Biochemical Pharmacology, Vol. 25, pp. 523–526. Pergamon Press, 1976. Printed in Great Britain.

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

RICHARD A. ORTEZ

Department of Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77025, U.S.A.

(*Received* 13 *August* 1974; *accepted* 15 *July* 1975)

Abstract— The effect of histamine on lung cAMP metabolism in normal and *Bordetella pertussis*-vaccinated mice was investigated. Histamine was shown to elevate lung cAMP in both types of animals in a dose-dependent manner. Pertussis-vaccinated mice, however, showed a marked shift to the left in the histamine dose–response curve. The ED_{50} for normal and pertussis-vaccinated mice was 8·0 and 0·25 mg/kg respectively. The histamine-induced rise in cAMP was maximum 1 min after injection and diminished thereafter. The effect of histamine on pulmonary cAMP may be mediated indirectly, as it occurred *in vivo* but not *in vitro*.

Vaccination of certain strains of mice with whole cells, or cell fractions, of the bacterium *Bordetella pertussis* induces in those animals an increased sensitivity to histamine [1–3]. This hypersensitivity to histamine is one observation that led to the suggested use of the pertussis-vaccinated mouse as an animal model for the study of bronchial asthma [4]. 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 [5, 6] led to the speculation that a beta-adrenergic blockade may be involved [4]. Investigations of spleen adenosine 3′,5′-monophosphate (cAMP) metabolism in normal and pertussis-vaccinated mice has supported that concept [7, 8]. Histamine-induced death in pertussis-vaccinated mice is preceded by cyanosis and rapid labored breathing suggestive of possible respiratory impairment. Because histamine is a potent constrictor of bronchial smooth muscle [9] and because recent evidence suggests that it may act in part through cAMP [10, 11], the present investigation was undertaken to evaluate the effects of histamine on cAMP metabolism in lungs of normal and pertussis-vaccinated mice. A preliminary report of these studies was given at the 1974 meeting of the Federation of American Societies for Experimental Biology [12].

MATERIALS AND METHODS

Animals. Female CFW mice (20–24 g) were obtained from Carworth Animal Farms, New York, N.Y., U.S.A., caged in groups of six to ten, and fed *ad lib.*

Sensitization. Merthiolated suspensions of *B. pertussis* (4×10^{10} cells/ml, Eli Lilly & Co., Indianapolis, Ind., U.S.A.) were diluted 1 : 2 in sterile, pyrogen-free physiological saline. The diluted vaccine (0·5 ml) was injected intraperitoneally (i.p.) in a single dose to sensitize the mice. The animals were sacrificed 5 days after vaccination.

Challenge and collection of samples. Histamine diphosphate was dissolved in pyrogen-free physiological saline before each experiment. Mice received the indicated doses of histamine base in a volume of 0·025 ml/g, i.p. Control animals received an equivalent volume of saline. The animals were killed by cervical dislocation after a sharp blow to the base of the skull, unless otherwise indicated [13]. After sacrifice, the tissues were removed, frozen rapidly between blocks of dry ice and subsequently analyzed for cAMP content.

Extraction and measurement of cAMP. In most of the studies, cAMP extraction was accomplished by the procedure described by Armstrong *et al.* [14], modified by the addition of an aluminum oxide (0·25 g/ml for 30 min) adsorption step between Tris neutralization and application to the Dowex 2 column [15]. After lyophilization and reconstitution in 100 mM acetate buffer (pH 4·0), cAMP was determined by the method of Gilman [16], modified by the use of charcoal to adsorb out free [^{3}H]-cAMP as described by Brown *et al.* [17]. Radioactivity was assayed in Bray's counting solution in a Beckman liquid scintillation system [18]. In some studies, cAMP extraction and determination were performed using the procedures outlined by Robison *et al.* [19] with comparable results.

