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ANTAGONISTIC ACTIONS OF PROSTAGLANDINS E_2 AND F_2 ALPHA ON THE ISOLATED LUNGS OF THE FROG, *RANA ESCULENTA* L.

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Abstract—1. Isolated lungs of the frog, *Rana esculenta* L., when incubated in amphibian Ringer solution for 30 min, produced a prostaglandin E_2-like substance (27.1 ± 3.8 ng/g w.w.), as determined by bioassay on the isolated rat stomach strip.

2. The release of PGE_2-like substance from skin, heart and bowel is also reported.

3. The activity of synthetic prostaglandins E_2 (PGE_2) and F_2alpha (PGF_2alpha) on the muscular contractility of frog isolated lungs was investigated: PGE_2 and PGF_2alpha relaxed and contracted respectively in a dose-dependent manner this preparation, a result similar to that obtained in mammals.

INTRODUCTION

The antagonistic actions of PGE_2 and PGF_2alpha on mammalian bronchial muscle have been shown by several investigators: as a rule PGF_2alpha contracts, and PGE_2 relaxes preparations from numerous species, including man (Anggard and Bergstrom, 1963; Cuthbert and Gardiner, 1981). Since lung tissue can synthesize and degrade both PGE_2 and PGF_2-alpha, a functional relationship between the two prostanoids and their involvement in the control of bronchial muscle tone has been suggested, but the physiological meaning of this relationship is still unclear (Sweatman and Collier, 1968).

During a survey on the presence and biological activities of prostanoids on isolated smooth muscle preparations of lower vertebrates, it was noticed that the isolated lungs of the common frog (*Rana esculenta* L.) both produced PGE_2-like material and responded to PGE_2 and PGF_2alpha in a fashion qualitatively identical to the one reported in mammals.

MATERIALS AND METHODS

Animals

Frogs, *Rana esculenta* L., of either sex, weighing 40–50 g, were maintained at room temperature in slowly circulating tapwater till use. They were kept fasting prior to experimentation. Male Wistar rats (200–250 g) were used for the bioassay.

Incubation, PG extraction and bioassay

The animals were pithed and lungs, heart, skin and intestine were rapidly removed and incubated at room temperature (20°C) in a saline (weight:volume 1:1) of the following composition (g/l): NaCl 6.5; KCl 0.14; $CaCl_2$ 0.12; $NaHCO_3$ 0.2. After 30 min, 1 ml of the incubation medium was removed, 0.250 ml of saturated NaCl solution added, acidified with 0.5 M citric acid and twice extracted with an equal volume of ethyl acetate.

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The organic phase was then evaporated in a stream of N_2. The residue was dissolved in 0.2 ml of Krebs bicarbonate and PGE_2-like activity tested by bioassay. The bioassay was performed by measuring the changes in isotonic tension (resting tension: 2 g) of a rat stomach strip superfused with Krebs bicarbonate (flow: 5 ml/min; Temp. 37°C). Synthetic PGE_2 (Upjohn, Kalamazoo) was used as reference standard.

Isolated lung preparation

The lungs were longitudinally cut and mounted into a 10 ml organ bath filled with saline (see above) continuously bubbled with 95% O_2 + 5% CO_2. Contractions were recorded using an isotonic transducer connected to a polygraph.

The tissues were placed under an initial tension of 0.5 g and left to equilibriate for 60–90 min. PGE_2 and PGE_2alpha, dissolved in saline, were added to the bath and kept in contact with the tissue for 3 min; the strip was then gently washed by dilution. In all the preparations, rapid washing or any slight mechanical stimulation caused a sustained and prolonged increase in muscle tension. Results are expressed as per cent of the maximal response elicited either by PGE_2 or PGF_2alpha in each preparation (±SEM). Regression analysis was carried out using the least-squares method.

RESULTS AND DISCUSSION

Frog lungs have been previously shown to generate prostanoids from endogenous polyunsaturated fatty acid stores (Christ and Van Dorp, 1974; Nomura and Ogata, 1976). The results shown in Fig. 1 confirm these findings and compare the PGE_2-like yields obtained in a few tissues under identical experimental conditions.

Synthetic PGE_2 and PGF_2alpha induced respectively relaxation and contraction of the isolated frog lungs (Fig. 2). This response was very rapid for PGE_2 and slower for PGF_2alpha (latency of *ca* 1 min); in both cases it was concentration-related. Arachidonate failed to elicit any motor response (not shown in figure).

The stimulatory action of PGF_2alpha and the inhibitory one of PGE_2 demonstrate a close parallelism with the response exhibited by the mammalian

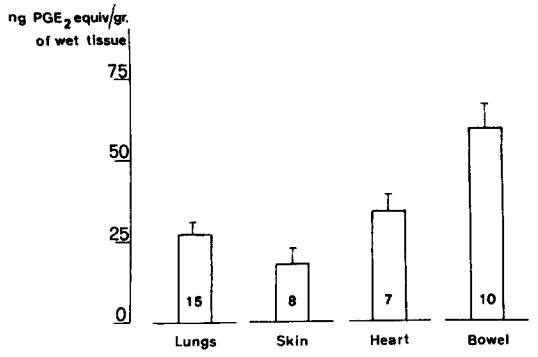

Fig. 1. Release of PGE_2-like substance by several organs of the frog *Rana esculenta*.

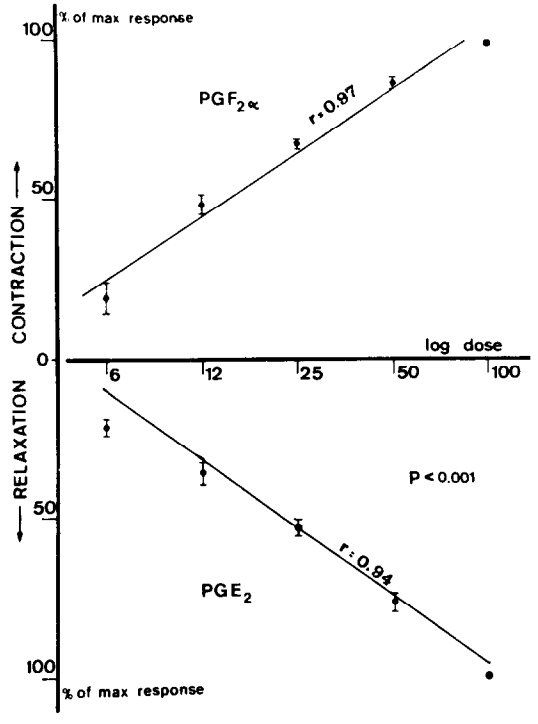

Fig. 2. Isolated lung preparation of *Rana esculenta* is contracted by $PGF_{2\alpha}$ (upper panel) and relaxed by PGE_2 (lower panel). Threshold: 6 ng/ml of bath. Vertical bars: SEM. Number of animals: six.

lung in spite of the large structural and functional differences between the two classes.

Modern Anura possess sac-like lungs with ridged, highly vascularized internal walls, inside which smooth muscle elements are enclosed (Goldie *et al.*, 1983). From a physiological standpoint this structure is essentially designed to supplement rather than to replace the exchange of gases through the skin. An important distinctive feature from mammalian lung is therefore the absence of a bronchial tree, which in mammals is the major control site of airway resistance and is very likely an important target of prostanoid action.

Our findings show that the pattern by which smooth muscle respond to PGE_2 and PGF_2alpha, whatever the function served, has been established early in evolution. As remarked by Johanssen and Reite (1967), this feature is shared by other neurohumoral substances such as acetylcholine, norepinephrine, epinephrine and serotonin.

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