COMPARATIVE BIOASSAY OF PROSTAGLANDIN E_2 AND ITS THREE PULMONARY METABOLITES

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The relative potencies of prostaglandin E_2 and its metabolites 15-keto PGE_2, 13,14-dihydro-15-keto-PGE_2 and 13,14,dihydro-PGE_2 were investigated on isolated smooth muscle preparations. These were rat stomach strip, colon and uterus, chick rectum, guinea-pig ileum, trachea and pulmonary artery and rabbit aorta and pulmonary artery. 15-keto PGE_2 was equiactive or 1-1.8 times more potent than prostaglandin E_2 in relaxing guinea-pig trachea but otherwise the three metabolites were less active than prostaglandin E_2 on all preparations.

Introduction Prostaglandins of the E series are metabolized by guinea-pig lung homogenates to yield two types of metabolites; these are 15-keto-13,14-dihydro- and 13,14-dihydro-derivatives (Änggård, 1971). The metabolic sequence involves the initial formation of a 15-keto-prostaglandin E metabolite. Although this metabolite is not seen when prostaglandin E_2 is completely metabolized by guinea-pig lung homogenates, it is the sole product of metabolism by swine lung homogenates.

Metabolism of prostaglandin in guinea-pig lung results in its biological inactivation (Piper, Vane & Wyllie, 1970) and since this inactivation is extremely efficient (90-95%) it has generally been assumed that the pulmonary metabolites of prostaglandins have little biological activity. It has been shown that 13,14-dihydro-15-keto-PGE_1 has little spasmogenic activity on many tissues. However, 13,14-dihydro-PGE_1 shows appreciable biological activity, especially when tested on guinea-pig, rat and rabbit blood pressure (Änggård, 1966; Pike, Kupiecki & Weeks, 1967). Dawson, Lewis, McMahon & Sweatman (1974) have reported that the 15-keto metabolite of prostaglandin $F_{2\alpha}$ is a potent bronchoconstrictor agent and appears to possess greater biological activity than the parent on several isolated smooth muscle preparations. We have therefore examined the effects of the three pulmonary metabolites of prostaglandin E_2 (15-keto-PGE_2, 13,14-dihydro-15-keto-PGE_2 and 13,14-dihydro-PGE_2) on a variety of isolated smooth muscle preparations.

Methods The tissues commonly used for detection and bioassay of prostaglandins, rat stomach strip, chick rectum and rat colon, were arranged for superfusion in banks of three. Their order was varied from experiment to experiment. The tissues were superfused at 5 ml/min with Krebs solution which was warmed to 37°C and gassed with a mixture of 5% CO_2 and 95% O_2. A combination of antagonists to 5-hydroxytryptamine, catecholamines, acetylcholine and histamine were infused into the Krebs solution superfusing the tissues to make them more specific for prostaglandins (Piper & Vane, 1969). Prostaglandin E_2 and its metabolites were given as random 2 min infusions into the superfusing Krebs solution. A dose cycle of 10-15 min was adopted. Contractions of the assay tissues were measured auxotonically by Harvard smooth muscle transducers and displayed on a Watanabe 6-channel pen recorder. The sensitivity of the recorder was adjusted to a suitable level for the first experiment and then kept unaltered for the remaining experiments. The metabolites were also tested on several other smooth muscle strips superfused by the method described. These were guinea-pig ileum, trachea and pulmonary artery, and spirally cut strips of rabbit aorta and pulmonary artery. Female rats were brought into oestrus by subcutaneous injection of stilboestrol 1 mg/kg 18 h before use. Uteri were divided into two horns and superfused at 5 ml/min with de Jalon's solution.

The purity of prostaglandin E_2 and its metabolites was checked by thin layer chromatography in the A1 and A111 systems of Gréen & Samuelsson (1964).

Results All three metabolites were active on the rat stomach strip, chick rectum and rat colon (Figure 1). The 13,14-dihydro metabolite had roughly 0.2, 15-keto-prostaglandin E_2 0.1, and 13,14-dihydro-15-keto-PGE_2 about 0.01 times the activity of the parent substance on all three tissues. No differential responses of the tissues were seen. The reactivity of the tissues was unchanged by their position in the cascade.

