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PHARMACOLOGIC BLOCKADE OF THE EFFECT OF HISTAMINE ON LUNG CYCLIC AMP LEVELS IN NORMAL AND PERTUSSIS-VACCINATED MICE

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Abstract—The pertussis-mediated increase in the ability of histamine to stimulate murine pulmonary cAMP accumulation was shown to appear as early as 1 day, peak by 4 days, and return to control values by 10 days after vaccination. This effect was inhibited by tripelennamine, propranolol and high doses of atropine, but not by prednisone, indomethacin, metiamide or guanethidine. Pulmonary cAMP accumulation after exogenous epinephrine administration was marked and equivalent between control and vaccinated mice. Histologic examination showed a marked polymorphonuclear leucocyte accumulation in the lungs of pertussis-vaccinated mice that followed a temporal course of development comparable to the cAMP abnormality. These data are interpreted to mean that the increase in cAMP after histamine challenge is not responsible for death and may in fact represent a compensatory effort to prevent death. Further, this phenomenon is likely to be related to the accumulating leucocytes.

Vaccination of certain strains of mice with whole cells or cell fractions of the bacterium *Bordetella pertussis* induces an increased sensitivity to histamine [1–3]. The biochemical basis of this histamine hypersensitivity is unknown. However, the ability to mimic the pertussis effect with acute administration of agents such as propranolol and dichloroisoproterenol [4, 5] led to the speculation that a beta-adrenergic blockade was involved [6]. I have recently reported [7, 8] that pertussis-vaccinated mice respond to intraperitoneally injected histamine with a rapid increase in pulmonary adenosine 3′,5′-monophosphate (cAMP) and show increased sensitivity when compared to non-vaccinated animals. In an effort to determine the mechanism of this pertussis-mediated phenomenon, attempts were made to regulate it with defined pharmacologic agents. The results of such experiments are reported here.

MATERIALS AND METHODS

Animals. Outbred female Swiss mice (22–26 g) obtained from Carworth Animal Farms, New York, N.Y., and Timco Breeding Laboratories, Inc., Houston, TX, were housed in groups of six to eight, and provided food and water *ad lib.*

Histamine sensitization. Unless otherwise stated, mice were rendered hypersensitive to histamine by an intraperitoneal (i.p.) injection of 1×10^{10} whole killed *B. pertussis* cells (courtesy of Eli Lilly & Co.) in a volume of 0.25 ml 5 days before sacrifice.

Histamine challenge and tissue collection. Histamine challenge was accomplished by an i.p. injection of 1.5 mg/kg of histamine base (as the diphosphate salt) in a volume of 0.025 ml/g 1 min before the animal was sacrificed by cervical dislocation. After sacrifice the lungs were rapidly removed and frozen between blocks of dry ice and subsequently analyzed for cAMP content.

Extraction of cAMP. Cyclic AMP was extracted from lung using the following modification of the procedure described by Murad *et al.* [9]. Tissue samples were homogenized in 10–20 vol. of 0.4 N perchloric acid with a Brinkmann polytron tissue homogenizer for 30 sec at a setting of 5. An aliquot (0.2 ml) was removed for protein determination by the method of Lowry *et al.* [10]. Cyclic AMP was extracted from a second aliquot (1 ml) after addition of approximately 2000 cpm [^{3}H]cAMP to allow determination of final nucleotide recovery. The sample was centrifuged (1600 *g* for 10 min) and the supernatant neutralized with 1 N KOH. After 30 min in an ice bath (4°) and centrifugation (1600 *g* for 10 min) to remove the resulting precipitate, the neutralized supernatants were applied to 0.6 × 3 cm Dowex-1-formate ion exchange columns previously equilibrated with 0.1 N formic acid. After sample application the columns were washed with an additional 6 ml of 0.1 N formic acid. Cyclic AMP was eluted with 6 ml of 2 N formic acid, lyophilized, reconstituted in an appropriate volume of water and measured. Cyclic AMP recovery averaged 70–80 per cent.

