Interaction between Bronchoconstrictor Stimuli on Human Airway Smooth Muscle[1]

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In healthy human subjects, the simultaneous aerosol administration of histamine and methacholine results in a pronounced decrease in maximum flow rates on partial expiratory flow-volume (PEFV) curves. When given alone in the same concentrations, these drugs produced no or minimal decreases in flow rates. The results suggest an interaction of histamine and cholinergic stimuli on airway smooth muscle (ASM). This mechanism might explain many experiments where vagal blockade diminished or abolished ASM response to histamine and other stimuli, simply by interfering with histamine–cholinergic interaction at the ASM level. These findings confirm similar findings of animal *in vitro* experiments. The experiments clearly confirm the sensitivity and value of assessing drug effects prior to a deep breath. Flow-rate changes after a full inspiration, taken from the maximum expiratory flow-volume (MEFV) curve, show either no relationship to the concentration of inhaled methacholine or significantly less effect than that seen on the PEFV curve.

In spontaneously breathing guinea pigs, airway smooth muscle responses to a contracting agent, histamine, are potentiated by drugs which enhance cholinergic stimuli (physostigmine) and block β-adrenergic stimuli (propranolol) while they are inhibited by drugs which block cholinergic stimuli (atropine) and stimulate β-adrenergic receptors (isoproterenol) (1,2). These findings led to the hypothesis that autonomic transmitter substances from vagal and adrenergic nerve endings, and from circulating catecholamines, interact with histamine at the smooth muscle cell level, leading to enhancement or inhibition of the contractor action of histamine as a function of the balance of the autonomic stimuli. Experiments with isolated tracheal muscle of guinea pigs have given support to this hypothesis (3,4). Indirect support for such a mechanism of interaction in man is derived from experiments in which propranolol potentiated airway constrictor responses to cigarette smoke in healthy persons (5). This result suggested that physiologic levels of β-adrenergic stimulation normally protect airway smooth muscle, in part, against the effect of constrictor agents. The present study demonstrates, in man, a potentiation of histamine responses by simultaneous administration of methacholine, an acetylcholine analog, and thus provides support for interaction between cholinergic stimulation and histamine on smooth muscle. In addition, the results confirm the superiority of maximum expiratory flow rate measurements without maximum inspiration [partial expiratory flow-volume (PEFV) curve] over maximum expiratory flow-volume (MEFV) curves in detecting and quantitating airway constrictor responses in man.

SUBJECTS AND METHODS

We studied seven healthy adult subjects aged 21 to 32 years, all nonsmokers who denied present respiratory symptoms. To assess airway caliber, we recorded partial

expiratory flow-volume (PEFV) curves with a pneumotachograph–electronic integrator device (6) and a Brush 500 high performance XY recorder (Gould, Inc.).

On each occasion the subject inspired to about 65% of forced vital capacity (FVC) rather than to total lung capacity (TLC) and next performed a fast expiration from that volume level to residual volume. The record of this expiration is the PEFV curve. The subject then inspired to TLC and performed a full forced expiratory maneuver, during which the maximum expiratory flow-volume (MEFV) curve was recorded. The response characteristics of the XY recorder are sufficient for accurate reproduction of MEFV and PEFV curves, in comparison with curves recorded on a storage oscilloscope, if one chooses excursions of 1 in. for 2 liters/sec on the flow axis, and 1 in. for 1 liter on the volume axis. From each set of curves we measured expiratory flow rates at 40% of FVC, i.e., at TLC minus 60% of the control FVC. These were measured on PEFV [MEF 40%(P)] as well as MEFV (MEF 40%) curves. In addition, a time marker set at 1 sec on the MEFV curve allowed reading of the $FEV_{1.0}$ value for each blow.

Aerosol Administration

We used a D30 aerosol generator (7) which produced 0.25 ml/min of aerosol at a disruptive pressure of 15 psi. More than 95% of the particles produced by this generator have a diameter of 0.3 μm or less; their mean size is about 0.05 μm (7). The aerosol was temporarily stored in an open tube on the inspiratory side of a Collins J valve. The concentrations of solutions used were determined for each subject by determining the threshold dose for both histamine and methacholine. Concentrations around the threshold dose were selected for each of the two drugs. The threshold dose was defined as the concentration of each drug which caused a decrease of MEFV 40%(P) greater than 0.3 liters/sec.

