Parainfluenza 3 Infection Blocks the Ability of a Beta Adrenergic Receptor Agonist to Inhibit Antigen-induced Contraction of Guinea Pig Isolated Airway Smooth Muscle

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Research Article

Guinea pigs, actively sensitized to ovalbumin, were inoculated by nasal insufflation with parainfluenza 3 or virus growth medium 4 d before performing in vitro pharmacological studies on tracheal and bronchial smooth muscle. In each airway segment, cumulative dose-response effects of ovalbumin were obtained in the absence and presence of a maximally effective concentration of a beta adrenergic receptor agonist, sulfonterol. Sulfonterol shifted the dose-response curve to the right and reduced the maximum smooth muscle contractile response to ovalbumin. Virus infection did not alter the dose-response effects of ovalbumin. However, the magnitude of the inhibitory effects of sulfonterol was smaller in segments taken from animals inoculated with virus. Blockade by virus infection of the inhibitory effect of sulfonterol was reversed when the concentrations of beta agonist were increased. Sulfonterol did not alter the dose-response effects of histamine at any of the concentrations that markedly antagonized the effects of ovalbumin. Virus infection did not alter the sensitivities to sulfonterol or papaverine in producing relaxation in either airway segment. The magnitude of relaxation produced by papaverine was significantly larger in bronchial rings taken from animals infected with virus for 4 d, but there was no alteration by virus of the dose-response effects of histamine or carbachol. In experiments measuring antigen-induced release of slow reacting substance of anaphylaxis and histamine from minced lung, virus […]

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sulfonterol to inhibit the release of slow reacting sub-
stance of anaphylaxis and histamine was not affected
by virus infection.

These results demonstrate that infection of guinea
pigs with respiratory virus results in a selective block-
ade of the beta adrenergic-mediated inhibition of
antigen-induced contraction of airway smooth muscle.
The guinea pig may serve as a useful model in physio-
logical studies of virus-induced asthma.

INTRODUCTION

Respiratory virus infections can exacerbate bronchial
asthma (1, 2) and enhance airway reactivity to smooth
muscle contractile substances (3, 4). The mechanisms
of these effects of virus infection have been suggested
to result from antagonism of beta adrenergic or other
inhibitory responses (5, 6), epithelial damage resulting
in sensitization of rapidly adapting airway epithelial
receptors (3), and enhancement of the release of
mediators of the allergic response (7). The develop-
ment of an animal model in which virus-induced
changes in lung function can be systematically ex-
amined is lacking. This report describes our studies
on pharmacological responses of isolated airway
smooth muscle from guinea pigs infected with para-
influenza 3 virus with emphasis on beta adrenergic
inhibitory effects.

Sulfonterol (8) is used as the beta agonist in these
experiments. It is a partial agonist (9) and the ability
of beta agonists to inhibit the contractile effects of

agonists acting on airway smooth muscle is limited by the beta receptor reserve (10). Therefore, sulfonterol does not antagonize effects of contractile agonists by action on smooth muscle cell beta receptors. The partial agonist property of sulfonterol is of value since antagonism of antigen-induced contraction provides a measure of beta agonist ability to activate beta receptors which modulate mediator release.

METHODS

Female, albino guinea pigs (Bio-Lab Inc., St. Paul, Minn.), weighing 300–500 g were treated with ovalbumin (10 mg/kg, i.p.) on days 1, 3, and 5. Beginning 21 d after the last injection, animals were inoculated with virus or virus growth medium as described below and subsequently killed with an injection of pentobarbital (90–100 mg/kg, i.p.). After exsanguination, the trachea and left and right main bronchi were removed, trimmed of excess tissue, and prepared for in vitro studies. The trachea was cut in spiral fashion (11), divided into proximal and distal halves, and each half mounted vertically in a tissue bath. Each main bronchus was trimmed of excess tissue and suspended as a ring (about 5 mm in length) on stainless steel stirrups in a tissue bath (12). Usually, one tracheal spiral and one bronchus from each animal served as control and the other as treated tissue. Each 10-ml water-jacketed (37–38°C) tissue bath contained a Krebs bicarbonate solution, gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Initial tensions on the tracheal strips and bronchial rings were adjusted to 5 and 2 g, respectively, and maintained at that level before inducing contraction in the tissues. The values used for initial tension were predetermined in separate length-tension studies using carbachol as the contractile agonist. The tissues were allowed to equilibrate for 1.5–2 h before addition of any drugs. Mechnical responses were recorded on Grass model 5D, 7C, or 79B polygraphs (Grass Instrument Co., Quincy, Mass.) via force transducers (FT-03).

