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PULMONARY PATTERNS OF ADENOSINE-3′, 5′-CYCLIC MONOPHOSPHATE ACCUMULATIONS IN RESPONSE TO ADRENERGIC OR HISTAMINE STIMULATION IN *BORDETELLA PERTUSSIS*-SENSITIZED MICE

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Abstract—Susceptible strains of mice when injected with *Bordetella pertussis* vaccine develop a marked hypersensitivity to histamine and other agents of pharmacologic interest. The possibility was raised that this hypersensitivity is due to a reduced responsiveness of adenylate cyclase to adrenergic stimulation in various tissues of the pertussis-sensitized organism. This paper describes experiments showing that, in contrast to some other tissues, the resting level of adenosine-3′,5′-cyclic monophosphate (cAMP) in lungs, as well as the capacity of the pulmonary cAMP system to respond to adrenergic activation, as effected by naturally occurring or synthetic catecholamines, is unaltered by the pertussis sensitization of the animals. Another finding here-in described is the pulmonary cAMP rise in both normal and pertussis-sensitized mice after histamine administration. Using beta-adrenergically blocked and adrenalectomized mice, evidence was obtained indicating that the increased amounts of cAMP after histamine may have resulted from an adrenergically mediated effect.

Live or killed *Bordetella pertussis* cells or components thereof appear to be unique in their ability to markedly reduce the normally high resistance of mice to endogenously released or exogenously administered histamine. This increase in sensitivity to histamine reaches a maximum approximately 5 days after the injection of the pertussis organisms, at which time a 20- to 300-fold increase in sensitivity may be found. The hypersensitivity then declines, and the animals apparently regain a normal state [1–3].

Pertussis vaccination in mice also produces hypersensitivity to serotonin, acetylcholine, bradykinin and the slow-reacting substance of anaphylaxis, though the time-course of development, as well as the disappearance of sensitivity, may differ slightly from that to histamine [1–3].

In addition to its obvious significance to microbiologists and clinicians, the phenomenon appears to attract growing interest in several other disciplines. For neuropharmacologists and neurophysiologists, it represents an acquired hypersensitivity to naturally occurring agents which, in a broad sense, may be commonly regarded as chemical organizers of neurohumoral integration. To immunopharmacologists and allergists, it represents, paradoxically, a unique experimental model of nonimmunologically acquired hypersensitivity to the established pharmacological mediators of immune reactions. Unlike antigen-induced hypersensitivity, which is mediated through a specific antibody response, this reaction is induced by another substance of undetermined nature. It is relatively transient; no anamnestic response is apparent and the histamine and serotonin sensitivity is not transferable by lymphoid cells or serum, the classical agents of the immune response [3]. The hypersensitive state may be duplicated in normal mice by pharmacologic beta-adrenergic blockade, a fact that led to the postulate that pertussis vaccination results in an impairment of the adenylate cyclase system to respond to adrenergic activation [4–9]. Indeed, subsequent investigations revealed a decreased stimulation of adenosine-3′,5′-cyclic monophosphate (cAMP) synthesis by adrenergic agents in several tissues and cell systems of the pertussis-sensitized organism [10–15].

This paper reports experiments showing that, in contrast to some other tissues of the pertussis-sensitized mouse examined so far, the capacity of the lung to synthesize cAMP is unaltered to adrenergic, and even magnified, to histamine stimulation. Since the pertussis-sensitized mouse has acquired in recent years a growing popularity as an animal model for the study of the basic abnormality in bronchial asthma [3], this combination of findings is of major potential importance.

MATERIALS AND METHODS

A total of 808 female mice, weighing 20–25 g each, was employed throughout this investigation. Animals were paired and one-half of each group was sensitized with *B. pertussis* vaccine (Eli Lilly & Co.). Each mouse received an intra-peritoneal (i.p.) injection of the vaccine diluted in 0.9% NaCl to a volume of 0.35 ml containing 7.2×10^9 killed organisms. Five days later, portions of groups of control and vaccinated animals were injected with 70 mg/kg of histamine base to establish the actual development of hypersensitivity. Throughout this paper, hypersensitivity to histamine indicates that a standard i.p. injection of histamine without visible effect in control animals is lethal in 80–100 per cent of the vaccinated animals. In all experiments, each animal within the sensitized group expired within 15 min of a histamine challenge, while control mice were alert and appeared healthy.

