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EFFECTS OF DIBUTYRYL-3′,5′-CYCLIC ADENOSINE MONOPHOSPHATE, PHOSPHODIESTERASE INHIBITORS AND PROSTAGLANDIN E_1 ON COMPOUND 48/80-INDUCED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS *IN VITRO*

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Abstract—The release of histamine from rat peritoneal mast cells by compound 48/80 was inhibited by high concentrations of ^6N-2′*O*-dibutyryladenosine-3′,5′-cyclic-monophosphate (DB-c-AMP). Several other adenine nucleotides were ineffective at comparable concentrations. The histamine release promoted by compound 48/80 was also inhibited by prostaglandin E_1 (PGE_1) and the phosphodiesterase inhibitors, theophylline, reserpine, diethylaminoethylreserpine and perphenazine. With the exception of theophylline, these inhibitors themselves produced a less dramatic release of histamine. Although the concentrations required for inhibition of histamine release are quite high, the data obtained for DB-c-AMP, phosphodiesterase inhibitors and PGE_1 can be viewed as circumstantial evidence in support of a role for c-AMP in the histamine release process of the mast cell.

A MAJOR store of histamine in mammals is known to reside in the cytoplasmic granules of tissue mast cells, where the amine appears to be bound in a heparin–protein complex. Because of the probable participation of histamine in certain allergic and drug reactions, the mechanism of its release from mast cells in response to various agents such as compound 48/80, dextran or antigen–antibody complexes has been studied in detail.[1–9] Rat peritoneal mast cells can be readily employed for investigations *in vitro* of the mode of binding and mechanism of release of histamine, since these cells can be obtained easily.*

Goth *et al.*[9,10] have recently reported the release of histamine *in vitro* from rat peritoneal mast cells by the polymer dextran, but only in the presence of the phospholipid phosphatidyl-L-serine. Baxter *et al.*[11] have also recently reported some very interesting observations concerning the characteristic anaphylactoid response produced in rats by the administration of dextran, which led to their postulate that cyclic-3′,5′-adenosinemonophosphate (c-AMP)[12] might be involved in the mast cell histamine release process. Lichtenstein and Margolis[13] had previously postulated a possible

* Studies on peritoneal mast cell suspensions indicate that the so-called "selective" or "specific" release of histamine[1,2] from mast cells by the polymeric amine, compound 48/80, or release in response to certain antigen–antibody reactions, involves the following: (1) attachment of the releasing agent to the cell membrane;[7] (2) selective and extremely rapid degranulation of the cell by an energy-requiring "exocytosis", liberating, without disruption of the cell membrane, only histamine-containing granules;[1–4] and finally (3) liberation of histamine from a histamine–heparin–protein complex by a process of ion exchange with cations of the extracellular fluid.[5–8]

inhibitory role of c-AMP in the allergen-promoted release of histamine from human leukocytes. Baxter *et al.*[11] have observed that the dibutyryl derivative of c-AMP (DB-c-AMP), epinephrine, isoproterenol and the c-AMP phosphodiesterase inhibitor theophylline can protect rats against the characteristic anaphylactoid response and also effectively block the normal elevation of blood histamine found after the administration of dextran. However, any interpretation of the possible inhibitory role of the adenyl cyclase system is unfortunately complicated by the similar inhibitory effects of glucose. The observations of Baxter *et al.* stimulated our study of the effects *in vitro* of adenine nucleotides and known c-AMP phosphodiesterase inhibitors on the release of histamine by dextran or compound 48/80 in the rat peritoneal mast cell system.

