Histamine Release from Rat Mast Cells by Dextran: Effects of Adrenergic Agents, Theophylline and Other Drugs[1] (36826)

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Dextran, when injected intravenously into normal rats of most strains, produces swelling of the paws, hypotension and prostration (1–3). The basis of this occurrence is obscure. However, the reaction has many characteristics of an allergic reaction, and is mediated by a release of histamine, serotonin and perhaps other vasoactive factors from the mast cells (4). Histamine is also released in a specific manner from rat mast cells upon contact with dextran *in vitro* (5). In the present study, after evaluating various parameters of the dextran reaction, we determined the effects of selected drugs—many of which are known to inhibit histamine release under other conditions and to enhance cyclic AMP in certain cells—on the quantity of histamine released by dextran from rat peritoneal mast cells *in vitro*.

Methods. Cells were obtained from large male Sprague-Dawley rats, after decapitation, by washing out the peritoneal cavity with 15 ml of buffer solution to which had been added 5 μg/ml of heparin. Any cell suspensions that contained blood were discarded. After confirming by use of the Ficoll-gradient procedure (6) that nearly all of the histamine was in mast cells, we employed the peritoneal cells without fractionation. The cells were strained through silk and used promptly, usually after centrifugation and resuspension in an equal volume of fresh buffer. Cells from several rats were pooled for each experiment, one rat supplying enough cells for 10 or 12 tubes. The buffer consisted of 9 parts solution of NaCl, 154 m*M*; KCl, 2.7 m*M*; and $CaCl_2$, 0.9 m*M*, and 1 part Sorenson's phosphate buffer, pH 6.8 (KH_2PO_4, 33 m*M* and Na_2HPO_4, 33 m*M*), with the addition of 1 mg/ml human serum albumin. Dextran and drug solutions were made with the buffer. pH of the solutions was adjusted to 6.8 with 0.1 *N* NaOH if necessary.

Histamine release studies were made as follows: Into a series of siliconized tubes, each already containing 1.0 ml of buffer alone or drug solution, 1.5 ml aliquots of the cell suspensions (containing about 10^5 mast cells and about 2 μg of histamine) were added. After preincubating the cells with the drugs for 12 min at 25° with periodic shaking, 1.0 ml of the buffer (control tubes) or of dextran solution was introduced (bringing volume in each tube to 3.5 ml). Drug concentrations are given on the basis of the 3.5 ml volume. The final dextran concentration was 3.8 mg/ml. Phosphatidyl serine sufficient to give a concentration of 7 μg/ml was included in the final addition of dextran or buffer solution. This was because of Goth, Adams and Knoohuizen's observation (7) that phosphatidyl serine exhances histamine release by dextran. After the dextran was added, each tube was immediately shaken and incubated for 15 min at 25°. The tubes were then chilled in ice, centrifuged at 4°, and the supernatant solutions were decanted. Supernatant solutions and residual cells, respectively, were made to 11.0 ml in 0.4 *N* perchloric acid, and cleared by centrifugation.

Histamine was determined on 1.0 ml aliquots by using the fluorometric method of Shore, Burkhalter and Cohn (8), and using phosphoric acid to stabilize the fluorescent

[1] Preliminary reports of this work [Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30**, 500 (1971)] and of a related *in vivo* study [Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 618 (1970)] have been presented before the American Society for Pharmacology and Experimental Therapeutics.

complex at pH 2 (9). The drugs did not interfere, and histamine assays could be made directly, without employing the extraction procedure described by Shore, Burkhalter and Cohn (8). Blank readings obtained with resuspended cells and the usual buffer came to only 1–5% of the readings obtained after adding dextran. If the cells were used in the original fluid, or albumin in the buffer was reduced, or Ca^{2+} was omitted (as practiced in a few auxiliary experiments), blanks were higher but varied little within an experiment. None of the principal drugs affected the blanks, and duplicate tubes arranged in nonconsecutive order always gave highly consistent results. Since the quantity of histamine released by the dextran varied considerably from one experiment to another (*i.e.*, with the use of different cell suspensions), effects of the drugs were expressed in percent inhibition of histamine release, calculated in each experiment from the quantities of histamine released by dextran in the presence of, and in the absence of, each drug solution, after appropriate blanks had been subtracted.

