Release of prostaglandins and rabbit aorta contracting substance (RCS) from guinea-pig lung by slow reacting substance of anaphylaxis (SRS-A)

DINAZ M. ENGINEER, PRISCILLA J. PIPER & P. SIROIS

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, London

Guinea-pig lungs previously sensitized to ovalbumin respond to challenge by the release of several mediators including histamine, SRS-A, RCS (thromboxane A_2 (TXA_2) and prostaglandins G_2 and H_2), releasing factor for RCS (RCS-RF) and other prostaglandin-like substances (Piper & Vane, 1969).

SRS-A was prepared from indomethacin-treated isolated perfused lungs from sensitized guinea-pigs as previously described (Engineer, Piper & Sirois, 1976). Histamine was removed and SRS-A concentrated by adsorption onto activated charcoal and elution with 80% ethanol. After evaporation under vacuum the SRS-A was freeze-dried.

Lungs from either sensitized or unsensitized guinea-pigs were perfused with Tyrodes solution via the pulmonary artery at 5 ml/min and the effluent superfused over strips of smooth muscle from guinea-pig ileum, rabbit aorta, rabbit coeliac artery, rat stomach strip and chick rectum. The assay tissues were blocked with mepyramine and hyoscine.

When crude SRS-A was injected directly over the assay tissues it caused contraction of guinea-pig ileum and rat stomach strip but when injected into the pulmonary artery all the assay tissues contracted showing the passage of SRS-A through the pulmonary circulation and the release of RCS and prostaglandin-like substances from the lungs. Following injection of SRS-A into the lungs, RCS-induced contraction of rabbit aorta was matched by injecting PGG_2 over the tissues. Repeated injections of SRS-A released similar amounts of RCS from lungs. Any RCS-RF which might have been present in the samples of SRS-A was destroyed by boiling for 15 min (Flower, Harvey, Moncada, Nijkamp & Vane, 1976) followed by centrifugation for 5 minutes. The biological activity of SRS-A and its ability to release RCS and prostaglandins was unchanged by boiling. Incubation of SRS-A with arylsulphatase for 1 h destroyed the biological activity (Orange, Murphy & Austen, 1974); if the enzyme activity was terminated by boiling, the release of RCS was prevented, but in the absence of boiling there was sometimes a small release, probably due to contamination by RCS-RF. The release of RCS and prostaglandin-like substances by SRS-A was prevented by treating the lungs with indomethacin (1–5 μg/ml) or with dexamethasone (4–40 μg/ml). Furthermore, treatment of the lungs for 15 min with the SRS-A antagonist FPL-55712 (for ref. see Augstein, Lee, Sheard & Tattersall, 1973) (0.1–1.0 μg/ml) also prevented the release of RCS by SRS-A.

These results (a) strengthen the hypothesis that SRS-A released in anaphylaxis (Engineer *et al.*, 1976) might in turn release prostaglandin-related materials to inhibit further release of mediators, and (b) could explain the mechanism of action of steroid and non-steroid anti-inflammatory drugs in acute immunological reactions.

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The relationship between prostaglandin-like substances and SRS-A released from immunologically challenged lungs

J.R. BOOT, A.D.J. BROCKWELL, W. DAWSON & W.J.F. SWEATMAN

Lilly Research Centre Limited, Erl Wood Manor, Windlesham, Surrey, England

Although the biological activities of certain prostaglandins (PGs), particularly of the F series and slow reacting substance in anaphylaxis (SRS-A) on bronchial smooth muscle are similar, they appear to derive from two different biological precursors. PGs, thromboxanes and hydroxy fatty acids are formed from arachidonic acid by two enzymes, a cyclo-oxygenase and a lipoxygenase, both of which are inhibited by the competitive substrate, eicosatetraynoic acid (TYA). This compound reduced the PGs released on immunological challenge of guinea-pig lungs but did not modify SRS-A release, in contrast to disodium cromoglycate which had the

reverse effect (Dawson & Tomlinson, 1974).

Walker (1973) has shown that some non-steriodal anti-inflammatory compounds increase SRS-A release, although these compounds are known to be inhibitors of PG synthetase (cyclo-oxygenase) under many conditions (Vane, 1971). The relationship between PG and SRS-A release has been further analysed using these compounds in preparations derived from passively sensitized human lung and actively sensitized guinea-pig lung (Sheard, Killingback & Blair, 1968; Brocklehurst, 1960).

Indomethacin (1 μg/ml), TYA (10 μg/ml) and aspirin (25 μg/ml) inhibited a microsomal preparation of PG synthetase from guinea-pig lung by 50%, whilst phenylbutazone (10 μg/ml) was virtually ineffective. It was not possible to produce a consistent preparation of this enzyme from human lung. At the same concentrations, the three anti-inflammatory compounds potentiated the release of SRS-A by at least 50% from both human and guinea-pig lungs, but had little effect on histamine release. TYA had no effect on SRS-A or histamine release.

$PGF_{2\alpha}$ and thromboxane B_2 (TXB_2) increased the release of SRS-A from challenged guinea-pig lung whilst PGE_2 reduced it (Table 1). The levels of the parent PGs released on challenge are low, but that of TXB_2 reaches 1 μg/ml (Dawson, Boot, Cockerill, Mallen & Osborne, 1976). It appears that the most important member of the PG family in the modulation of SRS-A release is TXB_2. The data further lead to the speculation that compounds such as indomethacin may preferentially inhibit the formation of parent PGs, leaving the thromboxane pathway from arachidonic acid relatively untouched. PGE_2 and $PGF_{2\alpha}$ are known to modify the adenyl and guanyl cyclase systems in lung tissue, which have been shown to regulate mediator release. Experiments to examine the effects of TXB_2 on the cyclic nucleotides and to analyse PG and Tx release in the presence of non-steroidal anti-inflammatory drugs are in progress.

Table 1 Effect of prostaglandin-like substances on SRS-A release from immunologically challenged guinea-pig lung. ($n=9$)

concentration (μg/ml)	% change in SRS-A release (mean ± s.e. mean) in the presence of PGE_2	$PGF_{2\alpha}$	TxB_2
0.1	0		0
1.0	−20 ± 15	+21 ± 12	+49 ± 22
10.0	−29 ± 6	+40 ± 10	+63 ± 34

+ = increased release; − = reduced release

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Hydrostatic pulmonary oedema is not a stimulus for prostaglandin synthesis in isolated, perfused lungs

ELIZABETH SCOTT, J. VAAGE & T. WIBERG (introduced by A. UNGAR)

Institute of Physiology, University of Oslo, Oslo, Norway

Isolated lungs can be induced to synthetize and release prostaglandins (PGs) by a variety of mechanical and chemical stimuli (Piper & Vane, 1971). It has been suggested that the stimulus for PG synthesis may be distortion of cell membranes (Piper & Vane, 1971). Said & Yoshida (1974) have reported release of PGs during formation of oedema in isolated cat lungs. In their experiments oedema was induced by prolongation of perfusion time. This is, however, a non-specific method of induction. Thus PG synthesis might occur secondary to cellular injury, which also might be responsible for the pulmonary oedema.

In the present experiments we caused lung oedema by elevating the hydrostatic pressure in the pulmonary circulation in order to study the effect of vascular distention and increased extravascular lung water on pulmonary PG-synthesis.

Isolated lungs were prepared as described by Hauge, Lunde & Waaler (1966). The following combinations of species and perfusates were used: 5 rabbit lungs – horse plasma; 2 cat lungs – horse