Institutional Review Board for Human Subjects Research

Johns Hopkins Asthma & Allergy Center
Room 3-B79
5501 Hopkins Bayview Circle
Baltimore, MD 21224
Phone: (410) 550-1853
Fax: (410) 550-0877

JOHNS HOPKINS
BAYVIEW MEDICAL CENTER

IRB PROTOCOL APPROVAL NOTICE

TO: Alkis Togias, MD
Associate Professor, Medicine

FROM: Gary Briefel, M.D.
Chairman - IRB

DATE: **September 18, 2000**

RE: **RPN NO.: AAC00-07-26-02, entitled, Mechanisms of Deep Inspiration-Induced Airway Relaxation (with Alvin Sanico, Robert Brown)**

I am pleased to inform you that at the convened meeting of **09/18/2000** the IRB voted to approve the above-referenced protocol. Approval of the protocol and the consent form(s) is for the period of **09/18/2000 to 09/18/2001.** As principal investigator of the project, you are responsible for fulfilling the following requirements of approval:

1) The co-investigators listed on the application should be kept informed of the status of the project.

2) Changes, amendments, and addenda to the protocol or the consent form must be submitted to the IRB for re-review and approval prior to the activation of the changes. The RPN number assigned to the project should be cited in any correspondence.

3) Adverse events should be reported to the IRB promptly. New information that becomes available which could change the risk:benefit ratio must be submitted promptly for IRB review. The IRB and outside agencies must review the information to determine if the protocol should be modified, discontinued, or continued as originally approved.

4) Only consent forms with a valid approval stamp may be presented to subjects. All consent forms signed by subjects enrolled in the study should be retained on file. The IRB conducts periodic audits of protocol records, and consent documentation is part of such audits.

5) Federal regulations require review of an approved study not less than once per 12-month period. **Therefore, a renewal application must be submitted to the IRB office by 08/28/2001, in order to allow sufficient time for review of the renewal application to be completed prior to the anniversary of the original approval date.** Failure to submit a renewal application in a timely fashion will result in termination of the study, at which point new subjects may not be enrolled and currently enrolled subjects must be taken off of the study.

CC: P&T

Enclosure

CLINICAL INVESTIGATION CONSENT FORM

The Johns Hopkins Medical Institutions
(The Johns Hopkins Hospital
The Johns Hopkins Bayview Medical Center, etc.)
Date/Revision: **July 24, 2000** Application No:
Title of Research Project:
Mechanisms of Deep Inspiration-Induced Airway Relaxation

Patient I.D. Plate

Explanation of Research Project to Subject

PURPOSE OF THE STUDY
The goal of this study is to find out how the tubes that carry air into the lungs (the airways) can stay open, even when we breathe all types of irritating chemicals. This is something that happens in the normal lung. However the airways of patients with asthma do get narrow. Because you have healthy lungs we are asking you to be part of this study.

PROCEDURES
You will be asked to visit the Johns Hopkins Asthma and Allergy Center 7-9 times. There will be two phases in this study. During the first phase (3-5 visits) you will get a methacholine test. Methacholine is a substance that is used to find whether somebody has asthma. When patients with asthma breathe methacholine, they get a mild asthma attack with chest tightness, some coughing and even mild shortness of breath. When healthy people breathe methacholine, they may develop the same symptoms only when they do not take deep breaths. In the first 1-4 visits you will be asked not to take deep breaths; therefore you may get the above symptoms. During the next visit, you will be asked to take deep breaths either before or after you get the methacholine. Each of these visits will last approximately 30-40 minutes.
For the second phase of the study you will be asked to come to the Asthma and Allergy Center 4 times. During each of the visits you will be asked to have the same methacholine test you had in the first phase of the study. This time however, you will get another medication called hexamethonium or a placebo (inactive solution) to breathe, immediately before you have the methacholine test. Each of these visits may last up to 4 hours.
Hexamethonium is a medication that has been used during surgery, as a part of anesthesia; this is capable of stopping some nerves in your airways from functioning for a short period. The question that is being asked is whether hexamethonium can reduce the ability of your lungs to resist the asthma-like effects of methacholine.
Before and while you get hexamethonium or saline you will also be asked to blow several times through a machine that measures the amount of nitric oxide gas that is normally produced in your lungs.
RISKS
A) Methacholine may make you get some symptoms that are like those that patients with asthma get such as cough and chest tightness. These go away on their own within a short period of time. If you do not want to wait until the symptoms go away on their own, a lung spray will be given to you, which will take the symptoms away within a few minutes.
B) Hexamethonium. This medication, when you breathe it, may reduce your blood pressure and may make you feel dizzy especially when you stand up. This effect may last up to 3 hours. During the visit you receive hexamethonium, you will be connected to a heart monitor and we will measure your blood pressure very often. You will also have an IV (a small tube in your vein) placed only as a precaution. If your blood pressure become too low, we may ask you to lay flat for as long as the problem persists or you may have to receive fluids through your IV.
You should not participate in this study if you have high or low blood pressure, any illness from your heart or blood vessels, any kidney problems, a history of allergy to anesthetic medications or if you are pregnant. If you are female, you may participate in this study only after we make certain that you are not pregnant. A pregnancy test will be performed prior to the beginning of the study.
BENEFITS
You are participating in this study as a healthy volunteer. Therefore, there is no benefit for you from doing this. The information that we will get from this study may help us understand some of the problems behind asthma.
For your time and effort you will be compensated with up to $365, that is $25 for each of the first phase (30-40 minute) visits you complete and $60 after each of the second phase (4hour) visits you complete.
ALTERNATIVES TO PARTICIPATION
None; this study does not include treatment for any condition and you are participating as a healthy volunteer.

THIS CONSENT FORM CONTINUES ON THE NEXT PAGE

QUESTIONS YOU MAY HAVE ABOUT THE RESEARCH STUDY:

This consent form explains the research study. Please read it carefully. Ask questions about anything you do not understand. If you do not have questions now, you may ask later. During the study, you will be told any new facts that could affect whether you want to stay in the study. If the study relates to a health problem you have, we will explain what other treatment could be given outside the research. You should understand those options before you sign this form. If you have questions you should call the principal investigator Alkis Togias, MD, at (410) 550-2189.

PRIVACY INFORMATION:

We will keep the study information private to the extent possible by law. However, State law requires us to report certain contagious diseases or if we find information about child abuse. Also, under certain conditions, people responsible for making sure that the research is done properly may review your study records. This might include people from Johns Hopkins, the National Institutes of Health, the Food and Drug Administration, or the sponsoring company (if any). All of these people are also required to keep your identity confidential. Otherwise, the information that identifies you will not be given out to people who are not working on the study, unless you give permission.

IF YOU ARE HURT BY BEING IN THE STUDY:

If you think you have been hurt by being in the study, or not treated fairly, you should call the Joint Committee on Clinical Investigation at (410)955-3008, or the Johns Hopkins Bayview Medical Center Institutional Review Board for Human Research (410) 550-1853 to receive help or advice, including help finding medical care if needed.

The Johns Hopkins University, The Johns Hopkins Hospital, the Johns Hopkins Bayview Medical Center, _____, and the Federal government do not have any program to pay you if you are hurt or have other bad effects which are not the fault of the study doctors.

JOINING OF YOUR OWN FREE WILL (Volunteering for the study):

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. All normal treatment options will still be available to you.

WHAT YOUR SIGNATURE MEANS:

Your signature below means that you understand the information given to you about the study and in this consent form. If you sign the form it means that you agree to join the study.

WE WILL GIVE YOU A COPY OF THIS CONSENT FORM.

APPROVED FOR: ✓ Adults Only ___ Adults and Children ___ Children Only

NOT VALID WITHOUT THE COMMITTEE OR IRB STAMP OF CERTIFICATION

APPROVED
OCT 2 2000
Adults only
JHBMC - IRB

VOID ONE YEAR FROM ABOVE DATE
RPN NO. AAC00-09-26-02
(Revised 05/2000)

Subject's signature (including children, when applicable) Date

Signature of Parent or Guardian (when applicable) Date

Signature of Investigator or Approved Designee Date

Witness to Consent Procedures * Date

*Optional unless subject is illiterate, or unable to sign

NOTE: **A COPY OF THE SIGNED CONSENT FORM MUST BE KEPT BY THE PRINCIPAL INVESTIGATOR AND A COPY OF THE CONSENT FORM MUST BE PLACED IN THE PATIENT'S MEDICAL RECORD**

Addendum
09/18/00

JOHNS HOPKINS
Asthma & Allergy Center
Johns Hopkins Bayview Campus
Experimental Pulmonary & Nasal
Physiology Laboratory
Alkis Togias, M.D.
Unit Office 7
Telephone 410/550-2191
FAX 410/550-2193
CLINICAL IMMUNOLOGY DIVISION

Dear Dr. Briefel,

I would like to thank the IRB for for reviewing our RPN # AAC-07-26-02.

I would like to respond to questions you had regarding this RPN.

1) The hexamethonium will be provided from the pharmaceutical/chemical company Fluka. We have obtained a certificate of analysis from the company. The hexamethonium that will be utilized is 99.6% pure as assessed by thin layer chromatography with the contaminants being inorganic salts. The procedure for the preparation that will be followed each time a new solution is prepared is described in detail below. The hexamethonium will be suspended in sterile isotonic saline under a chemical fume hood. This solution will be then passed through a 0.2 micron sterile filter. Every solution that will be made will also be tested for endotoxin content with a standard Limulus test. These procedures will ensure safety for human use.
2) A physician will be present throughout the duration of the administration of hexamethonium.
3) Other than for individuals who already belong to our volunteer database, who have already participated in other studies and have expressed the willingness to participate in more studies, we will not directly solicit participation in the study from employees or students.
4) We agree with the pregnancy test as a necessity for the recruitment of females in the reproductive age and a urine pregnancy test will be performed on all female volunteers prior to acceptance to the study. Please note that the consent form has been updated to include this as well and is included with this letter.

We look forward to hearing from you soon.

Alkis Togias, M.D.
Associate Professor of Medicine

Institutional Review Board for
Human Subjects Research

Johns Hopkins Asthma & Allergy Center
Room 3-879
5501 Hopkins Bayview Circle
Baltimore, MD 21224
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JOHNS HOPKINS
BAYVIEW MEDICAL CENTER

Research Application
Pending: 08/07/2000

August 10, 2000

Alkis Togias, MD
Associate Professor, Medicine
Room 3B.65B
JHBMC-JHAAC

RE: RPN AAC00-07-26-02, entitled, Mechanisms of Deep Inspiration-Induced Airway Relaxation

Dear Dr. Togias:

The IRB has reviewed the above-referenced protocol, and the following questions were raised in the review. In order to complete the review as quickly as possible, we request your response within two weeks of receipt of this letter. Responses may be sent by e-mail to jhbmcirb@jhmi.edu or faxed to the IRB office at 410-550-0877. If you wish to communicate by E-mail, please restate the questions.

1. If the hexamethonium is not a FDA approved product, the protocol should describe the source of the hexamethonium and how it will be made safe for human use.

2. Will a physician be present throughout the infusion?

3. The protocol indicates that students, staff, and employees may be subjects. JHBMC-IRB guidelines stipulate that recruitment of your employees and students should be through advertisements and not direct solicitation. Please acknowledge that you will follow the requirement.

4. If pregnancy would be a contraindication to participation, we believe that a pregnancy test should be performed prior to enrollment.

Upon receipt of your response, the review will continue.

Sincerely yours,

Gary Briefel, M.D.
Chairman, IRB
Room 3B-79, AAC

GB:cps

JOHNS HOPKINS
Asthma & Allergy Center
Johns Hopkins Bayview Campus
Alkis G. Togias, M.D.
Unit Office 7
Telephone 410/550/2189
Fax 410/550/2193
CLINICAL IMMUNOLOGY DIVISION

July 25, 2000

Gary Briefel, M.D.
Chairman, JHBMC IRB
Johns Hopkins Asthma and Allergy Center
5501 Hopkins Bayview Circle
Room 3B79
Baltimore, MD 21224

Dear Dr. Briefel:

We are hereby submitting a new RPN application entitled: "Mechanisms of Deep Inspiration-Induced Airway Relaxation".

We look forward to hearing from you soon.

Sincerely,

Alkis Togias, M.D.
Associate Professor of Medicine

AT/jk

encl:

Revised 02/04/2000 ADOBE PDF FILE

APPLICATION NUMBER: AAC 00-07-26-02
FOR OFFICE USE ONLY

(1)

APPLICATION FOR A NEW HUMAN SUBJECTS RESEARCH PROJECT
JCCI, JHBMC-IRB, HCGH
(Website: http://infonet.welch.jhu.edu/research/jcci or /jhbmc-irb)

Title of Project: **Mechanisms of Deep Inspiration-Induced Airway Relaxation**

Principal Investigator: **Alkis Togias, MD** Department: **Clinical Immunology**

Must be Faculty Member
Title of Investigator: **Associate Professor of Medicine**

Signature of Investigator: _____ (*Date of Signature*): 7/25/00

Mailing address: **5501 Hopkins Bayview Circle**

Phone: **(410) 550-2189** Fax: **(410) 550-2193** Beeper: **(410) 748-4868** E:-Mail: **atogias@jhmi.edu**

Department Chair signature (*when applicable*): _____ (*Date of Signature*): _____

Co-Investigators: **Alvin Sanico, M.D. , Robert Brown M.D.**

Study Location: (*JHH, JHBMC, HCGH, GRC, NIDA, JHAAC, JHGC, BPRU, etc.*) **JHAAC**

INTRODUCTION: The goal of the application process is to ensure that IRBs are provided sufficient documentation to determine that research studies are ethically sound. Each section of the application form asks for information that addresses specific issues of the research that pertain to human subjects issues. It is the intent of the application form to guide the investigator to areas that must be addressed when designing the protocol in order to maximize the protection of subjects.

In order to approve research involving human subjects, the IRB must determine that the research design is sound and minimizes risks to subjects, that informed consent is sought from the subject (*or their legally authorized representative*), that adequate monitoring of subjects will be performed to ensure safety, that vulnerable subject populations (*as defined in the Federal Regulations*) receive additional protections, and that subject confidentiality is protected.

ANSWER ALL OF THE FOLLOWING QUESTIONS:

YES/NO

☑ ☐ Will marketed drugs or diagnostic reagents be administered?

☐ ☑ **IF YES,** append a copy of the sponsor's protocol, if any.

Will investigational new drug(s) be administered? **IF YES,** *supply the following information:*

DRUG NAME(s):_____ IND #: _____ held by: Sponsor ☐ Investigator ☐

(If IND is held by the Sponsor, provide a copy of the investigator's brochure, the sponsor's protocol, and a Drug Data Sheet) (*Drug Data Sheet may be found on the JCCI and JHBMC-IRB guidelines Website*) To retrieve this PDF form click here *(If IND is held by the investigator, provide a copy of the IND application submitted to the FDA.)*

☐ ☑ Will medical devices be used in the study?

IF YES: *Identify below type of device:*

☐ DEVICE NAME(s):_____ IDE #: _____ held by: Sponsor ☐ Investigator ☐

(If IDE, is held by the sponsor; provide a copy of the investigators brochure and the sponsor's protocol. If IDE is held by the investigator, provide a copy of the IDE application submitted to theFDA.)

☐ For a device with an IDE , indicate Device Category assigned by the FDA: A ☐ B ☐

☐ 510K device (*Provide a copy of the FDA letter confirming the 510K status*)

☐ Non-significant risk device study. (*Provide justification of the non-significant risk determination.*)

☐ Marketed device. (*Provide a copy of approval from the JHH Clinical Engineering Department or the JHBMC Engineering Department for use of the device in the hospitals.*)

☐ ☑ Will Clinical Imaging Services be utilized? *(including extended time during clinically indicated procedures)*:

IF YES: *Identify type :*

☐ Ionizing radiation - Include an RCU 5 form *(Form available on the JCCI and JHBMC-IRB website) or click here*

☐ Ultrasound or other imaging tests?