After control or drug treatment sequences, the mice were decapitated by a scissor cut which also opened the thorax enabling removal of the heart and lungs to liquid nitrogen within 3 sec. Tissues were stored at −85° until dissected and weighed in a cryostat at −40°. Extraction was carried out in a cold room with 0.5 M perchloric acid. Extracts were centrifuged, supernatants decanted, neutralized with 2 M K_2CO_3, recentrifuged, and the neutralized supernatants stored at −85°. Measurement of cAMP was accomplished on unchromatographed extracts using the protein binding method of Gilman [16]. It was demonstrated that concentrations of ATP, adenine, adenosine, adenosine-5'-monophosphate and cyclic GMP, at least 10-fold greater than that found in tissue, did not interfere with the cAMP determination.

Dose–response curves were obtained by injecting L-isoproterenol hydrochloride (Sigma I-3878) or L-epinephrine bitartrate (Sigma E-4375) as the salt. Histamine dihydrochloride (Sigma H-7250) was injected as the base. In dose–response sequences, lung samples were taken 5 min after i.p. drug administration. In time-course studies, animals were injected i.p. with histamine dihydrochloride (70 mg/kg of the base) or isoproterenol hydrochloride (1.75 mg/kg of the salt), and lungs removed at varying time periods after injection. Pharmacologic beta-adrenergic blockade was established by 0.68 mg/kg of DL-propranolol as base (Sigma P-0884), diluted in 0.9% NaCl and injected i.p. in a volume of 0.2 ml.

In the adrenalectomized series, mice were maintained on 0.9% NaCl until sacrifice. Before experimentation commenced on post-operative day 6, the animals were given three i.p. injections of hydrocortisone-21-phosphate (Sigma H-4251) in single doses of 25 mg/kg. These were administered at 12-hr intervals with sacrifice and/or drug treatment at 3 hr after the last injection.

Throughout these studies, statistical comparisons were based on standard *t*-tests for unpaired data [17].

RESULTS

The effects of the histamine hypersensitive state on the pattern of responsiveness of the pulmonary cAMP system to adrenergic activation were explored by studying: (1) the basal cAMP levels, (2) the time course of cAMP response to isoproterenol or histamine, and (3) the changes in cAMP levels under conditions of exposure to progressively increasing doses of isoproterenol, epinephrine or histamine. While in all these experiments the patterns of cAMP reactivities of pertussis-sensitized animals were compared to normal controls, in separate sets of experiments an attempt was made to examine the effects of (4) prior beta-adrenergic blockade by propranolol on subsequent epinephrine responsiveness or (5) subsequent histamine responsiveness and (6) elimination of the adrenal medullary catechol store on histamine responsiveness.

Comparison of basal cAMP levels in lungs of control and pertussis-sensitized animals demonstrated that sensitization did not alter basal cAMP levels (Table 1). The time course of cAMP response to isoproterenol showed that at 1 min after isoproterenol administration, cAMP levels were increased approximately 4.5-fold (Fig. 1) and remained elevated at 5 min with a beginning decline toward baseline at the 10-min time interval. Changes in lungs of pertussis-sensitized animals followed much the same pattern, however, with a possible tendency for a more rapid return to baseline (Fig. 1).

Based on the peak-range of cAMP responses as determined in the time-course studies, the next experiments were designed to allow an optimum 5-min interval to elapse between drug administration and removal of lungs for the biochemical assays. As illustrated in Fig. 2, isoproterenol was used in progressively increasing amounts. The dose–response curves so obtained in the control groups closely paralleled those in the pertussis groups with the only observable divergence at the highest isoproterenol dose (500 mg/kg) level. This difference, however, could not be conceived as physiologically or pathologically meaningful, since this amount of isoproterenol was found to be sufficient to kill approximately 50 per cent of the animals.

Essentially the same patterns of cAMP responses were found to apply to epinephrine, as shown in Fig. 3. The integrity of at least some of the beta-adrenergic functions of pertussis lungs so shown was further supported by the demonstration of the beta-adrenergic specificity of the pulmonary cAMP rise after epinephrine. As seen in Table 2, it was found that propranolol (0·68 mg/kg) lowered basal cAMP levels within 15 min by approximately 50 per cent and the response to 0.5 mg/kg of epinephrine was effectively blocked, while to 5 mg/kg significantly reduced.

In subsequent experiments, it was examined whether pertussis sensitization alters the reactivity of the pulmonary adenylate cyclase system to histamine, that is, the agent itself toward which the increased

Table 1. Basal levels of cAMP in whole lung from CFW mice after pertussis sensitization*

	Number of animals	cAMP (pmoles/mg tissue)
Control	62	1.65 ± 0.39†
Pertussis-sensitized (5 days)	66	1.52 ± 0.37

* Mice were sensitized by an i.p. injection of *B. pertussis* vaccine (Eli Lilly & Co.) diluted in 0.9% saline to a volume of 0.35 ml containing 7.2×10^9 killed pertussis cells. Lungs were removed 5 days later. Measurement of cAMP was by the protein binding method of Gilman [16].