The dextran used was dextran 2000 (Pharmacia, av mol wt 2×10^6). Phosphatidyl serine, of bovine origin and 95% pure, was obtained from Supelco, Inc. or from Nutritional Biochemicals Corp. Crystalline human serum albumin was purchased from Pentex, Inc. Crystalline *l*-isoproterenol HCl and *l*-norepinephrine bitartrate were kindly supplied by Dr. S. Archer of Sterling-Winthrop Research Inst. *l*-Epinephrine base was supplied by Dr. H. M. Crooks of Parke Davis & Co., phenoxybenzamine HCl by Smith Klein & French Laboratories, and prostaglandin E_1 by the Upjohn Co. N^6,$O^{2'}$-dibutyryl-adenosine-3′,5′-monophosphate Na salt was obtained from Boehringer Mannheim.

Results. In over 50 experiments made as described under Methods, dextran released 15 to 60% (av 35%) of the histamine in the cells. Cells from germ-free rats reacted similarly. If phosphatidyl serine was omitted from the dextran solution, the release was more variable and averaged less than 10%, although phosphatidyl serine alone was without effect.

The dextran concentration employed (3.8 mg/ml = 2×10^{-6} *M*) gave maximal histamine release, although 1 mg/ml was almost as effective. Satisfactory release was most consistently achieved at the adopted 25° temperature. Release at 37° was variable; virtually no release occurred at 2, 12 or 45°. Cumulative release at 25° reached a plateau after about 5 min, well within the usual 15-min incubation period. Release was maximal at pH 6.8 to 7.2. The dextran reaction required Ca^{2+} but not Mg^{2+}, as previously noted (10). It did not require complement, since it occurred with thoroughly washed cells in the absence of serum. It appeared to be noncytotoxic, as evidenced by the normal appearance of the treated cells, except for degranulation, and by the exclusion of trypan blue (11) from most of them.

Effects of drugs. The release of histamine by dextran was decreased by each of several catecholamines, as shown in Fig. 1. The inhibition was incomplete, but was induced by fairly low concentrations of the drugs. Isoproterenol (10^{-8} *M*), epinephrine (10^{-7} *M*), and norepinephrine (10^{-6} *M*) each inhibited the release by almost 50%; higher concentrations usually were somewhat less effective. The β-adrenergic blocker propranolol (10^{-5} *M*) virtually abolished the inhibitory effect of isoproterenol, whereas the α-adrenergic blocker phenoxybenzamine (10^{-5} *M*) did not (Fig. 1). The blocking agents alone were without significant effect in the concentrations employed.

Prostaglandin E_1 in fairly high concentration (10^{-5} *M*) also decreased the histamine release (Fig. 1). Glucagon and insulin (3×10^{-7} to 3×10^{-6} *M*) did not significantly affect release. Acetyl choline (10^{-12} to 10^{-5} *M*) did not alter the release, or sometimes the higher concentrations produced slight inhibition.

Theophylline produced a dose-related inhibition of histamine release, 50% inhibition occurring with about 2×10^{-5} *M* concentration of the drug, and over 80% inhibition with 10^{-3} *M* (Fig. 2) .When isoproterenol and theophylline were employed in combination, the effects of the two drugs were additive, but not clearly synergistic (Table I). It was

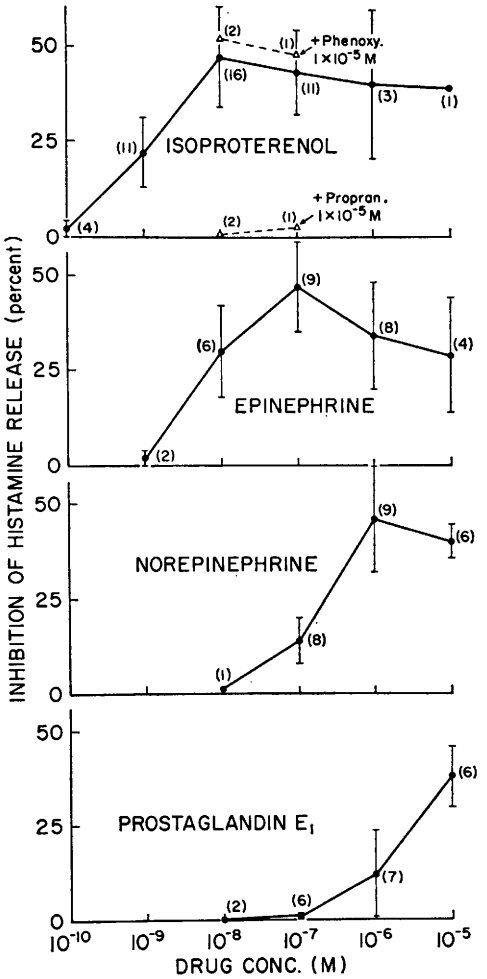

FIG. 1. Inhibition by various catecholamines and prostaglandin E_1 of dextran-induced histamine release from rat mast cells. The effects of adding propranolol and phenoxybenzamine (individually) in addition to isoproterenol, are shown in the upper panel. Mean value ± SD, and number of experiments, are shown for various concentrations of the drugs.

determined that even in the presence of inhibitory drugs (isoproterenol, theophylline), histamine release still reached completion in about 5 min.