MATERIALS AND METHODS

Cell preparation and histamine assay. Mixed peritoneal cells were obtained from male Sprague–Dawley rats (200–300 g) essentially according to the method of Uvnäs and Thon,[14] as modified by Moran *et al.*[3] The animals were anesthetized with ether, then slowly injected intraperitoneally with 10–15 ml of a specially prepared buffered physiological solution warmed to 37°. The buffered solution consisted of the following: NaCl, $1{\cdot}54 \times 10^{-1}$ M; KCl, $2{\cdot}7 \times 10^{-3}$ M; $CaCl_2$, 9×10^{-4} M; D-glucose, $5{\cdot}6 \times 10^{-3}$ M; human serum albumin, 1·0 g/l. (Pentex Inc.); and 10% by volume of a Sorensen buffer containing $Na_2HPO_4 . 7\ H_2O$(3×10^{-2} M) and $NaH_2PO_4 . H_2O$ ($3{\cdot}5 \times 10^{-2}$ M). The pH of the solution was adjusted finally to 7·0 with 1 N NaOH. After massaging the abdomen gently for 2 min, the animals were decapitated and exsanguinated, the abdominal wall was opened at midline, and the peritoneal fluid removed into a syringe by needle puncture of the peritoneal membrane. Chilled cell suspensions were centrifuged at about 200 *g* for 3–5 min, the supernatant fraction was carefully removed, and the cells at the bottom of the tube were resuspended in buffered medium. A pooled suspension from two or more rats was normally employed for a day's experiments (final cell suspension in 2 ml of solution per rat). Because of the consistent and uniform response of such cell mixtures to histamine-releasing agents, no effort was made to prepare homogeneous mast cell preparations by density gradient centrifugation procedures. For incubations, 200-μl volumes of the suspension in small glass centrifuge tubes were normally diluted to 400 μl with buffer containing releasing agents or other test compounds dissolved immediately before use. Adjustment of the pH of these solutions to 7·0 was made when necessary. Incubations were carried out at 37° with gentle agitation in a metabolic shaker. After incubations were completed, tubes were centrifuged at about 200 *g* for 3 min, and 50- and 100-μl aliquots of each supernatant fraction assayed for histamine according to the method of Shore *et al.*[15] Preliminary investigations revealed that no extraction procedures were necessary to remove interfering substances. Aliquots were diluted directly to 2·0 ml with water and 0·4 ml of 1 N NaOH was added, followed by 0·1 ml *o*-phthalyldialdehyde (10 mg/ml in methanol). After 4 min, samples were acidified with 0·2 ml of 3 N HCl and the fluorescence intensity was read at 450 mμ with activation at 350 mμ, employing an Aminco–Bowman spectrophotofluorimeter (American Instrument Company). Total histamine content of cell suspensions was determined, using the same procedure for assay of supernatants obtained by dilution of the suspension with equal volumes of water and 20% trichloroacetic acid, followed by centrifugation to remove the protein precipitate. Results could be expressed as the percentage of total histamine released

per milligram of protein (protein determined by the method of Lowry *et al.*[16]) or per million mast cells, according to the procedure of Bray and van Arsdale.[17] Possible interference of test compounds with the fluorimetric assay procedure was investigated and eliminated by employing standard histamine solutions. All data recorded in the tables in this paper were compiled by averaging two histamine assays on individual incubations, then averaging the values when obtained for several separately prepared incubations (usually five, as indicated in the tables). In addition, all experiments were repeated at least twice on different days using newly prepared cell preparations, with essentially parallel results. In all data given in this paper, values for spontaneous histamine release (controls) are given. For incubations containing test compounds, total percentage histamine release is given; that is, the control value has not been subtracted.

Materials. The following materials employed in these studies were obtained from the sources indicated: compound 48/80 (Burroughs Wellcome Company); dextran polymer (molecular weight, approximately two million), histamine dihydrochloride and theophylline (Nutritional Biochemicals Corp.); reserpine and *o*-phthalyldialdehyde (OPT) (Aldrich Chemical Company); adenosine, adenosine-3′,5′-cyclic-monophosphate (c-AMP), adenosine-5′-triphosphate . 2 K(5′-ATP) and DL-*N*-isopropylarterenol . HCl (isoproterenol) (Calbiochem Corp.); adenosine-5′-monophosphate (5′-AMP) (Pabst Chemical Company); adenosine-5′-diphosphate . 1 Na (5′-ADP) (Sigma Chemical Company);[6] *N*-2′-*O*-dibutyryladenosine-3′,5′-cyclic-monophosphate . 1 Na (DB-c-AMP), (Boehringer Mannheim Corp.); 1-diethylaminoethylreserpine-(DEAE-reserpine) bitartrate (kind gift of Dr. Jean Comar, Lavril Laboratories, Paris, France); perphenazine as an injectable solution containing 5 mg perphenazine per ml (Trilafon; Schering Corp.); prostaglandin E_1 (PGE_1; kind gift of the Upjohn Pharmaceutical Company).

