

Effect of beta-adrenergic stimulation on experimental canine anaphylaxis in vivo

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We studied beta-adrenergic modulation of antigen-induced release of histamine and changes in lung function in natively allergic, anesthetized dogs. After thoracotomy and bilateral cervical vagotomy, samples of peripheral lung frozen in situ were obtained for measurement of adenosine cyclic 3',5'-monophosphate (cAMP) and histamine. In 11 dogs with beta-adrenergic blockade (propranolol), subsequent Ascaris suum antigen (intravenous) decreased lung tissue histamine $21.5\% \pm 5.3\%$ (mean $\pm$ SE), with marked physiologic changes of anaphylaxis. In six other dogs, with no pharmacologic treatment before antigen, A. suum decreased lung tissue histamine only $11.5\% \pm 1.9\%$. In six other dogs, terbutaline (1 mg/kg, i.v.) increased concentration of cAMP in peripheral lung, indicating stimulation of beta-adrenergic receptors, and inhibited response to A. suum. When a beta-adrenergic agonist was given 15 sec prior to A. suum, cAMP doubled and inhibition was incomplete. When a beta-agonist was given 150 sec prior to A. suum, cAMP increased fivefold and inhibition was complete. (J ALLERGY CLIN IMMUNOL 71:62, 1983.)

In 1936, Schild demonstrated that epinephrine inhibited antigen-induced release of histamine from sensitized guinea pig lung in vitro.^{1, 2} Subsequent studies in vitro have suggested that not only histamine but other mediators of anaphylaxis are also inhibited by adrenergic agonists and that this inhibition is mediated by cAMP.³ The physiologic interpretation of

these studies is complicated, however, by the fact that many anatomic and neurohumoral relationships are disrupted in these in vitro preparations. Our study was designed to examine the effect of beta-adrenergic stimulation on antigen-induced release of lung tissue histamine and changes in lung function in natively allergic dogs in vivo.

For these experiments we used mongrel dogs with positive cutaneous reactivity to *Ascaris suum* antigen. We measured the release of histamine to reflect immunologically mediated degranulation of mast cells. We measured static lung compliance and airflow resistance to reflect the response of airway smooth muscle to the mediators of anaphylaxis released from mast cells. In additional experiments, we measured cAMP levels in peripheral lung as an index of the degree of beta-adrenergic stimulation.^{4, 5} Beta-adrenergic stimulation was produced by administration of terbutaline, a beta-adrenergic receptor agonist with affinity predominantly for beta₂ receptors.⁶

MATERIALS AND METHODS

Drugs and chemicals

Propranolol HCl was obtained from Sigma Chemical Co., St. Louis, Mo. Terbutaline sulfate (Bricanyl) was kindly supplied by Dr. T. Tencek, Astra Pharmaceutical Products, Framingham, Mass. Evan's blue was obtained from J. T.

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Supported in part by U.S.P.H.S. Pulmonary SCOR Grant HL-19156 and Program Project Grant HL-24136. Dr. Mjörndal was the recipient of a Merck International Fellowship in Clinical Pharmacology. Dr. Chesrown was supported by a California Heart Association Fellowship and the Parker B. Francis Foundation. Dr. Lazarus was the recipient of NIH Pulmonary Young Investigator Award HL-19604.

Received for publication Feb. 24, 1982.

Accepted for publication Aug. 9, 1982.

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This work was presented in part at the American Society of Clinical Investigation in San Francisco, Calif., April 1978.

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Abbreviations used

- cAMP: Adenosine cyclic 3',5'-monophosphate
cGMP: Guanosine cyclic 3',5'-monophosphate

Baker Chemical Co., Phillipsburg, N.J. All other chemicals used were of the highest chemical grade commercially available.

Antigens

A. suum antigen was prepared as follows: *A. suum* worms were collected fresh from slaughterhouse hogs and stored on ice. All subsequent procedures were performed at 4°C. The worms were washed thoroughly in distilled water, immersed in a 0.1M phosphate buffer, pH 7.4, and homogenized with a Waring blender. The homogenate was centrifuged at $100 \times g$ for 15 min, the surface lipid layer was removed, and the aqueous supernatants were recentrifuged for 30 min at $50,000 \times g$. These supernatants were then filtered, and the protein concentration in the resulting aqueous extract was estimated according to the method of Lowry et al.⁷ and adjusted to 5 mg of protein/ml. The antigen was stored in 50 ml aliquots at -70°C until used.

Bovine serum albumin, used as a nonspecific control protein for skin testing, was obtained as a 5% sterile solution, pH 7, from Sigma Chemical Co.