In vitro experiments. Chopped lung preparations were prepared and incubated as described by Palmer [10, 11]. Normal and pertussis-vaccinated mice were killed and their lungs removed and placed in cold (4) Krebs Ringer bicarbonate buffer (pH 7·4) containing glucose (1·8 mg/ml). The tissue was washed to remove excess blood and chopped on a McIlwain tissue chopper into 1-mm cubes. The tissue fragments were washed three times and then preincubated at 37 in a Dubnoff metabolic shaker incubator for 45 min. The incubation fluid was changed two times during this period. Sample gassing with an O_2 CO_2 mixture (95 : 5) was maintained throughout all the above operations. At the end of the preincubation period, the test agents, dissolved in the same buffer, were added and the incubation was continued for an additional 15 min. The incubation was terminated by acidification (0·4 N final concentration) with perchloric acid and homogenization of the total contents of

Table 1. Effect of histamine on cyclic AMP levels in tissues of normal and pertussis-vaccinated mice*

Tissue	Normal control	Normal + histamine	Pertussis control	Pertussis + histamine
Spleen	10·3 ± 0·9	8·8 ± 1·2	6·7 ± 1·1	6·4 ± 0·6
Heart	4·7 ± 0·7	6·4 ± 0·7	5·3 ± 0·8	6·5 ± 0·4
Abdominal muscle	9·2 ± 0·6	9·3 ± 0·6	8·7 ± 0·5	10·6 ± 0·7
Kidney	6·7 ± 0·8	9·1 ± 1·3	8·0 ± 1·5	9·3 ± 1·8
Skeletal muscle	4·5 ± 0·2	5·8 ± 0·4	6·6 ± 1·2	7·5 ± 0·7
Small intestine	17·6 ± 1·9	13·6 ± 0·7	21·1 ± 2·5	13·0 ± 0·5
Liver	4·5 ± 0·6	5·7 ± 0·3	6·2 ± 0·3	7·3 ± 0·3
Lung	15·3 ± 1·3	17·9 ± 1·5	12·7 ± 1·0	28·7 ± 2·4

* Mice received 25 mg/kg of histamine base (as the diphosphate salt) i.p. 20 min prior to sacrifice. The animals were killed by enclosure in a chloroform-saturated environment followed by cervical dislocation. Values of cAMP are expressed as pmoles/mg of protein ± S. E. M. and represent the mean of 12 determinations. Cyclic AMP was extracted and measured by the method of Robison *et al.*[19].

the incubation vessel in a Polytron tissue homogenizer. Cyclic AMP was determined as described above. Cell-free adenylate cyclase activity measurements were performed using the procedure of Thompson *et al.* [20].

Protein determination. Protein was determined by the method of Lowry *et al.* [21] using bovine serum albumin fraction V as standard.

Reagents. Histamine diphosphate, epinephrine-HCl, cAMP, theophylline and Dowex 2 (100–200 mesh) were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.: [^{3}H]cAMP from Schwarz/Mann, Orangeburg, N.Y., U.S.A.; aluminum oxide (neutral, activity grade I) from M. Woelm, West Germany; and the remainder of the chemicals from Fisher Scientific Co., Pittsburg, Pa., U.S.A.

RESULTS

Tissue cAMP levels after pertussis vaccination and/or histamine challenge. Cyclic AMP levels were determined for a number of tissues after pertussis vaccination and/or histamine challenge. The results shown in Table 1 reveal that the normal resting cAMP content of the tissues examined ranged from 4·5 pmoles/mg of protein in liver and skeletal muscle to 17·6 pmoles/mg of protein for small intestine. Histamine challenge of normal animals elicited no significant alteration in the cAMP content of any of the tissues examined except small intestine where a significant ($P < 0·05$) drop in cAMP content was observed. Pertussis vaccination caused no significant alteration in the cAMP content of any of the tissues examined except spleen [7, 8]. The combination of pertussis vaccination and histamine challenge elicited no alterations in cAMP content of any of the tissues examined except lung, where an approximate 100 per cent increase from 15·3 ± 1·3 to 28·7 ± 2·4 pmoles/mg of protein occurred. This latter value was significantly different by the Student *t*-test ($P < 0·05$) from lung cAMP levels observed under other experimental conditions.

Time of the histamine-induced cAMP accumulation in lung. To study the relationship between time after histamine challenge and lung cAMP elevation, animals were sacrificed at various time intervals after an 8 mg/kg i.p. injection of histamine. Figure 1 shows that the increase in cAMP seen in pertussis-vaccinated mice was greater at 1 min after histamine injection and diminished throughout the remainder of the observation period. A similar rapid initial rise and subsequent decline could be seen in normal animals but was much less pronounced.

Effect of histamine dose on cAMP accumulation in lung. Normal and pertussis-vaccinated mice received doses of histamine ranging from 0·13 to 64 mg/kg. Two min later they were killed and the lung cAMP content was determined. It is apparent from these results (Fig. 2) that the dose–response curve for vaccinated mice has been shifted to the left with a change in the ED_{50} from 8·0 to 0·25 mg/kg.