EXPERIMENTAL AND RESULTS

Compound 48/80. Preliminary experiments with compound 48/80 indicated that histamine release from peritoneal cell mixtures occurs extremely rapidly, as reported by Moran *et al.*[3] in their studies with isolated rat peritoneal mast cells. Using 5-min incubation times with compound 48/80, the dose–response curve obtained paralleled quite closely that obtained by these workers[3] and by Uvnäs and Thon[4] using isolated mast cells, with release approaching maximal values at 48/80 concentrations of about 1 μg/ml. Values for percentage histamine released with any given suspension were found to be quite reproducible and reliable at concentrations of 48/80 between 0·5 and 2·0 μg/ml. However, as indicated in the tables and in the data of others,[2–4] values for percentage histamine release at a given 48/80 concentration varied considerably from suspension to suspension.

High molecular weight dextran polymer. Employing the methods and solutions described above and a dextran polymer with an average molecular weight of about two million, no histamine release *in vitro* from mast cells above control values of about 7 per cent was observed over a period of 60 min at 37°, even at a concentration of 2·5 mg/ml of dextran. In contrast, 43 per cent histamine release was observed for the same cell suspension when another portion of it was incubated with compound 48/80 (0·75 μg/ml) for 5 min. The experiment was repeated once with similar negative results. Baxter *et al.*[11] report a dramatic elevation of plasma histamine *in vivo* with

the same dextran. A recent report by Goth *et al.*[10] indicates that the presence of phosphatidyl-L-serine (PS) may be a requirement for histamine release *in vitro* promoted by dextran. Details of this work have not yet appeared.* We have not succeeded in observing this effect under our experimental conditions, using a dextran of average molecular weight 2,000,000 (2·5 mg/ml) and a commercially available PS at a concentration as high as 50 μg/ml (incubation time, 45 min at 37°). However, several known differences in procedure exist. Goth employed dextrans of average molecular weights 40,000–150,000 (Pharmacia) and a different commercial preparation of PS (Supelco). In addition, glucose was not present in the incubation medium and cells were not washed and resuspended as in our experiments. We have, however, observed the inhibition of 48/80-induced histamine release by PS reported by Goth.[10] Employing a

TABLE 1. EFFECT OF DB-c-AMP ON 48/80-INDUCED HISTAMINE RELEASE*

Concn of DB-c-AMP (M)	Concn of 48/80 (μg/ml)	% Histamine released†
		6·2 ± 0·5 (3, P < 0·001)
	1·0	52·5 ± 1·7 (5)
$8·5 \times 10^{-3}$		4·6 ± 0·3 (5, P < 0·001)
$8·5 \times 10^{-3}$	1·0	25·1 ± 1·6 (5, P < 0·001)
$8·5 \times 10^{-4}$	1·0	36·6 ± 3·5 (5, P < 0·02)
$8·5 \times 10^{-5}$	1·0	44·5 ± 2·3 (5, P < 0·1)

* Preincubation with DB-c-AMP for 20 min, then 48/80 for 5 min (see text for details). All data in this table were obtained from one pooled cell suspension from five rats. However, parallel results were obtained in five additional experiments with different cell preparations (see discussion in text of paper).

† Each value is expressed as the mean ± 1 standard deviation. In parentheses are the number of separate incubations prepared. Duplicate histamine assays were performed on each incubation and the results averaged. The P value shown expresses the probability of the chance occurrence of differences from the value for 48/80 release alone. Values shown are for total histamine released; that is, spontaneous or control release values have not been subtracted from data obtained in the presence of releasing agents or inhibitors.

* Dr. J. H. Baxter has also observed the stimulatory effect of PS on dextran-induced histamine release *in vitro* (personal communication).

cell suspension which exhibited 6 per cent spontaneous release over a 20-min period at 37°, incubation with compound 48/80 (1·5 μg/ml) for 5 min resulted in 75 per cent total release. Preincubation for 15 min with PS(50 μg/ml) before the addition of 48/80 reduced the total histamine release to 16 per cent.