† Mean ± standard deviation.

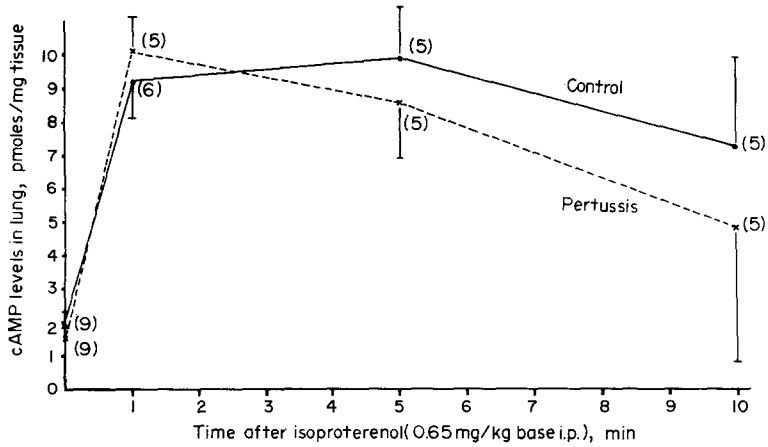

Fig. 1. Time course of cAMP changes after isoproterenol administration in lungs from pertussis-sensitized CFW mice. Animals were sensitized by an i.p. injection of pertussis vaccine (Eli Lilly & Co.) diluted in 0.9% NaCl to a volume of 0.35 ml containing 7.2×10^9 killed organisms. Five days later, animals received an i.p. injection of isoproterenol HCl in 0.9% NaCl with 0.01 M HCl. Tissues were frozen in liquid nitrogen at indicated time intervals. Figures in parentheses indicate the number of mice at each point. cAMP was measured by the protein binding method of Gilman [16], using duplicate determinations for each animal. Data are plotted as means ± the standard deviation.

susceptibility of the animal is manifested. In the first approach, time-course studies were conducted employing a standard dose of 70 mg/kg of histamine base, that is the dose adopted for testing the actual development of hypersensitivity. While the length of observation was 30 min in controls, it was only 10 min in the sensitized animals, since none of the latter survived for more than 12 min. As shown in Fig. 4,

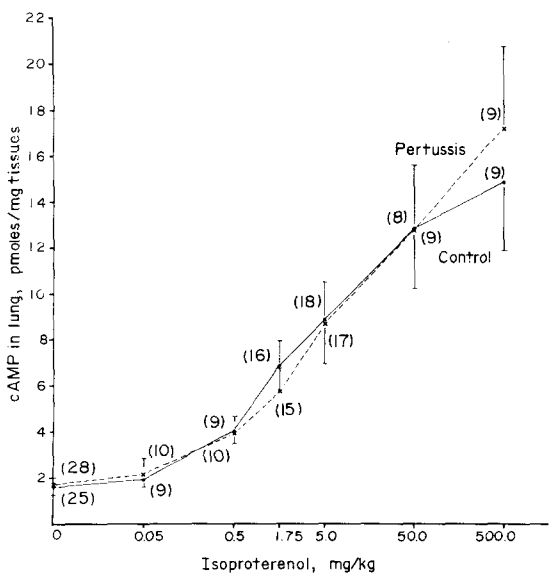

Fig. 2. Dose–response relationships of the isoproterenol-induced cAMP response in lungs from pertussis-sensitized CFW mice. Animals were sensitized as in Fig. 1 and 5 days later received an i.p. injection of isoproterenol HCl in 0.9% NaCl with 0.01 M HCl. Lungs were removed 5 min after isoproterenol administration. Figures in parentheses indicate the number of mice in each group. cAMP was measured by the protein binding method of Gilman [16], using duplicate determinations. Data are plotted as means ± the standard deviation.

control animals responded within 1 min with cAMP-level increases to a maximum of 163 per cent above baseline. Increases were smaller at 5 (148 per cent), 10 (138 per cent) and 15 (152 per cent) min and by 30 min levels were back to baseline (101 per cent). In animals sensitized with pertussis, the 1-min mean cAMP level (Fig. 4) was 326 per cent above that of baseline, which is twice the increase that occurred in control mice. cAMP levels remained in the same range at 5 min (313 per cent) and were still highly elevated (256 per cent) at 10 min.