Measurement of cAMP. Cyclic AMP was measured using a modification of the radioimmunoassay as described by Steiner *et al.* [11]. The assay mixture contained: 50 μl of unknown or standard nucleotide; 50 μl of 200 mM sodium acetate buffer, pH 6.2, containing 0.2 mg/ml of bovine serum albumin; 50 μl [^{125}I]succinyl-cAMP-tyrosyl methyl ester (containing approximately 18,000 cpm); and 50 μl of goat anti-cAMP antibody appropriately diluted in distilled water to maximally bind 30–40 per cent of the added radioactivity. After 12–16 hr of incubation at 4° the reaction was stopped by the addition of 50 μl of 10% pig plasma and 1 ml of 95% ethanol [12]. The resulting precipitate was collected by centrifugation (1600 *g* for 30 min) and counted in a Beckman radioimmuno analyzer. The concentrations of the unknowns are

estimated from a simultaneously determined cyclic AMP standard curve using a logit probit analysis of the data.

Reagents. Histamine diphosphate, cAMP, O^2-monosuccinyl adenosine 3',5'-cyclic monophosphate tyrosyl methyl ester, and prednisone were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; AG 1-X8 ion exchange resin (200–400 mesh formate form) from Bio-Rad, Richmond, CA, U.S.A.; [^{3}H]cAMP from New England Nuclear, Boston, MA, U.S.A.; ^{125}I from Schwarz/Mann Co., Orangeburg, NY, U.S.A.; and atropine sulfate from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Propranolol was a gift from Ayerst Laboratories, New York, N.Y., U.S.A.; tripelennamine and guanethidine from CIBA, Summit, NJ, U.S.A.; indomethacin from Merck, Sharp & Dohme; metiamide from Smith, Klein & French; and *B. pertussis* vaccine from Eli Lilly Co. Goat anti-cAMP antibody was produced in collaboration with Biotek Research, Inc., St. Louis, MO, U.S.A. All other reagents were purchased from either Fisher Scientific Co., Pittsburgh, PA, U.S.A.; Mallinckrodt Chemical Works, St. Louis, MO, U.S.A.; or J. T. Baker Chemical Co., Phillipsburg, NY, U.S.A.

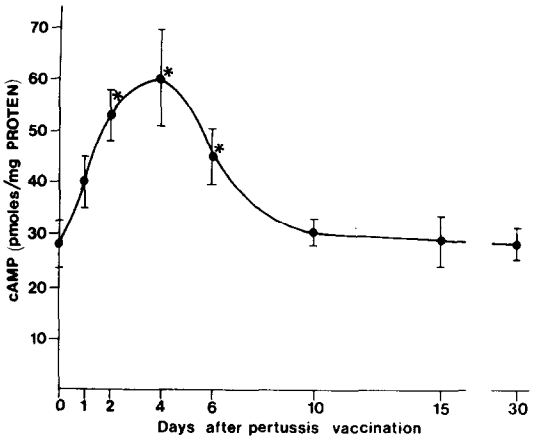

Fig. 1. Temporal development of the pertussis-induced histamine-mediated pulmonary cAMP abnormality. Animals received 1×10^{10} whole *B. pertussis* cells i.p. At the indicated number of days after vaccination, they were challenged i.p. with 1.5 mg/kg of histamine base (as the diphosphate salt) 1 min before they were sacrificed and lung cAMP was determined. Values are expressed as pmoles cAMP/mg of protein ± S.E.M. of twelve determinations. An asterisk indicates significant at $P < 0.05$ as determined by Student's *t*-test. Zero time = non-vaccinated animals.