The metabolites were inactive on rabbit aorta (2 experiments) and rabbit and guinea-pig pulmonary arteries (1 experiment each) in doses up to 1 μg/ml. Prostaglandin E_2 was inactive in

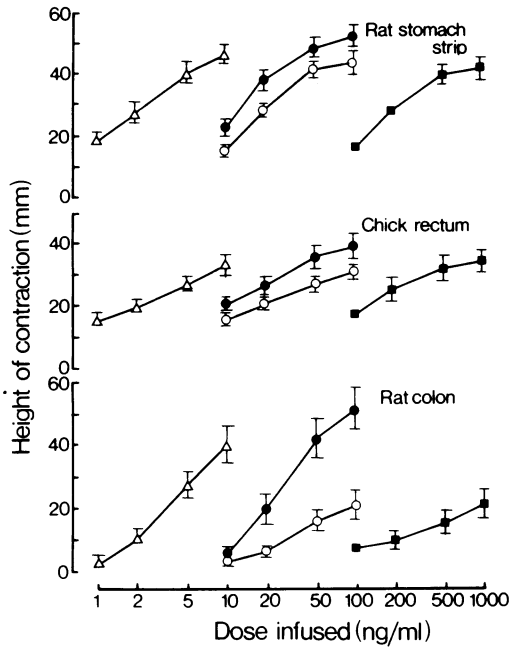

Figure 1 Comparative bioassay of prostaglandin E_2 (PGE_2) (△) and its pulmonary metabolites 13,14-dihydro-PGE_2 (●), 15-keto-PGE_2 (○) and 13,14-dihydro-15-keto-PGE_2 (■) on rat stomach strip, chick rectum and rat colon. Prostaglandin E_2 or metabolites were given as random 2 min infusions into the Krebs solution superfusing the assay tissues. The height of contraction of the assay tissues was plotted against the log dose of agonist. Each point is the mean of 8-10 experiments. Vertical bars show s.e. mean.

doses up to 100 ng/ml. 13,14-dihydro-PGE_2 was about 0.1 times as active as the parent compound on guinea-pig ileum, but the keto- and dihydro-keto-metabolites were inactive in doses up to 1 μg/ml (4 experiments). Prostaglandin E_2 contracted guinea-pig ileum with a threshold dose of 10 ng/ml.

On guinea-pig trachea, 13,14-dihydro-PGE_2 and 13,14-dihydro-15-keto-PGE_2 showed the same relative potency as on the prostaglandin assay tissues, i.e. approximately 0.1-0.2 and 0.01 times the potency of the parent respectively in relaxing this tissue. However, 15-keto-PGE_2 was 1.3 times as potent as the parent in relaxing guinea-pig trachea (4 experiments, range 0.8-1.8).

The 15-keto and 13,14-dihydro-15-keto metabolites were inactive on rat uterus in doses up to 1 μg/ml but 13,14-dihydro-PGE_2 was 0.4 times as active as prostaglandin E_2 (6 experiments, range 0.4-0.5).

Discussion The results show that two of the three pulmonary metabolites of prostaglandin E_2 have marked activity on the three assay tissues commonly used to detect parent prostaglandins. Moreover, the ratio of potency of parent and metabolites is roughly the same on all three tissues, making identification by differential assay impossible. For this reason, distinction between a relatively low level of prostaglandin E_2 and a higher level of 15-keto-PGE_2 or 13,14-dihydro-PGE_2 in biological fluids is not possible by bioassay alone. Hence, results indicating a 'release of prostaglandin' by bioassay alone without further identification should be regarded with caution. In addition the apparently high degree of inactivation of prostaglandin E_2 by isolated perfused lungs may in fact be even higher, since some of the residual activity of the effluent from the lungs may be due in part to the intrinsic activity of the metabolites formed.

Mathé & Levine (1973) have reported the release of high levels of prostaglandin metabolites from guinea-pig lung during anaphylaxis. The relatively high potency of 15-keto-PGE_2 in relaxing the guinea-pig trachea suggests that it may have a bronchodilator action when released in the lung. Since Dawson *et al.* (1974) have reported that 15-keto-$PGF_{2\alpha}$ is a more potent broncho-constrictor agent than is prostaglandin $F_{2\alpha}$ several of the biological effects of prostaglandins released in anaphylactic shock (Piper & Vane, 1969) may be due in part to high levels of prostaglandin metabolites.

13,14-dihydro-PGE_2 has about 0.2 times the activity of prostaglandin E_2 on rat stomach strip, chick rectum and rat colon, but nearly 0.4 times the parent prostaglandin's activity on the rat uterus. Since the pulmonary metabolism of prostaglandins is increased in late pregnancy (Bedwani & Marley, 1974), relatively more of this metabolite may be formed and might contribute to the induction of labour by intravenous infusion of prostaglandin E_2.

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