Dose-response effects of contractile and relaxant substances were obtained in a cumulative manner (13). When ovalbumin was the contractile substance, an additional response after the peak response from cumulative addition could usually be evoked by increasing the bath concentration to 1 mg/ml. This additional contractile response to ovalbumin was less marked or nonexistent in bronchial rings. In all cases, the maximum degree of contraction that could be elicited in each tissue was taken to be that produced by addition of carbachol, 1 mM, or barium chloride, 10 mM, at the end of the experiment. When relaxation was measured, the maximum degree of relaxation in each tissue was taken to be that produced by addition of papaverine, 1 mM, at the end of the experiment. In each airway segment, carbachol, 0.1 μM was used to induce tone 15 min before adding a relaxant substance. Responses to the contractile and relaxant substances were usually calculated as a percentage of the maximum response produced by carbachol and papaverine, respectively.

($\pm$)-Sulfonterol (8) was used as the beta receptor agonist in these studies and was allowed to interact with the tissues for 15 min prior to obtaining dose-response effects of contractile substances. This was adequate time for relaxant effects of each concentration to reach a constant maximum and for peak inhibitory effects to be attained. Before addition of contractile substances, the initial tension exerted on each tissue was returned to its presulfonterol level. In preliminary experiments, this procedure did not alter the results and maximum inhibition of ovalbumin-induced contraction occurred with 1 μM sulfonterol.

Usually, one cumulative dose-response curve to a contractile substance was obtained on a single tissue. Dose-response curves on paired tissues treated identically were superimposable. Ovalbumin did not produce contraction in tissues from unsensitized animals.

In separate experiments, the lung parenchyma was dissected from major airways and blood vessels and minced finely with scissors. The minced lung tissue from each animal was divided into six to eight samples of 300–400 mg wet wt each and incubated in test tubes containing 5 ml of Krebs bicarbonate as previously described (9).

Ovalbumin-induced release of slow reacting substance of anaphylaxis (SRS-A)[1] and histamine was obtained by incubating each sample with a single concentration of antigen. One sample from each animal was used to determine the degree of spontaneous release of these substances. The amount of histamine released by samples exposed to ovalbumin was corrected by the spontaneous histamine release determined in each experiment. The spontaneous histamine release did not exceed 2% in any sample. SRS-A was not spontaneously released from any of the samples.

The histamine content in the supernate and pellet was determined, as previously described (9, 14). The SRS-A content in the supernate was assayed on the isolated guinea pig ileum by a modification of the procedure described by Chakravarty (15). SRS-A in the supernate was assayed in the presence of mepyramine, 1 μM, atropine, 1 μM, and sotalol, 0.1 mM to block histamine H_1, muscarinic, and beta-adrenergic receptors, respectively. 1 U of SRS-A was defined as that amount which caused a contraction with a peak height equal to that produced by 5 ng/ml of histamine-free base. The slowly developing contractions produced by the addition of aliquots of the samples to the 10-ml tissue bath were presumed to be due to SRS-A since the response was destroyed by prior incubation of the samples with arylsulfatase (16) and antagonized by prior incubation of the tissues with FPL55712, 0.1 μM (17).

The effects of sulfonterol on ovalbumin-induced release of SRS-A and histamine were determined by incubating each sample with a single concentration of the beta agonist for a fixed period of time before adding a submaximal concentration (1×10^{-2} mg/ml) of antigen. In each experiment, one sample served as control (without sulfonterol).

Analyses. 50% effective dose (ED_{50}) values for contractile and relaxant substances were obtained visually from a plot of log concentration vs. percent of the maximum response produced by each substance. This method of analysis was performed to determine the concentration of agonist required to produce a contraction or relaxation at the midpoint of its effect and hence provide a measure of the position of the dose-response curve along the log dose axis. The term "potency" refers to the ED_{50} value and the "log shift" refers to the degree of change of the ED_{50} value of ovalbumin produced by sulfonterol.