☐ JHH Radiology will provide the Imaging? Attach a "Notification of Research Project Requiring JHH Imaging Services" form *(A copy of the JHH Radiology Research Policy document may be obtained from the JCCI or JHBMC-IRB web site)* *To retrieve this PDF form click here*

☐ ☑ Will samples be tested in a laboratory/facility that does not have CLIA *(Clinical Laboratory Improvement Amendments)* certification?

IF YES: *Provide information, on whether the results will be given to the patient/subject, patient's primary physician or used for patient diagnosis or management.*

☐ ☑ Will any infectious or biohazardous agents or specimens (*defined as any specimen involving blood or body fluids*) be obtained?

IF YES: *Contact the Biosafety Division, Health, Safety & Environment (410-955-5918).*

☐ ☑ Do any of the participating faculty (*or their immediate family, staff, or students*) have a financial *interest (royalty, equity, or consulting*) in the sponsor and/or products used in this project?

IF YES: *Submit a written statement of disclosure to the designated official for review of conflict of interest at the investigator's institution of primary appointment.*

☑ ☐ Is any support for the project (*monetary, drug supplies, etc.*) anticipated?

IF YES: Provide Name of sponsor (*federal or non-federal*): NIH

Provide Name of sponsor supplying only drugs/devices/equipment: _____

Provide start date: 09/15/2000 Sponsor protocol/grant number (*if known*): HL 61277

If funding will be from any agency of the Public Health Service, attach a copy of the Human Subjects Section from the Method of Procedures section of the grant application.

☐ ☑ Will one of the Clinical Research Centers be utilized?

IF YES: Identify center(s) below:

☐ JHH Adult inpatient unit
☐ JHH Adult outpatient unit
☐ JHH Pediatric inpatient unit
☐ JHH Pediatric outpatient unit
☐ General Clinical Research Center at JHB
☐ KKI GCRC

PRIMARY SUBJECT POPULATION(s) TO BE ENROLLED:

Age range : 18-65

- [x] Males
- [x] Females
- [ ] Children*
- [x] Adults
- [ ] Inpatients
- [ ] Outpatients
- [x] Non-patient volunteers
- [x] Employees
- [x] Staff (*JHH or JHBMC*)
- [x] Students
- [ ] Subjects with Mental Disorders
- [ ] Handicapped ***
- [ ] Prisoners***
- [ ] Pregnant Women***
- [ ] Fetuses (*or fetal tissue*)***
- [ ] Cognitively impaired subjects***

* *Children:* *Research involving children as subjects must be classified into one of the following categories. Information should be included in the application to support a risk category and include a statement to support that the benefit to children outweighs the risks of the research procedures:*

- [ ] Research not involving greater than minimal risk **(*45 CFR 46.404*)
- [ ] Research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects (*45.CFR 46.405*)
- [ ] Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition. (*45.CFR 46.406*)
- [ ] Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children. (*45.CFR 46.407*)

** *The regulations state: "Minimal Risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests."*

*** *These categories are considered to be vulnerable populations. The application should justify inclusion of these populations and include details on additional protections or consent processes that will be implemented to address the rights and welfare of these groups. (Please refer to the JCCI and JHBMC IRB Guidelines for additional information.)*

SUBJECT ENROLLMENT:

Number of subjects to be enrolled at the Hopkins site(s): First year: 10 Total study: 10

Total number of subjects to be enrolled at all sites for multi-center trials: First Year: Total study:

ANY PROPOSED ADS FOR RECRUITMENT OF SUBJECTS/PATIENTS MUST BE REVIEWED BY THE COMMITTEE OR IRB PRIOR TO PUBLICATION IN THE NEWSPAPERS OR POSTING ON INSTITUTIONAL BULLETIN BOARDS. INCLUDE A COPY OF ADVERTISEMENT WITH THE NEW APPLICATION

CONSENT DOCUMENTATION: *(Identify Type of Consent)* *To go to the Consent Website click here*

- [x] A copy of the proposed consent form to be signed by all subjects is attached to the application; or
- [ ] A request is being made to use a verbal consent statement. (*attach a copy of the proposed consent script to be read to subjects*)
- [ ] A request is being made to waive the requirement to obtain prospective informed consent from subjects:
  Explain why the project could not be practically done if written consent were required. Explain whether consent is normally obtained for the treatment/ procedure. Explain why the waiver will not adversely affect the rights and welfare of subjects who participate. Explain whether or not subjects will be debriefed after their participation.
- [ ] A request is being made to waive the requirement for written informed consent for a project involving research in an emergency situation. **SUCH WAIVERS MAY BE GRANTED ONLY IN STRICTLY DEFINED CIRCUMSTANCES.** Please refer to the IRB guidelines and provide the JCCI/JHBMC IRB with the five points required justifying a waiver of consent in this research area. To go to the Waiver of Consent Website click here

Revised 10/01/1999 RTF VERSION

*APPLICATION NUMBER:*_____

(OFFICE USE ONLY)

PART II

APPLICATION FOR A NEW HUMAN SUBJECTS RESEARCH PROJECT

JCCI, JHBMC-IRB, HCGH

(Website: http://infonet.welch.jhu.edu/research/jcci or /jhbmc-irb)

The following sections of the application are in an outline format and should be attached to the New Application Form:
If the questions are not applicable, enter N/A below the question.
Appendices can be added after the questions to the Application.

I. SPECIFIC RESEARCH QUESTION(S) TO BE ADDRESSED:

A. Does inhaled hexamethonium inhibit the bronchoprotective or bronchodilatory effect of deep inspirations in healthy humans?
B. Does inhaled hexamethonium decrease the amount of exhaled nitric oxide (NO)?

II. RATIONALE:

Briefly state the problem, present knowledge relevant to it, the research hypotheses, and the goals of the proposed study as related to the research question(s). Indicate the importance of the research.

We have recently begun to understand the beneficial effect of deep inspirations on the human airways and have been able to describe two phenomena that are involved. One is bronchoprotection, the phenomenon where, following a period of quiet breathing, deep inspirations are able to protect the airways from spasmogenic stimuli. The other phenomenon is bronchodilation where, once airway constriction has occurred, deep inspirations are able to partially reverse it. We have been able to describe several characteristics of these phenomena, two of which are key. The first is that in healthy humans, bronchoprotection is stronger than bronchodilation whereas in asthmatics, bronchoprotection is either absent or minimized and bronchodilation is only minimally decreased. These observations indicate that the mechanisms of the two phenomena are different. The second characteristic is that physiologic bronchoprotection differentiates the airway hyperresponsive from the normoresponsive state. Therefore, understanding the physiologic mechanisms underlying bronchoprotection and bronchodilation may be key in understanding the nature of hyperresponsiveness.

We have theoretical reasons to believe that the NANC bronchodilator system, which releases the neurotransmitter nitric oxide (NO), may be responsible for bronchoprotection in healthy individuals. Our hypothesis is that there are stretch receptors, which are activated when a deep inspiration is taken, leading to the generation of a neural reflex that releases NO and alters airway smooth muscle towards reduced ability to contract or towards relaxation. Since NANC bronchodilator fibers are filtered through the bronchial wall parasympathetic ganglia, by administering a ganglionic blocker such as hexamethonium, we will be able to determine their effect on deep inspiration-induced bronchoprotection.

Through RPN # AC97-04-11-02 we had permission to utilize the ganglionic blocker hexamethonium to test the above hypothesis. However our more recent knowledge of these phenomena has led us to the necessity of revising the exact methodology of testing the above hypothesis to allow separate testing of bronchoprotection and bronchodilation. Also, having acquired the technical capability of measuring exhaled nitric oxide we will be able to determine what portion of the exhaled nitric oxide is neural in origin.

III. PROTOCOL:

A. STUDY PROCEDURES:

i. Provide a brief description of the study design, including the sequence and timing of study procedures.

To determine whether a subject fulfills the inclusion criteria beyond those met by our standard screening protocol (RPN# AAC-93-01-27-01), he/she will be asked to participate in the following protocol, which will involve between 3 and 5 visits: at the beginning of the first visit, conventional spirometry will be performed. Thereafter, the subject will be asked to refrain from taking deep breaths (and will be monitored visually) for 20 minutes. A single dose of methacholine will then be administered with tidal inhalations and, 3 minutes later, spirometry will be repeated. If this dose of methacholine does not induce >20 % reduction in FEV1, the subject will be asked to return to the laboratory after at least 24 hours to repeat the provocation with a higher single dose of methacholine. The doses of methacholine to be employed are 10, 20, 40 and 75 mg/ml. If, on the other hand, the single dose of methacholine results in the anticipated reduction in lung function, the subject will be immediately asked to take four slow deep inspirations by

inhaling through her/his nose and spirometry will be repeated thereafter. The difference in the reduction in FEV1 from baseline between the pre and the post deep inspiration evaluations will constitute the bronchodilatory effect of deep inspiration. Once the bronchodilatory effect of deep inspiration has been determined, the subject will be asked to return for an additional visit during which, following baseline spirometry and the 20 minute quiet breathing period, he /she will perform 5 deep inspirations prior to the administration of the same single dose of methacholine that was able to reduce FEV1 by > 20% in the previous visit. Three minutes after the administration of the methacholine, spirometry will be repeated. The bronchoprotective effect of deep inspiration will be determined from the difference between the reductions in FEV1 induced by methacholine during the visit where no deep inspirations preceded the spasmogen and during the visit where 5 deep inspirations preceded the spasmogen.

When a patient that fulfils all of the inclusion criteria is identified, he will be asked to enter a randomized, crossover, 4-visit protocol. During these visits, subjects will be premedicated with either hexamethonium, or its vehicle (normal saline), by inhalation. Lung function will be measured with spirometry before and after the administration of the study medication. Also, nitric oxide levels will be measured before the administration of the study medication as well as after the last dose of medication is administered.

The dosing of hexamethonium has to be done in a stepwise fashion to allow intermittent cardiovascular assessments. We will also perform a measurement of NO during these assessment intervals. Therefore the dosing of hexamethonium and saline will be done as follows:

-Routine measurement of NO and routine spirometry will be performed

-The patient will be given a 200mg dose of hexamethonium

-Cardiovascular assessment and measurement of NO will be performed

-The next doses of hexamethonium (200mg at every step) will be administered, with intermediate cardiovascular assessments and measurements of NO, until our safety cutoffs have been reached (reduction in sitting systolic blood pressure of ≤ 30mmHg or heart rate increase >30bpm), or a cumulative dose of 1000mg has been administered.

-Afterwards, routine spirometry along with nitric oxide measurement will be performed.

Thereafter, a 20-minute quiet breathing (no deep inspirations) period will be observed and the previously identified single dose of methacholine will be inhaled. On two occasions, (one with hexamethonium pretreatment and one with vehicle pretreatment) 5 deep inspirations will precede the administration of methacholine (evaluation of the effect of hexamethonium on the bronchoprotective ability of deep inspiration). On the other two occasions, one with hexamethonium pretreatment and one with vehicle pretreatment, deep inspirations will follow the methacholine (evaluation of the effect of hexamethonium on the bronchodilatory ability of deep inspiration).

During the entire study visit, the patient will remain sitting or supine in the laboratory, if he/she has received hexamethonium, for 3 hours or longer, if necessary. Subjects will also be connected to an ECG monitor and to an automated blood pressure cuff meter for continuous cardiovascular monitoring.

In addition, at the beginning of each study visit involving the administration of hexamethonium, an intravenous catheter attached to normal saline solution at 21 ml/hr will be introduced and kept in place for the entire visit.

ii. Describe the study duration and number of study visits required of research subjects.

A group of 10 healthy individuals who will have been fully evaluated as part of the routine screening protocol that is used in our laboratory (RPN: AAC93-01-27-01) will be asked to visit the laboratory on 7-9 occasions at the Johns Hopkins Bayview Asthma and Allergy Center. The screening visits (3-5) may be completed within 1-2 weeks. The main study arms may be completed within 2 weeks (4 visits). The exact time frame is also dependent on the volunteer's personal schedule. There is no absolute timeframe to complete all parts of the study although it is desirable that they be placed as close to each other as possible.

iii. Describe subject population. Briefly describe how subjects will be identified and recruited. List major inclusion and exclusion criteria. Any proposed exclusion based on gender (*women of childbearing potential*), age, or race must include the scientific justification for the exclusion.

Subjects will be identified by the screening procedure described from in the protocol. The human database of the Experimental Pulmonary and Nasal Physiology Laboratory of the Johns Hopkins Asthma and Allergy Center will be used to recruit volunteers.

The inclusion criteria include: age 18-65; gender: male and female; medical history: non-asthmatic, non-rhinitic, non-allergic, non-smoker, non-pregnant with no chronic illness and on no current medications; also, with no history of

cardiovascular, cerebral, pulmonary or renal disease; sitting systolic blood pressure ≥110 mmHg and <140 mmHg.

Subjects should also a) demonstrate > 20% reduction in FEV1 upon inhalation of a single dose methacholine bronchoprovocation in the absence of deep inspirations b) demonstrate an at least 60% bronchoprotective effect from 5 deep inspirations taken prior to the administration of the same single dose of methacholine, and c) demonstrate an at least 40% bronchodilating effect with 5 deep inspirations taken after the administration of the same single dose of methacholine.

iv. If research involves study of existing samples/records, describe how authorization to access samples/records will be obtained.

Not Applicable

B. DRUG/DEVICE STUDY DESIGN ISSUES:

i. Briefly, summarize preclinical and early human studies (*For IND/IDE studies only*).

Not Applicable

ii. Provide the rationale for choosing the drug dose or for choosing the device to be used.

The dose of hexamethonium chosen is based on the dose used by other investigators who utilized hexamethonium administration via inhalation. This dose and dosing method has been shown to be both safe and efficacious in producing ganglionic blockade.

iii. *If applicable*, provide definition of treatment failure or subject removal criteria.

Not Applicable

***(FOR TREATMENT STUDIES)*:**

i. *If applicable*, provide justification on why subjects will not receive standard care or have current therapy stopped.

Not Applicable

ii. Distinguish procedures that are experimental from those which are part of routine care.

All procedures will be performed for experimental purposes.

iii. *If applicable*, justify inclusion of a placebo or nontreatment group.

Not Applicable

iv. *If applicable*, justify blinding or not blinding the trial.

Not Applicable

v. Describe early stopping rules.

Subject may be withdrawn early if he/she does not tolerate the administration of methacholine or hexamethonium.

vi. Describe what happens with therapy when study ends.

At the end of the study the subjects will have no further obligations or need of further treatment or follow-up.

C. HUMAN BIOLOGICAL SAMPLES AND/OR GENETIC TESTING: *If human biological samples will be collected/studied as part of the protocol, provide information to address all of the following points*:

i. Will samples from living individuals be studied?

Not Applicable

ii. Will new samples be obtained or will pre-existing samples be studied?

Not Applicable

iii. Will identifiers or codes be retained that could link the identity of the subject to the sample?

Not Applicable

iv. Describe procedures to protect against unauthorized use and loss of confidentiality of the samples or inadvertent release of confidential information.

Not Applicable

v. Are there plans to contact subjects or to access their medical records?

The individuals participating in this study are recruited from the human database of the Experimental Pulmonary and Nasal Physiology Laboratory of the Johns Hopkins Asthma and Allergy Center, and they are routinely contacted for participation in various studies. The database contains medical information, which is accessible only by the investigators and only for the purposes of the study. Other medical records will not be accessed during this

study.

vi. Will specimens be collected for "banking" and future research?

