Use of a Technique for Measuring Pulmonary Resistance to Airflow for the Pharmacological Evaluation of Bronchodilator Agents

By ROBERT G. FAMILIAR, JOE R. WARDELL, JR., and LEON C. GREENE

A technique for measuring pulmonary resistance to airflow without the interference of compliance changes was used to obtain data on the bronchodilator activity of ephedrine sulfate and methoxyphenamine hydrochloride. The resting pulmonary resistance of cats was increased by injecting histamine (100 mcg., base, i.v.) or 5-hydroxytryptophan creatinine sulfate (25 mcg., base, i.v.) or by electrical stimulation (5 v., 1.5 msec., 15 c.p.s.) of the peripheral ends of both vagus nerves. The average maximum per cent inhibition of the increase in pulmonary resistance induced by the constrictor agents was used to calculate ED_{50}'s of the test drugs. Drugs were administered either intraduodenally or intravenously. The ability to obtain valid quantitative data for the evaluation of potential bronchodilator agents offers a distinct advantage over the methods presently employed.

MEASUREMENTS of lung volume changes with constant pressure inflations or tracheal pressure changes with constant volume inflations have been used to evaluate bronchodilator agents in the intact animal (1–3). At the ventilation frequencies used, these methods are sensitive to changes in lung stiffness and relatively less sensitive to changes in pulmonary resistance to airflow (4). Such measurements are only indirectly related to airway smooth muscle tone and can be influenced by other factors such as pulmonary vascular congestion, edema, or alveolar collapse. It must be appreciated also that no valid quantitative deductions can be made from these methods about airway smooth muscle.

Obviously, a method which measures changes in airway size separate from changes in lung stiffness or lung compliance would be advantageous in the evaluation of bronchodilator agents. With a technique previously described (5), it is possible to separate these parameters and measure pulmonary resistance without the interference of compliance changes. Pulmonary resistance includes both airway resistance and tissue resistance. Tissue resistance represents only a small part of the pulmonary resistance and does not vary markedly even in pathological states (6). Therefore changes in pulmonary resistance are a valid index of changes in airway resistance. This technique has been used in the past to study the action of bronchoconstrictor agents on the airways (7–9), but has not been used for comparative pharmacological evaluation of bronchodilator agents. To demonstrate the application of this technique, two bronchodilators, ephedrine sulfate and methoxyphenamine hydrochloride, have been studied for their ability to inhibit the increase in pulmonary resistance induced by histamine, serotonin, and vagal stimulation.

EXPERIMENTAL

Cats of either sex weighing 1.6–4.0 Kg. are anesthetized with sodium pentobarbital (30 mg./Kg. i.p.), paralyzed with gallamine triethiodide (40 mg. i.v.), and respired through a tracheal cannula at a constant tidal volume (50 ml.) and frequency of 28 breaths per minute. Transpulmonary pressure (P_{tp}) is measured by introducing a No. 12 Malecot catheter through the 5th or 6th intercostal space into the pleural space, and connecting it to one side of a Statham differential pressure transducer (type PM 131 TC). The other side of this transducer is connected to a hole in the side of the tracheal cannula. Airflow is measured with a Fleisch pneumotachograph (type 0.157) in conjunction with a Statham differential pressure transducer (type PM 5 TC). A second Statham differential pressure transducer (type PM 15 TC) is also connected to the pneumotachograph, and a volume signal is obtained by electrical integration of the airflow signal from this transducer. A schematic illustration of the experimental set-up is shown in Fig. 1.

Individual parameters of transpulmonary pressure, airflow, and volume are monitored *via* their respective amplifiers on the upper screen of an Electronics for Medicine recorder (model DR 8). The lower screen of the recorder is used to monitor pulmonary resistance utilizing the subtraction technique of Mead and Whittenberger (5).

Once the resting pulmonary resistance has been established, an increase in resistance is induced either by injecting histamine (100 mcg., base) or serotonin (25 mcg. base) i.v. or by electrically stimulating the peripheral ends of cut vagus nerves. In experiments where vagal stimulation is used, the animal is pretreated 1 hr. before stimulation with 3–5 mg. of neostigmine i.v. to improve the consistency of the response. Control experiments have shown that the responses obtained with any of the constrictor agents are quite consistent over at least a 2-hr. period.

Intraduodenal Administration of Test Drug—To administer a test drug intraduodenally, a midline incision, 4 cm. long, is made in the abdomen and the duodenum is exposed. Two control responses to the constric or agent of choice are made 3 min. apart. The drug is then injected directly into the duodenum, either in suspension (0.5% tragacanth), or solution (distilled water) in a volume of 4 ml. Postdrug responses to the constrictor agent are made at 10, 20, 40, 60, 90, and 120 min.