RESULTS

Temporal development of the pertussis-induced lung cAMP abnormality. At various time periods after pertussis vaccination, mice were challenged with histamine and the pulmonary cAMP content was determined as described above. Figure 1 shows that the histamine-induced increase in pulmonary cAMP is first apparent 1 day after vaccination, becomes statistically significant by day 2, reaches a maximum around day 4 and thereafter gradually declines until it has disappeared by day 10.

Effect of varying the dose of pertussis vaccine. Mice received i.p. varying amounts of *B. pertussis* cells suspended in 0.5 ml of physiological saline. Five days later they were challenged with histamine and the pulmonary cAMP content was determined as described above. Figure 2 shows a dose-dependent rise in lung cAMP content over the range of 2.5 to 20×10^9 pertussis cells.

Blockade of the histamine-induced increase in pulmonary cAMP. In an effort to determine the basis for histamine-induced cAMP accumulation, a number of pharmacologically active agents were examined for their ability to block the histamine-induced increase in pulmonary cAMP. All the agents tested except prednisone were diluted in physiological saline and administered i.p. as a single dose in 0.2 ml 60 min prior to histamine challenge and sacrifice. Prednisone was suspended in 0.5% sodium methyl cellulose and given subcutaneously in 0.2-ml volumes daily from the time of pertussis vaccination until the day before histamine challenge and sacrifice. Table 1 shows that neither prednisone (5–40 mg/kg), indomethacin (1.25 to 10 mg/kg), metiamide (5–40 mg/kg), atropine (5–20 mg/kg), nor guanethidine (1.25 to 10 mg/kg) was effective in blocking histamine-induced lung cAMP accumulation. On the other hand, tripelennamine (20

Table 1. Effect of drugs on histamine-induced cAMP accumulation in lungs of pertussis-vaccinated mice*

Drug dose	Prednisone	Indomethacin	Metiamide	Tripelennamine	Propranolol	Guanethidine	Atropine
Control†	21.7 ± 2.5	22.4 ± 4.2	39.9 ± 5.3	17.3 ± 3.2	16.5 ± 4.5	18.3 ± 5.0	24.9 ± 7.3
0	42.4 ± 6.4	45.0 ± 4.6	60.5 ± 10.1	46.5 ± 7.7	30.8 ± 5.6	35.7 ± 5.6	50.7 ± 6.3
0.625					26.3 ± 5.5		
1.25					19.5 ± 3.7	38.2 ± 5.9	
2.5					16.6 ± 2.9‡	46.9 ± 7.7	
5	47.9 ± 7.9	49.5 ± 6.8	58.4 ± 8.5	45.7 ± 11.2	12.7 ± 2.6‡	38.3 ± 5.8	43.9 ± 8.8
10	45.2 ± 6.7	43.8 ± 7.0	58.3 ± 7.6	46.2 ± 11.3		33.3 ± 5.1	44.3 ± 6.9
20	36.0 ± 5.0	55.7 ± 6.5	55.5 ± 7.5	27.1 ± 7.0‡			35.6 ± 8.5
40	42.2 ± 4.5	41.4 ± 6.2	61.7 ± 11.3	17.7 ± 4.3‡			31.1 ± 4.7‡

* Drugs with the exception of prednisone (see Results) were administered i.p. 60 min before histamine. Histamine challenge was brought about by an i.p. injection of 1.5 mg/kg of histamine base as the diphosphate salt 1 min before sacrifice. cAMP accumulation is expressed as pmoles/mg of protein ± S.E.M. of nine determinations. Mice were rendered hypersensitive to histamine by an i.p. injection of 1×10^{10} whole killed *B. pertussis* cells 5 days before drug challenge.

† Received neither drug nor histamine.

‡ Statistically significant ($P < 0.05$) from 0 drug.