In experiments with minced lung, the effect of each concentration of ovalbumin was usually calculated as a percentage of the histamine release from the tissue. The amount of histamine present in the supernate was expressed as a percentage of the total histamine content in each sample (supernate plus pellet). The amount of SRS-A released to the supernate was measured in terms of units per gram of tissue. These values were then converted to a percentage of the maximum percentage of histamine and maximum amount of SRS-A released. The ED_{50} values for ovalbumin were deter-

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[1] *Abbreviations used in this paper*: RMK, rhesus monkey kidney cells; SRS-A, slow reacting substance of anaphylaxis.
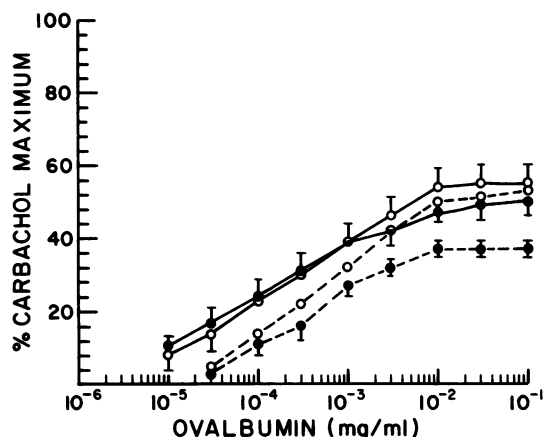
FIGURE 1 Log dose-response curves for ovalbumin in producing contraction of bronchial rings from actively sensitized guinea pigs. One main bronchus from each animal was treated with sulfonterol (---, 1 μM) and the other served as control (without sulfonterol) (——). Guinea pigs were inoculated with either parainfluenza-3 (O, eight paired experiments) or growth medium (●, eight paired experiments) before each experiment. Vertical lines indicate SEM. The data taken from these curves are summarized in Table I.

mined visually from a plot of percentage maximum release vs. log concentration. The effects of sulfonterol on ovalbumin-induced release of histamine and SRS-A were calculated as percentage inhibition of the release obtained in control samples. These values were converted to a percentage of the maximum inhibition of histamine and SRS-A release and the ED_{50} value for sulfonterol determined visually from a plot of percent maximum response vs. log concentration.

All ED_{50} values were converted to negative log values ($-\log ED_{50}$) and standard errors of the mean calculated for values obtained in each series of experiments. Differences between two means were determined using Student's paired or unpaired t test.

Infection of animals. Parainfluenza type 3 (P-3, HA-1 strain 47885, National Institutes of Health Research Reference Reagent) was maintained in rhesus monkey kidney cells (RMK) and suspensions were prepared by harvesting fluid and cell debris from infected cell cultures after 3–5 d of incubation at 33°C. The clarified suspension, obtained by centrifugation at 2,000 rpm for 30 min, contained 10^5–$10^{6.5}$ tissue culture infective dose$_{50}$/0.1 ml when determined in either RMK or Hela cells.

Guinea pigs, free of preexisting neutralizing serum antibody, were inoculated with 0.2 to 0.4 ml of virus suspension by nasal insufflation while under light ether anesthesia. Other animals (uninfected controls) were inoculated with fluids from virus-free RMK cultures that were prepared in the same manner as the virus suspensions. The inoculation procedure was performed twice in each guinea pig with 60 min intervening between treatment. Infected and uninfected animals were housed in separate rooms and killed on alternate days.

After extirpation of the trachea and bronchi for experiments as described above, the lungs were removed, placed in a sterile petri dish, and washed with 0.01 M phosphate-buffered saline (pH 7.2). Gross pathological features of each lung were noted and ~300 mg of parenchyma, with and without consolidation, were homogenized in Leibovitz L-15 medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) supplemented to 6% with agamma fetal bovine serum containing antibiotics (potassium penicillin G, 200 U/ml; streptomycin sulfate, 200 g/ml; gentamicin, 50 μg/ml; and amphotericin, 2 μg/ml). The virus was eluted from the lung by incubation in 2 ml of L-15 medium at 37°C for 1 h and the resulting suspension was clarified by centrifugation as described above. The amount of virus was determined after inoculation on Hela or RMK cultures and the isolates were identified as P-3 by neutralization using goat hyperimmune sera prepared with and tested against research reference strains of P-3 and parainfluenza type 1 (P-1, Sendai) virus obtained from the National Institutes of Health. All isolates were neutralized by P-3 sera and none by P-1 sera.

Only data obtained using virus-insufflated guinea pigs with established virus infection are reported here. Animals inoculated with virus growth medium were free of cultivatable virus.

In preliminary experiments, it was ascertained that the maximum degree of virus infection and changes in pharmacological effects, presumably resulting from the infection, occurred 4 d after nasal insufflation. Therefore, this time period was selected to explore further virus-induced changes.