In continuing the experiments, dose–response data were obtained for histamine, as plotted in Fig. 5. All doses of histamine caused a significant increase in pulmonary cAMP levels in both control and pertussis-sensitized animals. In the lower concentration range, histamine produced equivalent rises of cAMP in control and pertussis-sensitized animals. When higher doses of histamine were administered, the increases in cAMP levels of sensitized animals were greater than those observed in lungs of control mice. Thus, the maximum change in cAMP in control lung was 91 per cent above baseline, while the corresponding dose of histamine in lung of sensitized animals gave a value 333 per cent above the resting level.

The possibility that the above-mentioned pulmonary cAMP rise is an adrenergically mediated effect of histamine was then examined in propranolol-pretreated or adrenalectomized mice. The effect of propranolol on histamine-induced pulmonary cAMP changes is summarized in Table 3. It is seen that histamine caused again an increased accumulation of pulmonary cAMP with average increases somewhat greater in pertussis-vaccinated (145 per cent more than control) than in nonvaccinated (95 per cent) mice 5 min after histamine administration, but after 10 min they were approximately 30–34 per cent regardless of vaccination. In contrast, these increases were considerably smaller after treatment of mice with propranolol. Under these conditions, administration of histamine raised cAMP levels only 56 and 57 per cent at 5 min,

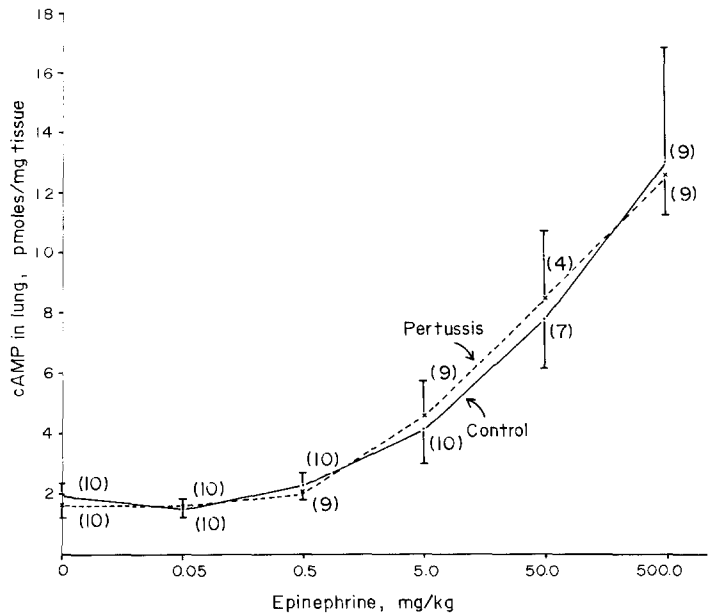

Fig. 3. Dose–response relationships of the epinephrine-induced cAMP response in lungs from pertussis-sensitized CFW mice. Animals were sensitized as in Fig. 1 and 5 days later received an i.p. injection of L-epinephrine bitartrate. Lungs were removed 5 min after epinephrine administration and frozen in liquid nitrogen for determination of cAMP by the protein binding method of Gilman [16]. Figures in parentheses indicate the number of mice in each group. Data are plotted as means ± the standard deviation.

and 4 and 15 per cent at 10 min relative to controls in nonvaccinated and vaccinated groups respectively. Thus, these histamine effects were about half or less than half as large as effects in the absence of propranolol.

Finally, the effects of bilateral adrenalectomy on the histamine-induced cAMP rise are shown in Fig. 6. As seen, basal cAMP levels were lower in adrenalectomized mice and the lung tissues did not respond by elevated cAMP levels after histamine challenge. Furthermore, the lack of response was apparently due to medullary catecholamine deprivation, since glucocorticoid replacement did not normalize the pulmonary basal cAMP level of the adrenalectomized mouse or restore the histamine response.

DISCUSSION

For reasons mentioned briefly above, and discussed extensively elsewhere [2, 3], the *B. pertussis*-induced hypersensitivity of mice served in this and several other laboratories as an experimental model for the analysis of the nature of the basic biochemical abnormality in bronchial asthma [18–24]. The hypersensit-

Table 2. Effect of propranolol on epinephrine-induced cAMP changes in lung from pertussis-sensitized CFW mice*

Treatment	cAMP (pmoles/mg tissue) Control	Pertussis-sensitized
None	1.98 ± 0.26† (10)‡	1.61 ± 0.40 (10)
Propranolol§	0.88 ± 0.18 (7)	0.95 ± 0.18 (8)
Propranolol plus epinephrine (0.5 mg/kg)‖	0.76 ± 0.14 (7)	0.99 ± 0.14 (9)
Propranolol plus epinephrine (5 mg/kg)	2.43 ± 1.16 (9)	3.21 ± 2.68 (9)
Epinephrine (0.5 mg/kg)	2.31 ± 0.37 (10)	2.03 ± 0.22 (10)
Epinephrine (5.0 mg/kg)	4.16 ± 1.20 (10)	4.59 ± 1.15 (9)

* Mice were sensitized by an i.p. injection of *B. pertussis* vaccine (Eli Lilly & Co.) diluted in 0.9% saline to a volume of 0.35 ml containing 7.2×10^9 killed pertussis cells. Lungs were removed 5 days later.