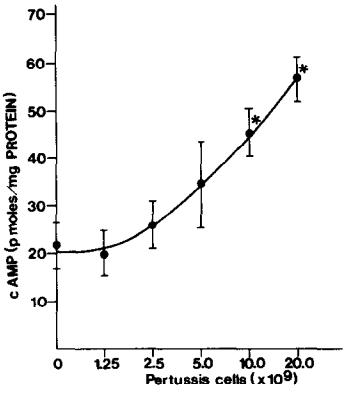

Fig. 2. Effect of increasing numbers of *B. pertussis* cells on histamine-mediated pulmonary cAMP accumulation. Animals received the indicated number of whole *B. pertussis* cells i.p. Five days later they received an i.p. injection of 1.5 mg/kg of histamine base (as the diphosphate salt) 1 min before sacrifice and lung cAMP determination. Values are expressed as pmoles cAMP/mg of protein ± S.E.M. of six determinations. An asterisk indicates significant at $P < 0.05$ as determined by Student's *t*-test.

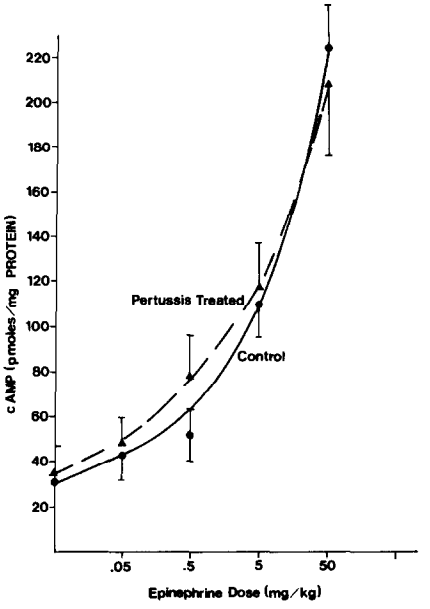

Fig. 3. Effect of exogenous epinephrine on lung cAMP levels in pertussis-vaccinated (▲) and control (●) mice. Animals received 1×10^{10} whole *B. pertussis* cells or an equivalent volume of physiological saline i.p. Five days later they received the indicated doses of epinephrine hydrochloride i.p. 1 min before sacrifice and lung cAMP was determined. Values are expressed as pmoles cAMP/mg of protein ± S.E.M. of thirteen determinations.

and 40 mg/kg), propranolol (2.5 and 5 mg/kg) and high doses of atropine (40 mg/kg) significantly ($P < 0.05$) inhibited the histamine-induced lung cAMP accumulation.

Effect of propranolol on histamine-induced death in pertussis-vaccinated mice. We tested the effect of propranolol, which blocked histamine-induced lung cAMP accumulation, on histamine-induced death in pertussis-vaccinated mice. Five days after pertussis vaccination, mice received 5.0 mg/kg of propranolol i.p. in 0.2 ml of physiological saline. Sixty min later they received varying doses of histamine, and mortality was determined 6 and 24 hr later. No differences existed between the 6- and 24-hr mortality rates. Table 2 shows that propranolol further reduced the minimal lethal histamine dose from 10.8 to 1.2 mg/kg of histamine base.

Effect of epinephrine on lung cAMP levels. In view of the ability of propranolol to block histamine-induced lung cAMP accumulation, it was of interest to test the direct effect of epinephrine on lung cAMP accumulation. Normal and pertussis-vaccinated animals received varying doses of epinephrine i.p. 1 min prior to sacrifice and pulmonary cAMP determination as described above. The results shown in Fig. 3 indicate that lung cAMP accumulation is the same in both normal and pertussis-vaccinated mice after exogenous epinephrine administration.

Table 2. Propranolol potentiation of histamine-induced death in pertussis-vaccinated mice

	Treatment	
Histamine dose*	Pertussis†	Pertussis† propranolol‡
Control	0/6§	0/6
0.4	0/6	0/6
1.2	0/6	1/6
3.6	0/6	2/6
10.8	1/6	4/6
32.4	3/6	5/6
97.2	4/6	5/6

* Histamine base (mg/kg) was administered i.p. as the diphosphate salt.