Drugs and solutions. The following drugs were used: chicken egg albumin-Grade V (ovalbumin), histamine dihydrochloride, papaverine hydrochloride, atropine sulphate (Sigma Chemical Co., St. Louis, Mo.); carbamylcholine chloride (carbachol, Aldrich Chemical Co., Inc., Milwaukee, Wisc.); barium chloride (AR grade, Mallinkrodt Inc., St. Louis, Mo.); (±)-sulfonterol hydrochloride (Smith, Kline and French Laboratories, Philadelphia, Pa.); (±)-sotalol hydrochloride (Regis Chemical Co., Morton Grove, Ill.); mepyramine maleate (ICN K & K Laboratories, Inc., Plainview, N. Y.) and FPL55712 (Fisons Ltd., Loughborough, England). The sign (±) refers to the racemic mixture. All drug solutions were prepared in 0.9% sodium chloride on the day of each experiment.

RESULTS

Characteristics of virus infection. Even though P-3 was recovered from the lungs of all guinea pigs 4 d after virus infection, the animals were without symptoms. Virus recovered from the lungs ranged from 10^2 to $10^{3.5}$ tissue culture infective dose$_{50}$/0.1 g tissue. Only in lungs from virus-infected animals could areas of consolidation be observed.

Effects of sulfonterol on ovalbumin-induced contraction. Dose-response curves to ovalbumin in producing a contractile response of the bronchi from guinea pigs insufflated 4 d previously with growth medium or virus suspension are illustrated in Fig. 1. The responses to ovalbumin in the paired tissues treated with sulfonterol, 1 μM, are also shown in this figure. The data taken from these and similar curves obtained on the trachea are summarized in Table I.

Ovalbumin was 10- to 30-fold less potent in the bronchi than in the trachea, and virus infection did not change the sensitivity ($-\log ED_{50}$) to ovalbumin in either segment. Virus infection also did not significantly alter the cumulative maximum response or the response to 1 mg/ml of ovalbumin in either segment.

TABLE I

Effects of Sulfonterol on Ovalbumin-Induced Contractions of Trachea and Bronchi from
Guinea Pigs with and without Parainfluenza 3 Infection

Sulfonterol treatment*	Ovalbumin −Log ED50 with SEM		Log shift§ with SEM	P^||	Percent carbachol Maximum¶ with SEM		Treated Percent Control** with SEM	P^||	Percent carbachol Maximum†† with SEM		Treated Percent Control** with SEM	P^||	n§§
Segment*	Control	Treated‡			Control	Treated‡			Control	Treated‡			
				cumulative					1 mg/ml				
Trachea													
1 μM													
Medium	5.06±0.19	3.52±0.22	1.54±0.16	<0.001	74±4	50±8	66±8	<0.005	79±5	51±8	64±8	<0.005	8
P-3	5.09±0.20	3.73±0.17	1.36±0.13	<0.001	85±2	71±6	85±7	>0.05	89±3	73±7	83±8	>0.05	7
10 μM													
Medium	5.23±0.10	4.38±0.07	0.85±0.11	<0.001	63±4	42±5	64±6	<0.001	73±3	54±5	74±6	<0.005	13
P-3	5.34±0.13	4.17±0.17	1.18±0.15	<0.001	67±3	44±5	65±8	<0.005	79±3	62±5	80±7	<0.02	11
Bronchi													
1 μM													
Medium	3.97±0.28	3.31±0.21	0.65±0.22	<0.025	50±4	39±3	76±6	<0.005	62±3	42±3	69±6	<0.005	8
P-3	3.66±0.29	3.20±0.23	0.46±0.22	>0.05	55±5	51±4	97±7	>0.4	57±5	55±6	99±10	>0.95	8
0.1 mM													
Medium	3.37±0.16	2.82±0.12	0.55±0.19±	<0.01	65±4	52±5	84±12	>0.1	71±3	57±5	86±9	>0.05	7
P-3	3.39±0.07	2.91±0.12	0.48±0.10	<0.005	69±6	44±6	63±4	<0.001	72±5	58±7	80±8	<0.05	7

* Guinea pigs were inoculated with virus growth medium or parainfluenza 3 (P-3) and killed 4 d later.
‡ One tracheal spiral and bronchial ring from each animal was treated with sulfonterol and the other served as control tissue (without sulfonterol). Sulfonterol was in contact with the tissues for 15 min prior to adding ovalbumin.
§ Log shift = (control ovalbumin -log ED50) − (treated ovalbumin -log ED50).
|| Significance of control vs. treated values.
¶ Maximum cumulative response to ovalbumin expressed as a percentage of the maximum contraction to carbachol.
** Maximum contraction to ovalbumin in treated curve expressed as a percentage of the maximum contraction to ovalbumin in control curve.
†† Maximum response to 1 mg/ml ovalbumin expressed as a percentage of the maximum contraction to carbachol.
§§ Number of paired observations.

