Br. J. Pharmac. (1976), **56**, 39–41

BIOASSAY OF HISTAMINE IN THE PRESENCE OF PROSTAGLANDINS

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1 An Amberlite XAD-2 column in a heated (37°C) jacket was incorporated in between two banks of bioassay organs superfused with Krebs bicarbonate solution in cascade. The column removed prostaglandins E_1, E_2 and $F_{2\alpha}$ and also rabbit aorta contracting substance (RCS) and possibly slow reacting substance of anaphylaxis (SRS-A).

2 The column gave free passage to histamine in concentrations up to 3000 ng/ml. The method described improves the accuracy of histamine bioassay in the presence of prostaglandins.

Introduction

The technique of Vane (1964) has been used for a simultaneous bioassay of histamine, slow reacting substance of anaphylaxis (SRS-A), prostaglandins and rabbit aorta contracting substance (RCS) in the effluent from the shocked perfused lungs of sensitized guinea-pigs (Piper & Vane, 1969, 1971; Palmer, Piper & Vane, 1973; Piper, 1974). The cat terminal ileum (Ferreira, Ng & Vane, 1973) and the mepyramine-treated guinea-pig trachea were used to differentiate between histamine and SRS-A, and prostaglandins and RCS were bioassayed by standard procedures (Ferreira & Vane, 1967; Piper & Vane, 1969).

We have observed that the peak concentration of histamine released during anaphylactic shock from perfused guinea-pig lungs, as indicated by the difference between the responses of the antazoline-free and antazoline-treated guinea-pig ileum (1.08 μg/ml, s.e. $\pm$ 0.21, $n=7$) is much higher than that determined spectrofluorimetrically by the method of Häkanson & Rönnberg (1974) (0.23 μg/ml, s.e. $\pm$ 0.04, $n=7$). One of the reasons for this discrepancy may be the presence of prostaglandins and RCS in the effluent.

Here we describe a technique, which enables histamine to be bioassayed by the guinea-pig ileum in the presence of relatively high concentrations of prostaglandins.

Methods

Two banks of the assay organs, each consisting of a rabbit aortic strip, a rat stomach strip and a guinea-pig ileum, were superfused with Krebs bicarbonate solution of the following composition (mM): NaCl 118.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ $7H_2O$ 1.17, $CaCl_2$ $6H_2O$ 2.5, $NaHCO_3$ 25.0 and glucose 8.4, gassed with 95% O_2 and 5% CO_2. Krebs solution (37°C) was dripped over the organs at a rate of 2.5 ml/min, or perfused through the isolated lungs of sensitized guinea-pigs before reaching the organs (Piper & Vane, 1969). All substances were infused directly over the organs at a rate of 0.2 ml/min except for ovalbumin (10 mg), which was injected into the pulmonary artery. The combined antagonists (Gilmore, Vane & Wyllie, 1968), minus mepyramine, were infused directly over the tissues. The movements of the assay organs (initial load 2–3 g) were recorded with Paton's auxotonic levers connected to Harvard transducers (type 386) and a Watanabe multirecorder.

Amberlite XAD-2 (Keirse & Turnbull, 1973) or glass wool (in control experiments) were used to fill the column (2 × 5 cm). The column was maintained at 37°C by a water jacket and placed between the two banks of the assay tissues (Figure 1). Before being used the Amberlite column was washed twice with 100 ml portions of distilled water and then perfused with Krebs (2.5 ml/min) for 15 minutes.

The following substances were used: prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn Co.), histamine dihydrochloride (Polfa), antazoline methanesulphonate (Polfa), mepyramine maleate (May and Baker) and Amberlite XAD-2 (BDH Chemicals Ltd).

Results

In our experiments the threshold concentrations of prostaglandins E_1 and E_2 which contracted the rat stomach strip were 0.5–2 ng/ml, and those of prostaglandin $F_{2\alpha}$ were 2–10 ng/ml. The guinea-pig ileum was usually contracted by histamine in concentrations of 5–10 ng/ml, and also by prostaglandins E_1 and E_2