Effect in vitro *theophylline, histamine and epinephrine on cAMP accumulation.* The results of additions of these agents *in vitro* to the chopped lung preparations are summarized in Table 2. Tissues from both normal and pertussis-vaccinated mice responded

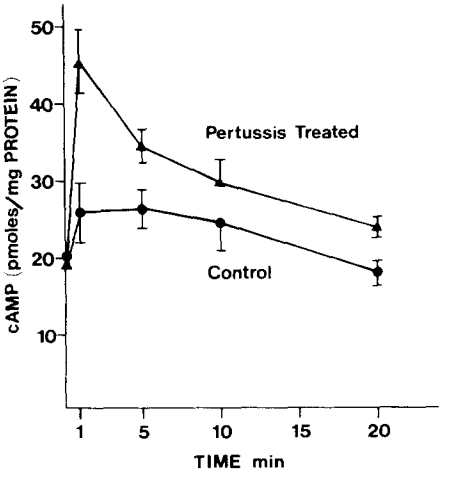

Fig. 1. Effect of histamine on cAMP content of lung tissue in normal and pertussis-vaccinated mice. Animals received 8 mg/kg of histamine base. At the times indicated, they were killed and lung cAMP was determined. The values are expressed as pmoles/mg of protein. Each point represents the mean ±S. E. M. 12 determinations. Key: (●) normal animals; and (▲) pertussis-vaccinated animals.

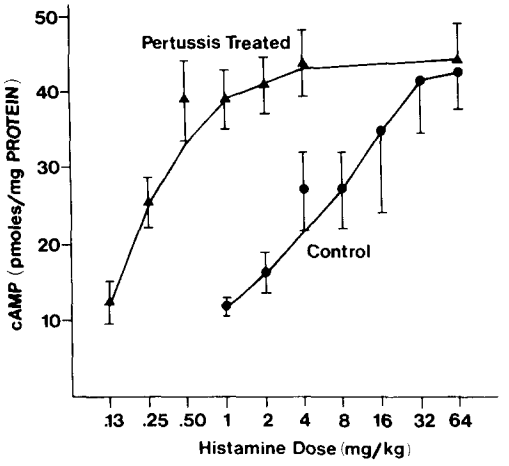

Fig. 2. Effect of varying doses of histamine on the cAMP content of lung tissue from normal and pertussis-vaccinated mice. The animals were killed 2 min after i.p. administration of histamine. Values are expressed as pmoles/mg of protein ± S. E. M. of 12 determinations. Key: (●) control animals; and (▲) pertussis-vaccinated animals.

to theophylline (3 mM) with approximate 150 per cent increases in cAMP. Neither tissue type responded to histamine (5×10^5 M). The addition of both theophylline and histamine acted synergistically in increasing cAMP levels in both tissue types by 250 per cent. Addition of epinephrine (5×10^{-5} M) caused a marked increase in cAMP in both tissue types. The same pattern of histamine and epinephrine stimulatory effects were seen when cell-free homogenates were measured for adenylate cyclase activity (Table 3).

DISCUSSION

The current studies demonstrate that pertussis vaccination leads to a significant alteration in cAMP metabolism in lung tissue. While pulmonary cAMP levels of both normal and pertussis-vaccinated mice increase after histamine administration, the responses are quantitatively different. Pertussis-vaccinated mice respond to much lower doses of histamine. This increased sensitivity is most apparent from the 30-fold shift of the ED_{50} from approximately 8·0 to 0·25 mg/kg. In pertussis-vaccinated animals, the maximal stimulated levels remained unchanged but were reached with a fraction of the dose required in normal animals. Further, this accumulation of cAMP was shown to be a very rapid and relatively short-lived response. The maximum response was seen as early as 1 min after histamine administration and diminished rapidly thereafter. This may be an important consideration when attempting to correlate these changes with histamine-induced shock and death. Animals appear relatively unaffected at 1–2 min after histamine administration, a period when cAMP levels are highest, but appear to be in a pronounced state of shock after 15–20 min, a period coincident with a return in pulmonary cAMP levels to near normal. This might suggest that either the two events (elevated lung cAMP and death) are unrelated causally or the initial rise in cAMP sets off a chain of events which can persist and produce death after cAMP levels have returned to near normal.

