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Research Article

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Sympathetic Modulation of Biochemical and Physiological Response to Immune Degranulation in Canine Bronchial Airways In Vivo

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Abstract

The effect of sympathetic stimulation on bronchial smooth muscle contractile response after mast cell degranulation with Ascaris suum antigen was studied in 36 natively allergic dogs in situ. Bronchial smooth muscle response was measured isometrically in a single right middle lobe bronchus. A dose of antigen causing maximal release of mediator was administered to the bronchus through the bronchial arterial circulation. Serial plasma histamine concentrations were determined at 15-s intervals after intra-arterial (i.a.) administration of antigen. Samples of blood were obtained simultaneously from right heart and femoral artery, and arteriovenous difference (AVd) in histamine concentration across the bronchus was determined during mast cell degranulation. In nine dogs showing bronchial mast cell degranulation to antigen challenge, bronchial smooth muscle contraction was 22.3±2.95 g and the mean AVd in histamine concentration across the bronchus was 188±41.5 ng/ml. Six other dogs having muscarinic blockade with 0.75–1.0 mg/kg intravenous atropine were given i.a. antigen after 1 min of steady-state sympathetic stimulation with intravenous 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). Sympathetic stimulation during Ascaris suum antigen challenge caused complete inhibition of bronchial smooth muscle contractile response to i.a. antigen ($P < 0.001$), and a significant AVd in histamine concentration across the bronchus (9.8±16.0 ng/ml; $P < 0.01$ vs. control) was not detected. Peak plasma histamine concentration in control dogs was 1,138±237 ng/ml vs. 310±135 ng/ml in animals receiving sympathetic stimulation ($P < 0.01$). In four dogs undergoing systemic anaphylaxis to i.v. antigen, subsequent sympathetic stimulation with i.v. DMPP reduced bronchomotor tone to ~70% of base-line control. Exogenously induced sympathetic stimulation can substantially inhibit systemic mast cell degranulation to Ascaris suum antigen in allergic dogs. Maximal stimulation of the sympathetic nervous system causes substantial inhibition of respiratory mast cell secretion of histamine and bronchial smooth muscle contraction to circulating mediator.

Introduction

The sympathetic nervous system plays a potentially important role in the regulation of respiratory mast cell secretion (1–3) and bronchomotor response (4–7). Orange and co-workers (1) have shown that beta-adrenergic stimulation inhibited degranulation of mast cells in passively sensitized human lung strips. Brown et al. (4) have shown that beta-adrenergic stimulation of canine tracheal smooth muscle in situ partially or completely inhibited mast cell degranulation. However, mast cell degranulation was achieved by a nonimmune mechanism employing Compound 48/80 in these studies (4); immune degranulation could not be achieved in tracheal airways in situ. Barnett and co-workers (8) showed inhibition of mast cell degranulation to Ascaris suum antigen in peripheral airways by intravenous (i.v.) administration of isoproterenol in natively allergic dogs. However, in all prior studies, the pharmacological doses of isoproterenol required were substantial, and the potential for physiological inhibition of mast cell degranulation could not be assessed. Leff and co-workers (6, 7) have shown that neural sympathetic stimulation of tracheal smooth muscle in situ antagonized respiratory smooth muscle contraction caused by intra-arterially (i.a.)[1] administered histamine. These studies used exogenous histamine, and inhibition of smooth muscle contraction caused by the immune degranulation process could not be assessed.

A potential limitation to prior in situ studies of mast cell or respiratory smooth muscle response in central airways has been the use of trachea as a representative resistance airway (4–7). Recent reports (9, 10) have shown that the density of innervation and postsynaptic respiratory receptors in trachea differ substantially from the bronchial airways in which asthmatic bronchoconstriction occurs. Furthermore, the density of respiratory mast cells increases substantially from the trachea to the peripheral airways (11). Inhibition of tracheal smooth muscle response to mast cell degranulation, therefore, might not reflect the response in bronchial airways, which have a greater density of respiratory mast cells (11) and H_1-receptors (12).

The objective of this study was to determine the potential

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1. *Abbreviations used in this paper:* AA, Ascaris suum antigen; ACh, acetylcholine chloride; AF, active force; AVd, arteriovenous difference across the bronchus; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; [H], histamine concentration; i.a., intra-arterial; MAP, mean arterial blood pressure; Paw, lateral airway pressure; PtP, transpulmonary pressure.

inhibitory effects of physiological sympathetic stimulation on (*a*) respiratory and systemic mast cell secretion of mediator, and (*b*) respiratory smooth muscle response during immune degranulation in the bronchial airways of a living animal. To assess the importance of endogenous sympathetic stimulation in these systems, we used a method for selective perfusion and measurement of the smooth muscle contractile response of a single third order canine bronchus in situ (5). This preparation permits sequential, simultaneous analysis of the physiological and biochemical events of mast cell degranulation in a relevant resistance bronchus of the lung. In this study, we evaluated the effect of steady-state sympathetic stimulation on mast cell secretion of histamine and canine bronchial smooth muscle response in situ. We show for the first time that physiological sympathetic reserve is sufficient to substantially inhibit the consequences of mast cell degranulation in a major resistance airway.

Methods

Preparation of animals. 36 healthy mongrel dogs of either sex, weighing 13–30 kg, were anesthetized with 1.5–2.5 g alpha-chloralose (Aldrich Chemical Co., Milwaukee, WI) and 7.5–12.5 g ethyl carbamate (Aldrich) i.v. Additional anesthesia was administered every 90–120 min as required. Immediately after anesthesia, a low cervical tracheotomy was performed, and a #9 cuffed endotracheal tube was inserted to a position just above the carina. Dogs were ventilated with pure oxygen using a volume ventilator (Model 613; Harvard Apparatus Co., Inc., S. Natick, MA) set at a tidal volume equal to 15–20 ml/kg body weight. Ventilatory rate was adjusted to maintain P_{CO_2} between 35 and 45 torr and pH between 7.35 and 7.45. Supplemental sodium bicarbonate was given as needed to compensate for metabolic acidosis caused by chloralose anesthesia. Arterial blood gas measurements were made with an automated blood gas analyzer (Model 175; Corning Glass Works, Corning, NY). Samples of arterial blood were analyzed before each experimental manuever. Arterial P_{O_2} remained >400 torr throughout the experimental period.

Drugs for i.v. administration were injected through a catheter inserted into the inferior vena cava through the femoral vein. For determination of arteriovenous difference (AVd) in histamine concentration across the bronchus (see below), a catheter was placed in the femoral artery (Fig. 1); a catheter also was placed at the pulmonary outflow tract of the right heart through an incision into the right ventricle and secured by a purse-string suture. Another catheter was inserted through the right internal jugular vein or caudal vena cava to a position just proximal to the azygos inflow to the superior vena cava (Fig. 1).