Animals

Mongrel dogs weighing 21 to 30 kg were selected randomly from the University of California, San Francisco, vivarium and observed to be free of upper respiratory disease. None of these animals was immunized with a specific antigen, but all had positive cutaneous reactivity to a dilution of 1:1000 or higher of *A. suum* antigen and negative cutaneous reactivity to bovine serum albumin.⁸ On this basis, we assume these dogs had a naturally occurring allergy to common canine parasites (*Toxocara canis*), which cross-reacted with *A. suum* antigen and which was mediated by a reaginic antibody as described previously.⁸

Experimental procedure

The experimental procedure (following the National Research Council's guide for care and use of laboratory animals), including the surgical preparation of the animals, the method of obtaining lung tissue samples, the purification and assay of cyclic nucleotides, and the method for the assay of histamine, has been described previously.⁹

In brief, dogs were anesthetized with chloralose (50 to 100 mg/kg, i.v.) and urethane (250 to 500 mg/kg, i.v.), placed in the supine position, and mechanically ventilated with pure oxygen through a cuffed endotracheal tube, using a constant volume respirator (Harvard Apparatus Co.). They were paralyzed with gallamine triethiodide (2 mg/kg intermittently, i.v.). The expiratory port of the respirator was connected to an underwater seal to maintain transpulmonary pressure at 5 cm H₂O at end-expiration. Respiratory frequency and tidal volume were set to maintain the CO₂

concentration at the end of expiration between 4.5% and 5.5%. All animals had bilateral cervical vagotomy to block possible vagally mediated reflex effects.

A sternotomy and a transverse thoracotomy bilaterally at the level of the fifth intercostal space were performed to sample lung tissue. Lung samples were obtained from within 3 cm of the edge of the lobe with clamps cooled in liquid nitrogen. Catheters were inserted into both femoral arteries, one to monitor arterial blood pressure continuously and the other to sample blood for measurement of histamine, pH, P_aCO₂, and P_aO₂. Another catheter was introduced via a femoral vein and positioned in the right ventricle for administration every 60 to 90 min of supplemental chloralose (10 to 15 mg/kg), urethane (50 to 75 mg/kg), sodium bicarbonate (0.25 to 0.50 mEq/kg), and saline (2.5 to 7.5 ml/kg), as well as for the delivery of *Ascaris* antigen. No tissue samples were obtained within 30 min of a dose of anesthetic. Femoral arterial blood pressure and transpulmonary pressure (using an esophageal balloon to approximate pleural pressure) were recorded continuously in all dogs. Total lung resistance was measured by a forced oscillation technique and static lung compliance was measured by inflating the lungs with 400 ml of pure O₂ and measuring the change in transpulmonary pressure as described previously.¹⁰ Measurements were made immediately before and then 1, 3, and 5 min after the administration of antigen. We obtained six samples of lung tissue for measurement of histamine before antigen, and another six samples 5 to 8 min after injection of antigen. Blood samples for plasma histamine were obtained before antigen and 1, 3, 5, and 8 min after injection.

Effect of beta-adrenergic stimulation

Our initial experiments were designed to examine the effects of antigen challenge during different degrees of beta-adrenergic stimulation produced by pharmacologic blockade, physiologic stimulation, or pharmacologic stimulation of the beta-adrenergic receptor.

Eleven dogs were treated with propranolol to induce beta-adrenergic blockade. Due to the short half-life of propranolol in the dog,¹¹ this drug was given by infusion to maintain a steady-state level (1.5 mg/kg as an intravenous bolus followed by a continuous infusion of 20 µg/kg · min for at least 20 min before antigen challenge). *A. suum* antigen (20 ml of an aqueous extract containing 5 mg protein/ml) was injected as a bolus into the catheter introduced via the femoral vein.

In six other dogs, no treatment was given prior to antigen. On the basis of previous studies in our laboratory, we assumed these animals probably had endogenous stimulation of the beta-adrenergic receptor by circulating catecholamines.⁹ Samples of blood and lung tissue and physiologic measurements were obtained at the same time intervals described above. These animals were challenged with the same dose of *A. suum*.

In a third group of six animals, terbutaline (as administered above) was used to stimulate the beta-adrenergic receptor markedly. The beta-adrenergic agonist was injected as a bolus into the right side of the heart via the catheter

TABLE I. Basal values after pharmacologic intervention but before antigen challenge

	Treatment		
	Propranolol (n = 11)	None (n = 6)	Terbutaline (n = 6)
Tissue histamine (ng/mg)	20.7 ± 0.36*	20.0 ± 1.2	17.0 ± 1.6
Plasma histamine (ng/ml)	0.93 ± 0.2	1.1 ± 0.5	1.0 ± 0.5
Static lung compliance (cm H ₂ O/L · sec)	77.8 ± 8.0	123.0 ± 12.0	116.0 ± 9.9
Total lung resistance (cm H ₂ O/L · sec)	0.78 ± 0.09	0.88 ± 0.10	1.04 ± 0.19
Arterial blood pressure (torr)			
Systolic	104.0 ± 5.0	110.0 ± 6.2	101.5 ± 15.7
Diastolic	68.5 ± 5.4	79.2 ± 8.4	37.3 ± 11.0

*Data expressed as mean ± 1 SEM.

introduced into the femoral vein 150 sec prior to challenge with *A. suum* antigen. Except for the treatment with terbutaline prior to antigen, the remainder of the experiment was performed as described above.