† Mean ± the standard deviation.

‡ Figures in parentheses are the number of animals used (duplicate determinations of cAMP by protein binding assay of Gilman [16] for each animal).

§ DL-Propranolol HCl (0.68 mg/kg) was injected i.p. and animals were sacrificed 15 min later.

‖ Ten min after injection of propranolol, L-epinephrine bitartrate was injected i.p. and tissues were taken 5 min after epinephrine.

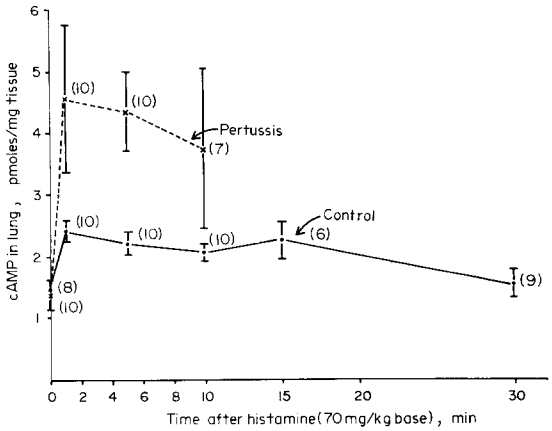

Fig. 4. Time course of histamine-induced cAMP changes in lungs from pertussis-sensitized CFW mice. Animals were sensitized as in Fig. 1 and 5 days later received an i.p. injection of 70 mg/kg of histamine as the base. Tissues were removed and frozen in liquid nitrogen at indicated time intervals. Figures in parentheses indicate the number of animals used to generate each point. cAMP was measured by the protein binding method of Gilman [16], using duplicate determinations. Data are plotted as means ± the standard deviation. Comparison of cAMP levels in lung from control vs pertussis-treated mice at 1, 5 and 10 min after histamine injection gave P values of < 0.001, < 0.001 and < 0.005 respectively.

ive state produced by the bacterial cells may be duplicated by pharmacological beta-adrenergic blockade, and, by several metabolic criteria, the pertussis-sensitized mouse behaves as though it were beta-adrenergically blocked.

Furthermore, since the postulated role of cAMP is that of an intracellular mediator of beta-adrenergic mechanisms, the possibility was raised that pertussis vaccination results in reduced cAMP formation in cells [3]. Subsequent investigations did in fact reveal a reduced cAMP synthesis to adrenergic stimulation in various tissues and cell systems of the pertussis-sensitized organism [10–15].

Under these conditions, and in view of the central role of pulmonary tissues in bronchial asthma, the present study was extended to include an examination of the possible involvement of cAMP in the pertussis-induced abnormality, using the lungs as the tissue of our inquiry.

Contrary to earlier experiences with other tissues [1–15], these investigations demonstrated that the resting level of cAMP, as well as the capacity of the pulmonary cAMP system to respond to adrenergic activation, as effected by naturally occurring or synthetic catecholamines, is unaltered by the pertussis sensitization of mice to histamine. In the interpretation of this conclusion, it is realized that the lung contains many diverse types of metabolically active cells that receive an abundant blood supply, any of which may possess an adenylate cyclase system capable of responding to a variety of stimuli. It must be noted, therefore, that the cells of pulmonary tissue which contained the elevated amounts of cAMP have not as yet been identified, and there is no evidence that smooth muscle, either vascular or bronchial, is involved in the response. Nevertheless, it is permissible to conclude that the integrity of at least some of the beta-adrenergic functions of lungs from pertussis-sensitized mice is preserved.