Bronchial smooth muscle response was measured in a 1-cm segment of right middle lobe bronchus. Details of this preparation have been described previously (5). Thoracotomy was performed into the right fifth intercostal space. The common bronchoesophageal artery was located and cannulated with PE50 (0.58 mm internal diam) tubing. The left bronchoesophageal artery, branches to the upper and lower lobes, and any muscular branches identified, were ligated, so that i.a. injection selectively perfused the bronchus (Fig. 1) (5). After cannulation, the bronchial segment was fixed for isometric measurement of bronchial smooth muscle response (5). The 1-cm bronchial segment was tethered between sutures attached on one side to a parallel bar and on the other to a force displacement transducer (Model FT.03; Grass Instrument Co., Quincy, MA) mounted on a rack and pinion (Fig. 1). The sutures were oriented at 180°, and bronchial resting force was preset to 35–40 g. This corresponded to a transverse length of 0.8 cm at which active force (AF) has been shown to be >95% of the maximum achievable response. Bronchial smooth muscle contractile response was assessed by subtracting the force achieved after administration of agonist or antigen from the initial resting force to give AF. Active

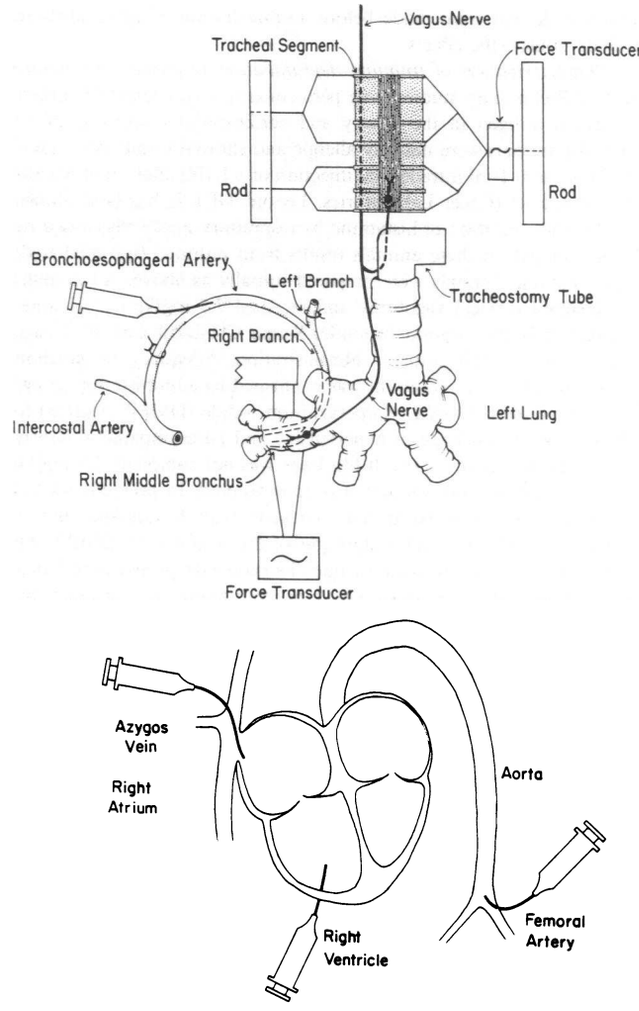

Figure 1. Schema of preparation. *Top,* third order bronchus is fixed isometrically; bronchoesophageal artery catheter permits selective delivery of antigen to bronchial airway. Isometrically fixed tracheal segment serves as control airway. *Bottom,* sites for withdrawal of blood samples during mast cell degranulation.

bronchial smooth muscle tension was thus expressed as grams force (g).

To assess the specificity of bronchial response in these studies, the response of a 2.6–3.8-cm segment of trachea was used as a control airway (5). The tracheal segment was fixed for isometric measurement in a manner similar to the bronchus (4, 6). Tracheal smooth muscle response was assessed as for bronchus. Active force was expressed as grams force and was normalized by dividing by the axial length of the tracheal segment, so that tracheal active force also was expressed for a 1-cm segment of tracheal airway (grams per centimeter).

Physiological measurements were monitored continuously on a polygraph (Model 7D; Grass Instrument Co.). Arterial blood pressure was measured through a 1.67-mm internal diameter catheter (PE 240) inserted into the abdominal aorta through the remaining femoral artery. Lateral airway pressure (Paw) was measured using a PM 5E±0.7 differential air pressure transducer (Gould, Inc., Santa Clara, CA) that was referenced to atmosphere. All animals had bilateral thoracotomy so that Paw was equal to transpulmonary pressure (PtP). PtP was maintained at 5 cm H_2O after thoracotomy to avoid atelectasis.

All drugs administered by i.a. injection into the bronchial circulation were dissolved in 37°C Krebs-Henseleit solution. Sham injections of

drug-free diluent were made before administration of all agonists to exclude nonspecific effects.

Characterization of immune degranulation response: preliminary studies. Preliminary studies were performed to characterize the effects of Ascaris antigen in the airway and cardiovascular response of 12 dogs. All animals were natively allergic and showed immediate hypersensitivity to 0.1-ml intradermal injection of ≤1:100 dilution of Ascaris antigen extract (Greer Laboratories, Lenoir, NC). It has been shown (13–15) that secretion of histamine by respiratory mast cells caused by Ascaris antigen in these animals results from immune (IgE-mediated) degranulation. Animals were prepared initially as above. All animals had bilateral cervical vagotomy and received 7.5 mg/kg i.v. hexamethonium (hexamethonium bromide; Sigma Chemical Co., St. Louis, MO) 45 min before antigen administration. Adequacy of ganglion blockade with hexamethonium was confirmed by administering 25 μg/kg i.v. 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Aldrich) to establish >95% blockade of hypertensive and parasympathetic airway contractile responses (7, 16). If blockade was not complete, 2.5 mg/kg i.v. additional hexamethonium was administered. In previous studies (6, 7), we have established that blockade with hexamethonium is complete for >90 min after abolition of the response to DMPP. All animals also received beta-adrenergic blockade with propranolol 2 mg/kg i.v. bolus plus 20 μg/kg per min continuous i.v. infusion (8, 16, 17).

Adequacy of bronchial perfusion through the arterial catheter was assessed by comparing the response obtained in bronchus and trachea to i.v. injection of 10^{-5} mol acetylcholine (ACh) (acetylcholine chloride; Sigma) with the response obtained to 10^{-7}–10^{-5} mol ACh administered i.a. into the bronchial circulation 7 min later (5). I.a. injection was considered to be selective (*a*) if bronchial contraction to i.a. ACh exceeded the response resulting from i.v. ACh, (*b*) if onset of bronchial contraction after i.a. injection preceded tracheal contraction by >7 s, and (*c*) if bronchial AF was >14 g (5, 10, 13). This preliminary assessment of the adequacy of the preparation was performed initially in all control and sympathetically stimulated animals (see below). These data also were compared with the response obtained after i.a. and i.v. Ascaris suum antigen extract (see below).