Intravenous Administration of Test Drug—Two control responses to the constrictor agent of choice are taken 3 min. apart. The test drug is then administered i.v., in saline. Postdrug responses to the constrictor agent are made at 30 sec., 5 min., 15

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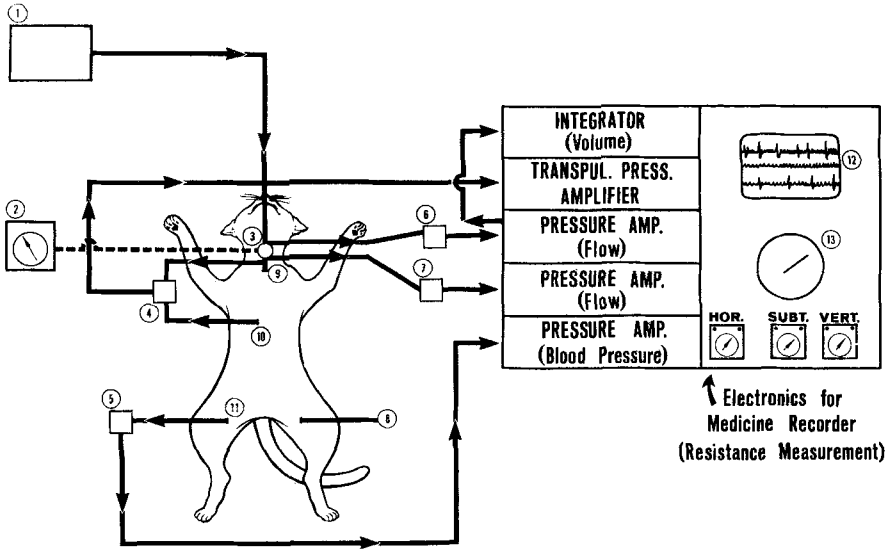

Fig. 1—Apparatus for measurement of pulmonary resistance. Key: 1, Harvard pump; 2, heater with rheostat; 3, pneumotachograph (A. Fleisch); 4, differential strain gauge (PM 131 TC 2.5); 5, pressure transducer; 6, differential pressure transducer (PM 15); 7, differential pressure transducer (PM 5); 8, intravenous catheter (injections); 9, tracheal cannula; 10, intrapleural catheter; 11, arterial cannula; 12, multitrace screen; 13, vector scope-resistance.

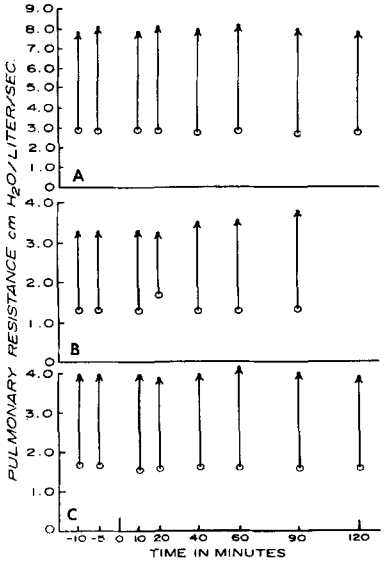

Fig. 2—Average control responses to: A, vagal stimulation (5 v., 1.5 msec., 15 c.p.s.) (two cats); B, histamine (100 mcg., i.v.) (three cats); and C, serotonin (25 mcg., i.v.) (three cats). Open circles represent pulmonary resistance preceding each administration of constrictor stimuli, while the dots on the top of the arrows represent pulmonary resistance 30 sec. after each administration. Distilled water was administered intraduodenally, 4 ml. vol. at zero time.

min., and at various intervals until the response returns to control values.

Evaluation of Data—The per cent inhibition of the increase in pulmonary resistance is calculated by the following formula. The test response is the increase in pulmonary resistance produced by the constrictor stimulus:

$$\% \text{ inhibition} = \frac{\text{av. predrug test response} - \text{postdrug test response}}{\text{av. predrug test response}} \times 100$$

The resting pulmonary resistance during the predrug period serves as the baseline for both the predrug and postdrug test responses. Postdrug test responses which are greater than the predrug value are considered zero per cent inhibition.

A dose-response curve is plotted for any test drug by obtaining a minimum of 3 to 5 individual responses for each dose, and calculating an average maximum per cent inhibition of pulmonary resistance increase. For intraduodenal administration each cat received only one dose of test drug. Several doses of a test drug, the number depending on the duration of individual responses, could be administered for intravenous evaluation. An ED_{50} and 95% Fieller limits are calculated by a method described by Finney (10).