Another important finding in the experiments herein described is the pulmonary cAMP rise in both normal and pertussis-sensitized animals after the administration of histamine. There are at least four lines of evidence indicating that the increased amounts of cAMP may have resulted from an adrenergically mediated effect. First, both neuronal release of norepinephrine and depletion of adrenal medullary stores of epinephrine following exposure of mice or murine tissues to histamine were previously demonstrated [6, 25, 26]. Second, the increases in cAMP levels were greatly reduced by propranolol which is known to block beta-adrenergic

Table 3. Effect of propranolol on histamine-induced cAMP changes in lung from pertussis-sensitized ICR mice*

Treatment and time	Nonvaccinated cAMP (pmoles/mg tissue)	Nonvaccinated Per cent of control	Vaccinated cAMP (pmoles/mg tissue)	Vaccinated Per cent of control
None (control)	1.64 ± 0.12† (9)‡	100	1.98 ± 0.24 (9)	100
Histamine (5 min)	3.20 ± 0.25 (8)	195	4.85 ± 0.41 (10)	245
Histamine (10 min)	2.13 ± 0.12 (9)	130	2.66 ± 0.23 (10)	134
Propranolol§ (15 min)	1.27 ± 0.13 (7)	77	1.29 ± 0.20 (9)	65
Propranolol (20 min)	1.29 ± 0.22 (7)	79	1.26 ± 0.17 (10)	64
Propranolol (15 min) + histamine‖ (5 min)	1.98 ± 0.19 (8)	121	2.03 ± 0.13 (9)	103
Propranolol (20 min) + histamine (10 min)	1.34 ± 0.17 (10)	82	1.45 ± 0.10 (9)	73

* Mice were sensitized by an i.p. injection of *B. pertussis* vaccine (Eli Lilly & Co.) diluted in 0.9% saline to a volume of 0.35 ml containing 7.2 × 10^9 killed pertussis cells. Lungs were removed 5 days later.

§ DL-Propranolol HCl (0.68 mg/kg) was injected intraperitoneally and animals were sacrificed or further treated 15 or 20 min later.

‖ Ten min after injection of propranolol, histamine dihydrochloride (70 mg/kg of the base) was injected intraperitoneally and tissues were taken 5 or 10 min after histamine.

† Mean ± the standard deviation.

‡ Figures in parentheses are the number of animals used (duplicate determinations of cAMP by protein binding assay of Gilman [16] for each animal).

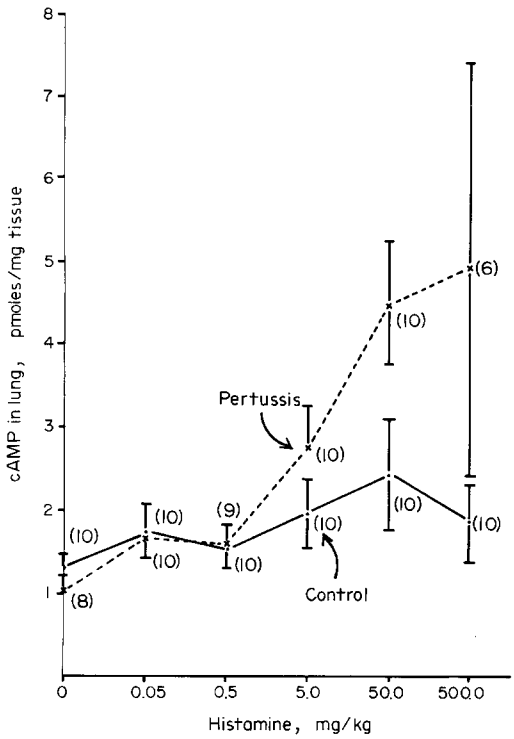

Fig. 5. Dose–response relationships of the histamine-induced cAMP response in lungs from pertussis-sensitized CFW mice. Animals were sensitized as in Fig. 1 and 5 days later received an i.p. injection of histamine. Lungs were removed 5 min after histamine administration and frozen in liquid nitrogen for determination of cAMP by the protein binding method of Gilman [16]. Figures in parentheses indicate the number of animals used to generate each point. Data are plotted as means ± the standard deviation. Statistical analysis of cAMP lung levels from control vs pertussis-treated mice provided P values of < 0.005, < 0.001 and < 0.005 at histamine doses of 5.0, 50.0 and 500.0 mg/kg respectively.

receptor sites. Third, resting cAMP levels were shown to be lower in adrenalectomized mice and the lung tissues did not respond by elevated cAMP levels after histamine administration, nor did glucocorticoid replacement normalize the basal pulmonary cAMP level of the adrenalectomized mouse or restore the histamine response. Fourth, in earlier experiments [6], the catecholamine-depleting efficiency of histamine was found to be considerably greater in the pertussis-sensitized than in the normal animals. For example, 5 mg/kg of histamine given to pertussis-sensitized mice produced a catechol depletion of adrenal medullary stores comparable to that caused by 500 mg/kg in normal mice. Consequently, these earlier findings could account for those results of the present experiments which showed that histamine produces a much larger pulmonary cAMP increase in pertussis-sensitized than in normal animals.