In six separate dogs, the dose of Ascaris antigen extract causing maximal bronchial contraction was assessed. 45 min after i.a. ACh, sham injection of Krebs-Henseleit diluent was administered i.a. into the bronchial circulation. After showing no response to the diluent in which the antigen was dissolved, 3 ml of Ascaris antigen extract was injected i.a. into the bronchial artery at 90-s intervals in increasing concentrations. An initial 3-ml dose of 1:100 was followed by 1:30 and 1:10 dilutions. A second 3-ml injection of 1:10 dilution of Ascaris antigen extract was given 90 s later as the final dose.

Samples of blood were obtained simultaneously for histamine assay from the right atrial orifice, right ventricle, and femoral artery (Fig. 1) 15 s before antigen, and 15, 30, 45, and 60 s after i.a. injection, and were assayed for histamine concentration [H]. The AVd in plasma [H] across the airway during bronchial mast cell degranulation was determined by subtracting the femoral artery [H] from right atrial [H] 45–90 s after antigen injection (see Results). The details and validation of this technique have been described in previous publications (4, 9, 13). Although the AVd in plasma [H] does not measure the absolute amount of histamine secreted from the bronchial airway, these preliminary studies have shown a direct correlation between the magnitude of the AVd and airway contraction. The physiological and biochemical responses from these studies were used to determine a single dose of antigen causing maximal release of mediator from the bronchus from i.a. antigen injection. This concentration was used in subsequent studies (see below).

To further assess the sequence and magnitude of airway and cardiovascular effects of systemic analphylaxis caused by Ascaris antigen, Ascaris antigen extract was administered i.v. in seven additional dogs. Animals were prepared identically as above, except that placebo injection was administered i.v. through the femoral venous catheter in 5.0 ml isotonic saline buffered to pH 7.4, and animals were not pretreated with either hexamethonium or propranolol. Samples were collected for assay of plasma [H] as above, 20 s before, and 20, 40, 60, 80, 100, 160, and 180 s after placebo injection. An i.v. injection of 5.0 ml Ascaris antigen extract (1:10 dilution) was administered 30 s after the final blood sample was obtained during sham injection, and blood samples for plasma [H] were obtained at the same intervals.

Immune challenge during sympathetic blockade. 14 animals that showed dermal sensitivity to ≤1:100 dilution of Ascaris antigen extract were prepared as in the preliminary studies where antigen was administered i.a. into the bronchial circulation (above). All animals had bilateral vagotomy, ganglion-blockade with hexamethonium, and beta-adrenergic blockade with propranolol as above. Plasma histamine samples for calculation of the AVd in [H] across the bronchus were obtained 15 s before placebo injection and 60 s after injection. ~90 s after sham injection, a dose of Ascaris antigen extract shown to cause maximal physiological and mast cell degranulation effects from preliminary studies (3.0 ml of 1:10 dilution; see Methods [above] and Results) was administered i.a. into the bronchial artery, and 5.0-ml blood samples from right atrial orifice, right ventricle, and femoral artery were obtained 15 s before and 15, 30, 45, 60, 120, and 180 s after i.a. injection for assay of plasma [H]. The AVd in plasma [H] across the bronchus was calculated as above (preliminary studies) 45–180 s after antigen injection.

Immune challenge during sympathetic stimulation. Six animals showing dermal reactivity to Ascaris antigen extract (≤1:100 dilution) were studied after vagotomy and muscarinic blockade with 0.75–1.0 mg/kg i.v. atropine. Adequacy of muscarinic blockade was confirmed by demonstrating complete attenuation of the bronchial contraction generated by 10^{-8}–10^{-6} mol i.a. ACh. This dose of atropine also has been shown to cause complete blockade of the parasympathetic stimulatory response to i.v. DMPP (7, 10). Because dogs possess no nonadrenergic inhibitory innervation to airways, autonomic stimulation with DMPP under these circumstances causes selective activation of the sympathetic nervous system (5, 6). In prior studies we have determined the specificity and time course effects of DMPP. These data indicate that DMPP does not alter the response to exogenously administered agonists, and the sympathetic modulation of the airway contractile response is restored to base line in <30 min (6, 7).

Sympathetic stimulation in these studies was accomplished by continuous i.v. infusion of 312 μg/kg per min DMPP (6). Sympathetic stimulation was regarded to be at steady-state when a stable plateau increase in arterial blood pressure was demonstrated (6). 1 min after arterial blood pressure reached plateau, a sham infusion of 3 ml Krebs-Henseleit solution was injected i.a. into the bronchial artery, followed 30 s later by 3 ml Ascaris antigen extract (1:10 dilution). Control specimens were obtained 15 s before DMPP infusion and 15 s i.a. before i.a. injection of antigen. Blood samples for plasma histamine analysis also were obtained 15, 30, 45, 60, 120, and 180 s after injection of antigen into the bronchial circulation from right atrial orifice, right ventricle, and femoral arterial catheters. Infusion of DMPP was terminated 240 s after administration of antigen, and animals were allowed to stabilize for ~90 min. For each animal, endogenous histamine responsiveness then was verified; dose-response curves were generated with 10^{-9}–10^{-5} mol i.a. histamine injected into the bronchial artery. These data were compared to bronchial response to histamine generated in four separate animals not receiving either DMPP or prior challenge with antigen or histamine.

At the end of all experiments, animals were sacrificed and adequacy of perfusion to the right middle lobe bronchus was established by postmortem injection of India ink. Autopsy also was performed to establish adequacy of cannula placement for histamine sampling.

Sympathetic stimulation after immune challenge. In four of the dogs receiving 5 ml (1:10 dilution) i.v. Ascaris antigen extract (see preliminary studies above), the effect of sympathetic stimulation in reversing airway contractile and cardiovascular hypotensive responses after anaphylaxis was studied. These animals were the four dogs from the initial group of six that showed the greatest bronchial contraction after i.v. antigen challenge (see Results). 1 min after stable maximal

increase in bronchial and tracheal contraction and maximal decrease in mean arterial blood pressure (MAP) was observed, 312 μg/kg per min i.v. DMPP was infused for 120 s. The degree of sympathetic reversal of the effects of anaphylaxis after antigen challenge was assessed by comparison to the initial prechallenge control values for airway and blood pressure response.

Plasma histamine assay. 5-ml samples of blood for histamine assay were drawn in disposable syringes coated with heparin. These were transferred immediately after each experiment and centrifugated at 4°C (at 1,300 *g*) for 10 min. The plasma supernatant for each sample was immediately frozen at −4°C. Samples were coded and assayed in single blind manner, so that the nature of each experiment was not known during assay. Histamine assay was performed by enzymatic isotope assay according to the methods of Beaven et al. (18) and Snyder et al. (19). We previously reported the details and validation techniques for this assay (4, 9) for respiratory histamine analysis. For the present studies, the sensitivity of the assay was determined from control samples containing known concentrations of histamine. Samples containing 0.55 ng/ml (5 pmol/ml) were statistically discernible from blank control samples ($P < 0.01$). Plasma samples were assayed in dilutions ranging from 1.0 to 55.0 ng/ml. The coefficients of variation determined from control samples over these ranges were 0.179 (1 ng/ml), 0.157 (11.0 ng/ml), and 0.140 (55 ng/ml).