Correlation between cAMP levels and response to antigen

The correlation between cAMP levels and the response to subsequent challenge with *A. suum* antigen was studied in three different groups of dogs, each consisting of six animals.

In one group of animals, no treatment was given prior to antigen challenge. Before the administration of antigen, three or four samples of lung tissue for cAMP were obtained. In this group, as well as in the other two groups of animals, physiologic measurements were performed and samples of lung tissue and blood were obtained at the same intervals described above. In the treated animals we studied the effect of varying the duration of beta-adrenergic stimulation prior to antigen challenge on the level of cAMP in lung tissue and the response of mast cells and airway smooth muscle to subsequent antigen challenge. In one group of treated dogs, terbutaline (1 mg/kg) was given 150 sec before challenge with *A. suum* antigen. Samples of lung tissue for cAMP were obtained before the administration of terbutaline and immediately before antigen.

In the other group of treated animals, *A. suum* antigen was given 15 sec or less after the administration of terbutaline. Samples of lung tissue for cAMP were obtained before terbutaline and immediately before antigen as described above. In addition, samples for lung tissue cAMP were obtained 45 sec after terbutaline.

Assay techniques

Cyclic nucleotides. The method for obtaining and purifying lung samples for the estimation of lung tissue concentrations of cyclic nucleotides has been described previously.⁹ Samples of lung tissue weighing 100 to 300 mg were

obtained with clamps cooled in liquid nitrogen. They were rapidly homogenized while still frozen. The homogenates were then purified and cAMP was isolated by column chromatography, as described by Murad et al.¹² cAMP was assayed by the method of Gilman,¹³ and cGMP was assayed according to the method of Steiner et al.¹⁴

Histamine. Lung samples (500 mg) were weighed while still frozen, then placed in 15 ml of 0.005M phosphate buffer, boiled for 10 min, homogenized for 20 sec, and boiled again for 5 min. The samples were centrifuged at 27,000 × g for 20 min and the supernatants were assayed for histamine by an enzymatic double-isotope dilution assay of Beavan et al.¹⁵ as modified by Nisam et al.¹⁶ Plasma samples for histamine concentration were prepared and assayed as described previously.¹⁶

Statistical analysis

Statistical analysis of most of the test results was performed with the Student's t test for paired samples within groups,¹⁷ indicated by p*, and with the Student's t test for unpaired samples between groups, indicated by p**. The results of the plasma histamine determinations and the total lung resistance measurements were analyzed with the Wilcoxon signed-rank test.¹⁸ For purposes of statistical analysis, maximal changes were analyzed regardless of the time at which they were observed in order to maximize the chances of detecting evidence of histamine release. When changes were compared at specific time intervals, the conclusions were similar.

RESULTS

Effects of beta-adrenergic stimulation

The basal values after pharmacologic intervention but before antigen challenge are summarized in Table I for propranolol-treated, terbutaline-treated, and untreated dogs. There were no significant differences by unpaired t tests between groups except for static lung

compliance in propranolol-treated dogs, which was significantly less than that in terbutaline-treated and untreated animals.

In 11 propranolol-treated dogs, intravenous administration of *A. suum* antigen decreased lung tissue histamine (Fig. 1). The mean peak arterial plasma histamine concentration increased from 0.93 ± 0.22 to 699 ± 123 ng/ml (mean $\pm$ SE, $p^* < 0.001$). The physiologic reaction in these animals was characterized by a decrease in static lung compliance from 77.8 ± 8.0 to 41.8 ± 5.0 ml/cm H₂O ($p^* < 0.01$, Fig. 1), an increase in total lung resistance from 0.78 ± 0.09 to 46.3 ± 19.4 cm H₂O/L $\cdot$ sec ($p^* < 0.05$), and hypotension (systolic blood pressure decreased from 104 ± 5.0 to 41.5 ± 5.2 torr [Fig. 1]; diastolic blood pressure decreased from 68.5 ± 5.4 to 21.0 ± 23.0 torr [$p^* < 0.05$]).