In both segments, sulfonterol shifted the dose-response curve to the right and depressed the maximum cumulative responses and responses to 1 mg/ml of ovalbumin. The magnitude of the effects of sulfonterol was smaller in the bronchi.

Infection with parainfluenza 3 decreased the ability of sulfonterol to inhibit ovalbumin-induced contractions in both airway segments. In neither the trachea nor the bronchi from infected animals did sulfonterol produce a statistically significant reduction of the cumulative maximum response or the response to 1 mg/ml of ovalbumin. Also, in the bronchi from infected animals, sulfonterol did not produce a statistically significant shift to the right of the ovalbumin dose-response curve.

The effects of increasing the concentration of sulfonterol are also summarized in Table I. With the larger concentrations, the inhibitory effects of virus infection on sulfonterol-induced changes in the responses to ovalbumin were not observed. In both segments from infected animals, the larger concentrations of sulfonterol produced statistically significant shifts of the dose-response curves to the right and reductions of the cumulative maximum responses and responses to 1 mg/ml of ovalbumin. Furthermore, the magnitude of the changes produced by sulfonterol was similar in tissues from uninfected and infected animals.

Effects of sulfonterol on histamine-induced contraction. Dose-response effects of histamine in producing a contractile response of the trachea and bronchi from animals not insufflated with growth medium or virus suspension are summarized in Table II.

Sulfonterol did not alter the position of the histamine dose-response curve along the log dose axis or the maximum response to histamine in either segment (Table II).

Histamine- and carbachol-induced contraction. Effects of histamine and carbachol obtained on paired bronchi from animals insufflated with growth medium or virus suspension 4 d previously are shown in Table III.

Histamine and carbachol were two $(P < 0.001)$ and fourfold $(P < 0.01)$ less potent, respectively, in the bronchi than in the trachea. Also, the maximum contractile response to histamine and the total grams of tension developed were larger in the bronchi $(P < 0.005)$. Virus infection did not alter the dose-response effects of histamine or carbachol in either segment.

Sulfonterol- and papaverine-induced relaxation. Effects of sulfonterol and papaverine obtained on paired trachea and bronchi from animals insufflated with growth medium or virus suspension 4 d previously are illustrated in Fig. 2. The data taken from these and similar curves obtained in the trachea are summarized in Table IV.

TABLE II

**Effects of Sulfonterol, 0.1 mM, on Histamine-induced Contractions
of Guinea Pig Trachea and Bronchi**

Segment*	Histamine			Percent carbachol			
	−Log molar ED50 with SEM			Maximum[‖] with SEM			
	Control	Treated‡	P§	Control	Treated‡	P§	n¶
Trachea	5.25±0.11	5.08±0.07	>0.05	72±5	68±3	>0.3	8
Bronchi	5.02±0.06	5.03±0.10	>0.9	96±2	92±2	>0.1	7

* Tissues were taken from guinea pigs that were not insufflated with virus or growth medium.
‡ One tracheal spiral and bronchial ring from each animal was treated with sulfonterol and the other served as control tissue (without sulfonterol). Sulfonterol was in contact with the tissues for 15 min prior to adding histamine.
§ Significance of control vs. treated values.
‖ Maximum cumulative response to histamine expressed as a percentage of the maximum contraction to carbachol.
¶ Number of paired observations.

In the tissues from uninfected animals, sulfonterol was equipotent and produced the same maximum degree of relaxation relative to that produced by papaverine in the trachea and bronchi. In both segments, sulfonterol was found to be a partial agonist. Papaverine was slightly less potent ($P < 0.05$) and produced a smaller total relaxation ($P < 0.001$) in the bronchi. In the trachea and bronchi, virus infection did not alter the potency or maximum response of sulfonterol. Although virus infection did not alter the potency of papaverine in either segment, the total degree of relaxation to papaverine in the bronchi was larger in the infected animals ($P < 0.001$).

Ovalbumin-induced release of SRS-A and histamine.

In tissues from infected and uninfected animals, maximum release of both SRS-A and histamine occurred 15 min after addition of submaximal concentrations of ovalbumin and this time period was chosen to obtain the antigen dose-response curves. Infection with parainfluenza 3 did not alter the sensitivity or maximum response to ovalbumin for release of either substance (Table V).

Inhibition of SRS-A and histamine release. In tissues from infected and uninfected animals, maximum inhibition of release of both SRS-A and histamine by sulfonterol occurred using a preincubation time of 15 min before antigen challenge and this time period was chosen to obtain sulfonterol dose-response curves.