Effects of adenine nucleotides and related compounds on histamine release by compound 48/80. A typical effect of the 3′,5′-c-AMP derivative ^{6}N-2′,*O*-dibutyryladenosine-3′,5′-cyclic-monophosphate(DB-c-AMP) upon the 48/80-induced release of histamine is summarized in Table 1. The percentage of histamine released (48/80 at 1·0 μg/ml for 5 min) was reduced from 53 to 25 per cent by preincubation of the cell suspension with DB-c-AMP ($8{\cdot}5 \times 10^{-3}$ M). A definite effect was also observed at one-tenth this concentration (37 per cent release). The data shown in Table 1 were obtained using one pooled cell suspension (five rats) with repeated incubations and histamine assays to show reproducibility and statistical significance of results. Since the percentage of histamine released varies considerably from one cell suspension to another with a fixed concentration of 48/80, data obtained with different pools of cells cannot be compared easily. However, in addition to the experiment shown in Table 1, five other major experiments were performed, each using a different pooled cell suspension, with a total of 22 various combinations of DB-c-AMP and 48/80. The results of all experiments closely paralleled the data reported in Table 1. The inhibitory effect of DB-c-AMP on histamine release by 48/80 was directly proportional to the DB-c-AMP concentration and inversely proportional to the amount of 48/80 employed in all these experiments. The observed inhibitory effect was found to be only slightly dependent upon preincubation time of the cells with DB-c-AMP prior to the addition of compound 48/80, the inhibition increasing slightly during preincubation periods of 20–60 min. Viability of cell suspensions after preincubation with these rather high concentrations of DB-c-AMP (approximately 10^{-2} M, 20 min) was demonstrated by repeated washing of the cells with fresh buffer, followed by resuspension of cells.

TABLE 2. REMOVAL BY WASHING OF THE INHIBITORY EFFECT OF DB-c-AMP ON 48/80-INDUCED HISTAMINE RELEASE*

Conditions of incubation	% Histamine released
Control (cells alone, 55 min)	9·8
Buffer alone (50 min); 48/80 (5 min)	35·6
DB-c-AMP alone (55 min)	7·9
DB-c-AMP (50 min); 48/80 (5 min)	21·3
DB-c-AMP (20 min); one 10-min wash; fresh buffer (20 min); 48/80 (5 min)	26·7
DB-c-AMP (20 min); two 10-min washes; fresh buffer (10 min); 48/80 (5 min)	34·3
DB-c-AMP (20 min); three 10-min washes; 48/80 (5 min)	33·0

* 48/80 (0·6 μg/ml); DB-c-AMP (1×10^{-2} M). Each percentage figure given here represents the average of duplicate histamine assays on a single incubation mixture. The term "wash" refers to the centrifugation of cells, careful decantation, then resuspension and incubation for a 10-min period in fresh buffer.

By means of this procedure, the histamine-releasing effects of newly added compound 48/80 could be nearly fully restored after two 10-min wash periods (see Table 2).

The effects observed for DB-c-AMP could not be duplicated with 3′,5′-c-AMP itself using similar experimental conditions and concentration levels, possibly because of decreased ability of this compound to cross the cell membrane. Likewise, the following substances in concentrations as high as 10^{-2} M failed to inhibit significantly the release of histamine induced by 48/80: 5′-AMP, 5′-ADP, 5′-ATP, adenosine, pyrophosphate, phosphate and glucose. In fact, certain of these compounds themselves appeared to produce significant release above control values. Typical results of these experiments are shown in Table 3. Data on DB-c-AMP and theophylline have been included in this table for purposes of comparison. Experiments listed in the table have been repeated twice for each compound, employing different pooled cell suspensions, with essentially parallel results.