Statistical analysis. All values are expressed as mean±standard error for each group. Comparisons were made by unpaired two-tailed *t* test (20). Comparisons of probabilities of degranulation were made by Fisher's Exact Test (20). Statistical significance was claimed whenever $P < 0.05$.

Results

Verification of the preparation and preliminary studies. Adequacy of perfusion to the bronchus was corroborated by i.a. injection of ACh into the bronchial artery cannula. Bronchial contraction to 10^{-5} mol i.a. ACh was 22.6±1.59 g and preceded tracheal contraction of 25.0±1.93 g/cm by 9.14±0.4 s.[2] Intravenous 10^{-5} mol ACh caused simultaneous contraction of both trachea and bronchus in all dogs. For all dogs studied, selective perfusion of the third order segmental bronchus and its two daughter bronchi was shown by postmortem injection of India ink. Postmortem examination also revealed proper placement of femoral artery, right atrial orifice, and right ventricle cannulae for every dog used in these experiments. Adequacy of muscarinic blockade before autonomic stimulation with DMPP was corroborated by demonstration of complete blockade of the response to i.a. ACh.

The effect of increasing doses of Ascaris antigen extract on bronchial contractile response and mast cell degranulation was determined from four of six dogs demonstrating regional and systemic anaphylaxis during cumulative administration of Ascaris antigen i.a. Fig. 2 shows that bronchial contraction occurred for concentrations of antigen as low as 1:100 dilution and that maximal physiological response, peak AVd in [H], and peak plasma [H] occurred with 3 ml 1:10 dilution of Ascaris antigen. A second challenge with the highest (1:10) dilution did not cause a statistically significant increase in AVd in [H], peak plasma [H], or bronchial active force (Fig. 2). In subsequent studies, this dose (3 ml i.a. of 1:10 dilution) was considered to be the single dose of antigen extract approximating the maximal physiological and mast cell degranulating

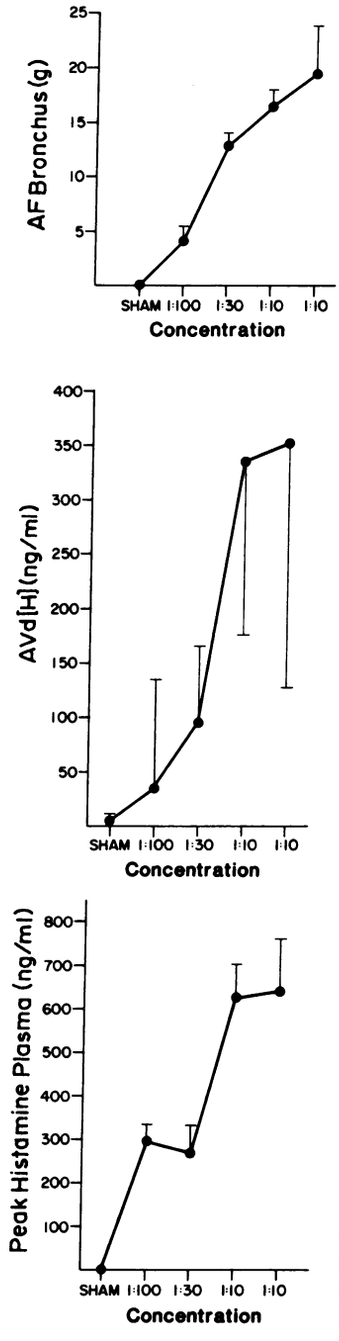

Figure 2. Response to increasing cumulative doses of Ascaris suum antigen (AA) administered i.a. in 3 ml bolus injection. Dose is given as concentration of purified extract. Both the AVd in [H] across the bronchus and the eventual peak plasma histamine concentration are elicited with 3 ml 1:10 dilution of i.a. AA. The slight increase in bronchial AF after a repeat 3-ml dose of 1:10 dilution of AA was not statistically significant. Points on concentration-response curve are mean±SE.

effects in the bronchus. For subsequent studies, where precise timing of the responses to immune degranulation was required, this dose (1:10 dilution) was used.

In two animals, no response was obtained to initial challenge with the highest concentration of i.a. Ascaris antigen (1:10) (Table I). In these animals, there was no change in MAP and no bronchial or tracheal contraction; circulating plasma histamine was not detectable in either dog (Table I). These data contrast with the data obtained from the other weak/nonreactive bronchial responses, for which weak bronchial contraction was obtained simultaneously with tracheal contraction (see below; and Discussion).

I.v. administration of 5.0 ml Ascaris antigen extract (1:10 dilution) caused simultaneous contraction of both tracheal and

2. Although tracheal and bronchial contraction are approximately equal, the force of bronchial contraction is ~70% maximal (5); force of tracheal contraction is only about 25% maximal (4, 5, 10). This is due to the substantially greater muscle mass of the trachea.

Table I. Effect of 3 ml (1:10 Dilution) Ascaris Antigen Administered into the Bronchial Circulation of 14 Consecutive Dogs with Cutaneous Hypersensitivity

Response	Animal no.	AF (Bronchus)	Onset bronchial contraction	AF (trachea)	[H] right heart	[H] femoral artery	Initial MAP	MAP after antigen (Nadir)	Maximal plasma [H]
		g	*s*	*g/cm*			*mmHg*	*mmHg*	
Weak/nonreactive bronchial	1	0	NA	NA	0	0	132	132	0
	3	8.6	50	1.6	44	128	88	16	1,333
	6	6.2	90	NA	116	339	80	32	383
	9	0	NA	NA	0	0	80	80	0
	10	4.2	75	7.8	55	7	92	40	163
Mean±SE		3.80±1.69	NA	NA	43.0±21.3	94.8±65.3	94.4±9.62	60.0±20.7	376±248
Reactive bronchial	2	15.8	40	4.6	915	279	80	16	1,042
	4	34.0	30	36	1,015	164	100	20	1,816
	5	24.0	30	18	435	380	100	20	586
	7	18.6	20	10.4	258	146	88	24	485
	8	16.0	80	4.8	233	214	110	24	1,071
	11	15.4	20	7.2	234	145	80	40	473
	12	39.0	20	3.8	670	7	140	32	700
	13	23.8	25	5.1	811	386	100	30	1,495
	14	14.3	30	11.4	1,045	201	100	45	2,577
Mean±SE		22.3±2.95	32.8±6.30	11.26±3.44	624±113	214±40.2	99.8±6.06	27.9±3.24	1,138±237

NA, no assessment; immune degranulation did not occur.

bronchial airways (Fig. 3). This contrasted with the response obtained with i.a. injection of Ascaris antigen where bronchial contraction preceded the tracheal contractile response by 19.2±4.6 s (see below; and Fig. 3). An AVd in [H] was not demonstrated in animals receiving antigen i.v. (mean AVd = 21.3±135 ng/ml; $P > 0.50$ vs. placebo control injection; not different from zero response). In later studies, where antigen was administered directly into the bronchial artery, a specific bronchial response was considered to have occurred only if bronchial contraction was >10 g, preceded tracheal contraction by >9 s, and was accompanied by a mean AVd in [H] across the bronchus >20 ng/ml. Where tracheal and bronchial contraction occurred simultaneously and no AVd in [H] was obtained, bronchial contraction was considered to have resulted predominantly or exclusively from degranulation of nonrespiratory mast cells after antigen reached the systemic circulation (see Discussion).