Not Applicable

vii. Describe procedures for obtaining consent for future studies of existing samples *(if applicable).*

Not Applicable

viii. If genetic testing will be conducted: describe plans for contact of relatives of an existing proband (*include any proposed written contact letter or materials).*

Not Applicable

ix. Describe plans for disclosure of test information (*To whom will information be disclosed and by whom*?).

Not Applicable

x. Will genetic counseling be provided prior to disclosure?

Not Applicable

D. CONDITIONS WHEREBY THE SUBJECT MAY BE REMOVED, IF ANY, FROM THE STUDY: (*i.e. behavior, noncompliance with study rules, study termination*).

1.Adverse reaction to any of the drugs to be utilized in the study (methacholine, hexamethonium)

2.Noncompliance or inability to perform satisfactory spirometry

E. PRIMARY OUTCOME VARIABLES:

1. Bronchoprotection: The difference in the methacholine-induced reduction in FEV1 from baseline between the hexamethonium and the vehicle treatment visits, on the days where deep inspirations preceded the administration of the spasmogen.

2. Bronchodilation: The difference in the methacholine-induced reduction in FEV1 from baseline between the hexamethonium and the vehicle treatment visits, on the days where deep inspirations followed the administration of the spasmogen.

3. Nitric Oxide Concentration: The difference in the concentration of exhaled nitric oxide between baseline and post-hexamethonium administration.

F. FOR INVESTIGATOR INITIATED STUDIES:

i. Provide a brief description of the statistical plan and sample size justification.

Paired parametric statistics will be employed to compare the bronchoprotection and bronchodilation changes when hexamethonium and saline are used. The same method will be utilized to compare the difference in exhaled nitric oxide concentration when the above agents are used.

Out of a pool of 18 healthy individuals on whom bronchoprotection data are available, we have obtained an average bronchoprotection of 80.8% with a standard deviation of 10.6%. In the proposed study, 10 subjects will allow us to detect a reduction in bronchoprotection by hexamethonium as small as 13% with α=0.05 and β=0.80.

ii. Provisions for interim data analysis *(i.e. Data Safety Monitoring Board information, etc.).*

Not Applicable

G. CONFIDENTIALITY: Describe procedures to be used to protect confidentiality of data collected and stored for research purposes. If sensitive information (*illicit drug use, illegal activity, etc.*) will be collected, the application should indicate whether a Certificate of Confidentiality would be obtained. See the JCCI/JHBMC Guidelines for information on how to obtain a Certificate.

Confidentiality of collected data is strictly observed by allowing access to such data only to the investigators of the study. No information, other than what is necessary for the purpose of the study , will be recorded.

IV. RISKS:

A. Medical risks (*If applicable, detail major and minor risks, expected frequency, and steps taken to minimize the risks*)

1.Methacholine Bronchoprovocation: In the presence of deep inspirations, non-asthmatics do not develop bronchospasm with methacholine inhalation. In the absence of deep inspirations, airflow obstruction does develop but it is fully reversible either spontaneously (within an hour) or with the use of bronchodilator. Repetitive provocations with increasing single methacholine doses of methacholine allow us to establish the minimum single dose of Mch that induces the desired reduction in FEV1, thus reducing the risk for excessive airflow limitation.

2.Hexamethonium Inhalation: Hexamethonium is an agent that has been used through the intravenous route for years in anesthesia. Currently, other agents are used more frequently for ganglionic blockade. In the two studies noted above, in which hexamethonium was used by inhalation, reductions in the blood pressure were noted. However the effects dissipated spontaneously within 2-3 hours after the local administration. Accordingly, we will monitor blood pressure and heart rate in our subjects until values return to baseline. Subjects will not be allowed to stand up during this period and when they do, this will be done under careful monitoring to detect orthostasis. If any subject becomes symptomatic from reduction in blood pressure, the study will be discontinued and the subject will remain in the supine position± lower limb elevation. We can also provide blood pressure support with intravenous fluids. Emergency cart will be available in the room where the study takes place and a physician will be present throughout the study.

B. Legal risks (*if applicable*, detail risks that would be associated with breach of confidentiality)
No legal risks are anticipated

C. Financial risks (*if applicable, provide a statement regarding the source of payment for research related costs drugs, tests, etc.)*
The finances of the study are covered by NIH grant RO 1HL61277

V. BENEFITS: Briefly describe probable benefits to the individual subject and/or society.

We anticipate no benefit to the subjects from participating in this study. However data collected, may provide better understanding of the mechanisms of bronchoprotection and bronchodilation, and the origin of exhaled nitric oxide.

VI. COMPENSATION FOR SUBJECTS: (*if applicable*):

A. Possible total compensation
Volunteers will be compensated with $25 for each of the initial screening visits that they complete and $60 for each of the challenge visits they complete, with a possible total of $365.

B. Proposed bonus
None

C. Describe any proposed reductions or penalties for not completing the protocol
In the event that a subject drops out of a study, he/she will be compensated according to the number of visits that they complete. They will not be penalized for discontinuing a study.

VII. CONSENT ISSUES:

A. Consent setting (*Who will obtain consent, where and when will consent be obtained, and how much time will subjects be afforded to make a decision to participate?)*

Consent will be obtained by one of the investigators at the study site.

B. Comprehension (*Will an assessment of consent material be conducted to assure the subjects (guardians) understand the information.*)

The consent will be using standard language according to IRB guidelines, such that no particular assessment needs to be anticipated.

C. Special Consent Provisions (*If some or all subjects will be cognitively impaired, or have language/hearing difficulties, describe how capacity for consent will be determined.*)

The procedures involved in this study require some skill from the subjects' standpoints, such that good communication between the subject and the study personnel is desired. For subjects who are unable to read, the entire consent form will be read out to them and discussed in details before their consent is obtained.

REFERENCES

1) **P. Sterk et al. Limited Maximal Airway Narrowing in Nonasthmatics Subjects. Role of Neural Control and Prostaglandin Release. Am Rev Respir Dis 1985; 132:865-870.**

2) **Holtzman. M et al. Effect of ganglionic blockade on bronchial reactivity in atopic subjects. Am Rev Respir Dis 1980; 122:17-25.**

3) **O'Byrne et al. The effect of inhaled hexamethonium bromide and atropine sulphate on airway responsiveness to histamine. JACI 1985;76(1):97-103**

4) **Ichinose et al. Nonadrenergic Bronchodilation in Normal Subjects.Am Rev Respir Dis 1988; 138:31-34**

mited Maximal Airway Narrowing in Nonasthmatic Subjects

le of Neural Control and Prostaglandin Release[1-3]

J. STERK, E. E. DANIEL, N. ZAMEL, and F. E. HARGREAVE

SUMMARY In nonasthmatic subjects with normal airway responsiveness to methacholine, maximal airway narrowing is limited to a mild or moderate degree. We investigated whether the maximal response plateau or the position of the dose-response curve is due to functional inhibition by neurogenic mechanisms or to prostaglandin release. Four nonasthmatics inhaled doubling concentrations of methacholine up to 256 mg/ml (67 mg delivered during tidal breathing), followed by 4-fold-increasing doses of salbutamol up to 80 mg/ml (24 mg during tidal breathing) on 5 separate days. On each day 30 min before the test, the subjects inhaled (using a dosimeter) saline, propranolol (11 mg), or hexamethonium (910 mg) or, 2 h before the test, ingested indomethacin (75 mg) or placebo. The response to methacholine was measured from volume history standardized partial and complete maximal expiratory flow-volume curves, as FEV_1 and the flows at 40% of the control FVC ($\dot{V}_{40p}$ and $\dot{V}_{40c}$). Compared with saline, on average, baseline $\dot{V}_{40p}$ was 18% lower after propranolol and 18% higher after hexamethonium. Indomethacin did not affect baseline values. There was no systematic difference between the 5 days in the dose of methacholine to cause a 10% fall in FEV_1 or a 40% fall in $\dot{V}_{40p}$, or in the maximal response with FEV_1, $\dot{V}_{40p}$, and $\dot{V}_{40c}$, or in $\dot{V}_{40p}/\dot{V}_{40c}$ at 256 mg/ml methacholine. We conclude that limited maximal airway narrowing to methacholine in nonasthmatics is not due to a change in adrenergic, cholinergic, or ganglion-transmitted-nonadrenergic inhibitory activity nor to the release of prostaglandins. Furthermore, the bronchodilatory effect of a deep inspiration is not caused by a neurogenic reflex mechanism or the release of prostaglandins.

AM REV RESPIR DIS 1985; 132:865–870

Introduction

irway responsiveness to inhaled histae or methacholine has generally been ressed as a point on the linear part he dose-response curve that represents position of the curve. The position he curve is shifted to the left in asthics compared with that in nonasthics (1). Recently, it was recognized t the dose-response curves in asthics also differ from those in nonasthics in the degree of airway narrowthat is possible. The curve in nonasthics shows a maximal response plateau elatively mild degrees of airway narving, indicating that airway narrowis limited (2–4). The maximal reonse plateau is reproducible and benes greater as the position of the curve fts to the left (4). In asthmatics, there-e, the maximal response plateau is ater or, more usually, cannot be meaed (3). These observations raise the ssibility that airway narrowing in nasthmatics is limited by one or more tent inhibitory mechanisms that are sent or less effective in asthmatics. Possible mechanisms that might inbit airway narrowing to methacholine nonasthmatics include tachyphylaxis methacholine, bronchodilation proced by a deep breath, the effect of circlating catecholamines, ganglionically nsmitted nonadrenergic inhibitory acity, and release of dilator prostaglanns. The first 2 are not responsible (4). this study, we examined the other posbilities. Inhalation tests with methachoe were performed in 4 nonasthmatic bjects to determine the position of the se-response curve and the maximal reonse plateau after pretreatment, on parate days, with inhaled propranolol, haled hexamethonium, ingested indoethacin, or placebo. The propranolol roduced considerable inhibition of airay beta-receptors, the hexamethonium aused inhibition of ganglion transmis-

Methods

Subjects

Four nonasthmatic, nonsmoking adults volunteered to participate in the study (table 1). They had no current or past history of respiratory disease (5) and their FEV_1 was greater than 80% of predicted (6). They were nonatopic, as indicated by no wheal and flare responses to prick skin tests with 16 common allergen extracts, and had no symptoms of respiratory infection for 2 wk. All subjects gave written informed consent, and the project was approved by the Research Committee of St. Joseph's Hospital.

Study Design

The subjects came to the laboratory on 5 separate days within a 3-wk period at the same time of day. The study design is shown in figure 1. On each day the subjects were pretreated with either inhaled propranolol, hexamethonium, or saline (during the 30 min prior to the test), or ingested indomethacin or placebo (120 min prior to the test), in random order. A methacholine inhalation test was then carried out by using a standardized procedure (7). After each methacholine inhalation test the subjects inhaled increasing doses of salbutamol, with the exception of on the placebo pretreatment day when the salbutamol was immediately after each dose. Resuscitation equipment was at hand during each experiment.

Pretreatment

The inhaled pretreatment consisted of aerosolized solutions of propranolol chloride (Sigma Chemical Company, St. Louis, MO) (5 mg/ml in normal saline), hexamethonium bromide (Sigma) (600 mg/ml in distilled water), and normal saline (9 mg/ml NaCl). The aerosols were generated by a DeVilbiss 646 nebulizer, (DeVilbiss Co., Somerset, PA) with open vent, connected to a Rosenthal-French nebulization dosimeter (model 2A) in series with a compressed air cylinder. The driving pressure of the nebulizer was set at 138 kPa

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[2] Supported by grants from the Medical Research Council of Canada and the Netherlands Asthma Foundation.

[3] Requests for reprints should be addressed to

Nonadrenergic Bronchodilation in Normal Subjects[1,2]

MASAKAZU ICHINOSE, HIROSHI INOUE, MOTOHIKO MIURA, and TAMOTSU TAKISHIMA

SUMMARY To investigate whether bronchial C-fiber stimulation induced by capsaicin inhalation evokes nonadrenergic inhibitory (NAI) system bronchodilation, we studied partial and maximal expiratory flow-volume (PEFV and MEFV) curves in 5 normal subjects after inhalation of oxitropium bromide and propranolol. $PGF_{2\alpha}$ (1 mg/ml inhaled for 5 min) was administered to induce bronchoconstriction. Then aerosolized capsaicin was inhaled (2.4×10^{-9} mol) to stimulate bronchial C-fibers. $PGF_{2\alpha}$ produced significant bronchoconstriction; FEV_1 and flow during a PEFV curve at 30% forced vital capacity ($\dot{V}_{30p}$) decreased over a 15-min period. Capsaicin induced significant bronchodilation; $\dot{V}_{30p}$ increased for 2 to 6 min ($0.001 < p < 0.02$), and FEV_1 increased for 2 to 4 min ($p < 0.05$) when compared with saline-ethanol (vehicle of capsaicin) inhalation. After treatment with the ganglionic blocking agent hexamethonium, the significant bronchodilator response disappeared. These results suggest that the NAI system has a distinct bronchodilator action in human subjects *in vivo*, and that the bronchial C-fiber receptors may be involved in the reflex pathway for NAI system bronchodilation in humans.

AM REV RESPIR DIS 1988; 138:31-34

Introduction

Airway smooth muscle is controlled by excitatory and inhibitory nervous systems. It has been reported that activity of the cholinergic nervous system is enhanced in asthmatic patients compared with that in normal subjects (1). Thus, airway hyperresponsiveness may be explained by dysfunction of the inhibitory nervous system. The adrenergic and he nonadrenergic inhibitory nerves are known as the inhibitory nervous system o the airways. However, innervation of adrenergic inhibitory fibers was not ound pharmacologically and histochemically in human bronchial smooth muscle (2). Therefore, it is suggested that the onadrenergic inhibitory (NAI) system an inhibitory system operating only on ne smooth muscle of human airways.

Recently, it has been reported that mechanical irritation of the larynx produces ne NAI system bronchodilation found feline (3) and human airways (4). However, the exact reflex pathway of the NAI stem has yet to be shown. Our previous report showed that capsaicin evokes e NAI system reflex in feline airways) and suggested that C-fiber receptors e the major sensory receptors of the AI system reflex pathway. In this study, used capsaicin inhalation to see if it duces the NAI system bronchodilation humans *in vivo*.

Methods

Subjects

e healthy men (laboratory personnel 28 to of age) volunteered to participate in the dy. They were informed of the risks of the erimental protocol, and they gave their sent to join this experiment. None of the jects had a current or past history of re atory disease, and their FEV_1 was greater n 80% of predicted values. They had no iptoms of respiratory infection for 2 wk or to the test days.

Inhalation of Drugs

scarinic receptors were blocked by inhalation of oxitropium bromide (Ba253; Boehringer Ingelheim Biochemicals, Ridgefield, CT) by using a metered nebulizer delivering 0.2 mg puff. Subjects received 10 puffs for 5 to 10 min. On a separate day, to test whether Ba253 inhibits the muscarinic receptors, subjects inhaled methacholine (Sigma Chemical, St. Louis, MO) (50 mg/ml for 2 min) 20 min after Ba253 inhalation. Although methacholine inhalation alone significantly decreased in FEV_1 and $\dot{V}_{30p}$, pretreatment with Ba253 completely inhibited the metacholine-induced decrease in FEV_1 and $\dot{V}_{30p}$ (table 1).

Beta-adrenergic receptors were blocked by inhalation of propranolol chloride (Sigma) (5 mg/ml for 10 min). To test whether propranolol inhibits β-adrenergic receptors, subjects inhaled metaproterenol (Boehringer Ingelheim) (10 puffs over 5 min) using a metered nebulizer after heightening bronchomotor tone by inhalation of methacholine (Sigma) (50 mg/ml over 2 min). Although, metaproterenol inhalation alone significantly increased FEV_1 and $\dot{V}_{30p}$, pretreatment with propranolol completely inhibited the metaproterenol-induced increase in FEV_1 and $\dot{V}_{30p}$ (table 1).