Initially each subject performed three baseline PEFV and MEFV curves. The aerosol was then inhaled for 30 sec, and further recordings were made immediately after inhalation and again 2 and 4 min later.

On each day solutions of histamine, methacholine, and histamine and methacholine combined were inhaled. Two hours elapsed between inhalations. This routine was repeated on 2 subsequent days, the order of the aerosols being varied according to a Latin square arrangement to eliminate any possible bias due to circadian variation in airway reactivity (8). Neither the subject nor the person who analyzed the results was aware of the contents of the aerosol. In several subjects, solutions of normal saline were included in the protocol.

For each inhalation experiment, the average flow rates from the three control curves were used as the baseline value. The absolute difference between the flow rates after inhalation and the baseline value was obtained at each time interval and averaged for each of the three occasions that each drug was inhaled.

RESULTS

Recordings from a typical experiment are shown in Fig. 1. In the control experiment, maximum flow rates at low lung volumes on the MEFV curves were superimposed on those recorded during the preceding forced expiration, made from a lung volume of about 65% VC. Immediately after inhalation of the constrictor aerosol, maximum flow rates during the first forced expiration (from a lung volume of about 1 liter less than TLC) were greatly decreased. The resulting PEFV curve terminates at TLC minus 3.2 liters, i.e., residual volume is increased. Next, the subject inspired to TLC and performed the MEFV curve maneuver, during which flow rates at similar
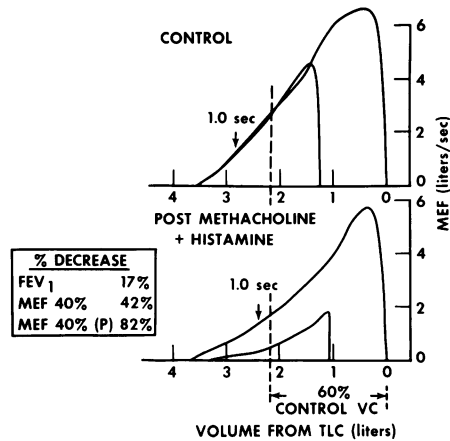
FIG. 1. Control and postexposure MEFV and PEFV curves in a healthy subject. The intersection of the curves and the vertical interrupted line indicate MEF 40% (at TLC less 60% of the control FVC). The vertical arrows indicate the position of the one-second marker.

volumes were less than control values but much higher than those on the immediately preceding PEFV curve. Thus, after a single maximum inspiration, the flow rate decrement after a constrictor aerosol was greatly diminished. Most likely as a result of the maximum inspiration, measurements made from the MEFV curve (FEV$_{1.0}$ and MEF 40%) decrease less than the flow measurement from the PEFV curve [MEF 40%(P)], in comparison with control values (for numerical results, see box in Fig. 1 and, also, Table 1).

Individual subjects appeared to differ in the extent to which a deep inspiration abolished the effect of the constrictor aerosol on maximum flow rates. This difference is demonstrated by the relationship between the dose of methacholine (i.e., the concentration in the nebulized fluid) and the responses of FEV$_{1.0}$, MEF 40%, and MEF 40%(P) in Figs. 2 and 3. In one subject (Fig. 2) there was a high correlation between the methacholine dose and the response, no matter which of the three measures was used. However, the changes of MEF 40%(P) were greater than those of MEF 40%, and the latter were greater than the changes of FEV$_{1.0}$. In contrast, only changes of MEF 40%(P) correlated with the dose of methacholine in the subject of Fig. 3. The measurements recorded after a full inspiration (MEF 40% and FEV$_{1.0}$) showed no relationship to the concentrations of methacholine inhaled, suggesting that in this subject a deep inspiration often completely abolished the constrictor response. Three out of the seven subjects in the present study gave dose–response relationships similar to those in Fig. 3. Therefore, we used MEF 40%(P) measure-

TABLE 1

Relative Changes in Measures of Airway Responses in Two Subjects for All Challenges where MEF 40% (P) Decreased by 20–40% of Control Values

