

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

VALERIE A. ALABASTER and Y. S. BAKHLE, *Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London W.C.2*

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% CO₂ 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 bimalate (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).

This work was supported by a grant from the Wellcome Trust.

REFERENCES

- ALABASTER, V. A. & BAKHLE, Y. S. (1970). Removal of 5-hydroxytryptamine by rat isolated lung. *Br. J. Pharmac.*, **38**, 440P.
- BROCKLEHURST, W. E. (1962). Slow reacting substance and related compounds. *Progr. Allergy*, **6**, pp. 539–558. Basle: Karger.
- GADDUM, J. H., HEBB, C. O., SILVER, ANN & SWAN, A. A. B. (1953). 5-Hydroxytryptamine: pharmacological action and destruction in perfused lung. *Q. Jl exp. Physiol.*, **38**, 255–262.
- PEART, W. S. & ROBERTSON, J. I. S. (1961). The effect of serotonin antagonist (UML 491) in carcinoid disease. *Lancet*, **2**, 1172–1173.
- 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.
- STACEY, R. S. (1966). 5-Hydroxytryptamine in disease. *Handbook of Experimental Pharmacology: 5-Hydroxytryptamine and related Indolealkylamines*, p. 760, ed. Erspamer, V. Berlin: Springer-Verlag.
- THOMAS, D. P. & VANE, J. R. (1967). 5-Hydroxytryptamine in the circulation of the dog. *Nature, Lond.*, **216**, 335–338.
- VANE, J. R. (1969). Release and fate of vaso-active hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

Simplified thin-layer chromatography of prostaglandins in biological extracts

A. L. WILLIS, *Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN*

Commercially prepared plates for thin-layer chromatography are disposable and can be sectioned, allowing phosphomolybdic acid visualization of prostaglandin (PG) markers run on the same plate as a biological extract. The AI and AII systems (Gr  en & Samuelsson, 1964) were used with glass plates (Merck) precoated with 0.25 mm of silica gel, sometimes containing fluorescent indicator (plates backed with aluminium foil were less satisfactory). As recommended by Ramwell & Daniels (1969), tanks were not equilibrated before use.

For the AII system, the plates were saturated with ethanolic AgNO₃ (Fig. 1). The edges were wiped and the plates thoroughly dried and sealed in a black polyethylene envelope for up to 10 days before use.

Extract and markers were applied to the plate as in Fig. 1. After development, the plate was dried, cut and the marker PGs visualized (Gr  en & Samuelsson, 1964). Silica gel containing separated extract was scraped off and eluted. Zones from AII plates were eluted into acidified Krebs solution or into 3 ml of acidified 2% NaCl solution (pH 2.5–3.0 after elution). PG in the eluate was extracted twice into an equal volume of ethyl acetate and the dried extracts dissolved in 1 ml of Krebs solution for bioassay. Zones from AI plates were eluted into 1–4 ml Krebs solution and, after centrifugation, activity in the supernatant determined directly. Eluted activity was assayed in parallel (Willis, 1969) on isolated tissues superfused in cascade with Krebs solution.

Good separation was achieved: in the AII system, mean (\pm S.E.M.) R_s on eighteen plates were 0.79 ± 0.02 for PGE₁; 0.54 ± 0.02 for PGE₂ and 0.26 ± 0.01 for PGF_{2 α} , with no significant difference across the plates. Recovery was 60–80% in the AI and 30–50% in the AII system. PGF_{1 α} was only used on four occasions, when it ran just in front of PGE₂. Detection of PGF_{1 α} in extracts thus relied upon parallel assay and rechromatography in the AI system.