TABLE III

**Effects of Histamine and Carbachol on Trachea and Bronchi from Guinea Pigs
with and without Parainfluenza 3 Infection**

Treatment* Segment	Histamine		Carbachol		Maximum tension§ with SEM	n[‖]
	−Log molar ED50 with SEM	Percent maximum‡ with SEM	−Log molar ED50 with SEM	Percent maximum‡ with SEM		
Trachea						
Medium	5.49±0.05	76±2	6.99±0.04	100	2.3±0.2	6
P-3	5.52±0.08	79±3	7.04±0.04	100	2.3±0.3	7
Bronchi						
Medium	4.97±0.07	83±1	6.64±0.09	93±2	3.6±0.4	6
P-3	5.02±0.10	85±2	6.44±0.14	95±2	2.9±0.3	6

* Guinea pigs were inoculated with virus growth medium or parainfluenza 3 (P-3) and killed 4 d later.
‡ Maximum cumulative response to histamine or carbachol expressed as a percentage of the maximum contraction to barium chloride, 10 mM.
§ Maximum grams of tension developed in response to barium chloride, 10 mM, in both paired tissues.
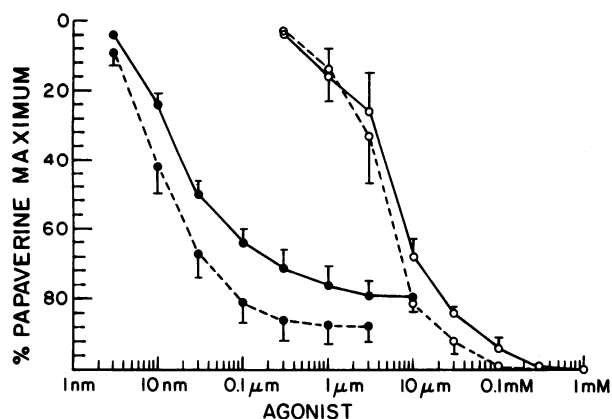‖ Number of paired observations.

FIGURE 2 Log dose-response curves for sulfonterol (●) and papaverine (○) in producing relaxation of bronchial rings from actively sensitized guinea pigs. Tone was induced in each bronchus by carbachol, 0.1 μM. One main bronchus from each animal was used to obtain the sulfonterol effects and the other to obtain the papaverine effects. Guinea pigs were inoculated with either parainfluenza-3 (---, 6) or growth medium (——, 6) 4 d prior to each experiment. Vertical lines indicate SEM. The data taken from these curves are summarized in Table IV.

Virus infection did not alter the sensitivity or maximum response to sulfonterol for inhibition of the release of either substance (Table VI).

DISCUSSION

Infection of guinea pigs with a respiratory virus, parainfluenza 3, resulted in diminution of the in vitro antianaphylactic effects in airway smooth muscle of a beta adrenergic receptor agonist, sulfonterol. In this regard, the data are consistent with previous observations using human granulocytes (5, 6) that respiratory viruses are capable of altering beta adrenergic responses. The present study has extended the virus-induced effect to include changes in physiological tissue responses of direct relevance to the state of allergic asthma.

The data demonstrate that virus infection results in a selective alteration of beta adrenergic responses mediating an inhibition of antigen-induced smooth muscle effects. The selectivity of the virus effect was found to be exerted in two directions. Virus infection blocked the ability of sulfonterol to inhibit ovalbumin-induced smooth muscle contraction (a) without causing a simultaneous change in the ability of sulfonterol to directly relax the precontracted airway segments and (b) without altering the ability of sulfonterol to inhibit the ovalbumin-induced release of histamine and SRS-A from minced lung.

In isolated airway smooth muscle, it is possible, using the techniques described here to examine at least two distinct populations of beta adrenergic receptors (18). Traditional studies on the abilities of beta agonists (e.g., isoproterenol) to relax isolated airway smooth muscle in a state of constant inherent or induced tone measure effects elicited via activation of beta receptors on the smooth muscle cell. However, isoproterenol (and sulfonterol) also blocks ovalbumin contractions at concentrations that do not functionally antagonize contractile responses to agonists that act

TABLE IV
Effects of Sulfonterol and Papaverine on Trachea and Bronchi from Guinea
Pigs with and without Parainfluenza 3 Infection