Ganglionic blockade was produced by inhalation of hexamethonium bromide (Sigma) (600 mg/ml over 10 min). The doses of hexamethonium were selected on the basis of previous work (6, 7). To verify the effectiveness of hexamethonium, we measured blood pressure and pulse rate before and after hexamethonium inhalation.

To produce bronchoconstriction, subjects inhaled $PGF_{2\alpha}$ (Ono Pharmaceutical Co., Japan) (1 mg/ml over 5 min). To study whether capsaicin causes airway dilation, 1 min after inhalation of $PGF_{2\alpha}$ subjects inhaled capsaicin (Sigma) (2.4×10^{-9} mol, 5 breaths). Because capsaicin causes cough, saline-ethanol (capsaicin vehicle, 5 breaths) was inhaled during voluntary coughing after inhalation of $PGF_{2\alpha}$. We asked subjects to voluntarily cough in a manner similar to the capsaicin-induced cough, that is, coughing to residual volume (RV) and then returning to FRC without taking a large breath.

All aerosols except Ba253 and metaproterenol were generated by a DeVilbiss 646 nebulizer (output, 0.3 ml/min) (DeVilbiss Co., Somerset, PA). The sizes of the particles produced by the nebulizers were measured under experimental conditions using a liquid droplet size analyzer (Malvern 2600 D) (8). The mass median diameter was 5.5 μm with a geometric standard deviation of 1.9 μm. The subjects inhaled the aerosols during tidal breathing at a fixed frequency of 12 breaths/min (durations of inspiration and expiration were the same).

Measurements

A partial expiratory flow-volume (PEFV) curve was measured from 60% of control FVC, and immediately after the PEFV maneuver, a maximal expiratory flow volume (MEFV) curve was measured from TLC. The flow-volume curves were measured using a dry rolling-seal spirometer (OST 80A; Chest Co., Japan). Before studies, 3 MEFV curves were

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TABLE 1

COMPLETENESS OF MUSCARINIC AND β-ADRENERGIC BLOCKADES

	Patient No.	No Pretreatment			Muscarinic Blockade			β-Adrenergic Blockade			
		Cont	MCh	Meta	Cont	Ba	MCh	Cont	Prop	MCh	Meta
FEV_1, L	1	4.98	4.68	4.90	5.00	5.04	5.02	4.92	4.90	4.62	4.65
	2	4.70	4.58	4.72	4.62	4.68	4.60	4.64	4.64	4.18	4.29
	3	4.52	4.28	4.48	4.48	4.54	4.48	4.50	4.50	4.20	4.20
	4	3.68	3.50	3.70	3.60	3.78	3.69	3.62	3.56	3.26	3.30
	5	3.94	3.68	3.89	3.88	3.88	3.80	3.90	3.90	3.68	3.68
	Mean	4.36	4.14	4.34	4.32	4.38	4.32	4.32	4.30	3.99	4.02
	SE	0.24	0.24	0.23	0.25	0.24	0.25	0.24	0.25	0.24	0.24
$\dot{V}_{30p}$, L/s	1	4.50	2.68	4.44	4.40	4.52	4.42	4.48	4.43	2.24	2.42
	2	4.48	2.00	4.38	4.54	4.48	4.38	4.60	4.58	1.60	1.80
	3	3.30	1.24	3.30	3.20	3.40	3.40	3.40	3.28	1.14	1.32
	4	2.04	1.00	2.00	2.10	2.20	2.20	2.00	2.00	0.90	0.90
	5	3.30	1.00	3.10	3.33	3.42	3.30	3.24	3.16	1.02	1.00
	Mean	3.52	1.58	3.44	3.51	3.60	3.54	3.54	3.49	1.38	1.49
	SE	0.46	0.33	0.45	0.45	0.43	0.41	0.47	0.47	0.25	0.28

Definition of abbreviations: $\dot{V}_{30p}$ = flow during a PEFV maneuver at 30% FVC; Cont = before inhalation of drugs; Ba, MCh, Prop, and Meta = after inhalation of Ba253, methacholine, propranolol, or metaproterenol, respectively.

obtained to insure reproducibility. The FVC did not differ more than 5%. The maximal FVC was used for the control FVC.

We measured FVC, FEV_1, and $\dot{V}_{30p}$ (flow during a PEFV at 30% FVC) for 15 min after $PGF_{2\alpha}$ inhalation and used these measurements for analysis.

Study Design

The subjects were tested at the same time on 3 separate days in random order within a 3-wk period. The study design is shown in figure 1. On each day the subjects were pretreated by inhaling either Ba253 and propranolol or hexamethonium combined with Ba253 and propranolol. Capsaicin, or its vehicle saline-ethanol, was then inhaled after inhalation of $PGF_{2\alpha}$.

Statistical Analysis

Data were expressed as mean ± SE. Statistical analyses were performed using Student's paired *t* test. Significance was accepted at $p < 0.05$.

Results

There were no significant differences between baseline FEV_1 and $\dot{V}_{30p}$ on each day (figure 2). Inhalation of $PGF_{2\alpha}$ caused rapid bronchoconstriction. FEV_1 significantly decreased from 4.33 ± 0.26 (SEM) to 3.79 ± 0.24 L (Day 1), 4.32 ± 0.22 to 3.93 ± 0.19 L (Day 2), and 4.33 ± 0.24 to 3.73 ± 0.20 L (Day 3) ($p < 0.01$), respectively. $\dot{V}_{30p}$ also significantly decreased 3.74 ± 0.45 to 2.09 ± 0.21 L/s (Day 1), 3.76 ± 0.62 to 2.31 ± 0.33 L/s (Day 2), and 3.74 ± 0.49 to 2.21 ± 0.31 L/s (Day 3) ($0.001 < p < 0.05$), respectively. There were no significant differences in FEV_1 and $\dot{V}_{30p}$ changes during the 3 test days. On Day 1, inhalation of saline-ethanol (with voluntary cough) did not cause bronchodilation; FEV_1 and $\dot{V}_{30p}$ decreased for 15 min (figures 3 and 4, open circles). On Day 2, inhalation of capsaicin caused significant bronchodilation; FEV_1 and $\dot{V}_{30p}$ increased significantly compared with Day 1, from 2 to 4 min ($p < 0.05$) and from 2 to 6 min ($0.001 < p < 0.02$), respectively (figure 3, closed circles). The significant bronchodilator effect induced by capsaicin disappeared after hexamethonium inhalation (figure 4).

Discussion

These results indicate that inhaled capsaicin has a significant bronchodilator effect on constricted human airways *in vivo* after muscarinic and β-adrenergic receptor blockade, and that the significant bronchodilator effect disappeared after ganglionic blocker inhalation. Thus, we conclude that the bronchodilation was probably due to the NAI system reflex.

In our experiments, the cumulative dose of hexamethonium delivered orally during inspiration was 900 mg (solution = 600 mg/ml, inhalation time = 10 min, nebulizer output = 0.3 ml/min, inspiration time = expiration time). This dose was almost comparable with the doses of previous reports (6, 7). At present, the blocking effect of hexamethonium on ganglia in the airways cannot be examined. We therefore investigated this effect on cardiovascular responses. Hexamethonium inhalation caused, on average, a drop in standing systolic pressure of 35 mm Hg (range, 30 to 45 mm Hg) and an increase in pulse rate of 30 beats/min (range, 20 to 40 beats/min). These changes were similar to those previously reported (7). Thus, we considered that the dose of hexamethonium employed in this study is adequate to block ganglionic neurotransmission in the airways.

In this study, inhalation of capsaicin induced coughing in all cases. To exclude the effect of coughing on bronchodilation, each subject voluntarily coughed (similar to capsaicin-induced coughing) during saline-ethanol inhalation on Day 1. However, on Day 1, bronchodilation was not observed (figure 3). Therefore, the bronchodilation observed in this study could not be explained by the coughing maneuver.

Capsaicin has been shown to stimulate nonmyelinated fibers (C-fibers) (9). Previous reports have shown that capsaicin causes airway smooth muscle contraction both by stimulating a vagal reflex (10, 11) or by the release of tachykinins (12, 13). There are several reports showing that capsaicin causes bronchoconstriction in dogs. Russell and Lai-Fook (14) reported that capsaicin injection into the right ventricle causes bronchoconstriction in dogs, whereas injection into the left ventricle did not. Coleridge and coworkers (15) reported that both pulmonary and bronchial C-fibers evoke tracheal contraction, but when capsaicin is injected into the left atrium any effects of stimulating bronchial C-fibers are masked by the reflex action of somatic afferents, which causes tracheal relaxation in dogs. Furthermore, Kaufman and coworkers (16) have confirmed and extended their findings by

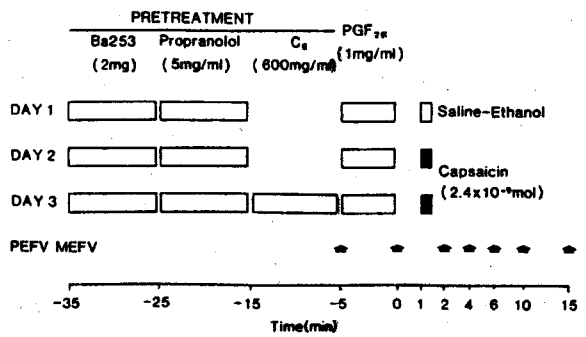

Fig. 1. Study design. Protocol 1 to 3 performed on 3 days in random order. (C_6 = hexamethonium; Ba253 = oxitropium bromide; PEFV and MEFV = partial and maximal expiratory flow volume maneuvers).

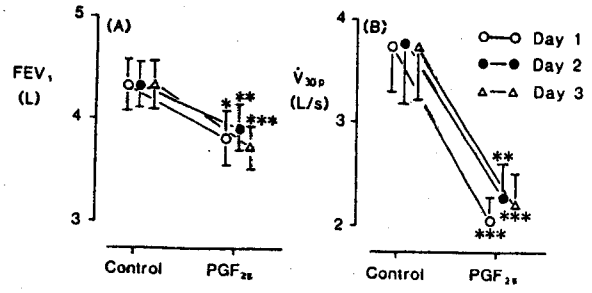

Fig. 2. Parameter changes induced by $PGF_{2\alpha}$ (1 mg/ml for 5 min) inhalation. On Days 1 and 2, each subject inhaled oxitropium bromide (0.2 mg, 10 puffs) and propranolol (5 mg/kg for 10 min) prior to the tests. On Day 3, each subject inhaled hexamethonium (500 mg/ml for 10 min) as well as oxitropium bromide and propranolol prior to the test. FEV_1 (A) and $\dot{V}_{30p}$ (B) decreased significantly after $PGF_{2\alpha}$ inhalation compared with control values. Each point represents the mean ± 1 SE ($\dot{V}_{30p}$ = flow during PEFV at 30% FVC). Significant differences from control values are indicated by a single asterisk ($p < 0.05$), double asterisks ($p < 0.02$), and triple asterisks ($p < 0.01$).

showing that chemical stimulation (capsaicin or bradykinin) of afferent endings in skeletal muscle causes reflex relaxation on tracheal smooth muscle in dogs without administration of atropine. The dog, however, lacks the NAI system in the airways, even though such nerves are present in the gastrointestinal tract; the tracheal muscle is innervated only by the adrenergic system for inhibition (17). The dilatory effect that they observed may not have been due to the NAI system, but possibly due to increased adrenergic activity or withdrawal of cholinergic tone (15, 16).

Previously, we reported that administration of capsaicin (inhalation or intravenous injection) after atropine and propranolol causes bronchodilation, which is blocked by hexamethonium *in vivo* in cats (5). Thus, we concluded that C-fiber receptors (both pulmonary and bronchial C-fibers) are major sensory receptors of the NAI system reflex pathway in cats. However, the NAI system reflex bronchodilation has not been reported in human airways. Fuller and coworkers (18) demonstrated that inhaled capsaicin causes a dose-dependent bronchoconstriction in humans. In their study, ipratropium bromide completely inhibited bronchoconstriction, suggesting that it was due to a cholinergic vagal reflex (through the C-fiber receptors) rather than on local release of tachykinin from nerves in the airways. The present study demonstrated that inhalation of capsaicin caused dilatory effects on airways constricted by $PGF_{2\alpha}$ in muscarinic and β-adrenergic blockade, and they were significantly reduced by a blocker of ganglionic neurotransmission, hexamethonium. These results suggest that the bronchodilator effect observed in the present study was probably due to the NAI system reflex through the C-fiber receptor.

Another possible receptor involved in inducing the bronchodilation in the present study may be the stretch receptor. Nadel and Tierney (19) and Hida and coworkers (20) reported that in the presence of pharmacologically induced bronchoconstriction a deep inspiration produces bronchodilation in both normal and asthmatic subjects. Then they reported that the bronchodilative mechanism is due to the stretch receptor. Full inspiration of the MEFV maneuver might modify the bronchodilation in this study. However, Hida and coworkers (20) reported that this effect lasts only 2 min. Then, in the present study, we measured pulmonary function parameters at intervals of 2 min. Furthermore, we measured a PEFV curve prior to a MEFV curve to avoid the effect of a full inspiration maneuver. FEV_1 and $\dot{V}_{30p}$ changed in the same manner. Therefore, it is unlikely that our results are influenced by the stretch receptor effect.

Other possible mechanisms in inducing the bronchodilation in the present study may be chemical mediators such as PGE_2, which is most frequently released by airway tissues (21). It is reported that the mediator release occurs slowly; the duration of release and action of this mediator is 30 to 120 min (22). However, the bronchodilation in this study occurred within 2 to 6 min after stimulation (capsaicin inhalation). The duration was almost comparable with our previous report (5). Thus, we considered that the bronchodilation observed in this study is not modified by these mediators.

In the present study, the NAI system effect in $\dot{V}_{30p}$ was more obvious than the FEV_1 changes. Possible explanations for this phenomenon may be as follows. The bronchoconstriction induced by $PGF_{2\alpha}$ was possibly slight in large airways but strong in small airways (23). $\dot{V}_{30p}$ would reflect changes in small airways more than changes in large airways. Then, $\dot{V}_{30p}$ decreased to almost 60% of control value. On the other hand, FEV_1 decreased slightly, which is almost 90% of control value. Thus, the dilatory effect of the NAI system may be more pronounced in $\dot{V}_{30p}$ than in FEV_1.