Immune challenge during sympathetic blockade. Selective bronchial degranulation was shown in 9 of 14 dogs receiving a single i.a. injection of 1:10 dilution Ascaris antigen extract. In these animals antigen injection caused 22.3±2.95 g bronchial contraction. Onset of bronchial contraction occurred 32.8±6.30 s after i.a. injection of Ascaris antigen (Fig. 4). Tracheal contractile force in the same nine animals was 11.3±3.44

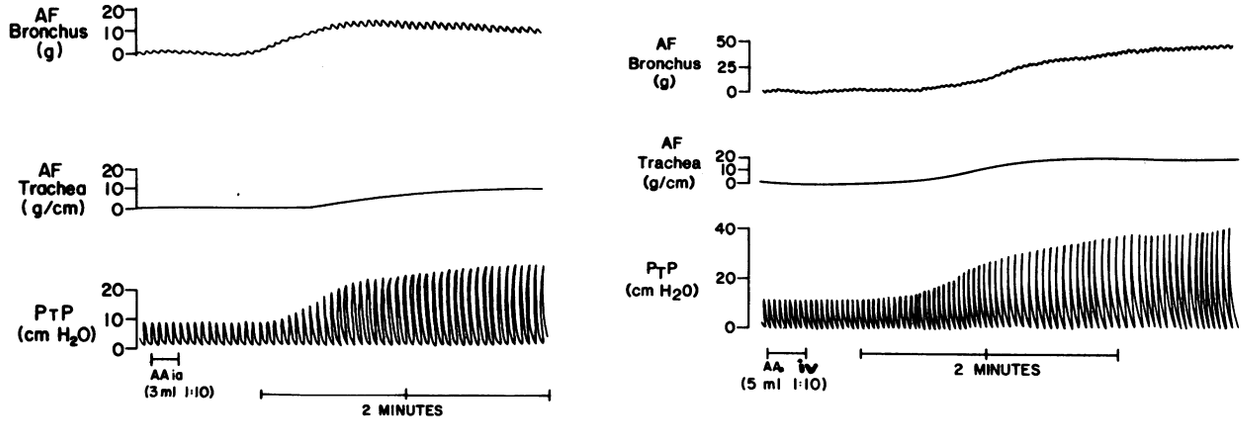

Figure 3. Response to Ascaris suum antigen (AA) administered i.a. (*left*) and i.v. (*right*): representative tracings. When administered i.a., bronchial contraction precedes tracheal contraction. When administered i.v., airways contract simultaneously. All points are mean±SE.

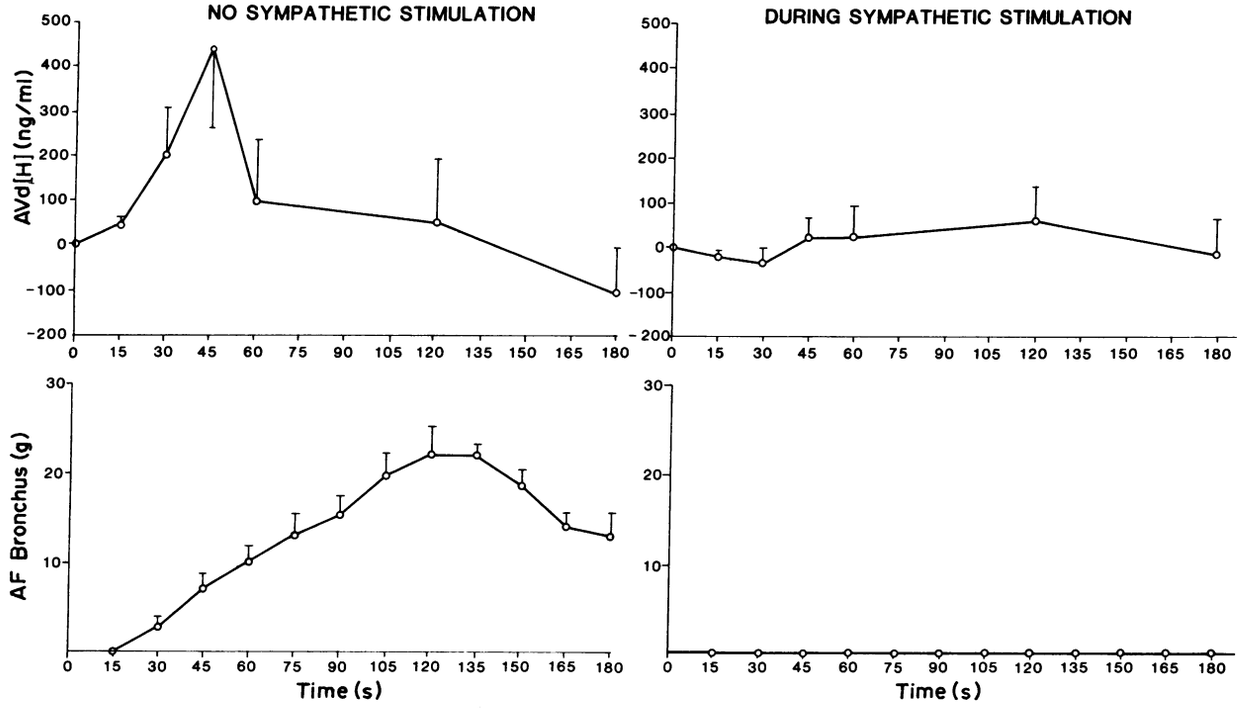

Figure 4. Time course of the arteriovenous difference in histamine concentration (AVd [H]) across the lung and active bronchial force (AF) for nine animals. Data are mean and standard errors at each time point. Onset of AVd occurred 30–45 s after antigen injection and persisted for ~1 min. Note that maximal AF occurs after peak gradient corresponding to the highest absolute histamine concentration (see text). Sympathetic stimulation (*right*) abolishes the AVd in [H] across the bronchus. There is no corresponding bronchial contraction during sympathetic stimulation. All points are mean±SE.

g/cm and occurred 57.5±6.41 s after antigen injection ($P < 0.001$ vs. bronchus). A circulatory wave of histamine corresponding to onset of bronchial contraction was shown sequentially in right atrial orifice, right ventricle, and lastly, femoral artery. An AVd in plasma [H] across the bronchus was demonstrated corresponding to onset of bronchial contraction in these same dogs (Fig. 4, Table I).