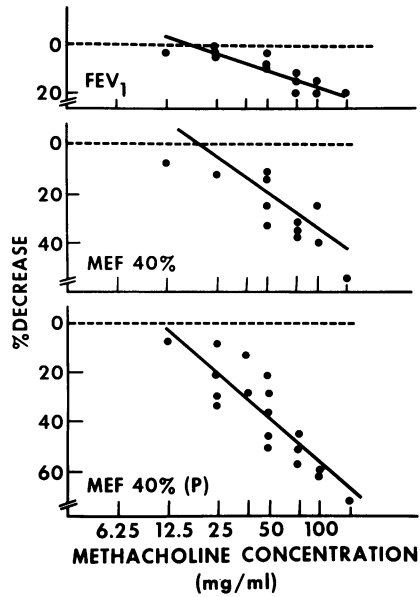|	Subject #1 (13 challenges)			Subject #2 (15 challenges)		
	Before	After	Decrease (%)	Before	After	Decrease (%)
FEV$_1$ (liters)	3.21	3.10	3.4	5.41	5.34	1.3
MEF 40% (liters/sec)	2.84	2.36	16.9	5.63	5.15	8.5
MEF 40%(P) (liters/sec)	2.81	2.07	26.3	5.02	3.65	27.3

FIG. 2. Dose–response relationships in a healthy subject to aerosol methacholine inhaled in various concentrations for 30 sec. The percentage decreases in FEV_1, MEF 40%, and MEF 40%(P) are shown. Linear regression analysis was performed for each measurement (shown as continuous line). The correlation coefficients for each analysis are: FEV_1, +0.83; MEF 40%, +0.76; MEF 40%(P), +0.84.

ments exclusively to assess the possible interaction between histamine and methacholine, since this was the only measure which gave dose-related responses in methacholine experiments in all subjects.

Interaction

The results of one subject are shown in Fig. 4. Histamine (5 mg/ml) and methacholine (12.5 mg/ml) inhaled separately, resulted in changes in flow rates
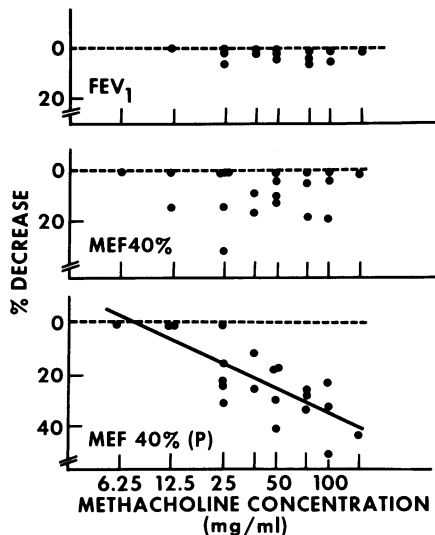

FIG. 3. As Fig. 2; the respective correlation coefficients are: FEV_1, 0.00; MEF 40%, 0.00; MEF 40% (P), +0.76.
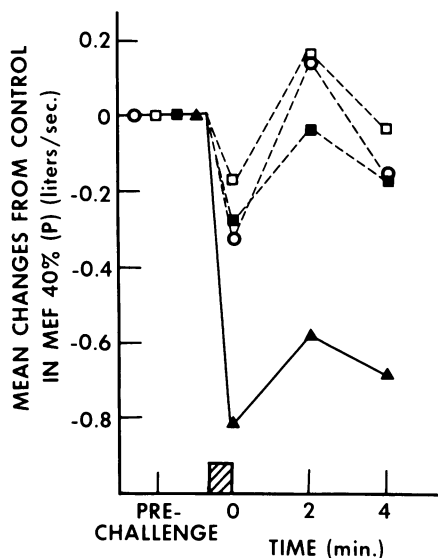
FIG. 4. Changes in flow rates on PEFV curves after 30-sec inhalation of histamine and methacholine administered separately and together in a healthy subject. (o---o) Saline control; (□---□) histamine alone; (■---■) methacholine alone; (▲—▲) histamine and methacholine together. The hatched area indicates the challenge.

similar to those seen after saline. However, histamine and methacholine administered together in the same concentrations resulted in a decrease in flow rates which was, at most time intervals, at least three times the algebraic sum of the changes to the drug administered separately. Similar results are shown in another subject in Fig. 5.