These findings and considerations provide sufficient justification for the postulate that the histamine-induced increase in pulmonary cAMP accumulation is an adrenergically mediated effect. Nevertheless, the possibility that some of the rise in cAMP level is due to more direct effects of histamine on pulmonary tissue cannot be entirely excluded, since propranolol did not eliminate the histamine-induced cAMP rise altogether. Furthermore, although the possible influence of species differences and dosage remains unresolved, cAMP elevations in guinea pig, rat and rabbit lung slices to added histamine *in vitro* have been reported by Palmer [27, 28].

Finally, the question arises whether there is any relation between the present findings and the mechanism of the pertussis-induced hypersensitivity to histamine. Increases in cAMP themselves cannot be responsible for the histamine hypersensitivity, since strong evidence is available that agents and stimuli known to cause intracellular accumulations of cAMP protect against rather than promote histamine susceptibility [3]. Also, propranolol, which was shown to reduce accumulations of cAMP, increases sensitivity to histamine.

However, another alteration of cyclic nucleotide metabolism, involving the excessive pulmonary accumulation of guanosine-3′,5′-cyclic monophosphate (cGMP) might be a more meaningful factor in the developmental mechanism of the pertussis-induced hypersensitivity. Cyclic GMP, in addition to being a proposed functional antagonist of cAMP in several tissues including lung [29], has also been shown by our laboratory to accumulate after histamine administration in the lungs of pertussis-vaccinated, but not nonvaccinated mice [30]. Furthermore, pharmacological beta-adrenergic blockade, which has

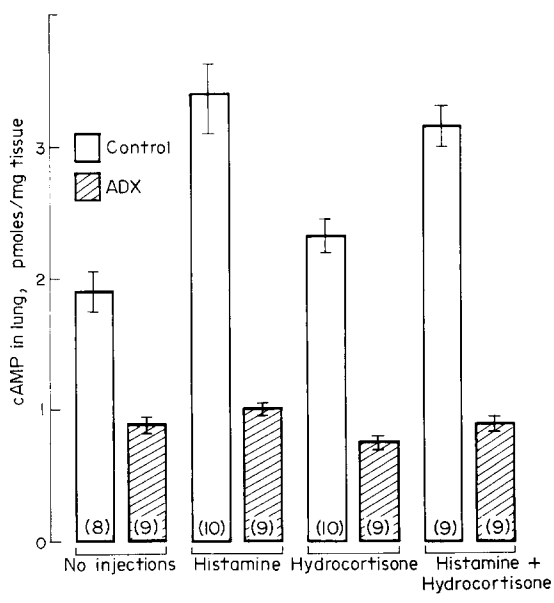

Fig. 6. Effect of bilateral adrenalectomy with or without hydrocortisone replacement on histamine-induced cAMP changes in lung from CD mice. Animals were maintained on 0.9% NaCl until sacrifice. Before experimentation commenced on post-operative day b, the mice were given three i.p. injections of hydro-cortisone-21-phosphate in single doses of 25 mg/kg. These were administered at 12-hr intervals with sacrifice and/or drug treatment at 3 hr after the last injection. cAMP was measured by the protein binding method of Gilman [16] and expressed as nmoles/g of tissue. The bars represent the means of cAMP levels in each group and the vertical lines are the standard deviations. Figures in parentheses indicate the number of animals used in each group.

been shown to duplicate the pertussis-induced histamine hypersensitivity [1–8], also caused increased accumulation of pulmonary cGMP after histamine [30]. These findings suggest involvement of cGMP in the pertussis-induced abnormality and, by implication, in the pathogenesis of bronchial asthma [3, 7, 31–38].