Six dogs received no treatment before administration of antigen. In these animals, intravenous *A. suum* decreased lung tissue histamine $11.5\% \pm 1.9\%$ ($p^* < 0.05$, Fig. 1), but less than in propranolol-treated dogs ($p^{**} < 0.001$). The mean arterial plasma histamine concentration increased from 1.1 ± 0.5 to 702.9 ± 562.6 ng/ml ($p^* < 0.05$), but the mean percent change (by the Wilcoxon signed-rank test) was less than that in propranolol-treated dogs ($p^{**} < 0.05$). Static lung compliance decreased from 123.0 ± 12.0 to 67.8 ± 9.4 ml/cm H₂O ($p^* < 0.01$), which was similar to the change observed in propranolol-treated dogs (Fig. 1). Total lung resistance increased from 0.88 ± 0.1 to 9.3 ± 5.7 cm H₂O/L $\cdot$ sec ($p^* < 0.05$), which was significantly less than the change observed in propranolol-treated dogs ($p^{**} < 0.05$). These biochemical and pulmonary physiologic changes were associated with hypotension, which was not significantly different from that observed in propranolol-treated dogs ($p^{**} > 0.05$).

In the six terbutaline-treated dogs, intravenous administration of *A. suum* antigen caused small but not significant biochemical and physiologic changes (Fig. 1).

Correlation between levels of cAMP and response to antigen

Terbutaline sulfate (1 mg/kg, i.v.) markedly increased the concentration of cAMP in peripheral lung tissue from 70.6 ± 4.2 to 365.8 ± 60.5 pmol/100 mg wet weight ($p^* < 0.05$) associated with a small but significant increase in cGMP from 1.1 ± 0.2 to 1.9 ± 0.2 pmol ($p^* < 0.05$). A typical time course of the terbutaline-induced change in lung tissue cAMP observed in one dog is illustrated in Fig. 2. cAMP increased very rapidly from 70 pmol/100 mg wet

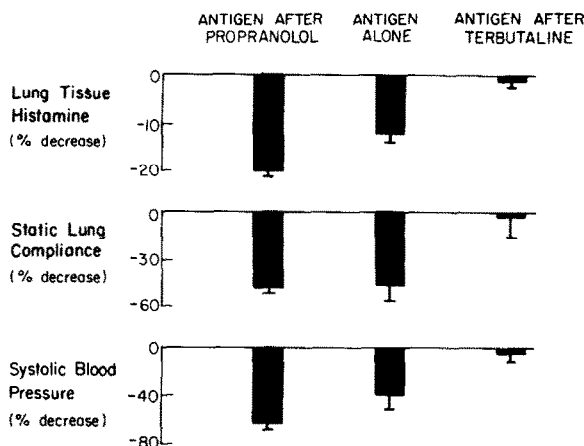


FIG. 1. Effect of beta-adrenergic stimulation on canine anaphylaxis. Eleven dogs received *Ascaris* antigen after beta-adrenergic blockade (propranolol), six dogs received *Ascaris* antigen alone, and six other dogs received *Ascaris* antigen after beta-adrenergic stimulation with terbutaline. All dogs had bilateral cervical vagotomy to block possible vagally mediated reflex effects on the reactions. The length of the bar represents the mean $\pm$ 1 SE.

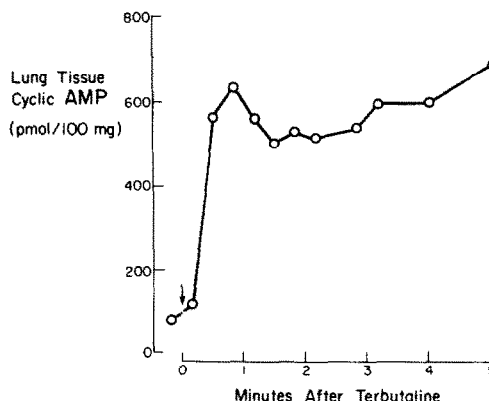


FIG. 2. Time course of effect of terbutaline sulfate (1 mg/kg, i.v.) administered at the arrow on the concentration of cAMP in peripheral lung of one dog.

weight to 8 times this basal level, with near maximal levels observed in less than 1 min.

The relationship between the concentration of cAMP and the subsequent biochemical and physiologic changes induced by *A. suum* antigen in control dogs and in terbutaline-treated dogs are summarized in Table II.