The mean data in the seven subjects are shown in Fig. 6. Histamine inhalation resulted in a slight but not significant increase in flow rates. Methacholine decreased flow rates slightly; the decrease compared to controls was significant ($p = 0.01$) immediately after inhalation but not significant later on. However, saline aerosol gave a similar response. The changes in flow rates after histamine and methacholine
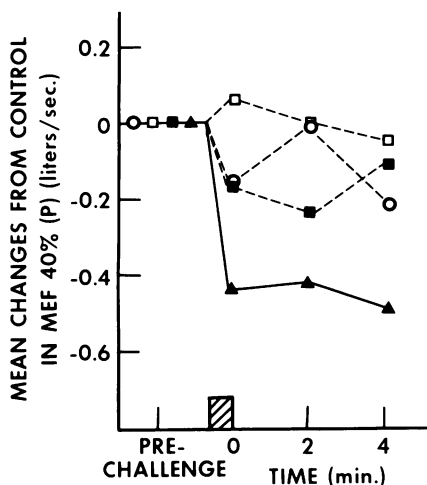

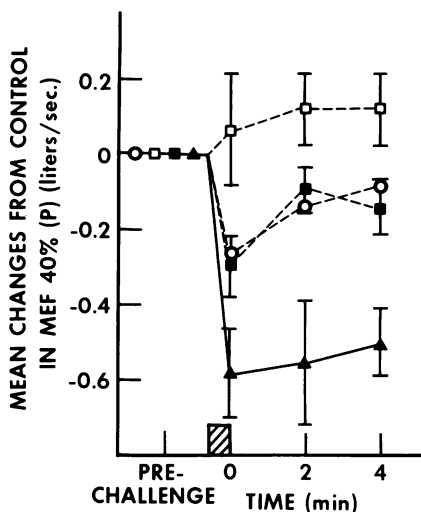FIG. 5. As Fig. 4; for another subject.

FIG. 6. As Fig. 4; for all seven healthy subjects. Values are shown as means ± SEM.

administered together were considerably greater than the algebraic sum of the effects of the drugs administered separately ($p < 0.005$ at 0 and 4 min; $p < 0.02$ at 2 min). For example, 4 min after the inhalation, the algebraic sum of the flow rate changes seen after the drugs given separately is +0.01 liters/sec, as compared to −0.49 liters/sec after the drugs given together.

Simultaneous administration of the two drugs was essential to the demonstration of interaction. Earlier experiments had failed to demonstrate any interaction when the drugs were administered sequentially.

DISCUSSION

We have shown that simultaneous administration of histamine and methacholine by inhalation of an aerosol results in a pronounced decrease of maximum expiratory flow rates at small lung volumes. When given alone in the same doses, the two drugs produced no or minimal decreases of these flow rates when compared to the effect of saline aerosol. After inhalation of the mixture, flow rates first rapidly decrease and then return to normal within 20 min. Since the reversion of flow rates to normal is not accompanied by expectoration of mucus, their reduction is most likely due to contraction of airway smooth muscle (ASM) with reduced airway caliber as a result. The fact that significant effects were observed with the two drugs together, while each drug given separately had at most minimal effects, points to a supraadditive type of interaction between the two agents.

Animal experiments support this conclusion. Histamine and methacholine both contract ASM of many species, including man (9), *in vitro*. In *in vitro* ASM preparations of guinea pigs, constrictor effects of histamine are potentiated by methacholine as well as by stimulation of cholinergic nerve fibers to the isolated trachea (3,4). In these preparations, potentiation of histamine effects can best be explained by interaction between histamine and cholinergic stimuli on smooth muscle cells in airways (10). The analogy between the interaction observed in *in vitro* ASM preparations and the potentiation of histamine-induced airway constriction by methacholine in the present experiments in intact man is striking. Thus, the simplest explanation of the

latter is that interaction between histamine and cholinergic stimuli on ASM occurs not only in isolated guinea pig trachea but also in the airways of intact man.

It has long been recognized that vagal efferent stimulation leads to airway constriction through contraction of ASM. In recent years several experiments, including those of Nadel, Gold, and their co-workers, have shown that vagal blockade by atropine, by cooling of the nerves, or by vagotomy reduces ASM responses to histamine in man (11) and to histamine and other airway constrictor stimuli in animals (12–15). These investigators interpret their results as indicating that the direct action of histamine on ASM is reinforced by a bronchoconstrictor reflex, with its efferent pathway in the vagal nerves. This hypothesis can explain why vagal blockade diminished the effect of histamine. However, the afferent pathways of the bronchoconstrictor reflex have not been clearly identified. The existence of "irritant receptors" in airways has been postulated from physiological experiments with irritants but these receptors have not been identified morphologically. Afferent stimuli might also originate in contracting airways, perhaps as a result of mechanical deformation of mucosal nerve endings (16).