† Whole *B. pertussis* cells (1×10^{10}) were administered i.p. 5 days before histamine challenge.

‡ Propranolol (5.0 mg/kg) was administered i.p. 60 min before histamine challenge.

§ Number dead/total number.

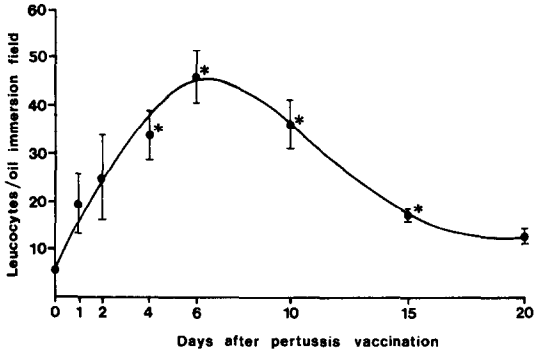

Fig. 4. Temporal development of the pertussis-induced accumulation of polymorphonuclear leucocytes in murine lungs. Animals received 1×10^{10} whole *B. pertussis* cells i.p. At the indicated number of days after vaccination, they were sacrificed by cervical dislocation. The lungs were removed and fixed in formalin, sectioned in a microtome-cryostat, stained with a hematoxylin–eosin-staining procedure, and examined microscopically. Values are expressed as polymorphonuclear leucocytes observed/oil immersion field ± S.E.M. for five fields on each of eight animals.

Accumulation of neutrophils in lungs of pertussis-vaccinated mice. At varying time periods after pertussis vaccination, mice were sacrificed and the lungs placed in formalin solution. After a minimal 24-hr fixation period the tissues were embedded in cryoform and 20 μm sections obtained on an International model CFT microtome-cryostat. The slices were stained with standard hematoxylin- and eosin-staining procedures [13] and examined microscopically. The most conspicuous histologic alteration observed was a marked accumulation of polymorphonuclear leucocytes (neutrophils) in the lungs of the pertussis-vaccinated mice. The temporal development of this accumulation is shown in Fig. 4. The number of neutrophils/oil immersion field increased approximately 10-fold over control values, with the peak increase occurring around day 6 after vaccination.

DISCUSSION

From the data presented in Figs. 1 and 2 and Table 1, it appears that the biochemical entity responsible for inducing histamine hypersensitivity as measured by pulmonary cAMP accumulation is probably a subfraction of the bacterial cell and not a host product synthesized in response to the bacterium. This conclusion is based on the facts that (1) the magnitude of the effect is linearly related to the mass of bacteria injected over a wide range of high doses, (2) the altered histamine response appears and subsides quite rapidly, and (3) prednisone, an immunosuppressive agent, did not block the development of the response.

From the data in Table 1, a number of conclusions can be made regarding the biochemical nature of the histamine-induced pulmonary cAMP accumulation. Using the H_1- and H_2-blocking agents, tripelennamine and metiamide, respectively, it appears that H_1 and not H_2 receptors are predominantly involved in this response. The doses of histamine antagonists used in these studies are consistent with those shown to protect pertussis-vaccinated mice from histamine-induced death [14] and modulate histamine-induced changes in blood pressure in the dog [15]. These latter studies demonstrate that the large variance between doses of H_1 and H_2 blockers required to inhibit responses *in vitro* may not apply to studies *in vivo*. Previously I had speculated that the action of histamine may be mediated through an intermediate [8]. The results obtained with indomethacin indicate that prostaglandins are probably not the intermediate. The ability of propranolol to markedly inhibit the histamine-induced lung cAMP accumulation supports the idea that catecholamines may be involved as Polson *et al.* [16] have suggested. However, I have failed to observe any increased pulmonary sensitivity to epinephrine either *in vivo* (Fig. 3) or *in vitro* [8]. This supports the suggestion that the abnormality might reflect an altered catecholamine release mechanism [17, 18]. Inability of guanethidine to prevent the histamine-induced cAMP accumulation suggests that norepinephrine released from nerve endings is probably not involved. On the other hand, while the failure of low doses (5–10 mg/kg) of atropine to block the cAMP accumulation suggests that muscarinic receptor activation is not involved, the effectiveness of higher doses (40 mg/kg) which can induce ganglionic blockade [19] supports the idea that neural or adrenal release may be involved. It is, however, very difficult to explain the high degree of organ specificity on the basis of blockade of adrenal release of epinephrine. The use of more specific ganglionic-blocking agents, such as hexamethonium, or studies in splanchnectomized animals should help clarify the intermediary role of adrenal release of catecholamines.