The six vagotomized control dogs had basal levels of cAMP of 69.9 ± 7.2 pmol/100 mg wet weight (Table II, left). Intravenous *A. suum* antigen caused typical changes of systemic anaphylaxis, with histamine release from the lung, constriction of airway

TABLE II. Effect of beta-adrenergic stimulation before and after *A. suum* antigen

	Minutes after terbutaline					
	Control		<0.25		2.5	
	Before	After	Before	After	Before	After
Tissue cAMP (pmol/100 mg wet wt)	69 ± 7.2	—	169.9 ± 39.6	(496.5 ± 43.6)*	365.8 ± 60.5	—
Tissue histamine (ng/mg)	20.0 ± 1.2	17.7 ± 1.1†	18.4 ± 1.6	18.3 ± 1.8	17.0 ± 1.6	17.1 ± 1.7
Plasma histamine (ng/ml)	1.1 ± 0.5	702.9 ± 562.6‡	2.4 ± 0.4	546.5 ± 413.1‡	1.0 ± 0.5	4.0 ± 1.9
Static lung compli- ance (ml/cm H ₂ O)	123.0 ± 12.0	67.8 ± 9.4†	102.3 ± 9.7	85.2 ± 11.7‡	116.0 ± 9.9	114.5 ± 10.4
Total lung resistance (cm H ₂ O/L · sec)	0.88 ± 0.10	9.3 ± 5.7‡	0.68 ± 0.10	0.95 ± 0.30‡	1.04 ± 0.19	1.06 ± 0.19
Arterial blood pressure (torr)						
Systolic	110.0 ± 6.2	47.8 ± 11.2‡	95.8 ± 6.0	61.3 ± 11.4‡	101.5 ± 15.7	95.5 ± 13.6
Diastolic	79.2 ± 8.4	22.7 ± 7.2‡	39.0 ± 16.6	23.5 ± 3.8‡	37.3 ± 11.0	33.7 ± 11.0

*The value in parentheses indicates the level of cAMP in lung tissue measured 45 sec after the administration of terbutaline.

†p < 0.01.

‡p < 0.05.

smooth muscle in central (increased lung resistance) and peripheral airways (decreased lung compliance), and systemic arterial hypotension.

In six other dogs, 15 sec after terbutaline sulfate (1 mg/kg, i.v.) lung tissue cAMP increased only 110% ± 43% (Table II, middle), but 45 sec after terbutaline, cAMP increased 470% ± 54% (p* < 0.001). After *A. suum* antigen, lung tissue histamine did not change significantly in these dogs (−0.25% ± 5.6%, p* > 0.05), but there was a marked increase in mean peak arterial plasma histamine from 2.4 ± 0.4 to 547 ± 413 ng/ml (p* < 0.05). Static lung compliance decreased from 102 ± 9.7 to 85.2 ± 11.7 ml/cm H₂O (p* < 0.05) and total lung resistance increased from 0.68 ± 0.10 to 0.95 ± 0.30 cm H₂O/L · sec (p* < 0.05). Systolic blood pressure decreased from 95.8 ± 6.0 to only 61.3 ± 11.4 torr (p* < 0.05), and diastolic blood pressure decreased from 39.0 ± 16.6 to only 23.5 ± 3.8 torr (p* < 0.05).

cAMP in peripheral lung tissue increased 385% ± 68% in six other dogs (p* < 0.01) 150 sec after terbutaline sulfate. *A. suum* antigen in these dogs caused no significant biochemical or physiologic changes.

To determine whether terbutaline-treated dogs were immunologically reactive, we waited 2 hr, treated these same animals with propranolol (cAMP decreased from basal values of 70.6 ± 4.2 to 57.4 ± 4.4 pmol/100 mg wet weight), and administered a second dose of *A. suum* antigen. This time, *A. suum* antigen produced

changes characteristic of anaphylaxis in all dogs (Fig. 3, right). Compared with the reaction to the first antigen challenge in the untreated animals, the reaction to the second injection of *A. suum* antigen was moderate: arterial plasma histamine increased from 2.9 ± 2.0 to 91.7 ± 58.6 ng/ml (p* < 0.05) and static lung compliance decreased from 84 ± 13 to 64 ± 13 ml/cm H₂O (p* < 0.05), whereas total lung resistance and arterial blood pressure did not change significantly.

A second challenge with *A. suum* antigen was also given to the group of animals that were not treated before the first antigen challenge. These dogs were also given propranolol 2 hr after the first antigen challenge to ensure that cAMP levels were decreased (to 60.3 ± 4.3 pmol/100 mg wet weight) below basal values. The reaction to the second antigen challenge in these animals was characterized by biochemical and physiologic changes similar to those in the terbutaline-treated group of animals (Fig. 3, left).

DISCUSSION

Beta-adrenergic modulation of anaphylaxis

Studies in vitro suggest that IgE-mediated release of mediators from sensitized lung can be modulated by beta-adrenergic stimulation¹⁻³ and that the effect of such beta-stimulation appears to be related to its effect on the level of cAMP in the lung.¹⁹⁻²¹ We have shown previously that release of histamine from dog lung in vivo is associated with degranulation of pulmonary mast cells^{16, 22} and that the reaction to *A.*

suum antigen probably represents a specific immunologic reaction mediated by IgE.^{8, 23} The present study indicates that the beta-adrenergic receptor modulates release of mediator from the mast cell, as well as lung function, during anaphylaxis.

