DEMONSTRATIONS

The release of rabbit aorta contracting substance (RCS) from chopped lung and its antagonism by anti-inflammatory drugs

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As well as the release of histamine, slow reacting substance in anaphylaxis (SRS-A) and prostaglandins, there is a release of rabbit aorta contracting substances (RCS) and of RCS-releasing factor when perfused isolated lungs from sensitized guinea-pigs are challenged (Piper & Vane, 1969). The release of all these substances has now been detected from chopped lung.

Lungs were removed from sensitized or unsensitized guinea-pigs and perfused with Krebs solution via the pulmonary artery until all the blood had been washed out. The lung tissue was then chopped into pieces about 2 mm^3 and washed again. The chopped tissue was placed in the barrel of a 10 ml plastic syringe and Krebs solution dripped through it at 5 ml/min. The effluent was superfused over one or more assay tissues selected from rabbit aorta (to detect RCS), cat terminal ileum (to detect histamine), rat stomach strip, rat colon, chick rectum (to detect prostaglandins), and guinea-pig trachea (to detect SRS-A). All tissues except cat terminal ileum were blocked with a combination of mepyramine, methysergide, propranolol, phenoxybenzamine and hyoscine to increase the specificity of the assay (Piper & Vane, 1969).

The release of histamine, SRS-A, prostaglandins and RCS were detected when ovalbumen (10 mg) was injected into the Krebs solution dripping through the chopped sensitized lung. The release of the same substances was also detected when chopped lung from sensitized or unsensitized guinea-pigs was stirred by manually moving a blunt nylon rod (3 mm diameter) vertically up and down the syringe barrel at a rate of about 1/s for 5/6 min.

When lung from unsensitized guinea-pigs was used, four consecutive stirrings of the choppings each released reproducible amounts of RCS, histamine and prostaglandins. The release by stirring could therefore be used to detect inhibitors of release. After an initial stirring of lung tissue to give a control contraction of rabbit aorta, indomethacin (0·01–1 μg/ml) infused into the Krebs solution bathing the lung tissue either reduced or completely blocked the release of RCS induced by subsequent stirrings of the lung tissue. Similar results were obtained with sodium aspirin (0·1–5 μg/ml). Indomethacin or aspirin superfused directly over rabbit aorta does not block the contraction produced by RCS. When indomethacin was washed out of the lung tissue, subsequent stirrings caused releases of RCS which gradually increased towards their original size. The doses of aspirin and indomethacin which antagonized the release of RCS were similar to those used in isolated perfused lungs (Piper & Vane, 1969).

It is interesting that the same mediators can be released by stirring choppings of sensitized or unsensitized lungs as are released by the anaphylactic response of this tissue. A common feature of both procedures might be damage to the cell membrane.

The fact that indomethacin and aspirin reduce the release of RCS induced by stirring suggests that some modification of this method could be used as a simple *in vitro* test for anti-inflammatory activity.

REFERENCE

PIPER, PRISCILLA J. & VANE, J. R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223**, 29–35.

The release of biologically active substances from isolated lungs by 5-hydroxytryptamine and tryptamine

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5-Hydroxytryptamine (5-HT) is removed from the circulation by the lung both *in vivo* and *in vitro* (Gaddum, Hebb, Silver & Swan, 1953; Thomas & Vane, 1967). Isolated lungs of the rat and guinea-pig perfused with Krebs solution remove more than 90% of 5-HT in low concentrations (5–50 ng/ml) from the pulmonary circulation, without causing a significant increase in pulmonary perfusion pressure or releasing other substances from the lung (Alabaster & Bakhle, 1970). The 5-HT removed by the lung was rapidly metabolized by a monoamine oxidase. Experiments have now been carried out with higher concentrations of 5-HT.

The lungs from guinea-pigs and rats were dissected free, inflated and perfused via the pulmonary artery with Krebs bicarbonate solution, maintained at 37° C and gassed with 5% CO_2 in oxygen. The rate of perfusion was kept constant at 8–10 ml/min. Isolated lobes of dog lung were perfused via a branch of the pulmonary artery in the same way. The effluent from the pulmonary circulation was used to superfuse up to six isolated assay organs. 5-HT was infused for 3–5 min into the pulmonary arterial cannula, and active substances in the lung perfusate were detected by a continuous bioassay technique (Vane, 1969). The tissues were chosen to detect the presence of 5-HT, histamine, kinins, prostaglandins, " slow reacting substance " (SRS: Brocklehurst, 1962), and " rabbit aorta contracting substance " (RCS; Piper & Vane, 1969). Combinations of antagonists were infused over the assay tissues to confirm biological activity and increase the specificity of the assay. Thus prostaglandins were detected in the presence of 5-HT by contractions of a rat stomach strip, rat colon and chick rectum in the presence of methysergide bimaleate (2×10^{-7} g/ml).

Infusions of 5-HT (0·05–1 μg/ml) into the pulmonary artery of rat isolated lungs produced a large rise in perfusion pressure associated with a release of substances from the lung. Substances detected in the lung perfusate included 5-HT (proportion not metabolized by the lung), prostaglandins, an SRS and other active substances. Similar results were also obtained with tryptamine (0·5–2 μg/ml) infused into the pulmonary artery. Results obtained from isolated lungs of rat, guinea-pig and dog have been compared.

These results may have clinical significance since venous plasma concentration of free 5-HT has been reported to be in the range of 0·1–2 μg/ml in acute conditions of carcinoid syndrome (Stacey, 1966; Peart & Robertson, 1961).

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