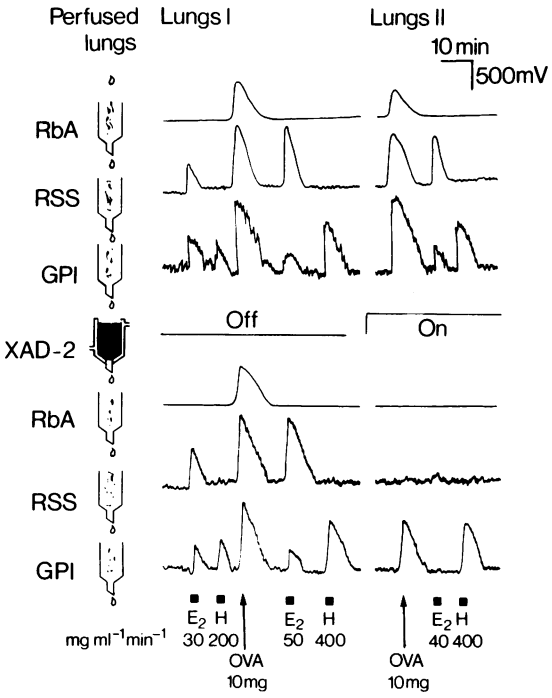

Figure 1 Isolated lungs of two sensitized guinea-pigs (lungs I and lungs II) were perfused with Krebs bicarbonate solution (2.5 ml/minute). The effluent was used to superfuse two banks of the assay tissues. Each consisted of a rabbit aortic strip (RbA), a rat stomach strip (RSS), and a piece of guinea-pig ileum (GPI). All tissues were blocked with combined antagonists minus mepyramine (Gilmore *et al.*, 1968). Calibration amounts (■) of prostaglandin E_2 (E_2) and histamine (H) were infused directly into the effluent at the top of the cascade. Antigen (ovalbumin 10 mg) was injected into the pulmonary artery (OVA). During the perfusion of lungs II the XAD-2 column was placed between the two banks of tissues. The difference in the responses of the guinea-pig ilea above and below the column shows the influence of the interfering substances on histamine bioassay by the upper guinea-pig ileum.

in concentrations of 10–20 ng/ml. As partially exemplified in Figure 1, prostaglandins E_1, E_2 and $F_{2\alpha}$ at 10–150 ng/ml and also RCS from shocked guinea-pig lungs are reduced to undetectable levels during one passage through XAD-2. Amberlite absorbs neither histamine (10–3000 ng/ml) nor catecholamines (10–100 ng/ml). The guinea-pig ileum pretreated with antazoline (0.5 μg/ml) or with mepyramine (1.0 μg/ml) and situated beneath the XAD-2 column was contracted neither by histamine nor by the effluent from shocked lungs (5 experiments). It seems likely therefore that SRS-A is also absorbed by XAD-2.

Using the above method we have found that the effluent from shocked lungs of sensitized guinea-pigs contains histamine in the range 225–700 ng/ml (470 ± 54 ng/ml, mean $\pm$ s.e., $n = 10$) together with a prostaglandin-like substance (43.1 ± 3.7 ng/ml prostaglandin E_2 equivalents).

Discussion

An Amberlite XAD-2 column removes prostaglandins, RCS and possibly SRS-A, but not histamine from the effluent from the shocked lungs of sensitized guinea-pigs, and therefore the guinea-pig ileum situated distal to the column registers the actual amount of histamine in the effluent. A similar absorbent column, but filled with aluminium oxide, has been proposed by us for the bioassay of prostaglandins in the presence of high concentrations of catecholamines (Korbut, 1975; Grodzińska, Panczenko & Gryglewski, 1975).

Differential assay of biologically active substances in a mixture is possible with organ cascades because of the selective sensitivity of the assay organs towards individual hormones (Vane, 1964). This may be further improved by administration of combined antagonists (Gilmore *et al.*, 1968). The procedure described here provides another way of facilitating the specific bioassay of hormones and autacoids in mixtures.

We thank the Trustees of The Wellcome Trust (London) for the generous grant of equipment, and Dr J. Pike, Upjohn Co., Kalamazoo, U.SA. for kindly supplying us with prostaglandins.

References

FERREIRA, S.H., NG, K.K. & VANE, J.R. (1973). The continuous bioassay of the release and disappearance of histamine in the circulation. *Br. J. Pharmac.*, **49**, 543–553.

FERREIRA, S.H. & VANE, J.R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature, Lond.*, **216**, 868–873.

GILMORE, N., VANE, J.R. & WYLLIE, J.H. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135–1137.

GRODZIŃSKA, L., PANCZENKO, B. & GRYGLEWSKI, R.J. (1975). Generation of prostaglandin E-like material by the guinea pig trachea contracted by histamine. *J. Pharm. Pharmac.*, **27**, 88–91.

HÄKANSON, R. & RÖNNBERG, A.L. (1974). Improved fluorimetric assay of histamine: condensation with *o*-phthalaldehyde at −20°C. *Anal. Biochem.*, **60**, 560–567.

KEIRSE, M.J.N.C. & TURNBULL, A.C. (1973). Extraction of prostaglandins from human blood. *Prostaglandins*, **4**, 607–617.

KORBUT, R. (1975). Bioassay of prostaglandins in the presence of high concentrations of catecholamines. *Pol. J. Pharmac. Pharm.* (in press).

PALMER, M.A., PIPER, P.J. & VANE, J.R. (1973). Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical or mechanical stimulation of guinea pig lungs. *Br. J. Pharmac.*, **49**, 226–242.

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

PIPER, P.J. & VANE, J.R. (1971). The release of prostaglandins from lung and other tissues. *Ann. N.Y. Acad. Sci.*, **180**, 363–385.

PIPER, P.J. (1974). Release and metabolism of prostaglandins in lung tissue. *Pol. J. Pharmac. Pharm.*, **26**, 61–72.

VANE, J.R. (1964). The use of isolated organs detecting active substances in the circulating blood. *Br. J. Pharmac.*, **23**, 360–373.

(Received April 25, 1975.
Revised July 1, 1975)