Dibutyryl cyclic AMP inhibited histamine release in a manner closely paralleling that of theophylline (Fig. 2). In about half of the experiments, the drug had been freshly extracted with ether, after acidification, to remove any free butyric acid. This procedure did not affect the results. After dibutyryl cyclic AMP, theophylline, or the catecholamines had been incubated with the cell suspensions, their inhibitory effects could be largely removed by washing the cells twice with fresh buffer before adding the dextran, indicating that the cells had not been irreversibly damaged by the drugs. The drugs did not themselves release histamine (except for high concn of glucagon).

Results obtained with miscellaneous agents are shown in Fig. 3. Propranolol (10^{-6} to 10^{-4} *M*) was without effect. Phenoxybenzamine, nicotinamide, glucose and ethanol, respectively, and in successively higher concentrations between 10^{-5} and 10^{-1} *M*, substantially inhibited histamine release. Colchicine (not shown), with the usual 25° preincubation, produced significant inhibition of the histamine release only in concentrations of about 10^{-3} *M* or greater. However, when preincubated with the cells for 20 mn at 0°, it exhibited mild inhibitory activity at 10^{-5} *M* concentration. Sodium fluoride (without dextran) in concentrations of 10^{-2} *M* or greater released most of the histamine of the cells.

Histamine in the medium has been shown to inhibit histamine release from human leucocytes by specific antigen (12). However,

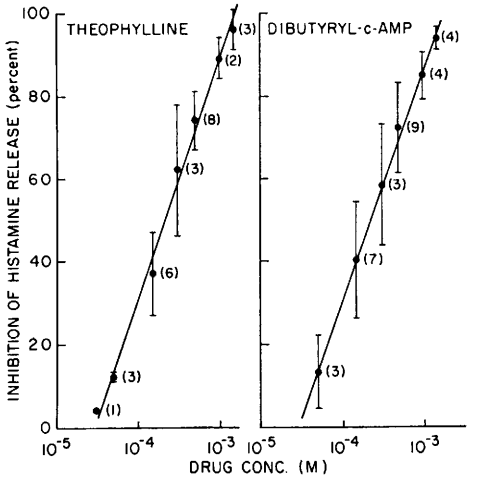

FIG. 2. Inhibition by theophylline (left) and by dibutyryl cyclic AMP (right) of dextran-induced histamine release. Mean values ± SD, and numbers of experiments are shown.

TABLE I. Inhibition of Dextran-Induced Histamine Release by Isoproterenol and Theophylline Employed Individually and in Combination.

Isoproterenol		Theophylline		
Concn (M)	Inhibition produced[a] (%)	Concn (M)	Inhibition produced[a] (%)	Inhibition by combination (%)
10^{-10}	0	5×10^{-5}	4	7 (4)[b]
10^{-10}	0	1.5×10^{-4}	27	25 (27)
10^{-9}	11	5×10^{-5}	4	26 (15)
10^{-9}	11	1.5×10^{-4}	27	41 (38)
10^{-8}	32	5×10^{-5}	4	47 (36)
10^{-8}	32	1.5×10^{-4}	27	65 (59)

[a] Dextran alone released 0.8 of 2.0 μg total cell histamine.
[b] The sum of individual effects is shown in parentheses.

the amount of histamine released by dextran and the effectiveness of the drugs in the present study were not influenced by the concentration of released histamine reached in the medium (up to about 3×10^{-6} M), nor in turn by the density of cell suspensions employed, as shown by two observations. When exogenous histamine was added to the cells with the dextran, in final concentrations of 10^{-7} to 10^{-5} M, the amount of additional histamine released by the dextran was not appreciably affected (Table II). In addition, in experiments employing tenfold diluted cell suspensions (which released one-tenth the usual amounts of histamine), the effects of the principal drugs remained well within the range of the results already presented. If preincubated with the cells for 12 min, however, exogenous histamine in concentrations of 10^{-6} M or greater did decrease histamine release by dextran.