The absence of sympathetic nerves in

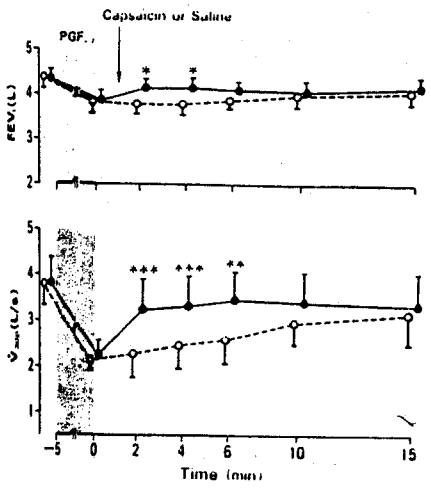

Fig. 3. The effect of capsaicin or saline-ethanol inhalation (with voluntary cough) on bronchoconstriction induced by $PGF_{2\alpha}$ inhalation. Open circles indicate the effect of saline-ethanol inhalation (on Day 1) and closed circles indicate the effect of capsaicin (on Day 2). Each point represents the mean ± 1 SE. Time 0 represents the point when $PGF_{2\alpha}$ inhalation was completed. Statistical difference between the capsaicin and saline-ethanol results at each point. Asterisk indicates $p < 0.05$

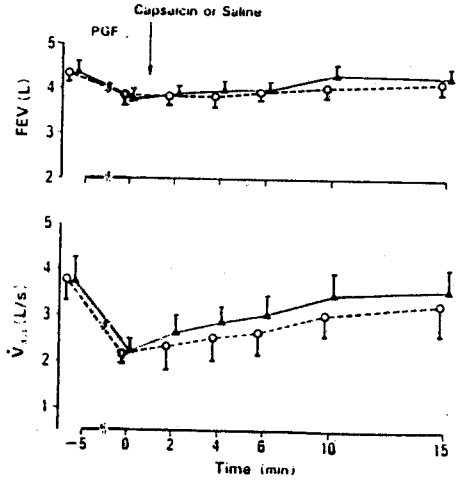

Fig. 4. The effect of capsaicin (after hexamethonium inhalation) or saline-ethanol inhalation (with voluntary coughing) on bronchoconstricton induced by $PGF_{2\alpha}$ inhalation. Open circles indicate the effect of saline-ethanol (on Day 1) and closed triangles indicate the effect of capsaicin (after hexamethonium inhalation) (on Day 3). Statistical difference between the capsaicin (after hexamethonium) and saline-ethanol results at each point. Double asterisks indicate $p < 0.02$; triple asterisks indicate $p < 0.01$

...way smooth muscle is sup- ... functional (2), histologic (8, 24), ... pharmacologic (25, 26) studies. The NAI system is suggested to be the only neural inhibitory pathway in human airway smooth muscle (2), and it innervates from the trachea to the smallest bronchi (27). One interesting role of the NAI system is its functional significance in humans. However, it is currently difficult to investigate the role of this system in humans, since the stimulant and blocker of the NAI system have not yet been established. However, as we report here, a specific stimulant of the sensory receptors can stimulate the NAI system. Thus, it would seem possible that stimulation of the sensory receptors would provide a useful tool to investigate the physiologic significance of the NAI system in asthmatic patients as well as in normal subjects. Further study should be done to address the question of whether its function may be impaired in asthma (28).

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The effect of inhaled hexamethonium bromide and atropine sulphate on airway responsiveness to histamine

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The degree of protection against inhaled histamine achieved by inhalation of the ganglion blocker hexamethonium bromide plus placebo, hexamethonium plus atropine sulphate, and placebo plus placebo was examined in six atopic subjects, four of whom had current asthma. Hexamethonium was administered until there was systemic evidence of ganglionic blockade with a postural drop in blood pressure of 31 ± 7.5 mm Hg (mean ± SD) (p = 0.01) and an increase in heart rate of 30 ± 3.1 bpm (mean ± SD) (p = 0.01). Atropine was inhaled in a dose (18 mg nebulized during tidal breathing) known to produce systemic inhibition of cardiac and salivary cholinergic (muscarinic) receptors. The airway effects were measured by FEV_1. Hexamethonium caused bronchoconstriction in all four subjects with asthma, which was reversed by atropine. The mean provocation concentration of histamine to provoke a 20% fall in FEV_1 was 2.97 mg/ml after premedication with placebo, it was not different at 2.84 mg/ml after hexamethonium alone, and it increased slightly to 5.31 mg/ml after both hexamethonium and atropine (p = 0.06). The results suggest that the main effect of inhaled histamine is not by reflex bronchoconstriction but rather through stimulation of H_1-receptors on airway smooth muscle. Therefore, histamine hyperresponsiveness in asthma is not primarily caused by a defect in the parasympathetic nervous supply to the airway. (J ALLERGY CLIN IMMUNOL 76:97-103, 1985.)

Increased airway responsiveness to inhaled histamine is usually found in symptomatic asthma.[1] The cause of this increased airway responsiveness to histamine is unclear. An abnormality in the autonomic nervous control of airway smooth muscle has been suggested as a possible mechanism.[2] Support for this hypothesis includes evidence that airway hyperresponsiveness to histamine can be partially blocked by premedication with atropine sulphate, a competitive inhibitor of the muscarinic receptors on smooth muscle.[3] In addition, inhalation of hexamethonium bromide, a competitive inhibitor of nicotinic cholinergic receptors in autonomic ganglia by atopic subjects without asthma, caused bronchodilation and reduced bronchoconstriction produced by histamine but not that produced by methacholine.[4] Both of those results are consistent with the suggestion that activity transmitted through the efferent parasympathetic vagal pathway is required for airway hyperresponsiveness to histamine and were interpreted to support an essential role of vago-vagal reflexes in airway hyperresponsiveness, i.e., increased activity in this pathway because of increased input or enhanced coupling of input to output.[4] However, in both studies the dose of histamine administered was limited; therefore, the degree of protection against histamine achieved by atropine or hexamethonium could not be determined. In comparable experiments, when histamine was administered in increasing doses to overcome the inhibition by atropine, the shift of the histamine dose-response curve was found to be small.[5] These results, in contrast, are not consistent with the hypothesis that the

Abbreviation used
PC_{20}: Provocative dose causing a 20% fall in FEV_1

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TABLE I. Subject characteristics

Subject	Gender	Age (yr)	Height (cm)	Atopic status*	FEV_1† (% Predicted)	Treatment
1	M	30	178	4	80	S 400
2	M	30	177	3	83	S not daily
3	F	36	152	4	94	S not daily
4	M	30	173	3	102	S not daily
5	M	36	180	2	91	Nil
6	M	27	191	4	91	Nil

S = salbutamol (microgram daily).
*Atopic status is the number of positive wheal-and-flare responses in prick skin tests with 16 common allergens extracts.
†Baseline FEV_1 expressed as percent predicted.[17]

parasympathetic nervous control of the airways plays a major role in promoting airway hyperresponsiveness to histamine.

Because of these divergent results we have attempted to clarify the importance of an abnormality of the parasympathetic nervous system in the pathogenesis of airway hyperresponsiveness to histamine. We have compared the effect of pretreatment with hexamethonium plus placebo and hexamethonium plus atropine with placebo plus placebo on the position of histamine dose–FEV_1 response curves. Hexamethonium and atropine were administered in large doses in an attempt to completely inhibit the parasympathetic input to the airways. Six atropic subjects were selected with a wide range of histamine responsiveness from severely increased to normal, and four of the six subjects had current symptoms of asthma.

SUBJECTS AND METHODS

Six subjects were chosen for study, all but one of whom were from the medical staff of the Firestone Regional Chest and Allergy Unit in Hamilton, Ontario. Subjects had a range of airway responsiveness to inhaled histamine and were considered able to give complete informed consent. Four subjects (nos. 1 to 4) had current symptoms of intermittent wheezing and dyspnea consistent with asthma. Two subjects (nos. 5 and 6) had never had symptoms of asthma. All subjects were atopic as judged by positive wheal-and-flare responses to prick tests with one or more of 16 common allergen extracts (Table I).

Subjects were studied on 3 days within a 2-week period. On each day, measurement of FEV_1 were made three times, and vital capacity was measured once. Then baseline blood pressure was measured sitting and standing, and baseline pulse rate counted at the wrist.

Subjects then inhaled hexamethonium bromide followed by placebo on one day, hexamethonium followed by atropine sulphate on another day, and placebo followed by placebo on another day. The medications were administered single-blind in random order. Then a histamine inhalation test was carried out.

The first inhalations of hexamethonium (800 mg/ml) or placebo on each day were administered from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.) attached to a Rosenthal-French dosimeter set to nebulize for 0.6 sec and producing an output of 9 μL per breath.[6] With the nose clipped, subjects were instructed to breathe tidally through a mouthpiece every 5 sec for 2 min. Thus, the total amount nebulized was 0.22 ml, producing a nebulized dose of hexamethonium of 170 mg; the estimated lung dose was 50 mg per 2 min of inhalation as determined from a previous study indicating that 30% of a nebulized radiolabeled aerosol inhaled from this nebulizer was deposited in both lungs.[6] At the end of the inhalation, FEV_1 was measured at 30 and 90 sec and every 2 min until no further change in FEV_1 occurred. Pulse rate was measured at 60 sec, and sitting and standing blood pressure was measured at 120 sec. The procedure was repeated until the pulse rate had increased by more than 20 bpm, and there was a postural drop in systolic blood pressure of more than 10 mm Hg or until a maximum of six inhalations were received (producing a maximum nebulized dose of hexamethonium of 1020 mg).

The second inhalation on each day of atropine (20 mg/ml) or placebo was administered immediately after the series of hexamethonium or placebo inhalations and was delivered from a Wright nebulizer operating at 50 psi to produce an output of 150 μL/min. It was delivered directly into a face mask held loosely over the mouth and clipped nose and inhaled by tidal breathing for 6 min. Thus, the nebulized dose of atropine was 18 mg and the estimated lung dose was 540 μg, again as determined from a previous study indicating that 3% of a nebulized radiolabeled aerosol inhaled from this nebulizer was deposited in both lungs.[6] This dose of atropine has previously been demonstrated to produce systemic effects of an increase in heart rate and a reduction in saliva output.[5, 7]

The FEV_1 was repeated after the second inhalation. Then subjects rested for 30 min, and the FEV_1, pulse, and sitting and standing blood pressure were measured to ensure that ganglionic blockade persisted. When the FEV_1 was within 10% of the baseline values, a histamine inhalation test was performed. This was at 30 min in five subjects. In the remaining subject the FEV_1 returned to baseline 90 min after the inhalation of hexamethonium and placebo, and there-

TABLE II. Effect of placebo, hexamethonium, and hexamethonium plus atropine on heart rate and blood pressure

Subject	HR	BP sitting	BP standing	HR	BP sitting	BP standing	HR	BP sitting	BP standing
	Placebo day baseline			After saline			After saline		
1	72	112/72	118/76	68	112/76	112/80	70	110/74	112/80
2	84	106/64	114/78	86	110/68	110/78	84	100/64	110/74
3	86	105/55	100/60	84	102/50	98/55	80	105/65	100/60
4	72	126/66	130/74	80	118/72	118/74	72	116/66	118/72
5	80	110/74	130/96	80	110/74	128/92	76	118/72	130/90
6	70	118/70	120/82	68	116/74	122/86	66	116/72	122/84
Mean	77.0	113/69	119/78	77.7	111/69	115/78	74.7	110/69	115/80
±SD	7.3	—	—	7.8	—	—	6.7	—	—
	Hexamethonium day baseline			After hexamethonium			After saline		
1	68	114/74	120/78	96	102/60	98/60	90	100/60	94/66
2	72	106/72	110/80	106	80/60	74/54	110	78/60	76/60
3	84	104/62	110/72	112	90/60	86/54	98	90/62	88/62
4	68	110/60	122/74	100	102/64	88/50	94	98/66	78/48
5	68	110/89	132/92	100	98/68	90/70	98	94/70	92/66
6	66	116/74	122/86	92	112/72	92/72	92	102/78	98/80
Mean	71.0	110/73	119/80	101.0	97/64	88/60	97.0	94/67	88/63
±SD	6.7	—	—	7.1	—	—	7.1	—	—
	Hexamethonium plus atropine day baseline			After hexamethonium			After atropine		
1	68	108/68	114/76	98	98/68	92/64	100	90/68	84/60
2	60	120/80	122/82	112	100/80	78/60	106	104/88	94/80
3	88	106/62	116/74	128	102/70	80/60	136	118/70	98/75
4	80	120/68	122/74	96	102/66	80/64	96	110/70	88/70
5	80	114/70	120/68	98	96/72	90/66	96	96/74	90/70
6	64	120/80	120/90	88	110/84	90/78	88	112/82	94/76
Mean	72.6	115/72	119/80	103.3	101/73	85/65	103.7	105/75.3	91.3/72.1
±SD	11.4	—	—	14.3	—	—	16.9	—	—

HR = heart rate; BP = blood pressure.

fore, the histamine inhalation test was performed at 90 min on that study day.

The histamine test was carried out by the method described by Cockcroft et al.[8] Inhalation of saline was followed by inhalation of histamine acid phosphate in twofold increasing concentrations (0.03 to 16 mg/ml). The response was measured by FEV_1 at 30 sec and 90 sec after each inhalation. Inhalations were continued until there was a decrease in FEV_1 of 20% or more from the lowest postsaline value. The concentration of histamine causing PC_{20} was obtained from the log dose-response curve by linear interpolation of the last two points.

Analysis

The data from this study form a randomized block design, and thus, a two-way analysis of variance[9] was used for statistical comparisons. Linear contrasts were used to highlight the nature of any differences found. Analysis of covariance[10] was used to adjust the observed treatment effect for differences in baseline FEV_1. PC_{20} data were analyzed under a natural logarithm transformation. Differences were considered significant if $p < 0.05$.

RESULTS

Inhalation of hexamethonium alone caused a mean ± SD drop in standing systolic blood pressure of 31 ± 7.5 mm Hg ($p = 0.01$) and an increase in pulse rate of 30 ± 3.1 bpm ($p = 0.01$) (Table II). The drop in standing systolic blood pressure and increase in pulse rate persisted throughout the period of the study. The mean ± SD dose of hexamethonium needed to produce this evidence of ganglionic blockade was 651 ± 128 mg. The addition of atropine did not further alter blood pressure or pulse rate.

Hexamethonium alone caused bronchoconstriction

TABLE III. Effect of placebo, hexamethonium, and hexamethonium plus atropine on FEV_1 and PC_{20}

	FEV_1 (L BTPS)				PC_{20} (mg/ml)
Placebo day					
Subject	Baseline	After saline	After saline	Prehistamine test	
1	3.28	3.27	3.38	3.18	0.08
2	3.25	3.26	3.30	3.42	3.10
3	2.33	2.22	2.22	2.25	1.50
4	3.95	4.12	3.96	4.15	5.00
5	3.67	3.74	3.73	3.70	17.70
6	3.92	4.20	3.90	4.18	20.90
Mean	3.40	3.46	3.42	3.51	2.97*
G SD	0.60	0.73	0.64	0.71	2.03†
Hexamethonium day					
	Baseline	After hexamethonium	After saline	Prehistamine test	
1	3.24	1.80	1.72	2.98	0.03
2	3.35	2.73	2.50	3.05	0.31
3	2.35	1.80	2.05	2.38	2.30
4	4.18	3.72	4.20	4.35	21.70
5	3.55	3.58	3.60	3.84	19.70
6	4.25	3.85	4.15	4.25	57.00
Mean	3.48	2.91	3.05	3.55	2.84
G SD	0.70	0.95	1.09	0.76	2.92
Hexamethonium plus atropine day					
	Baseline	After hexamethonium	After atropine	Prehistamine test	
1	3.30	1.77	2.75	3.00	0.06
2	3.15	2.72	3.38	3.37	1.50
3	2.36	1.80	2.40	2.38	6.30
4	4.27	3.82	4.45	4.25	23.30
5	3.70	3.72	3.80	3.98	23.80
6	4.06	4.08	4.15	4.58	71.20
Mean	3.42	2.98	3.49	3.59	5.31
G SD	0.69	1.04	0.80	0.83	2.57

L = liter; BTPS = body temperature, pressure, and saturated.
*Geometric mean.
†Geometric standard deviation.

in the four subjects with asthma with a maximum mean ± SD fall in FEV_1 of 0.77 ± 0.45 L ($p < 0.001$); the bronchoconstriction was partially or completely reversed by inhalation of atropine (Table III; Fig. 1). Hexamethonium had no significant effect on FEV_1 in the two subjects without asthma (nos. 5 and 6).

Hexamethonium caused a shift to the left in the histamine dose-response curves in two subjects with asthma (nos. 1 and 2) but either no change or a small rightward shift in the remaining four subjects (Table III; Fig. 2). Addition of atropine reversed the leftward shift in subjects 1 and 2 and provided a small additional rightward shift in subject 3. The mean PC_{20} histamine was the same after placebo (2.97 mg/ml) and after hexamethonium alone (2.84 mg/ml). After both hexamethonium and atropine it increased slightly to 5.31 mg/ml largely because of the reversal of the leftward shift in the histamine dose-response curves in subjects 1 and 2; however, this difference did not reach statistical significance ($p = 0.06$).