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REFERENCES

1. C. W. Fishel, A. Szentivanyi and D. W. Talmage, in *Bacterial Endotoxins* (Eds. M. Landy and W. Braun), p. 474. Rutgers University Press, New Brunswick, N. J. (1964).
2. A. Szentivanyi, in *Textbook of Immunological Diseases* (Ed. M. Samter), p. 356. Little, Brown & Co., Boston (1971).
3. A. Szentivanyi, *J. Allergy* **42**, 203 (1968).
4. C. W. Fishel, A. Szentivanyi and D. W. Talmage, *J. Immun.* **89**, 8 (1962).
5. C. W. Fishel and A. Szentivanyi, *J. Allergy* **34**, 439 (1963).
6. A. Szentivanyi, C. W. Fishel and D. W. Talmage, *J. infect. Dis.* **113**, 86 (1963).
7. R. G. Townley, I. L. Trapani and A. Szentivanyi, *J. Allergy* **39**, 177 (1967).
8. A. Szentivanyi, S. Katsh and R. G. Townley, *J. Allergy* **41**, 107 (1968).
9. S. Katsh, D. G. Halkias and A. Szentivanyi, *J. Allergy* **43**, 171 (1969).
10. R. A. Ortez, T. W. Klein and A. Szentivanyi, *J. Allergy* **45**, 111 (1970).
11. R. A. Ortez, T. W. Klein and A. Szentivanyi, *Fedn Proc.* **29**, 640 (1970).
12. R. A. Ortez, C. W. Fishel and A. Szentivanyi, *Fedn Proc.* **31**, 748 (1972).
13. C. W. Parker and S. I. Morse, *J. exp. Med.* **137**, 1078 (1973).
14. R. A. Ortez, *Fedn Proc.* **33**, 586 (1974).
15. R. A. Ortez, D. Seshachalam and A. Szentivanyi, *Biochem. Pharmac.* **24**, 1297 (1975).
16. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
17. G. W. Snedecor, in *Statistical Methods*, p. 91. Iowa State University Press, Ames, Iowa (1956).
18. C. W. Parker and J. W. Smith, *J. clin. Invest.* **53**, 48 (1973).
19. C. W. Parker, M. L. Baumann and M. G. Huber, *J. clin. Invest.* **53**, 1336 (1973).
20. C. W. Parker, M. G. Huber and M. L. Baumann, *J. clin. Invest.* **53**, 1342 (1973).
21. R. G. Coffey, P. J. Logsdon and E. Middleton, Jr., *J. Allergy clin. Immun.* **49**, 87 (1972).
22. P. J. Logsdon, E. Middleton, Jr. and R. G. Coffey, *J. Allergy* **50**, 45 (1972).
23. P. J. Logsdon, D. V. Carnright, E. Middleton, Jr. and R. G. Coffey, *J. Allergy clin. Immun.* **52**, 148 (1973).
24. E. Gillespie, M. D. Valentine and L. M. Lichtenstein, *J. Allergy clin. Immun.* **53**, 27 (1974).
25. A. Szentivanyi, D. G. Halkias and S. Katsh, *Proceedings of the Twenty-fifth Annual Meeting of the American Academy of Allergy* (abstr. 65). Bal Harbour, Fl., May 15–19, 1969.
26. A. Szentivanyi and C. W. Fishel, in *Pathogenese und Therapie allergischer Reaktionen. Grundlagenforshung und Klinik* (Ed. G. Filipp), pp. 588–683. Ferdinand Enke Verlag, Stuttgart, Germany (1966).
27. G. C. Palmer, *Biochim. biophys. Acta* **252**, 561 (1971).
28. G. C. Palmer, *Biochem. Pharmac.* **21**, 2907 (1972).
29. J. F. Kuo and W. N. Kuo, *Biochem. biophys. Res. Commun.* **55**, 660 (1973).
30. J. B. Polson, J. J. Krzanowski and A. Szentivanyi, *Res. Commun. Chem. Path. Pharmac.* **9**, 243 (1974).
31. A. Szentivanyi and C. W. Fishel, *Acta allerg.* **29** (suppl. 11), 26 (1974).
32. J. B. Polson, J. J. Krzanowski and A. Szentivanyi, *J. Allergy clin. Immun.* **53**, 100 (1974).
33. J. J. Krzanowski, J. B. Polson and A. Szentivanyi, *J. Allergy clin. Immun.* **53**, 100 (1974).
34. J. J. Krzanowski, J. B. Polson and A. Szentivanyi, *Pharmacologist* **16**, 212 (1974).
35. J. B. Polson, J. J. Krzanowski and A. Szentivanyi, *Pharmacologist* **16**, 212 (1974).
36. J. J. Krzanowski, J. B. Polson and A. Szentivanyi, in *Advances in Cyclic Nucleotide Research* (Eds. G. Drummond, P. Greengard and G. A. Robison), Vol. 5, p. 815. Raven Press, New York (1975).
37. J. B. Polson, J. J. Krzanowski and A. Szentivanyi, in *Advances in Cyclic Nucleotide Research* (Eds. G. Drummond, P. Greengard and G. A. Robison), Vol. 5, p. 815. Raven Press, New York (1975).
38. A. Szentivanyi and C. W. Fishel, in *Bronchial Asthma: Mechanisms and Therapeutics* (Eds. M. S. Segal and E. B. Weiss), Little, Brown & Co., Boston, in press.