Five animals (see above) demonstrated either no response to i.a. antigen ($n = 2$) or delayed onset of bronchial contraction that occurred simultaneously with tracheal contraction ($n = 3$). These animals were considered to be nonresponders and were excluded from the data pool (Table I). For nine animals responding to i.a. injection of antigen, MAP decreased by 76.5±6.75 mmHg after injection. The peak plasma [H] was 1,138±237 ng/ml. A mean AVd in [H] across the bronchus of 188±41.5 ng/ml was observed for 66.7±16.5 s after i.a. administration of antigen into the bronchial circulation (Table II).

Immune challenge during sympathetic stimulation. Intravenous infusion of DMPP caused an increase in mean systolic blood pressure from 132±4.3 to ≥220 mmHg in all animals. A stable plateau was achieved in all dogs within 1 min of starting the infusion. A significant AVd in [H] across the bronchus was not detected 60 s after sham injection in the sympathetically stimulated animals or in animals receiving autonomic blockade (above) (Table II). Injection of antigen extract i.a. 1 min after onset of systolic blood pressure plateau caused no change in bronchial or tracheal smooth muscle tone for the duration of sympathetic stimulation (Table II). For these six dogs, mean AVd in [H] across the bronchus was 9.8±16 ng/ml ($P < 0.002$ vs. nonsympathetically stimulated controls). Peak plasma [H] was 310±135 ng/ml ($P < 0.01$ vs. control) (Table II). The MAP corresponding to the peak plasma [H] was 94.0±19.3 mmHg ($P < 0.001$ vs. sympathetic stimulation; $P > 0.20$ vs. initial control MAP). 2 min after termination of DMPP infusion, MAP decreased to 45.3±11.1 mmHg ($P < 0.001$ vs. initial control).

All animals had a normal response to exogenous histamine

Table II. Effect of Sympathetic Stimulation of Bronchial Response to Antigen Challenge

	n	Onset of response	Bronchial contraction	AVd [H] after sham	AVd [H] after AA	Peak [H]
		s	*g*	*ng/ml*	*ng/ml*	*ng/ml*
Symp. block	9	32.8±6.30	22.3±2.95	0.67±9.54	188±41.5	1,138±237
Symp. stim.	6	—	0.0±0.0*	5.88±4.70	9.8±16.0‡	310±135§

* $P < 0.001$ vs. symp. block. ‡ $P < 0.002$ vs. symp. block. § $P < 0.01$ vs. symp. block. Symp. block = dogs not receiving sympathetic stimulation; Symp. stim. = dogs receiving Ascaris antigen 1 min after sympathetic stimulation. AA, Ascaris suum antigen 1:10.

administered at the end of each experiment. I.a. injection of 10^{-5} mol histamine caused 11.5±2.3 g contraction vs. 13.5±3.2 g in four animals not receiving sympathetic stimulation ($P > 0.50$).

Sympathetic stimulation after immune challenge. The ability of maximal sympathetic stimulation to reverse the cardiovascular and respiratory effects of anaphylaxis caused by i.v. administration of 5.0 ml i.a. Ascaris antigen extract was studied in the four dogs showing the greatest response to immune challenge (Fig. 5). Prechallenge MAP was 86.5±4.0 mmHg. During anaphylaxis, MAP decreased to 25.0±2.4 mmHg ($P < 0.001$) after i.v. bolus infusion of antigen; this corresponded to an increase in plasma [H] of 1,045±261 ng/ml ($P < 0.001$). Bronchial contraction (47.0±5.8 g) and tracheal contraction (35.1±11.2 g/cm) occurred simultaneously with the decrease in MAP. I.v. infusion of 312 μg/kg per min i.v. DMPP caused increase in MAP to 66±8.2% of control value before antigen challenge ($P < 0.001$). Bronchial AF decreased by 67±12% to 15.7±5.8 g ($P < 0.001$) and tracheal AF by 83.5±8.5% to 5.8±3.0 g/cm ($P < 0.001$). These values were sustained for the 2 min of DMPP infusion.

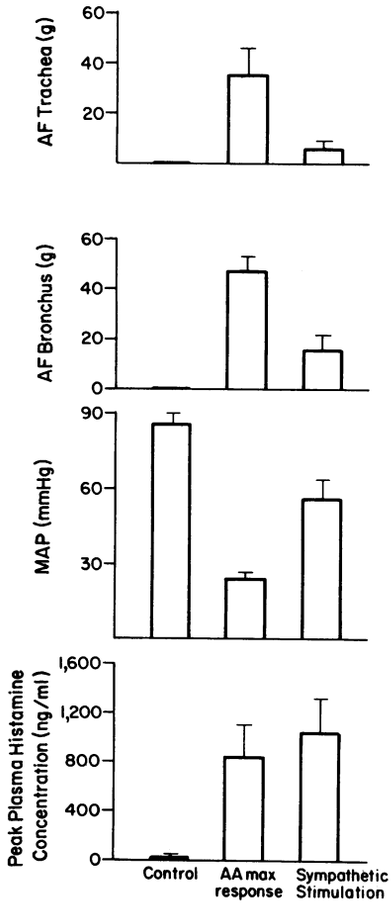

Figure 5. Reversal of airway contraction induced during anaphylaxis resulting from intravenous administration of 5 ml Ascaris suum antigen (AA). Note that both tracheal and bronchial tone are restored to near base-line levels, as is mean arterial blood pressure (MAP), while high plasma histamine concentrations persist. AF, active force of airway contraction. All points are mean±SE.

Discussion

This report is the first demonstration that the in situ reserve of the sympathetic nervous system is sufficient to potentially inhibit the consequences of immune degranulation in the bronchial airways of dogs. The effects on antigenic challenge in bronchial airways and during systemic anaphylaxis were studied during and after maximal exogenously induced sympathetic stimulation. These data show (*a*) that substantial but not complete inhibition of antigen-induced degranulation of mast cells and basophiles can be elicited during sympathetic stimulation, (*b*) abolition of the arteriovenous difference in [H] across the bronchus during maximal sympathetic stimulation, implying even greater inhibition of the airway (vs. systemic) degranulation response, and (*c*) complete inhibition of the airway contractile response to regionally secreted and circulating mediator during sympathetic stimulation. Note that these data assess the potential physiological reserve of sympathetic nervous system, and do not define the circumstances under which a homeostatic bronchodilator response is elicited. These data also do not define the extent to which the sympathetic secretory reserve can be recruited during immune challenge.

Also consider the implications of these findings in terms of the model employed. Antigen challenge was administered to natively allergic mongrel dogs. These animals have been shown previously to have no intrinsic airway hyperreactivity (14, 21). It also is presumed that histamine secretion caused by Ascaris antigen in these studies resulted from immune (IgE-mediated) degranulation. A linear relationship between concentrations of Ascaris antigen (1:100 to 1:10) and force of bronchial contraction was obtained (Fig. 2). Although it is not possible to exclude nonspecific degranulation of respiratory mast cells at the highest concentration of antigen, the onset of immune degranulation occurred substantially later (~30 s) and magnitude of histamine secretion was about twofold that which we have observed during nonimmune degranulation using this identical model (13). In two animals showing no physiological response to Ascaris antigen, no respiratory circulating histamine was detected (Table I).