The most convincing evidence for the existence of bronchoconstrictor reflexes comes from experiments in dogs, where slight contraction of an isolated cervical tracheal segment occurred within seconds after an injection of histamine into a bronchial artery (12). Unilateral challenges in dogs with separate ventilation of the two lungs suggest that vagally mediated reflexes determine a major degree of the ASM constrictor response to histamine and allergen challenge (13). However, similar experiments in two asthmatic humans have given negative results (17). Appropriate allergen challenge, sufficient to cause a moderate delay of nitrogen clearance on the challenged side, failed to produce a demonstrable effect in the contralateral unchallenged lung. As has previously been argued (13), these subjects were premedicated with opiates, scopolamine, and local anesthesia to the airway, and this premedication may have been sufficient to abolish all vagal activity.

The absence of a demonstrable effect in the unchallenged contralateral lung implies either that reflexes play an unimportant role in allergic airway reactions in man or that vagal blockade was present in these studies. If vagal blockade is accepted as the explanation of the absence of a contralateral response, then the moderate effect seen on the challenged side must be due entirely to the local action of released mediators of the allergic reactions, as it is hard to argue that reflex vagal activity would be abolished on one side only. Thus, although this study (17) does not allow any conclusions regarding the contribution of the reflex vagal activity to the allergic reaction, it implies that a moderate ASM response may be produced by the local action of released mediators alone. These same studies demonstrated a decreased sensitivity of the airways after the premedication in that it was necessary to use "allergen concentrations ten times larger than those needed to produce asthma . . . when the subjects were not anesthetized" (17). This decreased sensitivity might be explained by the reflex theory.

However, interaction between physiological levels of cholinergic stimuli (vagal tone) and histamine at the level of ASM cells offers an alternative hypothesis to explain this and many other experiments in which efferent vagal blockade diminishes or abolishes airway constriction induced by histamine or allergens. It is also a more comprehensive hypothesis than the reflex theory because it explains not only why vagal blockade reduces but also why physostigmine in guinea pigs intensifies histamine-induced airway constriction (2). The reflex theory cannot account for the

potentiation of histamine responses in isolated trachea–nerve preparations by constant levels of vagal nerve stimulation (3,4). The present results in intact humans are similar to those obtained with these isolated preparations. Thus, we believe that interaction between cholinergic stimuli and histamine may occur in intact humans. In this concept, vagal blockade reduces histamine-mediated responses by abolishing histamine–cholinergic interaction.

The mechanism of interaction between different chemical stimuli on ASM cells is not known. Interactions might occur at receptor sites on the cells. It may also involve common actions of chemical stimuli, mediated via different receptors, on intracellular processes involved in the contraction and relaxation of the smooth muscle filaments, such as intracellular transport of calcium or intracellular levels of cyclic nucleotides.

Since we measured maximum expiratory flow rates on PEFV curves, as well as on MEFV curves recorded immediately after a PEFV curve, our data give further information on the effects of a single deep inspiration on changes in airway caliber induced by bronchoconstrictor agents (18). In all subjects, flow rates decreased more on the PEFV curves than on the MEFV curves (Fig. 1). In three subjects, the effect of one deep inspiration was so marked that there was no relationship between inhaled methacholine concentrations and flow rates measured after a deep inspiration (Fig. 3). In the remaining four subjects, the inhaled methacholine concentrations correlated significantly with flow rates measured after a deep inspiration, but flow rates measured prior to the deep inspirations always showed the greatest change (Fig. 2). These differences in flow rate changes on MEFV and PEFV curves probably reflect an effect of lung volume history on ASM tone. Such a volume history effect, resulting in higher airway resistance at a given volume when previous volumes were small, has been demonstrated in normal subjects (19). It is abolished by atropine (20) and intensified by methacholine (18). The greater sensitivity of PEFV curves in assessing airway constrictor effects in our experiments may, in part, be due to this effect of methacholine. However, PEFV curves are also more sensitive than $FEV_{1.0}$ and MEFV curves in assessing responses in airway constrictor agents other than methacholine (5,21). Apparently, a single deep inspiration considerably increases airway caliber regardless of the agent which reduced it.

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