Whatever the nature and/or role of intermediates in histamine-induced accumulation of cAMP in lungs of pertussis-vaccinated mice, it seems certain that this alteration is not causally related to histamine-induced death in these animals. This conclusion is derived from the observation that prednisone, which is reported to be protective against histamine-induced death [14, 20], did not inhibit histamine-induced cAMP accumulation while propranolol, which was shown to facilitate histamine-induced death (see Table 2), had a pronounced inhibitory effect on the histamine-induced lung cAMP accumulation (see Table 1). If anything, it appears that elevated cAMP levels may be protective. This hypothesis is supported by the finding that epinephrine, which is also reported to protect pertussis-vaccinated animals against histamine-induced death [18], markedly elevated lung cAMP levels (see Fig. 3).

Lack of specific knowledge of the particular cell type(s) which respond to exogenous histamine with an increased cAMP accumulation makes the interpretation of these results difficult. Previously it was suggested that leukocytes, reported to be present in large numbers in the lungs of pertussis-vaccinated mice [21], may be involved [8]. The data represented in Fig. 4 support that idea by revealing a close temporal similarity between the accumulation and disappearance of polymorphonuclear leukocytes (neutrophils) and the appearance and disappearance of the abnormality in histamine-induced cAMP accumulation. Further evaluation of this hypothesis is currently underway in this laboratory using immunohistochemical methodologies [22].

Based on the available data, one could consider several mechanisms for the observed pulmonary cAMP abnormality. The simplest hypothesis would involve a direct interaction of histamine with an H_1 receptor on the surface of polymorphonuclear leucocytes to increase cAMP accumulation. This would tend to inhibit further mediator release as has been suggested [23]. This idea is supported by the parallel appearance of neutrophils and altered histamine-induced cAMP accumulation, inhibition by tripelennamine, and the organ specificity previously observed [8]. Preliminary data on neutrophil accumulation show the same organ specificity as observed for the cAMP abnormality. The propranolol data do not support this hypothesis but suggest that the histamine action is mediated via catecholamine release. However, an effect of propranolol on the histamine receptor has not been eliminated. One could have hypothesized that pertussis vaccination results in pulmonary β-adrenergic receptor sensitization with a subsequent exaggerated response to catecholamine, but this hypothesis seems unlikely due to the failure to observe such a sensitization either *in vivo* (Fig. 3) or *in vitro* [8]. A more complex hypothesis, suggested

by the atropine results, envisions two separately acting bacterial fractions, one of which can sensitize for histamine-induced adrenal release of epinephrine and a second which acts specifically to alter the lung such that polymorphonuclear infiltration is enhanced. This could result in an abnormal cAMP response to epinephrine possibly due to an increase in receptor numbers. The site of this cAMP synthesis and accumulation might be the leucocytes themselves or some other cell altered by the presence of the leucocytes. However, these data do not exclude the possibility that the accumulation of polymorphonuclear leucocytes results from, instead of causing, the observed changes in cAMP metabolism.

Finally, these data support the suggestion I made earlier [8] that the increased cAMP accumulation observed in lungs of pertussis-vaccinated mice after histamine administration might occur as part of a homeostatic adjustment to prevent further histamine release. As such these changes should be regarded as protective rather than detrimental.

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