Some canine strains do show nonspecific airway hyperreactivity (21). It also is possible to induce immune sensitization in dogs to specific antigen. However, the objective of this study was to determine the potential regulatory mechanisms modulating bronchoconstriction in atopic dogs that were not overtly asthmatic (21). Therefore, in this canine model, we chose to exclude canine strains with either endogenous airway hyperreactivity (21) or nonenvironmentally acquired allergy.

Because natively allergic dogs do not always manifest respiratory immune hypersensitivity (6, 7, 13–15), it was essential to establish criteria for assessing immune degranulation after antigen challenge. Studies were performed to show that the dose of antigen administered i.a. caused (*a*) maximal regional secretion of mediator (histamine) and (*b*) maximal physiological response in the airway (isometric bronchial contraction). This was the dose of antigen used in antigen challenge studies (Fig. 2). Because antigen was administered i.a. directly into the circulation of a major resistance bronchus, precise timing of the events of immune degranulation was possible (Tables I and II, Figs. 3 and 4) (13). By showing contraction in the bronchus that preceded contraction in a control (tracheal) airway in the same animal (Fig. 3), it was possible to distinguish

dogs having specific bronchial reactivity to i.a. administration of antigen. These results differed substantially from the response obtained with i.v. administration of antigen (Fig. 3), where antigen presumably was distributed simultaneously to tracheal and bronchial airways through the systemic circulation after intravenous injection. By demonstrating contraction of the bronchus that preceded contraction in a control (tracheal) airway (Figs. 3 and 5; Tables I and II), it was possible to distinguish dogs manifesting respiratory reactivity. Where equivalent quantities of agonist are administered, tracheal contraction always substantially exceeds bronchial contraction due to the greater muscle mass in the trachea (5). In these studies, i.a. injection of antigen caused substantially greater contraction in bronchus relative to the trachea (Fig. 3, Table I). An arteriovenous difference in [H] across the bronchus also was shown, and bronchial contraction preceded tracheal contraction in all animals reacting to i.a. injection of Ascaris antigen. In contrast, animals considered to be nonreactive showed none of these characteristics (Table I) (13). Use of the selective bronchial preparation in this study thus permitted: (*a*) precise timing of the response essential for assessing specificity of mast cell degranulation, (*b*) measurement of the response of a specific resistance bronchus analogous to that involved in asthmatic bronchoconstriction, and (*c*) estimation of the magnitude of the mast cell response in this (bronchial) airway during antigen challenge.

In dogs demonstrating regional airway reactivity to i.a. antigen extract, contraction of the control (tracheal) airway was observed (Fig. 3, Table I). Tracheal contraction, which had an onset equal to approximately one circulation time (Tables I and II), resulted either from washout of mediator from the bronchial circulation or from recirculation of antigen extract to the tracheal airway. It is less likely, however, that recirculating antigen caused degranulation of respiratory mast cells and/or basophiles in canine trachea. In preliminary studies, we and others (4, 9) were unable to show histamine secretion in the trachea in natively allergic dogs when intra-arterial Ascaris antigen extract was injected directly into the tracheal arterial circulation. However, completely selective mast cell degranulation (right middle lobe bronchus only) is not an essential requirement of this study, since endogenous sympathetic stimulation caused substantial inhibition (Table II) or substantial reversal of respiratory smooth muscle contraction (Fig. 5) for the duration of sympathetic stimulation.

Although this model does not permit specific assessment of the late events of immune degranulation, there are several potential advantages to the use of the preparation in these studies. By administering antigen i.a. directly into a third order bronchus, we were able to assess directly the early events of asthmatic bronchoconstriction in a major resistance bronchus of the lung, an airway that is predominant in the bronchoconstrictor response. This is especially important, since it recently has been shown that the physiological (5) and pharmacological (10) properties of bronchial smooth muscle differ substantially from tracheal smooth muscle or from smooth muscle in peripheral airways (12). The technique (rapid i.a. injection) used in this study also permitted precise timing of the events of immune degranulation, which is not possible with aerosol inhalation of antigen (14, 15, 22). I.a. administration of antigen directly into the bronchial airway permitted precise correlation between the physiological response and the events of immune degranulation. In these studies, immune degranulation was correlated to regional concentration in plasma histamine. This does not exclude the importance of other mediators of immune degranulation, e.g., slow reacting substance. However, preliminary studies indicate a minor role of leukotrienes in the early events of immune degranulation in natively allergic mongrel dogs (13).

To determine the potential role of the sympathetic nervous system in preventing immune degranulation in normal (non-hyperreactive [21]) natively allergic dogs, we used a dose of antigen that caused maximal regional bronchial contraction (Fig. 2) and a level of sympathetic stimulation shown in previous studies (6, 7) to approximate 100% of the sympathetic reserve. Infusion of DMPP permitted steady-state stimulation of all sympathetic ganglia (6, 7) and hence simultaneous stimulation of both adrenal secretion and sympathetic nervous innervation of the airways. This was essential, since we have shown previously that adrenal secretion is the predominant mechanism by which the sympathetic nervous system antagonized bronchoconstrictor stimuli (7). Studies have shown that pharmacological beta-adrenergic stimulation may prevent the effects of immune degranulation in vitro (3) and nonimmune degranulation in vivo (4). This has led to the theoretical assumption that sufficient beta-adrenergic stimulation might inhibit the airway smooth muscle contractile response to mediator (23) or the secretion of mediator (24) from respiratory mast cells. It has been assumed, however, that the degree of sympathetic stimulation required to prevent mast cell degranulation from immune challenge could not be accomplished physiologically, since the level of stimulation required to inhibit mast cell degranulation was approximately tenfold greater than that required to prevent bronchial smooth muscle contraction to secreted mediator (25).

In this report, we show for the first time that sufficient physiological reserve exists to prevent both respiratory mast cell secretion of histamine and respiratory smooth muscle contraction to secreted mediator. All animals receiving 1–2 min of sympathetic stimulation before administration of antigen showed subsequent reactivity to histamine. The dose of antigen administered caused maximal secretion of histamine from bronchial airways (Fig. 2). None showed bronchial smooth muscle contraction to antigen during sympathetic stimulation. The absence of an AVd in histamine (9.8±16.0 ng/ml) during sympathetic stimulation vs. control animals ([188±41.5 ng/ml]; $P < 0.002$) indicates that histamine secretion from the bronchus was substantially inhibited during the period of sympathetic stimulation. However, a substantial circulating concentration of histamine was demonstrated even during sympathetic stimulation (310±135 ng/ml vs. >1,100 ng/ml in nonsympathetically stimulated controls [Table 1]), indicating that sympathetic stimulation did not prevent entirely the degranulation of extrarespiratory mast cells. In nonsympathetically stimulated animals, systemic concentration of histamine < 300 ng/ml causes airway smooth muscle contraction (4, 13). Therefore, it is concluded that sympathetic stimulation caused inhibition of bronchial smooth muscle contraction as well as substantial inhibition of mediator secretion during immune challenge with Ascaris antigen. While the degree of sympathetic stimulation caused by DMPP may exceed the true physiological maximum, the level of antigenic stimulation also was extreme (anaphylaxis).