Thus, in the presence of vagal as well as beta-adrenergic blockade, *A. suum* antigen caused a marked decrease in the concentration of histamine in peripheral lung tissue with release of histamine into aortic plasma.

When no beta-adrenergic agonists or antagonists were administered prior to antigen challenge, the beta-adrenergic receptor was probably stimulated by endogenous physiologic levels of catecholamines (increased above basal values as a result of anesthesia and/or surgery), causing an increased level of cAMP in lung tissue, as suggested previously.⁹ Under these conditions, the release of histamine from lung as well as the increase in peak plasma histamine was less than in the beta-adrenergically blocked group of animals. Similar differences were found for the changes in pulmonary function and arterial blood pressure. When the beta-adrenergic receptor was stimulated by terbutaline sulfate for 150 sec there was no evidence of mast cell degranulation or contraction of airway smooth muscle in response to *A. suum* antigen, indicating that the immunologic response to antigen was blocked completely.

cAMP, adrenergic stimulation, and anaphylaxis

We measured concentrations of cAMP because the use of such measurements is a well-established technique for assessing the degree of stimulation of beta-adrenergic receptors.^{4, 5} The fact that terbutaline significantly increased concentrations of cAMP in peripheral lung tissue (Table II) indicates that the drug reached this region and stimulated beta-adrenergic receptors there. These conclusions were confirmed by the physiologic finding of decreased diastolic arterial blood pressure after injections of terbutaline, reflecting stimulation of beta₂ receptors in the vascular bed distal to the peripheral lung. When the concentration of cAMP in the lung was increased fivefold by treatment with terbutaline (a selective beta₂-adrenergic receptor agonist), histamine release into the blood from lung mast cells and all other sources was inhibited and contraction of airway smooth muscle was blocked completely. These findings are consistent with several in vitro studies suggesting that increased cellular levels of cAMP inhibit antigen-induced release of histamine and other mediators of anaphylaxis from mast cells.¹⁹⁻²¹

The interval between administration of a beta-adrenergic agonist and administration of antigen also

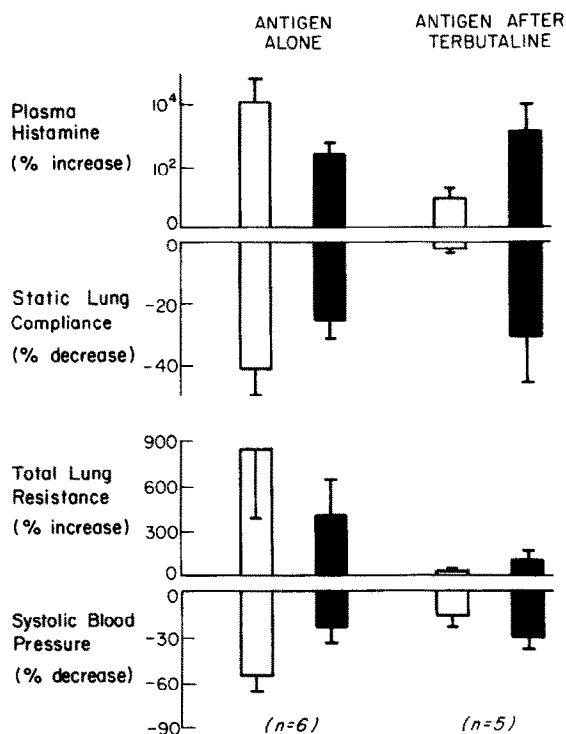


FIG. 3. Effect of sequential antigen challenge in dogs with cervical vagotomy (left) and in dogs with cervical vagotomy plus terbutaline (right). Open bars, First antigen challenge; solid bars, second antigen challenge 2.5 hr later. In the dogs that received terbutaline, propranolol was administered prior to the second antigen challenge to ensure that cAMP levels were not increased (see text for details).

appears to be important in determining the response of the mast cell and airway smooth muscle to antigen challenge. When *A. suum* antigen was administered less than 15 sec after terbutaline, the concentration of cAMP in lung tissue was only doubled. This degree of beta-adrenergic stimulation was apparently sufficient to inhibit the immunologic response of the pulmonary mast cells, since there was no significant change in the mean concentration of lung tissue histamine after antigen. When the duration of beta-adrenergic stimulation was only 15 sec, antigen still caused a marked increase in aortic plasma histamine; in contrast, when the duration of beta-adrenergic stimulation was prolonged for 150 sec prior to antigen challenge, plasma histamine levels did not increase above control values.