While it is obvious from these data that cAMP metabolism is significantly altered in lungs of pertussis-vaccinated mice, there is no evidence presented here which is incompatible with the suggestion that cGMP rather than cAMP could be involved in histamine-induced shock and death in pertussis-vaccinated mice [22]. It is possible that the increase in cAMP might occur as part of a homeostatic adjustment in response to histamine administration. Bourne *et al.* [23] have demonstrated that histamine causes an increase in leukocyte cAMP and have suggested that this may function as a negative feedback mechanism to limit further release of histamine and thus limit the inflammatory process. According to this concept, the increase in pulmonary cAMP may be the result of an attempt to avoid death, rather than a contributing cause of it.

Table 3. Adenylate cyclase activation *in vitro* with histamine or epinephrine*

	Normal	Pertussis
Control	174 ± 25	202 ± 10
Histamine		
(10^{-6} M)	164 ± 4	181 ± 11
(10^{-5} M)	167 ± 31	165 ± 10
(10^{-4} M)	172 ± 7	173 ± 1
Epinephrine		
(10^{-6} M)	308 ± 80	329 ± 26
(10^{-5} M)	377 ± 90	364 ± 21
(10^{-4} M)	442 ± 29	395 ± 26
NaF		
(10^{-2} M)	808 ± 74	709 ± 33

* Values are expressed as pmoles cAMP formed/min/mg of protein. Each value is the mean ± S. E. M. of four determinations.

Table 2. Effects *in vitro* of theophylline, histamine and epinephrine on chopped lung fragments from normal and pertussis-vaccinated mice*

	Normal	Pertussis
Control	14·7 ± 1·8 (16)	11·1 ± 1·1 (12)
Theophylline	37·4 ± 4·3 (4)	29·8 ± 4·8 (4)
Histamine	13·6 ± 2·0 (12)	11·9 ± 1·2 (6)
Theophylline + histamine	49·5 ± 6·7 (4)	39·5 ± 7·0 (4)
Epinephrine	90·2 ± 8·0 (14)	117·9 ± 18·1 (8)

* Values are expressed as pmoles cAMP/mg of protein ± S. E. M. Theophylline concentration was 3 mM while histamine and epinephrine were each 5×10^{-5} M. The numbers in parentheses represent the number of determinations.

Polson *et al.* [22] failed to observe these striking differences in cAMP metabolism between normal and pertussis-vaccinated mice. From our data it can be seen that the reasons for this could have been their selection of a dose of histamine (70 mg/kg) and time after administration (5 and 10 min) which were not conducive to detection of the difference.

The cell type(s) in the lung responsible for the increased cAMP cannot be deduced from the current studies. However, a reasonable candidate would be cells of the lymphoid series shown to infiltrate the lungs of pertussis-vaccinated mice in large numbers [24]. This would certainly be consistent with the hypothesis that the increased cAMP is the result of histamine acting in a negative feedback capacity to limit its own release. Failure to observe a similar histamine effect on cAMP in spleens of these animals, which also undergo a marked increase in lymphoid cell content, does not detract from this idea, since there is no evidence that the cell types accumulating in lung and spleen are similar either morphologically or functionally.

The failure of the administration of histamine *in vitro* to induce cAMP accumulation in either chopped lung or cell-free homogenates suggests that a direct interaction between histamine and the enzymes regulating cAMP metabolism (adenylate cyclase and phosphodiesterase) is not involved. This raises the possibility that histamine may be acting through some as yet unidentified intermediate. Failure to demonstrate a difference in the effect of epinephrine *in vitro* on cAMP metabolism in normal and pertussis-vaccinated mouse lung suggests that epinephrine may not be that intermediate as has been suggested [22, 25]. However, more extensive studies both *in vivo* and *in vitro* with a wide range of catecholamines as well as agonists and antagonists are needed to elucidate their role if any in this phenomenon. Another class of compounds which require evaluation in this regard are the prostaglandins, most especially those of the E and F series, which have been shown to regulate smooth muscle tone [26–28], modulate the inflammatory response [29], and influence cAMP metabolism [28–30].

Acknowledgements—This research was supported by a grant (HL-18250) from the U.S. Public Health Service. I am grateful to Ms Elizabeth Norris and Mrs. Susie Rubit for technical assistance, and to Drs. G. Alan Robison and Thomas F. Burks for helpful discussions.

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