Effects of adenosine-3′,5′-cyclic monophosphate phosphodiesterase inhibitors on histamine release by compound 48/80. In an effort to reduce the concentrations of DB-c-AMP required for inhibition of histamine release to levels approaching physiological (or to see an effect with c-AMP), the following phosphodiesterase inhibitors were investigated: theophylline, reserpine, 1-diethylaminoethylreserpine (DEAE-reserpine) and perphenazine. The effects of these agents on the mixed peritoneal cell

TABLE 3. EFFECT OF CERTAIN ADENINE NUCLEOTIDES AND OTHER AGENTS ON 48/80-INDUCED HISTAMINE RELEASE*

Test compound	% Histamine released				
	Control (spontaneous)	Test compound alone	Concn 48/80 (μg/ml)	48/80 alone	Test compound + 48/80
DB-c-AMP	12·5	8·0	1·8	88·1	58·9†
Theophylline	5·2	11·7	1·8	89·7	27·3‡
5′-AMP	5·2	11·0	1·8	89·7	95·0‡
5′-ADP	5·2	11·1	1·8	89·7	84·9‡
5′-ATP	5·2	17·1	1·8	89·7	87·1‡
3′,5′-c-AMP	5·2	8·0	1·8	89·7	97·0‡
Adenosine	3·9	4·3	0·9	59·9	57·9§
Pyrophosphate	10·4	23·1	0·6	44·1	40·6‖
	10·4	23·1	1·8	47·7	42·8‖
	10·4	23·1	2·7	56·5	46·0‖
	5·0	19·1	2·7	89·4	88·6¶
Phosphate	10·4	10·1	0·6	44·1	41·3‖
(10^{-2} M above buffer concn)	5·0	8·3	2·7	89·4	91·5¶
Glucose (10^{-2} M above buffer concn)	5·0	5·9	1·8	41·3	44·1**

* Preincubation with test compound (10^{-2} M) for 20 min, then 48/80 at concentration indicated for 5 min. Each percentage figure given represents the average of duplicate histamine assays on a single incubation mixture. Theophylline and DB-c-AMP have been included as effective reference inhibitors.

Symbols (†, ‡, §, ‖, ¶, **) denote tests performed on identical cell suspensions. Closely parallel results were obtained in two repeat experiments for each compound, employing different pooled cell suspensions.

system are shown in Tables 4 and 5. All of these compounds were found to be effective inhibitors, even in the absence of DB-c-AMP or c-AMP, and inhibitory concentrations appeared to be similar to those reportedly required for phosphodiesterase inhibition.[18] The data shown in Tables 4 and 5 were obtained on a single (but different) pooled cell suspension for each compound tested. However, in addition to the data presented, eight other experiments, each with a different pooled cell suspension, resulted in very closely parallel data showing the inhibitory effects of theophylline (a total of 32 different combinations of theophylline and 48/80). Two repetitive experiments on each of the other three phosphodiesterase inhibitors (Table 5), again using a new pooled cell suspension each time, resulted in completely consistent and parallel data. The inhibitory effects of theophylline could be removed and the response of the cells to 48/80 restored almost completely by one 10-min wash with fresh buffer as described previously (see Table 6). In addition to their dramatic inhibitory effects on 48/80-induced histamine release, reserpine, DEAE-reserpine and perphenazine (but not theophylline) were all observed to promote significant histamine release above control values (see Table 5). Attempts to demonstrate any potentiation of the effect of DB-c-AMP with less than completely inhibitory concentrations of a phosphodiesterase inhibitor such as theophylline were unsuccessful.

TABLE 4. EFFECT OF THE PHOSPHODIESTERASE INHIBITOR THEOPHYLLINE ON 48/80- INDUCED HISTAMINE RELEASE*

Concn of theophylline (M)	Concn of 48/80 (μg/ml)	% Histamine released†
		6·2 ± 0·5 (3, P < 0·001)
	1·0	52·5 ± 1·7 (5)
$1·5 \times 10^{-2}$		5·9 ± 0·5 (3, P < 0·001)
$1·5 \times 10^{-2}$	1·0	8·7 ± 0·5 (5, P < 0·001)
$1·5 \times 10^{-3}$	1·0	40·1 ± 2·1 (5, P < 0·005)
$1·5 \times 10^{-4}$	1·0	50·6 ± 3·2‡ (5)

* Preincubation with theophylline for 20 min, then 48/80 for 5 min. The same cell suspension was employed as that in Table 1. Closely parallel results were obtained in 8 separate experiments, each using a different pooled cell suspension (see text for discussion).

