 experiments).

These results suggest that SRS-A released in anaphylaxis may in turn release RCS and prostaglandins. They also strengthen the hypothesis that the prostaglandins then exert a negative feedback on further release of SRS-A. It is therefore not difficult to understand why inhibition of prostaglandin release by non-steroid anti-inflammatory drugs potentiates the release of SRS-A.

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Some properties of rabbit aorta contracting substance-releasing factor (RCS-RF)

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Rabbit aorta contracting substance-releasing factor (RCS-RF) was the name given by Piper & Vane (1969) to an unidentified factor distinct from slow reacting substance of anaphylaxis (SRS-A) found in the perfusate of immunologically shocked lungs from sensitized guinea-pig. RCS-RF injected into the pulmonary artery of perfused lungs from unsensitized guinea-pigs induced release of RCS, now thought to be a mixture of prostaglandin endoperoxides and their derivative, thromboxane A_2. We have now partially purified RCS-RF and describe some of its physiochemical and pharmacological properties.

Lungs from guinea-pigs sensitized to ovalbumin (Piper & Vane, 1969) were perfused with Krebs solution at 10 ml/min and shocked with ovalbumin (10 mg) injected into the pulmonary artery. Perfusate collected over the next 5 min was centrifuged at 5000 *g*, filtered, lyophilized and redissolved in distilled water to give about one tenth of the original volume. A batch of perfusate (about 50 ml) from a single lung contained 1000–2000 units of RCS-RF activity (defining 1 unit as that amount which gives the same release of RCS from perfused lung as 1 μg of arachidonic acid.)

The active principle was removed from the crude perfusate by adsorption onto Amberlite XAD-2 and was recovered from the resin in about 75% yield by elution, with ethanol-water (80:20, v/v), giving a preparation almost completely free of salts, proteins and histamine. The ethanol-water extract was dried *in vacuo* and washed three times with dry diethyl-ether.

The residue, when dissolved in distilled water, was stable for at least six weeks at −10°C.

The preparation was chromatographically free of fatty acids and prostaglandins but traces of phospholipid material remained. There was no prostaglandin-like activity when tested on the rat fundus strip, rat colon and chick rectum (Ferreira & Vane, 1967). High doses (5.0 U) contracted the guinea-pig ileum and rat stomach strip. However, RCS-RF was distinguished from SRS-A by its potent RCS releasing properties. Furthermore, RCS-RF activity was not destroyed by incubation with the enzyme arylsulphatase which selectively inactivates SRS-A (Orange, Murphy & Austen, 1974). Indeed, RCS-RF activity was often increased by incubation with arylsulphatase.

RCS-RF is insoluble in ether, chloroform, ethyl acetate, acetone and ethanol but is more soluble in methanol. It is very polar as judged by its behaviour in several lipid chromatography systems. It is destroyed by acid or alkali (2 h at pH 2 or pH 12) but not by mild base treatment (0.1 N NaOH for 30 minutes). It loses < 10% activity after boiling for 2 min but is substantially (> 75%) degraded after 10 min at 100°C. Passage through a dialysis membrane suggests that it is a small molecule (< 5000 m.w.).

The release of RCS-RF was not prevented by aspirin (200 μg/ml), indomethacin (2 μg/ml), dexamethasone (2 μg/ml), di-sodium cromoglycate (20 μg/ml), colchicine (5 μg/ml), diethyl carbamazine (1 mg/ml) or mepacrine (20 μg/ml) but the release of RCS by the factor was blocked by these substances (except di-sodium cromoglycate and colchicine) in the same doses. All but dexamethasone also blocked the conversion in perfused lungs of arachidonic acid to RCS, implying direct block of the cyclo oxygenase. Dexamethasone, however, blocked the release of RCS by RCS-RF without affecting release by arachidonic acid.

RCS-RF does not release RCS or prostaglandin from guinea-pig perfused kidneys (which only convert arachidonic acid to PGE_2). RCS-RF has no inflammatory action in the rat paw (8 u/rat) oedema test but does cause hyperalgesia. This is partially (80%) blocked by indomethacin (10 mg/kg). It does not aggregate human platelets (10 u/ml).

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The effects of prostaglandin endoperoxides and thromboxane A_2 on strips of rabbit coeliac artery and certain other smooth muscle preparations

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Rabbit aorta contracting substance (RCS) contracts all vascular tissue tested and is generated by lung and other tissues after several stimuli, including immunological shock and bradykinin or arachidonic acid injection (Piper & Vane, 1969; Vargaftig & Dao Hai, 1971; Palmer, Piper & Vane, 1973). RCS is unstable and is probably a mixture comprising mainly thromboxane A_2 (TxA_2; half-life 30 s) with some prostaglandin endoperoxides (PGG_2 and PGH_2; half lives approximately 5 min) (Hamberg, Svensson & Samuelsson, 1975). We have compared the effects on various smooth muscle preparations of PGG_2, PGH_2 and TxA_2 with the stable prostaglandins.

Tissues were superfused in cascade at 10 ml/min with Krebs solution at 37°C containing a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) plus indomethacin (1 μg/ml). As well as rat stomach strip, rat colon and chick rectum, strips of rabbit vascular tissue (cut spirally) were tested.

Prostaglandin endoperoxides were prepared by incubating microsomes of ram seminal vesicles with arachidonic acid at 20°C without co-factors. Endoperoxides were purified by low temperature column chromatography (Ubatuba, Moncada & Vane, unpublished). Thromboxane A_2 was prepared by incubation of PGG_2 or PGH_2 with horse platelet microsomes at 0°C (Needleman, Moncada, Bunting, Hamberg, Samuelsson & Vane, unpublished). TxA_2 was then rapidly ($\simeq$ 1 min) extracted by shaking (20 s) with two parts dry ether (0°C) at neutral pH, dried under nitrogen and redissolved in buffer immediately before testing.