The relationship between FEV_1 before histamine inhalation and PC_{20} was examined by use of analysis of covariance, and no effect of FEV_1 on the subsequent measurement of PC_{20} could be found.

DISCUSSION

These results indicate that inhalation of both hexamethonium and atropine in doses revealing systemic evidence of ganglionic and cholinergic blockade have only a small effect on histamine dose-response curves in subjects with and without asthma. Premedication with hexamethonium alone caused bronchoconstriction in the subjects with asthma but not in subjects without asthma. After recovery from the bronchoconstriction, it also produced a shift to the left in the

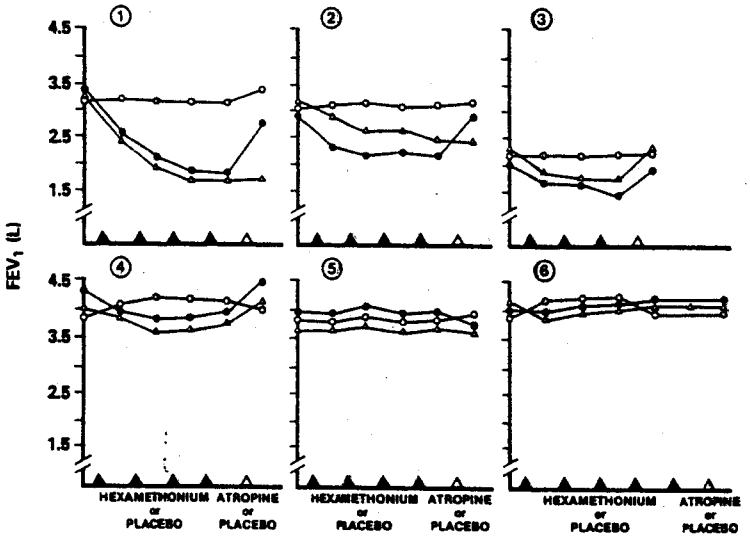

FIG. 1. Effect of placebo followed by placebo (o——o), hexamethonium followed by placebo (Δ--Δ), or hexamethonium followed by atropine (●------●) on FEV_1.

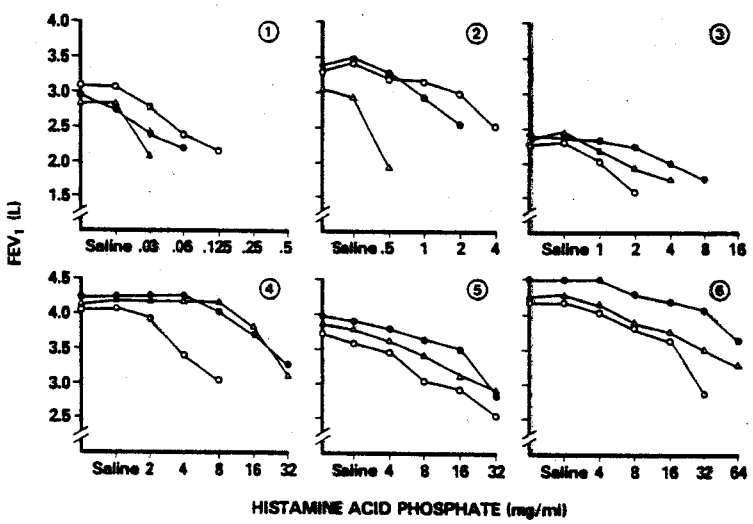

FIG. 2. Effect of placebo plus placebo (o——o), hexamethonium plus placebo (Δ--Δ), or hexamethonium plus atropine (●------●) on histamine dose-response curves.

histamine dose-response curves of two of the subjects with asthma and had either no effect or a small shift to the right in the remainder. Both the bronchoconstriction and the leftward shift and the dose-response curves were partially or completely reversed by atropine.

The ability of hexamethonium alone to inhibit histamine responsiveness varied between subjects from a tenfold leftward shift to a maximal 4.3-fold rightward shift in the histamine dose-response curves. The addition of atropine to hexamethonium increased the mean PC_{20} histamine; however, this was largely by reversing the leftward shift in the histamine dose-response curves induced by hexamethonium in subjects 1 and 2. The maximal rightward shift in the histamine dose-response curves after hexamethonium plus atropine was 4.7-fold. The degree of shift of the dose-response curves was not related to the underlying degree of airway responsiveness. Thus, although the vagal parasympathetic component of the response to histamine may vary between subjects, altered activity in the vagal parasympathetic pathway is not the major cause of the increased airway hyperresponsiveness to histamine found in asthma.

These data critically depend on the demonstration that hexamethonium was effective in causing gangli-

onic blockade and that atropine was effective in causing cholinergic blockade. Inhalation of hexamethonium was continued until postural hypotension and tachycardia occurred, indicating that blockade of the autonomic ganglia had occurred.[11] The addition of atropine in doses that have previously been demonstrated to produce systemic symptoms of cholinergic blockade[5, 7] did not add to the cardiovascular effects of hexamethonium, thus confirming that no additional cholinergic blockade could be achieved. Histamine inhalation tests were carried out 30 min after inhalation of hexamethonium and atropine in all but one subject on one study day. The persistence of ganglionic blockade was confirmed by evidence of postural hypotension and tachycardia both before and after the histamine inhalation test. The persistence of cholinergic blockade was assumed as the peak effect of atropine sulphate on several indices of cholinergic blockade has been demonstrated to be more than 30 min with a duration of action of more than 1 hr.[12]

We could not confirm the observation of Holtzman et al.,[4] that the response to inhaled histamine was abolished by hexamethonium. This difference may have occurred because these authors only used a limited number of doses of inhaled histamine so that the degree of rightward shift in the histamine dose-response curves could not be determined. In addition, they only studied subjects without asthma. By contrast, in the present study subjects inhaled histamine until a 20% fall in FEV_1 occurred on all study days, thus overcoming the inhibition produced by hexamethonium and atropine and allowing the degree of rightward shift in the histamine dose-response curves to be determined. In addition, we examined the degree of protection achieved by hexamethonium and atropine in both atopic subjects without asthma and subjects with asthma who were chosen to cover a wide range of responsiveness to histamine. Thus, our findings have general applicability to patients with hyperresponsive airways. Furthermore, we do not believe that the difference observed in these two studies is due to a difference in the dose of hexamethonium delivered to the subjects. Holtzman et al.[4] used two nebulized doses of hexamethonium (500 and 1000 mg) and observed postural hypotension and tachycardia in four of five subjects at the higher dose. In our study these signs of ganglionic blockade were used as the end point for the delivery of hexamethonium. In addition, the mean nebulized dose of hexamethonium needed to induce these changes was 651 mg, which was similar to the doses used by Holtzman et al.[4] Our data not only support the findings of Boulet et al.[5] who demonstrated that inhaled atropine alone had only a small effect on the response to inhaled histamine but also the observations that inhaled atropine elicit a minor degree of protection against the response to inhaled allergen,[13] exercise,[14] and inhalation of cold air[7] in subjects with asthma.

The observations that inhaled hexamethonium-induced bronchoconstriction in the four subjects with asthma and caused a leftward shift in the histamine dose-response curves in two of these were unexpected. Furthermore, the mechanisms of the bronchoconstriction and leftward shift of the histamine dose-response curve are obscure. Possible explanations include: (1) unmasking of excitatory muscarinic receptors in ganglia[15, 16] after blockade of nicotinic receptors by hexamethonium, thus allowing increased transmission through the ganglia leading to bronchoconstriction, (2) selective inhibition by hexamethonium of nicotinic receptors in sympathetic ganglia compared to nicotinic receptors in parasympathetic ganglia, which would explain our observations if there is a greater contribution to the control of airway resistence by sympathetic compared to parasympathetic nerve pathways in subjects with asthma, (3) selective inhibition by hexamethonium of nicotinic receptors on adrenal medullary cells, thus reducing the output of circulating catecholamines, and (4) activation of rapidly adapting irritant receptors by hexamethonium. All these possibilities, however, lack independent supporting evidence. The ability of atropine to antagonize the bronchoconstrictor effect of hexamethonium would be consistent with all the possibilities and does not distinguish among them. Whatever the mechanism of the bronchoconstriction, the addition of inhaled atropine to hexamethonium did not markedly reduce the baseline airway responsiveness to inhaled histamine in any subject. This indicates that the major effect of inhaled histamine occurs through stimulation of H_1 receptors on airway smooth muscle. In addition, the results, together with the observation that ganglionic blockade had no effect on methacholine-induced bronchoconstriction,[4] suggest that an abnormality in airway smooth muscle is the major cause of the increased airway responsiveness found in asthma.

We thank those subjects who participated in the study.

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The development of subsensitivity to chlorpheniramine

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To assess the development of subsensitivity to antihistamines, titrated prick skin test (PSTs) were performed to seven fivefold dilutions of histamine and either morphine or antigen at specific intervals during therapy. Ten subjects received chlorpheniramine, 24 mg per day, and placebo in a double-blind crossover study. Total wheal area was measured at baseline and after 1, 3, 7, 21, and 24 days. The dose of chlorpheniramine (or placebo) was doubled from days 22 to 24 to assess the response to dosage increase. Serum levels of chlorpheniramine were measured at days 3 and 21 in six patients. Maximal skin test suppression was observed on days 3 or 7. On day 21 there was significantly less ($p < 0.01$) suppression of all PSTs than on days 3 or 7. Mean serum chlorpheniramine was 48.7 ng/ml on day 3 and 36.1 ng/ml on day 21 (not significant). There was no significant correlation between changes in serum chlorpheniramine levels and changes in PST suppression. Doubling the dose of chlorpheniramine did not achieve the maximal suppression observed at days 3 or 7. We conclude that subsensitivity to antihistamines develops between 7 and 21 days of therapy and cannot be completely overcome by doubling the dose. The decreased effect does not appear to be due to induced metabolism but may be related to increased H_1 receptor number. (J ALLERGY CLIN IMMUNOL 76:103-7, 1985.)

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Effect of Ganglionic Blockade on Bronchial Reactivity in Atopic Subjects[1-3]

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SUMMARY

To determine the site in the parasympathetic pathway responsible for the increased bronchial reactivity in 5 atopic subjects, we studied the effect of premedication with aerosols of hexamethonium, a ganglionic blocking agent, and atropine, a postganglionic blocking agent, on the bronchomotor responses to histamine and methacholine aerosols. After 7 mg of aerosolized atropine, baseline specific airway resistance (SRaw) decreased, and the increases in SRaw produced by histamine and by methacholine were prevented in each subject ($p < 0.001$). After 1 g of hexamethonium, baseline SRaw was decreased to a similar level, and the increase in SRaw produced by histamine was again prevented in each subject ($p < 0.001$); however, the increase in SRaw produced by methacholine was not affected significantly in 3 subjects ($p > 0.5$) and was increased or decreased only slightly in 2 subjects ($p < 0.05$). These results suggest that bronchial hyperreactivity in atopic subjects may be due to a change in the characteristics of the efferent parasympathetic pathway at a site distal to the ganglion, possibly at the smooth muscle, and that bronchodilation caused by atropine and hexamethonium cannot, by itself, account for their effects on bronchomotor responses.

Introduction

Bronchial hyperreactivity to inhaled irritants is a characteristic feature of patients with asthma (1) and occurs in some subjects with allergic rhinitis (2–4). Possible causes for the exaggerated smooth muscle constriction in response to bronchoactive substances include an increased responsiveness of the smooth muscle itself or an abnormality in the autonomic nervous control of the smooth muscle (5). In patients with asthma, the increased bronchomotor response to histamine (6) and other irritants (6–9) has been prevented or reversed by atropine sulfate, implicating the involvement of parasympathetic pathways. It has been suggested that the increased bronchomotor response might be due to an increase in the sensitivity of vagal sensory endings in the airways (6), thus exaggerating reflex bronchoconstriction. Other work, however, has demonstrated that patients with asthma (10) and allergic rhinitis (2–4, 11, 12) also respond to inhaled cholinergic agonists with a greater-than-normal amount of bronchoconstriction. Studies in animals have shown little direct effect of cholinergic agonists on the rate of discharge from vagal sensory endings (13) and no significant effect of vagal blockade on acetylcholine-induced bronchoconstriction (14). These findings suggest that bronchial hyperreactivity may be due not only to an increase in the sensitivity of vagal sensory endings, but also to an increase in the respon-

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siveness at some other site in the vagal reflex pathway.

We selected for study a group of atopic subjects with a greater-than-normal bronchomotor response to histamine and methacholine. Because the increased response to histamine in these subjects could be prevented by atropine sulfate, parasympathetic pathways appeared to be involved in the exaggerated bronchomotor response to histamine and methacholine. To determine more precisely the site in the neural pathway that is responsible for bronchial hyperreactivity in this group of subjects, we studied the effect of hexamethonium chloride, a ganglionic blocking agent, on the bronchomotor responses to histamine and to methacholine. We reasoned that ganglionic blockade would prevent the bronchomotor response to histamine if histamine acts by stimulating the sensory pathway of a vagal reflex but should not prevent any direct effect of histamine on airway smooth muscle. Unlike histamine, cholinergic agonists such as methacholine may only act directly at the muscarinic receptor site on smooth muscle to cause bronchoconstriction, and the exaggerated response to methacholine might be due to an increase in the sensitivity of smooth muscle to parasympathetic stimulation. Hexamethonium should then have no effect on the bronchomotor response to methacholine. Alternatively, the exaggerated response to methacholine could be due to an increase in the sensitivity of vagal sensory endings, because vagal sensory endings are stimulated by bronchoconstriction. If this is the case, ganglionic blockade should diminish the response to methacholine. We therefore determined whether ganglionic neural transmission was necessary for the increased responses to histamine and methacholine in our subjects.

Methods

The subjects were 5 otherwise healthy, nonsmoking, adult volunteers, 4 men and 1 woman, 26 to 32 years of age, who were informed of the risks of the experimental protocol and who signed consent forms approved by the Academic Senate Committee on Human Experimentation of the University of California. Although none of the subjects had asthma or other pulmonary disease at the time of the study, one subject (Subject 1 in figure 3) had a family history of allergic rhinitis, and 4 subjects had a personal history of allergic rhinitis. All subjects had 2 or more positive responses to prick skin tests with 7 mixtures of allergens common to Northern California. None of the subjects was using antihistaminic or bronchodilator drugs at the time of the study, and none had symptoms suggesting a viral upper respiratory tract infection during the month before the study. Results from screening tests of pulmonary function (pulmonary function—spirometry), single-breath diffusing capacity of the lung for CO, single-breath O_2 test of distribution of inspired gas, and maximal expiratory flow-volume curve were normal in all subjects.

Before entry into the study, each atopic subject was shown to have a normal value for baseline specific airway resistance (SRaw) and a greater-than-normal bronchomotor response to inhalation of both histamine and methacholine aerosols: the increase in SRaw after inhalation of 10 breaths of either histamine aerosol (16 mg/ml solution) or methacholine aerosol (10 mg/ml solution) was more than 2 SD above the mean increase in SRaw produced by the same dose of histamine or methacholine in a group of 13 healthy, nonsmoking control subjects, 22 to 32 years of age, with no personal or family history of atopy, asthma, or other pulmonary disease and negative responses to allergen skin tests (figure 1). In addition, we compared the effect of atropine sulfate in atopic and control subjects on t motor response to this dose of histamine (f the atropine studies, atropine sulfate aeroso of body weight) was inhaled from a sol mg/ml 30 min before inhalation of histam Because atropine sulfate decreased the br response to histamine of atopic subjects to r (see figure 1 and *Results*), more detailed done as outlined later.