Source of plasma histamine and physiologic effects

In experiments without terbutaline, plasma histamine undoubtedly came from extrapulmonary as well as pulmonary mast cells. In the experiments when antigen was administered 15 sec after terbutaline, the increase in plasma histamine probably reflected de-

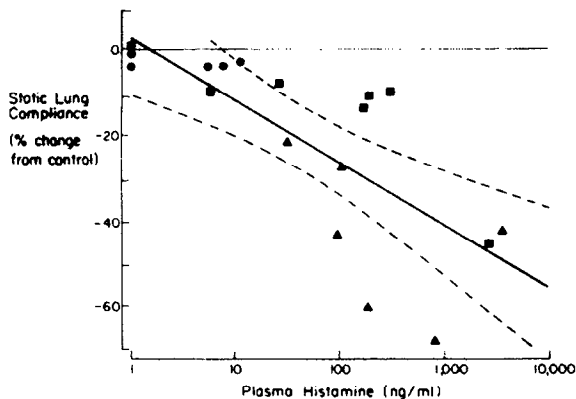


FIG. 4. Relation between static lung compliance and plasma histamine in six dogs that received antigen alone (Δ), six dogs that received antigen 15 sec after terbutaline ($\blacksquare$), and six other dogs that received antigen 150 sec after terbutaline ($\bullet$). $Y = -14.71X + 3.72$, where Y is static lung compliance expressed as a percent change from control and X is plasma histamine (ng/ml); $r = 0.75$. Solid line, Regression line; dashed lines, 95% confidence limits of the line.

granulation of extrapulmonary mast cells in which the duration of beta-adrenergic stimulation was insufficient to inhibit the response to antigen. This possibility was confirmed in two dogs in which histamine concentrations in mixed venous plasma samples were increased and similar to the values in arterial plasma leaving the lung in each animal. In previous studies²³ we found no significant increases in plasma histamine in arterial blood leaving the lung compared with mixed venous blood entering the lung unless there was an associated change in lung tissue histamine concentration. Thus when lung tissue histamine level was unchanged but arterial plasma histamine was increased, we concluded that the circulating histamine had been released from extrapulmonary mast cells.

Interestingly, peak arterial plasma histamine levels occurred later in dogs receiving beta-adrenergic stimulation for 15 sec than in control animals (3.6 vs 2.0 min), also consistent with release of histamine from extrapulmonary sites (which would have a shorter duration of beta-adrenergic stimulation than pulmonary mast cells prior to exposure to antigen). This hypothesis is consistent with the observation that whereas lung tissue cAMP 15 sec after terbutaline had increased $110\% \pm 43\%$, liver tissue cAMP in two of these dogs had increased only 25% and 30%, respectively. (Maximal increase in lung and liver tissue cAMP was approximately 425% within 60 sec of terbutaline administration.)

The dog has large concentrations of histamine in skin, liver, and gut, in addition to lung. Thus stimu-

lation of beta-adrenergic receptors on pulmonary mast cells for only 15 sec before antigen administration may have been sufficient to inhibit detectable histamine release from the lung but not from other sites (such as skin, liver, and gut), resulting in markedly increased aortic plasma histamine. Histamine (and other mast cell mediators) presumably caused contraction of smooth muscle in peripheral airways (decreased static lung compliance), passed through the pulmonary circulation, and was distributed to central airways by way of bronchial arteries (increased lung resistance) as well as to receptors for mast cell mediators on systemic blood vessels (decreased blood pressure).

The response of airway smooth muscle to antigen was inhibited completely in the dogs that received terbutaline 150 sec prior to antigen. In the animals given terbutaline only 15 sec before antigen, the physiologic response to antigen was also inhibited markedly, in spite of the increased aortic plasma histamine. The fact that the physiologic response was less severe than in the control dogs may have been due in part to the inhibition of local release of mast cell mediators in the lung. Alternatively, the duration of beta-adrenergic stimulation of smooth muscle may have been sufficient to inhibit the effect of circulating mediators even though the duration of beta-adrenergic stimulation was too brief to prevent mediator release. This alternative is consistent with the fact that beta-adrenergic stimulation increased tissue cAMP 470% in 45 sec and produced maximal levels of tissue cAMP within 1 min, well before peak plasma levels of histamine. It is also consistent with previous studies which demonstrated that histamine is not metabolized by lung and that histamine introduced into the circulation from exogenous sources in sufficiently large doses can cause physiologic changes in the lung and cardiovascular system similar to those observed in these dogs in which histamine was introduced into the circulation from endogenous sources.^{9, 24}

Thus the severity of the physiologic reaction (decreased lung compliance) appeared to correlate with the severity of the immunologic reaction (reflected by the release of histamine), regardless of whether pulmonary or extrapulmonary mast cells were partially or completely inhibited (Fig. 4).

