BIOASSAY OF HISTAMINE
IN THE PRESENCE OF PROSTAGLANDINS

R.J. GRYGLEWSKI & R. KORBUT
Department of Pharmacology, Copernicus Academy of Medicine, 16 Grzegorzecka, 31–531 Cracow, Poland

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 E₁, E₂ and F₂α 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. ± 0.21, n = 7) is much higher than that determined spectrophotometrically by the method of Håkanson & Rönnberg (1974) (0.23 µg/ml, s.e. ± 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, KH₂PO₄ 1.2, MgSO₄ 7H₂O 1.17, CaCl₂ 6H₂O 2.5, NaHCO₃ 25.0 and glucose 8.4, gassed with 95% O₂ and 5% CO₂. 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 300 ml portions of distilled water and then perfused with Krebs (2.5 ml/min) for 15 minutes.

The following substances were used: prostaglandins E₁, E₂ and F₂α (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 E₁ and E₂ which contracted the rat stomach strip were 0.5–2 ng/ml, and those of prostaglandin F₂α 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 E₁ and E₂.
in concentrations of 10–20 ng/ml. As partially exemplified in Figure I, 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 ± 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.

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### References


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