Airway resistance (Raw) and thoracic (Vtg) were measured using a constant-volu body plethysmograph (15, 16). Air flow w with a heated pneumotachograph (Fleisch differential transducer (Validyne DP 45). box pressures were measured with pressure (Validyne DP 7 and DP 45, respectively). output of these transducers entered carrier tors (Validyne CD 19), was amplified by amplifier (Tektronix 5418 N), and was displ ings on a single-beam storage oscilloscope D15). The slopes of the tracings were read d a protractor mounted to the oscilloscope fa

A solution of histamine (16 mg/ml) w daily from a stock supply of histamine diph was buffered with sodium bicarbonate to a p methacholine solution (10 mg/ml) was made a stock supply of methacholine chloride normal saline. All solutions were delivered from a glass nebulizer (DeVilbiss no. 40) eq a dose-metering device (17). This device co breath-activated solenoid valve and a timin series with a compressed O_2 source at 20 ps noid valve was set to remain open for 0.6 s f set of inspiration, during which time O_2 was flow through the nebulizer and dispersed an 0.008 ml of the solution with each breath. was delivered throughout the course of a ti tion starting at functional residual capacity. median droplet diameter of the aerosol un conditions was reported as 3.2 μm (18).

Bronchial reactivity was assessed in each ject by obtaining dose-response curves for and for methacholine. Dose-response curves lished by having the subjects inhale 10 brea successively increasing concentrations of hi methacholine aerosol administered at 5-min The initial concentration used was 0.5 mg mine/ml and 0.16 mg of methacholine/ml, quent concentrations were increased in 2- ments. For the baseline values and for the v inhalation of each dose of histamine or met Raw and Vtg were measured 5 times at 30- values of SRaw (Raw × Vtg) were calculated aged.

The dose-response curves for histamine a choline in the atopic subjects were determi each of 4 experimental conditions: after no tion and 30 min after premedication with 7 n pine, with 500 mg of hexamethonium, and v hexamethonium. Hexamethonium chloride w from a solution of 400 mg/ml, and measur

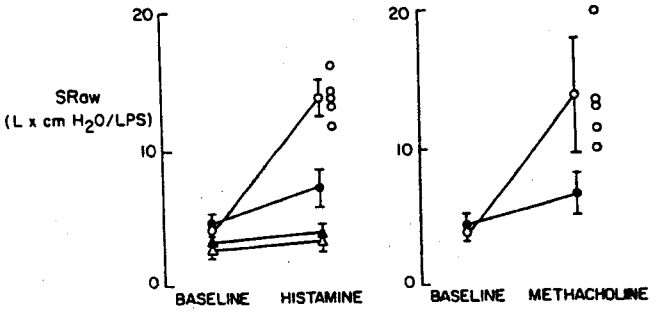

Fig. 1. Effect of inhalation of histamine and methacholine aerosols on specific airway resistance (SRaw) in 5 atopic (O) and 13 normal (●) subjects. For the normal subjects, each symbol represents the mean ± SD value for SRaw. For the atopic subjects, the baseline values for SRaw (reported as mean ± SD) were not significantly different from values in the normal subjects, but bronchomotor responses to inhaled histamine and methacholine aerosols were greater than those in the normal subjects (response in each atopic subject plotted with a separate symbol). After premedication with atropine (Δ), the response to inhaled histamine was similar in normal and atopic subjects. L = liter; LPS = liter per second.

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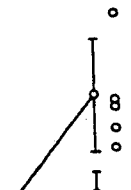

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sulfate in atopic and control subjects on the bronchomotor response to this dose of histamine (figure 1). For the atropine studies, atropine sulfate aerosol (0.1 mg/kg of body weight) was inhaled from a solution of 10 mg/ml 30 min before inhalation of histamine aerosol. Because atropine sulfate decreased the bronchomotor response to histamine of atopic subjects to normal levels (see figure 1 and *Results*), more detailed studies were done as outlined later.

Airway resistance (Raw) and thoracic gas volume (Vtg) were measured using a constant-volume, whole-body plethysmograph (15, 16). Air flow was measured with a heated pneumotachograph (Fleisch No. 2) and a differential transducer (Validyne DP 45). Mouth and box pressures were measured with pressure transducers (Validyne DP 7 and DP 45, respectively). The electric output of these transducers entered carrier demodulators (Validyne CD 19), was amplified by a dual-trace amplifier (Tektronix 5418 N), and was displayed as tracings on a single-beam storage oscilloscope (Tektronix D15). The slopes of the tracings were read directly from a protractor mounted to the oscilloscope face.

A solution of histamine (16 mg/ml) was prepared daily from a stock supply of histamine diphosphate and was buffered with sodium bicarbonate to a pH of 7.0. A methacholine solution (10 mg/ml) was made daily from a stock supply of methacholine chloride dissolved in normal saline. All solutions were delivered as aerosols from a glass nebulizer (DeVilbiss no. 40) equipped with a dose-metering device (17). This device consisted of a breath-activated solenoid valve and a timing circuit in series with a compressed O_2 source at 20 psi. The solenoid valve was set to remain open for 0.6 s from the onset of inspiration, during which time O_2 was allowed to flow through the nebulizer and dispersed an average of 0.008 ml of the solution with each breath. The aerosol was delivered throughout the course of a tidal inspiration starting at functional residual capacity. The volume median droplet diameter of the aerosol under similar conditions was reported as 3.2 μm (18).

Bronchial reactivity was assessed in each atopic subject by obtaining dose-response curves for histamine and for methacholine. Dose-response curves were established by having the subjects inhale 10 breaths each of successively increasing concentrations of histamine or methacholine aerosol administered at 5-min intervals. The initial concentration used was 0.5 mg of histamine/ml and 0.16 mg of methacholine/ml, and subsequent concentrations were increased in 2-fold increments. For the baseline values and for the values after inhalation of each dose of histamine or methacholine, Raw and Vtg were measured 5 times at 30-s intervals; values of SRaw (Raw × Vtg) were calculated and averaged.

The dose-response curves for histamine and methacholine in the atopic subjects were determined under each of 4 experimental conditions: after no premedication and 30 min after premedication with 7 mg of atropine, with 500 mg of hexamethonium, and with 1 g of hexamethonium. Hexamethonium chloride was inhaled from a solution of 400 mg/ml, and measurements of blood pressure in the supine and standing positions were made 15 min later. Atropine sulfate was inhaled from a solution of 10 mg/ml. The doses of hexamethonium were selected on the basis of preliminary studies, and the dose of atropine was selected on the basis of previous work (19, 20).

Each dose-response curve for histamine and for methacholine was obtained on a separate day in a random sequence to avoid drug interaction, and the subjects were unaware of the sequence of the aerosols. To avoid any diurnal variation in bronchomotor responses, all studies were performed at the same time of day for each subject.

We evaluated the changes in bronchial reactivity after premedication with atropine or hexamethonium for each atopic subject as follows: first, to linearize each dose-respone curve, we plotted the log of SRaw against the dose of histamine or methacholine administered and then calculated the regression equation (for example, figure 2). Using this method, the correlation coefficients for the responses under most conditions were greater than 0.93 ± 0.05 (mean ± SD), suggesting that the linearizing procedure was valid. For the responses after atropine, when the slopes of the dose-response curves were near zero, the correlation coefficients were appropriately lower (0.71 ± 0.31). Changes in bronchial reactivity could then be analyzed by comparing the slopes of the dose-response curves by analysis of covariance and the Newman-Keuls multiple range test (21). The significance of differences in baseline SRaw

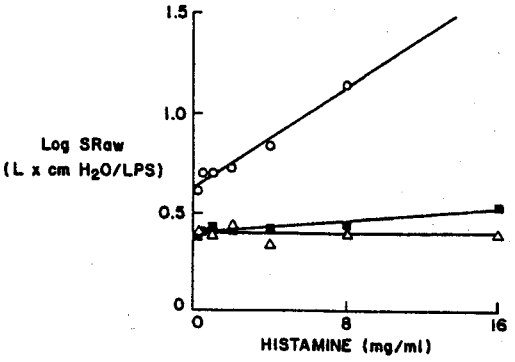

Fig. 2. Effect of increasing concentration of histamine aerosol on specific airway resistance (SRaw) in one atopic subject. The subject inhaled 10 breaths each of increasing concentrations of histamine aerosol administered at 5-min intervals; the initial concentration used was 0.5 mg/ml, and subsequent concentrations increased in 2-fold increments. Each point represents the mean of 5 measurements of SRaw obtained after inhalation of a given concentration of histamine. Responses to histamine were assessed in the control state before premedication (○) and 30 min after premedication with aerosols of both 7 mg of atropine (Δ) and 1 g of hexamethonium (■). Regression lines were calculated for each condition. Both atropine and hexamethonium decreased baseline SRaw to the same level and prevented the response to histamine. L = liter; LPS = liter per second.

was determined with "Student's" *t* test for paired data (21).

Results

Comparison with normal subjects. The bronchomotor response to inhalation of histamine aerosol was greater in each atopic subject than in the group of normal subjects: inhalation of histamine aerosol (10 breaths, 16 mg/ml) increased SRaw from a mean ± SD of 4.46 ± 1.15 to 7.23 ± 1.97 L · cm H_2O/L · s^{-1} in the 13 normal subjects, a mean increase of 2.77 ± 1.08 L · cm H_2O/L · s^{-1}. In the atopic subjects, inhalation of the same dose of histamine increased SRaw from 4.01 ± 0.51 (range, 3.30 to 4.40) to 13.74 ± 1.56 L · cm H_2O/L · s^{-1}, a mean increase of 9.73 ± 1.96 (range, 7.51 to 12.38) L · cm H_2O/L · s^{-1}. In each atopic subject, the baseline value for SRaw before inhalation of histamine was within the normal range (mean ± 2 SD), but the increase in SRaw after inhalation of histamine was more than 2 SD above the mean increase in SRaw in the normal subjects (figure 1).

Atropine sulfate decreased baseline SRaw and decreased the bronchomotor response to histamine in both groups of subjects; in the atopic subjects, the response to histamine was decreased to the same level found in the normal group: after atropine premedication, inhalation of histamine aerosol (10 breaths, 16 mg/ml) increased mean SRaw from 2.98 ± 0.75 to 4.01 ± 0.83 L × cm H_2O/L/s in the 13 normal subjects, a mean increase of 1.02 ± 0.55 L × cm H_2O/L/s (figure 1); in the atopic subjects, inhalation of histamine after atropine premedication increased mean SRaw from 2.84 ± 0.68 (range, 2.02 to 3.62) to 3.48 ± 1.11 L × cm H_2O/L/s, a mean increase of 0.63 ± 0.45 (range, 0.27 to 1.31) L × cm H_2O/L/s. Atropine decreased the value for baseline SRaw in almost every subject (in one atopic subject, there was no change) and decreased the bronchomotor response to histamine in every subject. Furthermore, after atropine premedication, the values for baseline SRaw and the increase in SRaw after inhaling histamine were no longer different for the 2 groups of subjects ($p > 0.18$).

The bronchomotor response to inhalation of methacholine aerosol was also greater than normal in each atopic subject: inhalation of methacholine aerosol (10 breaths, 10 mg/ml) increased mean SRaw from 4.48 ± 1.26 to 6.61 ± 1.65 L × cm H_2O/L/s in the 13 normal subjects, a mean increase of 2.14 ± 0.69 L × cm H_2O/L/s; in the atopic subjects, inhalation of the same dose of methacholine increased mean SRaw from 3.78 ± 0.64 (range, 2.81 to 4.46) to 13.81 ± 4.19 L × cm H_2O/L/s, a mean increase of 10.03 ± 3.89 (range, 6.42 to 20.83) L × cm H_2O/L/s. In each atopic subject, the baseline value for SRaw was within the normal range, but the increase in SRaw after inhalation of methacholine was more than 2 SD above the mean increase in SRaw in the normal subjects (figure 1).

Histamine. Premedication with either hexamethonium or atropine aerosol prevented the bronchomotor response to histamine (figures 2 and 3). In each of the 5 subjects, hexamethonium chloride decreased the baseline SRaw and decreased the bronchomotor response to inhalation of histamine aerosol: baseline SRaw decreased from a control value of 3.75 ± 0.94 L × cm H_2O/L/s (mean ± SD) to values of 2.76 ± 1.11 ($p < 0.01$) and 2.81 ± 0.95 L × cm H_2O/L/s ($p = 0.01$) after premedication with 500 mg and 1 g of hexamethonium aerosol, respectively. There was no significant difference between the mean values for baseline SRaw after each of the 2 doses of hexamethonium aerosol ($p > 0.8$). After premedication with 500 mg of hexamethonium aerosol, the slope of the dose-response curve to inhalation of histamine was decreased markedly in 2 subjects ($p < 0.001$), decreased slightly in 2 subjects ($p < 0.005$), and not affected significantly in one subject ($p > 0.1$) when compared to the control value. After premedication with 1 g of hexamethonium aerosol, the slope of the dose-response curve to inhalation of histamine was decreased markedly in each subject when compared to the control value ($p < 0.001$; figure 3, top). Thus, in 3 subjects (Subjects 1, 2, 3), premedication with 1 g of hexamethonium prevented the bronchomotor response to inhalation of histamine more completely when compared to 500 mg of hexamethonium ($p < 0.001$), although both doses produced similar decreases in the baseline SRaw.

Orthostatic increases in heart rate and decreases in arterial blood pressure developed in 4 subjects after inhalation of the higher dose of hexamethonium aerosol. The mean postural decrease in systolic blood pressure for the group was 15 ± 9 mm Hg. No other side effects due to autonomic ganglion blockade were observed.

Atropine sulfate also decreased the baseline SRaw and decreased the bronchomotor response to inhalation of histamine aerosol: baseline SRaw decreased from a mean ± SD control value of 3.75 ± 0.94 L × cm H_2O/L/s to 2.74 ± 0.75 L × cm H_2O/L/s after premedication with 7 mg of atropine aerosol ($p < 0.01$). There was no significant difference between the mean value for baseline SRaw after premedication with a the mean value after either dose of nium ($p > 0.15$). After premedicatior pine aerosol, the slope of the dose-res to histamine was decreased markedly i ject when compared to the control 0.001; figure 3, top) and, for the whole, was similar in magnitude to the histamine after premedication with 1 methonium ($p > 0.2$).