In additional studies, maximal sympathetic stimulation also substantially reversed airway smooth muscle contraction

after systemic anaphylaxis induced by intravenous administration of Ascaris antigen (Fig. 5). These studies also were performed under conditions of maximal sympathetic stimulation and maximal antigenic degranulation. These data show the potential of the sympathetic nervous system to reverse substantially the effects of antigenic stimulation on airway smooth muscle response. However, these data do not define the homeostatic role of the sympathetic nervous system in regulating bronchomotor tone. During anaphylaxis, the spontaneous sympathetic response shown in these studies was not enough to restore completely arterial blood pressure or bronchomotor tone to base-line prechallenge levels (Tables I and II). These data indicate that, although substantial sympathetic reserve exists to inhibit both mediator secretion and bronchomotor response, the extent to which this reserve is used and the physiological factors evoking sympathetic activation during antigen challenge remain incompletely defined.

We conclude that prior in vitro studies demonstrating inhibition of the mast cell degranulating response after beta-adrenergic stimulation are of potential physiological importance in vivo. Our data show that sufficient sympathetic reserve exists in atopic dogs to substantially inhibit immune degranulation of bronchial mast cells. The precise physiological homeostatic role of the sympathetic nervous system in regulating mediator secretion and bronchomotor response remains to be defined.

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References

1. Orange, R. P., W. G. Austen, and K. F. Austen. 1971. Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5' adenosine monophosphate. *J. Exp. Med.* 134:136s–148s.

2. Kaliner, M., R. P. Orange, and K. F. Austen. 1972. Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. IV. Enhancement by cholinergic and alpha-adrenergic stimulation. *J. Exp. Med.* 136:556–567.

3. Kaliner, M. 1977. Human lung tissue and anaphylaxis. I. The role of cyclic GMP as a modulator of the immunologically induced secretory process. *J. Allergy Clin. Immunol.* 60:204–211.

4. Brown, J. K., A. R. Leff, M. J. Frey, B. R. Reed, S. C. Lazarus, R. Shields, and W. M. Gold. 1982. Characterization of tracheal mast cell reactions in vivo: inhibition by a beta-adrenergic agonist. *Am. Rev. Respir. Dis.* 126:842–848.

5. Hendrix, S. G., N. M. Munoz, and A. R. Leff. 1983. Pharmacological and physiological response of canine bronchial smooth muscle in situ. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 54:215–224.

6. Leff, A. R., N. M. Munoz, and S. G. Hendrix. 1982. Sympathetic inhibition of histamine-induced contraction of canine trachealis in vivo. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 53:21–29.

7. Leff, A. R., and N. M. Munoz. 1981. Selective autonomic stimulation of canine trachealis with dimethylphenylpiperazinium. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 51:428–437.

8. Barnett, D. B., S. E. Chesrown, A. F. Zbinden, M. Nisam, B. R. Reed, H. R. Bourne, K. L. Melmon, and W. M. Gold. 1978. Cyclic AMP and cyclic GMP in peripheral lung: regulation in vivo. *Am. Rev. Respir. Dis.* 118:723–733.

9. Leff, A. R., J. K. Brown, M. Frey, B. Reed, and W. M. Gold. 1983. Biochemical and physiological effects of compound 48/80 on canine trachea in vivo. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 54:720–729.

10. Leff, A. R., N. M. Munoz, and S. G. Hendrix. 1983. Comparative distribution of smooth muscle postsynaptic contractile responses in canine trachea and bronchus in vivo. *J. Pharmacol. Exp. Ther.* 224: 259–264.

11. Guerezon, G. M., P. D. Pare, M.-C. Michoud, and J. C. Hogg. 1979. The number and distribution of mast cells in monkey lung. *Am. Rev. Respir. Dis.* 119:59–66.

12. Drazen, J. M., and M. W. Schneider. 1977. Comparative response of tracheal spiral and parechymal strips to histamine and carbachol. *J. Clin. Invest.* 61:1441–1447.

13. Garrity, E. R., N. P. Stimler, N. M. Munoz, R. Fried, and A. R. Leff. 1983. Response of bronchial smooth muscle to mast cell degranulation in situ. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 55:1803–1810.

14. Chiesa, A., D. Dain, G. L. Meyers, G.-F. Kessler, and W. M. Gold. 1975. Histamine release during antigen inhalation in experimental asthma in dogs. *Am. Rev. Respir. Dis.* 111:148–156.

15. Nisam, M. R., A. Zbinden, S. Chesrown, D. Barnett, and W. M. Gold. 1978. Distribution and pharmacological release of histamine in canine lung in vivo. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 44:455–463.

16. Leff, A. R., and N. M. Munoz. 1981. Evidence for two subtypes of alpha-adrenergic receptors in canine airways. *J. Pharmacol. Exp. Ther.* 270:530–535.

17. Evans, G. H., A. S. Nies, and D. G. Shand. 1973. The disposition of propranolol III. Decreased half-life and volume of distribution in man, monkey, dog, and rat. *J. Pharmacol. Exp. Ther.* 186:114–121.

18. Beaven, M. A., S. Jacobsen, and Z. Horakova. 1972. Modification of the enzymatic isotope assay of histamine and its application to measurement of histamine in tissues, serum, and urine. *Clin. Chim. Acta.* 37:91–103.

19. Snyder, S. H., R. J. Baldessarini, and J. Axelrod. 1966. A sensitive and specific enzymatic isotopic assay for tissue histamine. *J. Pharmacol. Exp. Ther.* 13:544–549.

20. Zar, J. H. 1974. Biostatistical Analysis. Prentice Hall, Inc., Englewood Cliffs, NJ. 105–107, 518–542.

21. Hirshman, C. A., H. Downes, D. A. Leon, and J. A. Peters. 1981. Basenji-greyhound dog model of asthma; pulmonary responses after β-adrenergic blockade. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 51:1423–1427.

22. Gold, W. M., G. L. Meyers, D. S. Dain, R. L. Miller, and H. R. Bourne. 1977. Changes in airway mast cells and histamine caused by antigen aerosol in allergic dogs. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 43:271–275.

23. Drazen, J. M. 1978. Adrenergic influences of histamine-mediated bronchoconstriction in the guinea pig. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 44:340–345.

24. Sullivan, T. J., P. K. Parker, S. A. Einsen, and C. W. Parker. 1975. Modulation of cyclic AMP in purified rat mast cells. II. Studies on the relationship between intracellular cyclic AMP concentrations. *J. Immunol.* 114:1480–1485.

25. Lazarus, S. C., S. E. Chesrown, M. J. Frey, B. R. Reed, T. O. Mjorndahl, and W. M. Gold. 1979. Experimental canine anaphylaxis: cyclic nucleotides, histamine, and lung function. *J. Appl. Physiol.* 46: 919–926.