† See second footnote, Table 1.

‡ Difference from 52·5 was not statistically significant.

TABLE 5. EFFECTS OF PHOSPHODIESTERASE INHIBITORS ON 48/80 INDUCED HISTAMINE RELEASE*

Test compound	Concn of test compound (M)	Concn of 48/80 (μg/ml)	% Histamine released†
1-DEAE-reserpine			6·0 ± 0·2 (3, P < 0·001)
		1·0	64·5 ± 4·3 (5)
	8×10^{-5}		43·4 ± 1·7 (5, P < 0·001)
	8×10^{-5}	1·0	45·1 ± 1·9 (5, P < 0·001)
Reserpine‡			5·8 ± 0·4 (3, P < 0·001)
		0·9	55·8 ± 2·2 (5)
	3×10^{-4}		18·5 ± 0·6 (5, P < 0·001)
	3×10^{-4}	0·9	24·3 ± 1·1 (5, P < 0·001)
Perphenazine			5·1 ± 0·5 (3, P < 0·001)
		1·0	72·8 ± 2·3 (5)
	6×10^{-5}		54·3 ± 1·2 (5, P < 0·001)
	6×10^{-5}	1·0	59·5 ± 2·1 (5, P < 0·005)

* Preincubation with test compound for 20 min, then 48/80 for 5 min. Data for each compound were obtained using a different cell suspension. Also, closely parallel results were observed using different pooled cell suspensions in two repeat experiments for each test compound.

† See the second footnote, Table 1.

‡ Compound was partially undissolved at concentration employed.

TABLE 6. REMOVAL BY WASHING OF THE INHIBITORY EFFECT OF THEOPHYLLINE (*T*) ON 48/80-INDUCED HISTAMINE RELEASE*

Conditions of incubation	% Histamine released
Control (cells alone, 55 min)	13·5
Buffer alone (50 min); 48/80 (5 min)	65·5
T (50 min); 48/80 (5 min)	13·5
T (20 min); one 10-min wash; fresh buffer (20 min); 48/80 (5 min)	49·6
T (20 min); two 10-min washes; fresh buffer (10 min); 48/80 (5 min)	49·6
T (20 min); three 10-min washes; 48/80 (5 min)	52·2

* 48/80 (0·75 μg/ml); T ($1·5 \times 10^{-2}$ M). See footnote to Table 2 for details of washing procedure and explanation of percentage figures.

Effect of prostaglandin E_1 on histamine release by compound 48/80. Recent reports have indicated that incubation of blood platelets *in vitro* with PGE_1 (10^{-5}–10^{-7} M) or PGE_1 plus theophylline (10^{-2}–10^{-3} M) increases endogenous levels of c-AMP.[19–24] Employing the mixed peritoneal mast cell system, the present authors have observed inhibitory effects upon 48/80-induced histamine release (5 min with 48/80 at 1·0 μg/ml) when cells were preincubated for 15, 30 or 60 min with PGE_1. For maximum effects, preincubation times of at least 30 min appeared to be required. However, rather high concentrations of PGE_1 were needed to obtain significant inhibitory effects (i.e. 3×10^{-5} M; see Table 7). No significant inhibition of histamine release was observed

TABLE 7. EFFECT OF PROSTAGLANDIN E_1 (PGE_1) ON 48/80-INDUCED HISTAMINE RELEASE*

Concn of PGE_1 (M)	Concn of 48/80 (μg/ml)	% Histamine released†
		5·8 (1, $P < 0·001$)
	1·0	72·7 ± 1·8 (5)
3×10^{-4}		5·8 ± 0·4 (2, $P < 0·001$)
3×10^{-4}	1·0	46·7 ± 1·1 (5, $P < 0·001$)
3×10^{-5}	1·0	56·5 ± 3·0 (5, $P < 0·01$)
3×10^{-6}	1·0	75·0 ± 4·3‡ (5)

* Preincubation with PGE_1 for 30 min, then 48/80 for 5 min. Data were derived from a pooled cell suspension different from any shown in Tables 1–6. Closely parallel data were obtained in each of two additional experiments using different pooled cell suspensions.