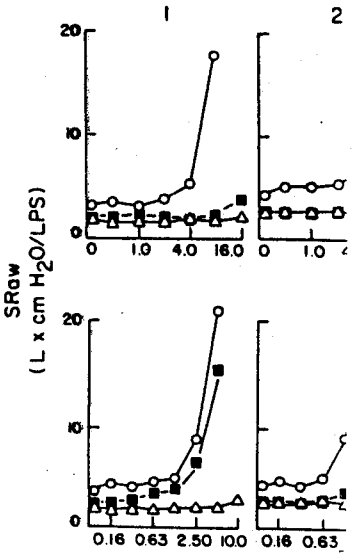

Fig. 3. Effect of hexamethonium chl mine (upper graphs) and methacholine as figure 2. Initial concentrations were with atropine (△) decreased baseline vented the responses to histamine and a similar value and prevented the resp

Methacholine. The control response t of methacholine aerosol was similar in to the control response to histamine. tion with atropine sulfate decrease SRaw and prevented the bronchomot to methacholine. Hexamethonium cl decreased the baseline SRaw but did the bronchomotor response to inh methacholine aerosol: baseline SRaw from a mean control value of 3.95 ± 1 H_2O/L/s to 2.98 ± 1.36 L × cm H_2(premedication with 1 mg of hexa aerosol ($p = 0.01$). There was no sign ference between these mean values f SRaw and the respective values obtai the studies with histamine ($p >$ premedication with 1 g of hexametho sol, the slope of the dose-response curv

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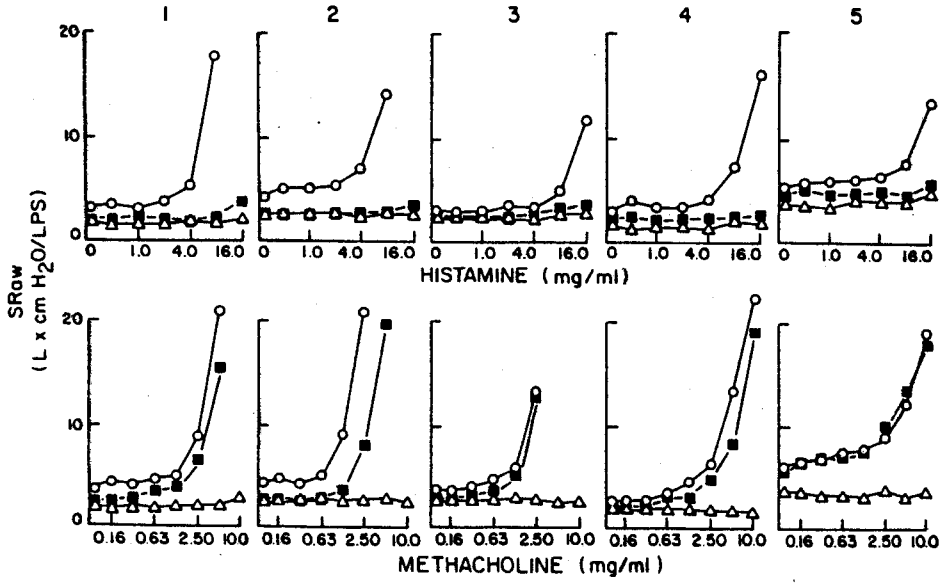

Fig. 3. Effect of hexamethonium chloride and of atropine sulfate on the dose-response curve for histamine (upper graphs) and methacholine aerosols (lower graphs) in each of 5 atopic subjects. Same protocol as figure 2. Initial concentrations were: histamine, 0.5 mg/ml; methacholine, 0.16 mg/ml. Premedication with atropine (△) decreased baseline specific airway resistance (SRaw) from control values (○) and prevented the responses to histamine and to methacholine. Hexamethonium (■) decreased baseline SRaw to a similar value and prevented the response to histamine, but did not prevent the response to methacholine.

line SRaw after premedication with atropine and the mean value after either dose of hexamethonium ($p > 0.15$). After premedication with atropine aerosol, the slope of the dose-response curve to histamine was decreased markedly in each subject when compared to the control value ($p < 0.001$; figure 3, top) and, for the group as a whole, was similar in magnitude to the response to histamine after premedication with 1 g of hexamethonium ($p > 0.2$).

Methacholine. The control response to inhalation of methacholine aerosol was similar in magnitude to the control response to histamine. Premedication with atropine sulfate decreased baseline SRaw and prevented the bronchomotor response to methacholine. Hexamethonium chloride also decreased the baseline SRaw but did not prevent the bronchomotor response to inhalation of methacholine aerosol: baseline SRaw decreased from a mean control value of 3.95 ± 1.18 L × cm H_2O/L/s to 2.98 ± 1.36 L × cm H_2O/L/s after premedication with 1 mg of hexamethonium aerosol ($p = 0.01$). There was no significant difference between these mean values for baseline SRaw and the respective values obtained during the studies with histamine ($p > 0.3$). After premedication with 1 g of hexamethonium aerosol, the slope of the dose-response curve to inhalation of methacholine was not affected significantly in Subjects 1, 4, and 5 ($p > 0.5$), decreased slightly in Subject 2 ($p < 0.001$), and increased slightly in Subject 3 ($p < 0.05$) when compared to the control value (figure 3, bottom).

Atropine sulfate decreased the baseline SRaw and prevented completely the bronchomotor response to inhalation of methacholine aerosol: baseline SRaw decreased from a mean ± SD control value of 3.95 ± 1.18 L × cm H_2O/L/s to 2.48 ± 0.72 L × cm H_2O/L/s after premedication with atropine aerosol ($p < 0.01$). There was no significant difference between the mean value for baseline SRaw after premedication with atropine and the mean value after hexamethonium ($p > 0.2$). After premedication with atropine, the slope of the dose-response curve to methacholine was decreased markedly in each subject when compared to the control value ($P < 0.001$; figure 3, bottom).

Discussion

This study demonstrates that pretreatment with an aerosol of hexamethonium chloride prevents the exaggerated bronchomotor response to inhalation of histamine in atopic subjects but has no significant effect on the response to methacholine. The bronchomotor responses of our

atopic subjects to histamine and methacholine were greater than the responses of a group of normal subjects, but the response to histamine in the atopic subjects was no different from the response of the normal subjects after each group was pretreated with an aerosol of atropine sulfate. Thus, while the small direct effect of histamine was the same for the 2 groups of subjects, the neural component of the response to histamine was increased greatly in the atopic subjects. This finding suggests that bronchial hyperreactivity to histamine is mediated through parasympathetic pathways but does not identify the site(s) in the neural pathway responsible for the increased bronchomotor response. That the increased response to methacholine persists despite ganglionic blockade further suggests that bronchial hyperreactivity to methacholine is due to a change in the efferent parasympathetic pathway at a site distal to the ganglion.

Although we delivered hexamethonium by aerosol, the occurrence of orthostatic decreases in arterial blood pressure implies that the drug was absorbed into the systemic circulation and caused blockade of sympathetic ganglia. Blockade of sympathetic β-adrenergic and α-adrenergic activity might also have effects on airway smooth muscle tone. Blockade of possible β-adrenergic bronchodilator activity by hexamethonium could have increased both baseline airway resistance and the response to bronchoactive substances (22, 23). In fact, hexamethonium had the opposite effect in our subjects. Blockade of possible α-adrenergic bronchoconstrictor activity, however, could have decreased both baseline airway resistance and the response to bronchoactive substances (24). The importance of adrenergic effects in our subjects is unknown.

In animals (25, 26) and in healthy humans (27, 28), a mild degree of resting tone is present in airway smooth muscle, and this tone is maintained by vagal nervous activity (29). Thus, in our subjects, muscarinic blockade with atropine or ganglionic blockade with hexamethonium caused mild bronchodilation. It is possible that changes in baseline airway caliber could influence the subsequent response to inhaled bronchoconstrictor agents, so that the decreased bronchomotor responses to methacholine and to histamine after pretreatment with atropine or hexamethonium were due to decreases in baseline airway resistance rather than to a neural mechanism. However, changes in baseline airway caliber cannot explain our results for the following reasons: both atropine and hexamethonium caused equivalent degrees of bronchodilation, yet only atropine prevented the bronchomotor response to inhalation of methacholine aerosol; hexamethonium had no significant effect on the response to methacholine despite the decrease in baseline resistance. In addition, 2 separate doses of hexamethonium (500 mg and 1 g) caused the same degree of bronchodilation, but the higher dose was more effective at preventing the increase in resistance caused by histamine, implying that blockade of a specific neural mechanism, not changes in airway geometry, was the cause of the hexamethonium effect.

Although inhaled stimuli may have multiple effects on the airway, it is possible to determine the relative importance of direct effects on airway smooth muscle from effects mediated through nervous pathways. For example, histamine can cause bronchoconstriction by stimulating airway smooth muscle directly (30); it can also stimulate vagal sensory endings in the airways and cause reflex bronchoconstriction (31), and may have additional interactions at ganglionic (32) or efferent sites (14, 33) in the parasympathetic pathway. Direct and neural effects may occur in varying degrees under different conditions. Previous studies have used atropine (6, 20, 34, 35) and hexamethonium (36) in humans and interruption of conduction in the vagus nerves in animals (31) to abolish the neural component of the response to histamine. The bronchomotor response to histamine would then be decreased markedly if neural effects predominated over direct effects on airway smooth muscle. Some studies have concluded that the effect of histamine mediated through parasympathetic pathways may predominate in patients with asthma or obstructive airway disease (6) and in previously healthy subjects who develop transient bronchial hyperreactivity after exposure to ozone (20, 35) or during an upper respiratory viral infection (34), whereas other studies have found little parasympathetic effect of histamine in asthmatic subjects (37–39). Variations in the dose and mode of delivery of the drugs and in the type of subjects may explain some of the differences: for example, other workers have used lower doses of atropine (37), different types of nebulizers (39), and selected patients with more severe asthma for study (37–39). We used atropine (in a dose sufficient to abolish the effect of methacholine) to decrease markedly the effect of histamine and suggest that a neural mechanism predominated in our subjects with allergic rhinitis under the conditions of our study. Hexamethonium caused the same decreases in baseline airway resistance and in the response to histamine, thus implying blockade of parasympathetic ganglia in ways and confirming a neural effect of hi

In one study of patients with asthma, t chomotor response to histamine was dec pretreatment with chlorpheniramine, s an important role for H_1 receptors in the (40). Because these receptors are located smooth muscle, where they mediate direc of histamine, and in the neural pathway (sensory endings), where they mediate the r fects of histamine (41), this finding is co with the concept that histamine causes b constriction through neural pathways.

It is relatively easy to determine the pre neural mechanisms in human subjects. It difficult to determine the specific mechani is responsible for the exaggerated respo histamine and to muscarinic agonists. possible sites in the parasympathetic pathw be involved: vagal sensory endings, nodose sory ganglion, central nervous system, motor ganglia, release of acetylcholine fr motor end plate, and the smooth muscle m nic receptor sites. In addition, various fe mechanisms may decrease or increase the of bronchoconstriction. For example, stim of the airways causes reflex bronchoconst (31, 42, 43), which in turn stimulates vag sory endings (44), creating a "positive fee loop" for increasing the contractile resp Thus, an increase in the sensitivity at any the vagal reflex pathway could be responsib the exaggerated response to cholinergic ago To determine more precisely which site is re sible, we delivered hexamethonium, a drug occupies receptor sites on the postsynaptic brane of ganglion cells and interrupts n transmission at the parasympathetic ganglia. response to methacholine was unchanged by h methonium, unlike the studies with histami which the same dose of hexamethonium vented a similar increase in airway resista ganglionic neural transmission was therefore necessary for the response to methacholine. T findings suggest that inhaled methacholine directly at the smooth muscle muscarinic rece site to cause bronchoconstriction and that the creased bronchomotor responses are due t change in the sensitivity or the responsiveness the smooth muscle to parasympathetic nerv activity. Dose-response curves in isolated air smooth muscle may be necessary to determine exact mechanism (5).

One possible explanation for the increase

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...uled stimuli may have multiple ef... ...irway, it is possible to determine the ...rance of direct effects on airway ...le from effects mediated through ...ways. For example, histamine can ...constriction by stimulating airway ...directly (30); it can also stimulate ...endings in the airways and cause ...constriction (31), and may have ad... ...tions at ganglionic (32) or efferent ...in the parasympathetic pathway. ...ural effects may occur in varying ... different conditions. Previous ...ed atropine (6, 20, 34, 35) and hex... ...36) in humans and interruption of ...the vagus nerves in animals (31) to ...ural component of the response to ...e bronchomotor response to hista... ...en be decreased markedly if neural ...inated over direct effects on airway ...e. Some studies have concluded that ...histamine mediated through para... ...athways may predominate in patients ...or obstructive airway disease (6) and ...healthy subjects who develop tran... ...al hyperreactivity after exposure to ...) or during an upper respiratory viral ..., whereas other studies have found ...pathetic effect of histamine in asth... ...s (37–39). Variations in the dose and ...very of the drugs and in the type of ... explain some of the differences: for ...er workers have used lower doses of ...), different types of nebulizers (39), ... patients with more severe asthma for ...). We used atropine (in a dose suffi... ...ish the effect of methacholine) to de... ...edly the effect of histamine and sug... ...eural mechanism predominated in our ...n allergic rhinitis under the conditions ...y. Hexamethonium caused the same ... baseline airway resistance and in the response to histamine, thus implying adequate blockade of parasympathetic ganglia in the airways and confirming a neural effect of histamine.

In one study of patients with asthma, the bronchomotor response to histamine was decreased by pretreatment with chlorpheniramine, suggesting an important role for H_1 receptors in the response (40). Because these receptors are located both on smooth muscle, where they mediate direct effects of histamine, and in the neural pathway (e.g., on sensory endings), where they mediate the reflex effects of histamine (41), this finding is compatible with the concept that histamine causes bronchoconstriction through neural pathways.

It is relatively easy to determine the presence of neural mechanisms in human subjects. It is more difficult to determine the specific mechanism that is responsible for the exaggerated response to histamine and to muscarinic agonists. Several possible sites in the parasympathetic pathway may be involved: vagal sensory endings, nodose or sensory ganglion, central nervous system, vagal motor ganglia, release of acetylcholine from the motor end plate, and the smooth muscle muscarinic receptor sites. In addition, various feedback mechanisms may decrease or increase the degree of bronchoconstriction. For example, stimulation of the airways causes reflex bronchoconstriction (31, 42, 43), which in turn stimulates vagal sensory endings (44), creating a "positive feedback loop" for increasing the contractile response. Thus, an increase in the sensitivity at any site in the vagal reflex pathway could be responsible for the exaggerated response to cholinergic agonists. To determine more precisely which site is responsible, we delivered hexamethonium, a drug that occupies receptor sites on the postsynaptic membrane of ganglion cells and interrupts neural transmission at the parasympathetic ganglia. The response to methacholine was unchanged by hexamethonium, unlike the studies with histamine in which the same dose of hexamethonium prevented a similar increase in airway resistance; ganglionic neural transmission was therefore not necessary for the response to methacholine. These findings suggest that inhaled methacholine acts directly at the smooth muscle muscarinic receptor site to cause bronchoconstriction and that the increased bronchomotor responses are due to a change in the sensitivity or the responsiveness of the smooth muscle to parasympathetic nervous activity. Dose-response curves in isolated airway smooth muscle may be necessary to determine the exact mechanism (5).

One possible explanation for the increase in bronchial reactivity to both methacholine and histamine is an increase in the number of or binding affinity of the muscarinic receptors on bronchial smooth muscle. In this case, the bronchomotor response to methacholine and other muscarinic agonists would be increased because they act by stimulating the smooth muscle receptors directly, and atropine should block the effect by competing for the receptor sites. For histamine and other inhaled irritants that cause reflex bronchoconstriction by stimulating vagal sensory endings, the reflex response could also be augmented by an increase in the sensitivity of smooth muscle to acetylcholine released at the motor nerve endings. This explanation is based on the assumption that the increased bronchomotor responses to histamine and to methacholine are due to the same mechanism. In fact, previous work suggests that the increased response to histamine may be due to changes at other sites in the vagal reflex pathway. It has been proposed that the increased bronchomotor response to inhaled irritants may be due to an increase in the sensitivity of vagal sensory nerve endings in the airways responsible for initiating reflex bronchoconstriction. This hypothesis has been supported by studies of other responses of the respiratory system that are influenced by afferent vagal activity, such as the pattern of breathing (45) and the threshold for cough (34). For example, the threshold dose of citric acid that produced cough in subjects with viral respiratory infections was significantly lower than that in subjects without infection, suggesting that the low threshold for stimulation of vagal sensory endings (cough receptors) was responsible for the exaggerated bronchomotor responses in these subjects. At present, however, it is unclear whether the sensory endings responsible for cough are also responsible for regulating smooth muscle tone. Furthermore, such a mechanism may be more important under conditions associated with airway epithelial damage, such as viral infection or exposure to oxidizing pollutants (20, 35), than in our subjects with allergic rhinitis. Under any condition, epithelial damage could cause bronchial hyperreactivity by increasing airway permeability, thereby increasing the amount of inhaled materials that reach "target cells" (e.g., sensory nerve endings and smooth muscle). The importance of such an effect in our subjects is unknown.

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