GUINEA PIG BRONCHUS AS A MODEL FOR ICOSANOID STUDIES

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Abstract—Strips of guinea pig bronchus dissected from the upper, middle, and lower lobe and divided in two segments referred to as external and internal bronchi, were analyzed for their reactivity to several icosanoids. The external bronchi produced much more contractile force than the internal bronchi, and the reactivity to the agonists was different. The order of potency of prostanoids and histamine on the external bronchus was U44069 = histamine > PGF$_2$ and on the internal bronchus U44069 > histamine > PGF$_2$. The internal bronchus did not react to PGE$_2$, whereas this agonist produced a dose-dependent relaxation on the external bronchus. The order of potency of leukotrienes and histamine on the external bronchus was LTD$_4$ > LTC$_4$ > LTE$_4$ > LTA$_4$ > histamine and on the internal bronchus LTC$_4$ > LTD$_4$ > LTA$_4$ = LTE$_4$ > histamine. LTB$_4$ has a significant myotropic activity on guinea pig bronchus. Because of its sensitivity and characteristic responses to icosanoids, it is suggested that the guinea pig bronchus may be as suitable (and possibly more) a pharmacological preparation as the trachea or the parenchyma to study the bronchoreactivity.

INTRODUCTION

An array of chemical mediators are released from the lung tissue during asthma (for reviews see references 1 and 2) and are responsible for the main symptoms of this obstructive disease. The pharmacology of these mediators has been studied quite extensively in vitro in animal trachea and parenchyma strips (3–5) and to a certain extent in human bronchus and parenchyma (5–7). The use of these preparations as models of bronchial reactivity is debatable. The trachea response is a measure of large proximal airways, whereas the response of strips of lung parenchyma is the result of various contractile components including airway smooth muscles, vascular smooth muscles, and contractile interstitial cells (8–9).

In light of the possible contribution of prostaglandins, thromboxanes, and
leukotrienes to the physiopathology of bronchial asthma, and of the relevance of the bronchi as an important target for these mediators, we have done a preliminary pharmacological characterization of the effect of icosanoids on guinea pig upper, middle, and lower bronchi in order to obtain a reliable model of bronchoreactivity.

MATERIALS AND METHODS

Preparation of Guinea Pig Bronchi. Dunkin Hartley guinea pigs weighing 300–400 g were sacrificed by cervical dislocation. The thorax was cut open, and the trachea, lungs, and heart were removed and put in cold Krebs solution. The methods used for the preparation of strips of bronchi are shown in Figure 1. In brief, a metal rod (0.8–0.9 mm) is inserted in the trachea and right bronchus. The parenchyma around the main bronchi is dissected and the right and left bronchi are then cut from the trachea. Using a smaller metal rod (0.4–0.5 mm) as support, the bronchi are further gently dissected free of parenchyma and adhering connective tissues. Both bronchi are cut in spirals of approximately 1.5 mm width and 1.5 cm long. In early experiments, each bronchus was divided in three segments identified as external, median, and internal segments. The diameters of the external, median, and internal bronchi were around 1.5, 1.0, and 0.4 mm, respectively. However, for most of this work, each bronchus was divided in two segments (external and internal). The diameter of the bronchi corresponding to the external segments was around 1.0–1.5 mm, whereas the diameter of the internal segment was around 0.2–1.0 mm.

Superfusion of Guinea Pig Bronchi. The tissues were installed in the organ baths of a cascade superfusion system and perfused with oxygenated (95% O₂-5% CO₂) Krebs solution (5 ml/min; 37°C) as described by Sirois and Gagnon (10). The agonists were injected as boluses (10–50 µl) in the superfusion fluid, and the responses were recorded with Grass FT03C isometric transducers coupled to a Grass polygraph.

Drugs Used. Histamine hydrochloride was purchased from Sigma (St. Louis, Missouri). The following compounds were supplied as generous gifts: prostaglandin E₂, prostaglandin F₂α, tromethamine salt, and the thromboxane mimic U44069 (Dr. J. E. Pike, Upjohn Co., Kalamazoo, Michigan); leukotriene B₄ (Dr. J. Rokach, Merck Frost Lab., Montreal, Canada); leukotriene A₄, C₄, D₄, and E₄ methyl esters (Dr. S. Rakhit, Biomega Inc., Montreal, Canada). Leukotriene C₄, D₄ and E₄ methyl esters were hydrolyzed with a solution of Na₂CO₃ 5% for 1 h. LTA₄ methyl ester was hydrolyzed with a solution of ethanol: NaOH 1.0 N (1:1). Their concentration was evaluated by spectrophotometry.

RESULTS

Response of Bronchus Segments to Selected Icosanoids. The first series of experiments was designed to analyze and compare the response of different segments of bronchus to two potent lung agonists, histamine (1.8 × 10⁻⁸ mol; 2 µg) and leukotriene D₄ (4 × 10⁻¹⁰ mol; 200 ng). The doses of these agonists were chosen to obtain contractions which were not maximal. As shown in Figure 2, the external, median, and internal segments of bronchi (referring to segments of the main bronchi dissected approximately in three equal parts from the