† See the second footnote, Table 1.

‡ Difference from 72·7 was not statistically significant.

under these conditions using PGE_1 concentrations of 3×10^{-6} M. Likewise, the effect of PGE_1 (3×10^{-4} M) could not be potentiated by the addition of 10^{-3} M theophylline to the incubation medium. The data presented in Table 7 were obtained from one pooled cell suspension; however, two repetitive experiments, each using a different pooled cell suspension, resulted in almost identical percentage figures for histamine release. In addition, one of these experiments showed PGE_1 to be completely inhibitory at a concentration of about 10^{-3} M. Preincubation of cells with the β-agonist isoproterenol, which might be expected to exert an inhibitory effect upon release through stimulation of the adenyl cyclase system and elevation of intracellular c-AMP, was

found to have no detectable effect at concentrations of 10^{-6}–10^{-2} M (60-min preincubation time).

DISCUSSION

Baxter *et al.*[11] have presented data which supports their suggestion that c-AMP might be involved in the inhibition of dextran-induced histamine release in rats. The current studies involving 48/80-induced histamine release from mast cells in mixed peritoneal suspensions are also consistent with the hypothesis that intracellular mast cell c-AMP in some manner protects the cell against the release of the histamine-containing granules. However, on the basis of our observations to date, it would be presumptuous to attempt to implicate definitely a mast cell adenyl cyclase system as explaining the effects *in vitro* of DB-c-AMP, certain phosphodiesterase inhibitors or PGE_1 upon 48/80-induced histamine release. The evidence obtained is therefore of a circumstantial nature, especially since the effective concentrations of active agents (particularly DB-c-AMP, theophylline and PGE_1) are quite high. Although the observed effects may conceivably differ in an homogeneous mast cell system, the possibility seems doubtful, and in fact the use of a mixed cell system may be in some ways more similar to a natural situation *in vivo*.

The inhibitory effect of DB-c-AMP upon 48/80-induced histamine release from mast cells (Table 1), as opposed to the inactivity of 3′,5′-c-AMP, 5′-AMP, 5′-ADP and 5′-ATP in similarly high concentrations (Table 3), is consistent with the idea that DB-c-AMP may penetrate the cell membrane more readily than c-AMP itself, and seems to eliminate the possibility that inhibition may be occurring through electrostatic binding of the positively charged amine groups of the 48/80 with the negatively charged phosphate groups of the adenine nucleotides. Removal of the effect of DB-c-AMP by washing of the cells, which restores the effectiveness of 48/80, appears to discount the possibility of any gross damage of the cells by exposure to high concentrations of DB-c-AMP (Table 2).

The effective inhibition of 48/80-induced release observed with theophylline and three other phosphodiesterase inhibitors (reserpine, DEAE-reserpine and perphenazine; Tables 4 and 5) at concentrations similar to those required for phosphodiesterase inhibition seems more than coincidental, and it appears possible that these agents are acting by preventing destruction of intracellular c-AMP. The release of histamine above control values promoted by phosphodiesterase inhibitors other than theophylline may reflect some cell damage. Washing of the cells with restoration of 48/80 release and the absence of any histamine release promoted by theophylline alone, appear to eliminate the possibility of cell damage in the case of this inhibitor.

The inhibitory effect of prostaglandin E_1 (Table 7), although demonstrable only at rather high concentrations (i.e. 3×10^{-5} M), is also consistent with the elevation of intracellular c-AMP resulting from stimulation of the enzyme adenyl cyclase.

The mode of action of the agents discussed above might possibly be elucidated by definitive biochemical studies of c-AMP levels and demonstration of an adenyl cyclase system in homogeneous peritoneal mast cell preparations. Further studies with these and other agents are planned, in order to elucidate whether these compounds are interfering with the interaction of releasing agents with the cell membrane or with the energy-requiring process concerned with the expulsion of histamine-containing granules from the cell.

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