Immunologic specificity of antigen reaction

Conceivably, the apparent inhibition of the response to *A. suum* antigen in the terbutaline-treated dogs could be because these animals were immunologically unreactive. This possibility seems unlikely, since the dogs were selected randomly and were natively allergic to *A. suum*, as confirmed by a

markedly positive skin reaction to the antigen. In our laboratory, 90% of dogs with positive cutaneous reactivity to this antigen experienced anaphylaxis when challenged intravenously.²³ In addition, three of the six terbutaline-treated animals released small but significant amounts of histamine, suggesting that some mast cell degranulation occurred.

More direct evidence of reactivity of these dogs was provided by the fact that all terbutaline-treated dogs reacted to a second challenge with *A. suum*, administered 2.5 hr after the first challenge. Although the rate of elimination of terbutaline is rapid in the dog,²⁵ to ensure that cAMP levels did not remain increased, the animals were given propranolol, which decreased cAMP below control levels. The amount of histamine released after the second antigen challenge in the terbutaline-treated dogs was small compared with that in untreated, control dogs after the first antigen challenge (even smaller than that released in propranolol-treated dogs after their first antigen challenge, see Fig. 1). This suggests that the mast cells were partially inhibited in the terbutaline-treated dogs at the time of the second antigen challenge. The reasons for this inhibition are unknown; possibilities include persistent terbutaline in lung tissues, persistent terbutaline on the beta-adrenergic receptor, or persistent biochemical effects of beta-adrenergic stimulation in the mast cell despite the low levels of cAMP after propranolol.

In the untreated animals, other mechanisms may be responsible for the smaller amount of histamine released after the second antigen challenge. Receptor sites may have been occupied by substances released during the first reaction and only slowly removed,²⁶ or mediator stores may have been partially depleted by the first antigen challenge without sufficient time for repletion before the second challenge.^{24, 27, 28}

It is conceivable that nonimmunologic histamine release in these experiments was caused by a low-molecular-weight peptide found in some preparations of *A. suum*.²⁹ This seems unlikely, since the percent of randomly selected dogs reacting to our preparation of *A. suum* is similar to that reported by others who have attempted to purify the antigen³⁰ as well as those who did not.³¹ Furthermore, recent studies in our laboratory show that despite rigorous physicochemical purification, not all the nonspecific peptide is removed, as shown by rabbit antiserum directed against the peptide and by studies of histamine release. However, the peptide comprises less than 1% of the crude extracts of *A. suum* and has little biologic effect on canine mast cells. In our studies, 500 $\mu\text{g}/\text{ml}$ of the peptide was required to cause significant histamine release from canine lung in vitro. The dose of *A. suum*

used in these experiments in living dogs would result in approximately 250 $\mu\text{g}/\text{ml}$ of the antigen in the lung. This dose of antigen would contain 1/2000 of the dose of peptide that might be expected to cause significant histamine release by nonimmunologic mechanisms.³²

Terbutaline sulfate, a beta agonist

We have not been able to demonstrate a change in cAMP in peripheral canine lung after stimulation or blockade of the sympathetic nerves, although beta-adrenergic stimulation by circulating catecholamines probably does occur.⁹ Therefore we used terbutaline sulfate to stimulate beta-adrenergic receptors in lung tissue prior to antigen challenge. Studies in vitro suggest that the beta-adrenergic receptors involved in the inhibition of the immunologic release of histamine from guinea pig lung differ from beta-receptors in guinea pig atria³³ and appear to be related to receptors in guinea pig trachea.³⁴ Comparison of the effects of terbutaline with those of isoproterenol on histamine release and on pulmonary and vascular beta-adrenergic receptors in guinea pigs and dogs suggests that terbutaline acts on beta-adrenergic receptors on the mast cell in ways that are analogous to the type 2 beta-adrenergic receptor described by Lands.^{35, 36} The concentrations of terbutaline necessary to inhibit mast cells in these studies are very high. They probably cannot be attained when the drug is administered in the usual clinical doses by mouth or parenterally, but they may be attained in the airways when the drug is administered by aerosol.

As in previously reported studies of the modulation of antigen-induced reactions in isolated lung tissues,^{1-3, 19-21} our samples of canine lung contain a heterogeneous population of cells that may contribute in varying proportions to the total concentration of cAMP in lung tissue measured chemically. Our present methods do not provide definitive evidence regarding the specific type of cell in which the changes in cAMP occur. However, our results are consistent with similar studies in vitro,³ based on the correlation between total cyclic nucleotide levels and inhibition of a specific mast cell function (i.e., secretion of histamine). On the basis of similar circumstantial evidence, it has been concluded that changes in cyclic nucleotide levels in pulmonary mast cells paralleled those observed in fragments of lung tissue. Studies of isolated pulmonary mast cells or immunocytochemical detection of changes in cAMP levels in pulmonary mast cells in vivo will be necessary to determine the relationship between cAMP levels measured chemically in the whole lung, cAMP levels in the mast cells per se, and changes in mast cell function in vivo.

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