Treatment*	Sulfonterol		Papaverine		
	−Log molar ED50 with SEM	Percent maximum‡ with SEM	−Log molar ED50 with SEM	Maximum§ with SEM	n‖
Segment					
				g	
Trachea					
Medium	7.88±0.13	71±7	5.85±0.07	2.8±0.2	8
P-3	7.73±0.09	65±5	5.79±0.07	2.6±0.2	7
Bronchi					
Medium	7.74±0.03	79±4	5.45±0.16	1.3±0.1	6
P-3	7.93±0.09	88±5	5.58±0.17	2.2±0.1	6

* Guinea pigs were inoculated with virus growth medium or parainfluenza 3 (P-3) and killed 4 d later.
‡ Maximum cumulative response to sulfonterol expressed as a percentage of the maximum relaxation produced by papaverine, 1 mM.
§ Maximum grams of relaxation in response to papaverine, 1 mM, in both paired tissues.
‖ Number of paired observations.

TABLE V

Effects of Ovalbumin on Release of SRS-A and
Histamine from Guinea Pig Lung

	SRS-A		Histamine		
Treatment*	−Log ED50 with SEM	Maximum release‡ with SEM	−Log ED50 with SEM	Maximum release§ with SEM	n‖
Medium	3.76±0.13	45±10	3.25±0.18	32±3	7
P-3	3.97±0.15	56±11	3.04±0.17	32±4	8

* Guinea pigs were inoculated with virus growth medium or parainfluenza 3 (P-3) and killed 4 d later.
‡ Maximum release expressed as units per gram of tissue.
§ Maximum release expressed as percentage of total histamine content.
‖ Number of observations.

directly on the smooth muscle (18). These latter studies have been suggested to measure effects elicited via activation of beta receptors which modulate the release of anaphylactic mediators and which are presumed to be located on mast cells or other nonsmooth muscle cell types (18). The observations that virus infection antagonized sulfonterol-induced inhibition of ovalbumin-induced smooth muscle contraction, but did not alter the sulfonterol-induced relaxation of the precontracted smooth muscle would suggest that the beta response to inhibit mediator release is selectively affected by the virus.

The use of the minced lung preparation has allowed a means of examining, in a more direct manner, the effect of virus infection and beta agonists on mediator release. The observation that virus infection did not alter the ability of sulfonterol to inhibit the release of SRS-A and histamine creates a paradoxical situation because these mediators have been suggested to be primary mediators in the smooth muscle contractile

response to antigen (19, 20). How can parainfluenza 3 infection alter the ability of a beta agonist to inhibit antigen-induced smooth muscle contraction and not change the ability of the agonist to inhibit antigen-induced release of SRS-A and histamine? There are several possibilities that remain to be explored. (a) A mediator(s) other than SRS-A and histamine may also be involved in eliciting smooth muscle contraction and the ability of beta agonists to modulate the release of such a substance(s) could be selectively altered by the virus. Indirect evidence for contribution by another mediator(s) is provided by observations that inhibition of histamine release and smooth muscle contraction by isoproterenol are not strictly correlated (18) and inhibition of antigen-induced smooth muscle contraction by histamine H_1 and SRS-A receptor antagonists is not theoretically compatible with exclusive roles for these two mediators (20). (b) Properties of the mast cells or other cell types from which mediators are released may differ between upper and lower airways. Evidence for a heterogeneity in mast cells has been reviewed by Kaliner (21). Therefore, our data on mediator release from the lung parenchyma may not be readily extrapolated to events occurring in trachea and bronchi. The small amount of tissue available in the trachea and bronchi has precluded the use of these tissues to study mediator release by the present experimental design. (c), There may be two different types of beta response modulating mediator release in the guinea pig lung (9) and only one of these may be altered by virus. On the basis of relative potencies of different beta agonists, it has been suggested that the beta receptors that mediate a reduction in maximum antigen-induced histamine release have properties that differ from those that mediate a shift to the right of the antigen dose-response curve (9). The experimental design used in the present study has examined the former type of beta response to inhibit mediator

TABLE VI

Effects of Sulfonterol on Ovalbumin-induced Release of
SRS-A and Histamine from Guinea Pig Lung

	SRS-A		Histamine		
Treatment*	−Log molar ED50 with SEM	Maximum inhibition‡ with SEM	−Log molar ED50 with SEM	Maximum inhibition‡ with SEM	n§
Medium	7.53±0.16	50±5	7.99±0.13	29±4	9
P-3	7.64±0.19	52±6	7.80±0.13	24±4	10

* Guinea pigs were inoculated with virus growth medium or parainfluenza 3 (P-3) and killed 4 d later.
‡ Maximum inhibition expressed as a percentage inhibition of the release obtained in samples without sulfonterol.
§ Number of paired observations.

release and larger amounts of tissue will be required to examine the influence of virus on the latter beta response.