RESULTS

Control Responses to Individual Constrictor Stimuli—The pulmonary resistance increase induced by histamine, serotonin, or vagal stimulation was consistent over time. The average results of the control experiments, in which distilled water (4 ml.) was injected intraduodenally, are shown in Fig. 2.

Intraduodenal Administration of Test Drugs—Ephedrine was shown to inhibit the pulmonary resistance increase induced by histamine, serotonin, and vagal stimulation. ED_{50}'s have been obtained for ephedrine against each of the constrictor agents. The onset of activity was between 20 and 60 min.; the duration was usually greater than 90 min. The dose-response curves are shown in Fig. 3.

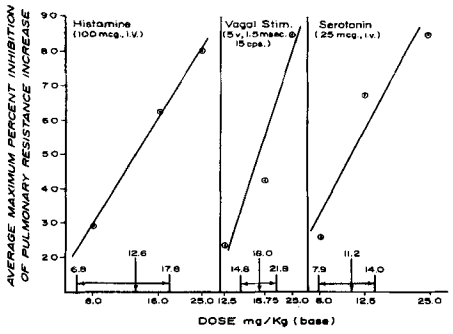

Fig. 3—Effect of intraduodenal ephedrine on the pulmonary resistance increase induced by histamine, serotonin, and vagal stimulation. Each point on the curves represents a minimum of three individual cats. ED_{50} (95% Fieller limits) in each case.

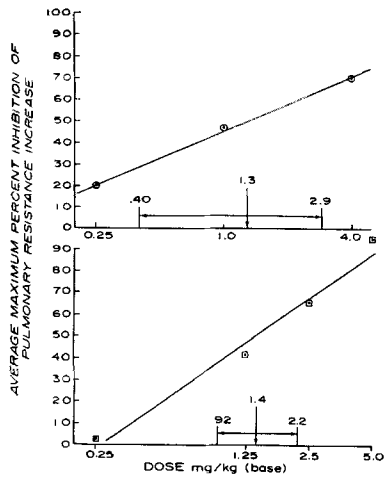

Fig. 4—Effect of intravenous ephedrine (top) and methoxyphenamine (bottom) on the pulmonary resistance increase induced by serotonin (25 mcg. i.v.). Each point on the curves represents a minimum of three responses. ED_{50} (95% Fieller limits) in each case.

Intravenous Administration of Test Drugs—To demonstrate the feasibility of using i.v. administration for drug evaluation, dose-response curves were obtained for ephedrine and methoxyphenamine against serotonin injection (25 mcg., i.v.). Both compounds were shown to inhibit the pulmonary resistance increase induced by this constrictor agent. The results are shown in Fig. 4.

DISCUSSION

The control of airway muscle has been frequently investigated. In the main, the studies fall into two categories. The first includes *in vitro* studies of the action of drugs and chemicals on excised airways or on denervated lung. Such methods are obviously unphysiological, the studies being limited to certain portions of the airways, and correlation with clinical conditions impossible. The second category includes *in vivo* studies involving the assessment of the relationship between inflation pressure and tidal volume during cyclical changes in pressure or volume. These methods measure all three pressures of breathing: elastic, resistive, and accelerative; therefore, changes in either compliance or resistance will affect the measurement. In fact, at the usual frequencies of ventilation, such measurements are relatively insensitive to changes in airway diameter (*i.e.*, resistive and accelerative forces) but are greatly influenced by alterations in lung stiffness (*i.e.*, elastic forces). Although lung compliance may change in the present method, this change is quite obvious (opening of pressure–flow trace) and correction can be made. In other words, the changes measured are due to changes in the size of the airways.

All of the agents used to induce the increase in pulmonary resistance have been implicated in hypersensitivity reactions (11–13). A more meaningful evaluation of potential bronchodilators can be made if they are compared to standard agents for their effect against all three of these constrictor stimuli, rather than any one.

The feasibility of using this method for intraduodenal drug evaluation is demonstrated in Fig. 2. The stable resting pulmonary resistance along with consistent responses to each constrictor stimulus over time makes the method ideal for this purpose. Some drugs may decrease an animal's resting pulmonary resistance in addition to inhibiting the response to a constrictor stimulus. Both effects are reflected in the calculations of drug activity since the predrug resting resistance is used as the baseline for postdrug test responses.

This method of drug evaluation offers a distinct advantage over the other methods since the measurements are not influenced to any great extent by factors such as pulmonary vascular congestion, edema, or alveolar collapse. In addition, as shown, the data can be quantitatively analyzed for a more valid comparison of test agents.

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