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Drug-Induced Effects on the Longitudinal Smooth Muscle of Rat Bronchus[1] (40847)

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Bronchial smooth muscle, studied *in vitro* as either a helical strip or a ring, responds to a variety of substances (1–5). Contractions or relaxations obtained as a result of these procedures are a function of circular smooth muscle surrounding the bronchial lumen. We questioned whether the bronchi contain smooth muscle in a longitudinal orientation that responds to drugs. This has been previously demonstrated in arteries and veins (6–9). Therefore, the present investigation was designed to determine whether drug-induced responses could be obtained from a relatively small section of bronchus (1–2 mm diameter) set up in an isolated tissue bath to record lengthening and shortening of longitudinal muscle. The terminal end of the rat main stem bronchus was chosen due to its ease in handling.

Methods and materials. Male Sprague–Dawley rats (Harlan Industries, Cumberland, Ind.), 200 to 300 g were killed by cervical dislocation. The lungs were excised and placed in a petri dish containing Krebs' bicarbonate solution of the following composition in millimoles per liter: KCl, 4.6; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; NaCl, 118.2; $NaHCO_3$, 24.8, and dextrose, 10.0. The overlying parenchyma was gently teased away with two surgical forceps to expose the airways. A 16-mm length of terminal main stem bronchus was isolated and split longitudinally with a scissor to form a sheet of tissue. The bronchus was secured at both ends with cotton thread and mounted longitudinally in 10-ml tissue baths containing Krebs' bicarbonate solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Contractions and relaxations were measured isometrically with a Grass FTO3 C force-displacement transducer and recorded on a Grass polygraph as changes in grams of force. Contractions were maximized with respect to applied force. A passive force of 1 g was applied to the tissue. For comparison, tracheal rings from the same animals were prepared by the method of Hooker *et al.* (5) and placed in similar tissue baths under identical conditions. Cumulative dose–response curves were determined for carbachol, serotonin, and KCl whereas single responses were obtained for bradykinin, papaverine, and nitroglycerin.

Upon completion of the experiments, the bronchi were fixed in formalin. Paraffin sections were prepared according to standard procedures (10), stained with hematoxylin and eosin, and examined under a light microscope. The following drugs were used in the present study: carbamylcholine (carbachol), 5-hydroxytryptamine creatinine sulfate (serotonin), and bradykinin triacetate (Sigma Chemical Company), potassium chloride (J. T. Baker Chemical Company); papaverine hydrochloride and nitroglycerin (Eli Lilly and Company).

Results and discussion. Carbachol contracted the longtitudinal rat bronchus to the greatest extent (Fig. 1). The ED_{50} was 2.4×10^{-6} *M;* a maximal force of 243 ± 29 mg was generated by 3×10^{-5} *M* carbachol. Serotonin and KCl also contracted this tissue, but to a lesser degree. A high concentration of bradykinin (10^{-5} *M*) produced only a 10% contraction relative to the maximal carbachol response. The four agonists were also tested on rings of trachea obtained from the same animals (Fig. 2). As with the longitudinal bronchus, carbachol proved to be the most effective contractile agonist tested; the ED_{50} was 3.2×10^{-7} M and the maximal force generated was 2.53 ± 0.16 g. The percentage maximal tissue response to serotonin was lower in the trachea than in the bronchus whereas KCl produced a larger percentage maximal re-

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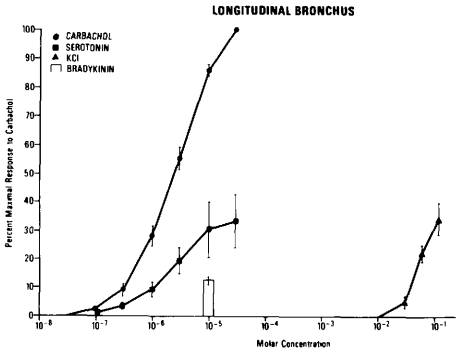

FIG. 1. Contraction of rat longitudinal bronchus by carbachol, serotonin, KCl, and bradykinin. Each point represents the mean response of 5 or more tissues ± SE.

chus. Interestingly, ED_{50} values were the same in both tissues for serotonin and KCl. Bradykinin was virtually inactive on the trachea. Neither the longitudinal bronchus nor the trachea contracted to prostaglandin $F_{2\alpha}$, histamine, or norepinephrine.

The carbachol-contracted rat bronchus responded to bronchodilator agents. An actual tracing is depicted in Fig. 3. In this case, the tissue relaxed in response to 3×10^{-4} M papaverine and to a similar concentration of nitroglycerin. Had the relaxant drugs not been added to the tissue baths, the response to carbachol would have slowly waned. This makes it difficult to obtain a dose–response curve by the cumulative dose method.

Independent microscopic examination of the tissues confirmed the specimens tested to be bronchi. Although some epithelial sloughing occurred, the bronchial smooth muscle appeared normal. No large pulmonary arteries or veins were seen in any of the preparations examined and therefore could not have contributed to the contractions or relaxations of the longitudinal bronchus.

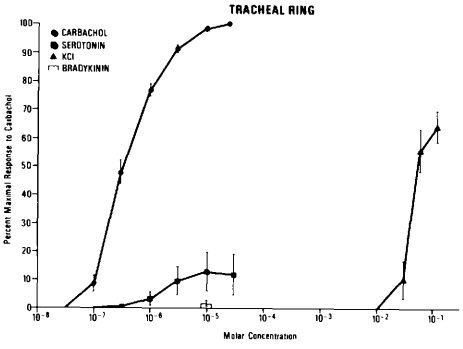

FIG. 2. Contraction of rat tracheal rings by carbachol, serotonin, KCl, and bradykinin. Each point represents the mean response of five or more tissues ± SE.

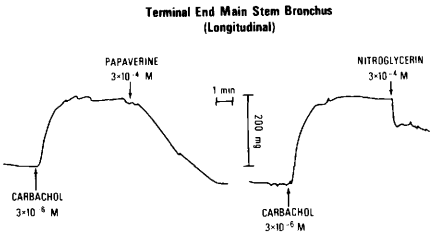

FIG. 3. Polygraph tracing showing papaverine and nitroglycerin-induced relaxations of carbachol-contracted rat longitudinal bronchus.

Our pharmacologic studies provided presumptive evidence that the airways not only constrict and dilate in response to drugs but they lengthen and shorten as well. Anatomical findings of Miller (11) are consistent with our observations. He characterized the smooth muscle winding around the bronchus as a "geodesic network" in which some of the muscle fibers run obliquely to give a partial longitudinal orientation. We propose that this muscle may represent a significant component of the total airway smooth muscle response and should be taken into consideration when determining the action of drugs on the bronchial tree. However, more quantitative *in vitro* and *in vivo* experiments are necessary before the contribution of the longitudinal smooth muscle can be properly ascertained.

Summary. Longitudinally oriented smooth muscle in the rat main stem bronchus contracts and relaxes to drugs *in vitro*. These actions would manifest themselves in the intact animal as lengthening and shortening of the airways and therefore could represent significant factors in the total airway response to drugs.

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