In addition to a selective effect on beta responses modulating antigen-induced smooth muscle contraction, virus infection resulted in an increase in the maximum degree of relaxation that could be elicited by papaverine in the bronchial rings. Because the dose-response effects of carbachol, used to induce tone in these experiments, were not altered by virus, it would appear that virus treatment results in a larger degree of inherent tone in the bronchi. This could also be a means by which respiratory virus infection results in altered pulmonary function.

The mechanism of the change in beta response by virus infection is not known, but appears to be of a competitive nature. This is demonstrated by the fact that increasing the concentrations of sulfonterol above 1 μM reversed the virus-induced changes in both airway smooth muscle segments. This occurred without a concomitant increase in the effect of sulfonterol in tissues from uninfected animals, demonstrating that a maximally effective concentration of sulfonterol to inhibit ovalbumin-induced contractions is 1 μM. Because sulfonterol is a partial agonist relative to isoproterenol, its maximum effects are produced when $[RA]/[R_t] = 1$, where $[RA]$ is the concentration of agonist-receptor complexes and $[R_t]$ is the total concentration of available receptors. Therefore, noncompetitive antagonism (e.g., resulting from a decrease in $[R_t]$) would have been observed as an inability to reverse the virus effect upon increasing the concentration of agonist. The observation that sulfonterol produced smaller maximum changes in the ovalbumin-induced effects in the bronchi suggests that there is a smaller $[R_t]$ in the more peripheral segment.

Even though it is tempting to suggest that parainfluenza 3 infection inhibits the beta response in guinea pig airways by altering the beta adrenergic receptors, other mechanisms could also be responsible. It is unlikely that diminution of the effect of sulfonterol by virus infection is related to changes in adrenergic neuronal density or alpha adrenergic receptors since sulfonterol neither releases endogenous norepinephrine nor activates directly the alpha receptors. Furthermore, it is possible to exclude changes in neuronal uptake, catechol-O-methyl-transferase, and monoamine oxidase since sulfonterol is not a substrate for these processes. However, an effect of virus infection to enhance extraneuronal amine uptake would lead to observations similar to those made in these experiments and a possible effect on this process needs to be examined.

Virus infection did not alter the sensitivities or the maximum degrees of smooth muscle contraction to ovalbumin, histamine, or carbachol in either airway segment. Also, neither the sensitivity nor the maximum response to ovalbumin for releasing SRS-A and histamine from minced lung were altered by virus infection. Ida et al. (7) reported that incubation with different respiratory viruses resulted in a larger release of histamine from human basophils. Since contraction of the airway smooth muscle by ovalbumin is presumably due to the release of some mediator(s), the inability of virus infection to alter contraction to the antigen indicates that there is no alteration of the antigen-antibody interaction or the subsequent events leading to release in the guinea pig airways. This was also confirmed by the observation that virus did not alter the ability of ovalbumin to evoke SRS-A and histamine release from minced lung. Furthermore, virus infection did not produce an alteration of the histamine (H_1) and muscarinic receptors or the events subsequent to activation of these receptors on the smooth muscle. Observations that isolated airway smooth muscles from the guinea pig have normal responses to these various contractile substances lend support to the postulate that increased sensitivity to the pulmonary effects of histamine in virus infected humans is due to sensitization of rapidly adapting vagal sensory fibers (3). Therefore, part of the refractoriness to beta agonists in patients with virus-induced asthma could also result from a greater degree of functional antagonism at the level of the smooth muscle (10).

It is interesting to note that ovalbumin was at least 12 times less potent in the bronchi than in the trachea. This large difference in potency was not observed for histamine and carbachol between the two segments. Therefore, the difference may be related to the mediator release process and could result from dissimilar antigen-antibody interactions or distribution or characteristics of immunological elements (e.g., mast cells). Since the number of mast cells has been shown to increase toward the periphery in the dog (22) and monkey (23) lung, the guinea pig could represent a species difference in pulmonary mast cell distribution. Alternatively, the contractile response of the guinea pig airway smooth muscle to antigen may also involve other cell types from which mediators can be released (18, 24). Regardless of the mechanism, the potency of ovalbumin in contracting the bronchi was more similar to its potency in evoking a release of SRS-A and histamine from guinea pig lung.

Observations that quantitative changes in the pharmacological effects of a beta agonist occur in isolated airways from guinea pigs infected with a respiratory virus suggest that this model has some similarities with virus-associated effects in humans and may be useful to explore further mechanisms of virus-induced asthma.

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