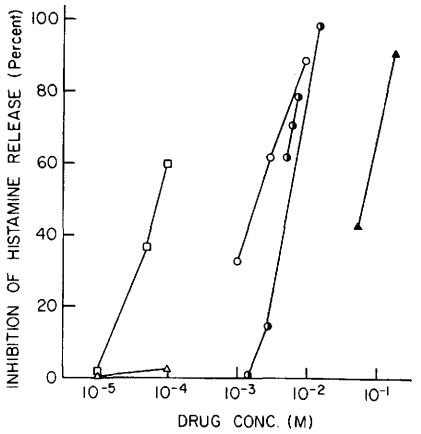

FIG. 3. Inhibition of dextran-induced histamine release by propranolol (△) phenoxybenzamine (□), nicotinamide (○), and glucose (◒), and ethanol (▲).

A number of additional experiments were made to explore the effectiveness of the drugs under altered experimental conditions. Drug effects obtained at temperatures between 25 and 37° were generaly similar to those which have been presented. The results were not appreciably changed by preincubating the cells with the drugs for 30 min instead of the usual 12 min. The effectiveness of the principal drugs did not vary greatly over the pH range of 6.8 to 7.2, or over the Ca^{2+} concentration range of 0.8 to 1.2 mM (Table III). Adding 1 mM Mg^{2+} did not influence the drug actions, nor was effectiveness of the drugs greatly influenced by varying the dex-

TABLE II. Effect of Adding Exogenous Histamine with the Dextran on the Amount of Histamine Released from Mast Cells by Dextran.

Added histamine (final concn)	Histamine released (concn in medium)
(M)	% of control
0	100 (2×10^{-6} M)
1×10^{-7}	92
1×10^{-6}	102
3×10^{-6}	94
1×10^{-5}	82

TABLE III. Effect of pH and Ca^{2+} Concentration of Medium on Histamine Released by Dextran, and on the Inhibitory Effect of Certain Drugs.

Conditions		Histamine released by dextran (% of total)	Inhibition of release by		
			Isoproterenol 1×10^{-8} *M*	Dibut.-cAMP 4×10^{-4} *M*	Theophylline 4×10^{-4} *M*
pH	Ca^{2+} (m*M*)		(%)	(%)	(%)
6.8	0.8	39	48	75	51
6.8	1.0	41	46	74	49
6.8	1.2	38	48	69	45
7.2	0.8	33	40	52	45
7.2	1.0	32	38	49	42

tran concentration from 0.5 to 4 mg/ml, or by reducing albumin in the buffer to 0.1 mg/ml.

Discussion. Why dextran should cause normal rat mast cells to release histamine is not understood. Antibodies against dextran have not been demonstrated in the rats, and the responsiveness of mast cells from germ-free rats to dextran indicates that sensitization to bacterial products plays no role. The fact that well washed mast cells still respond to dextran implies that dextran may act directly on the mast cells, rather than through production of a secondary serum factor such as has been postulated to mediate the dextran reaction *in vivo* (13). Some investigators have failed to obtain *in vitro* histamine release with dextran (14), which possibly explains why the dextran reaction has been studied relatively little *in vitro,* compared with the effects of less specific agents such as 48/80 and octylamine. Based on its species specificity and other characteristics, the action of dextran has been considered to resemble that of specific antigen in allergic histamine release (7). The present study extends this analogy.

Lichtenstein and Margolis (15) noted inhibitory effects of catecholamines, theophylline and dibutyryl cyclic AMP on antigen-induced histamine release from human leucocytes. Since the catecholamines have been shown under other circumstances to increase adenyl cyclase activity, and theophylline to inhibit phosphodiesterase, each thereby causing an increase in cyclic AMP (as presumably does also the addition of dibutyryl cyclic AMP), these investigators postulated that the histamine release was regulated by cyclic AMP. Others have observed similar effects of these and other cyclic AMP-active drugs on histamine release from cells or tissues by specific antigens (12, 16–20), by soluble immune complexes (21), and by the polymeric amine 48/80 (14, 18). Furthermore, several of these studies demonstrated that the drugs which inhibited histamine release also increased cyclic AMP in the mixed cell populations employed in the studies (12, 19). The present results with the catecholamines, theophylline and dibutyryl cyclic AMP, which resemble those discussed above, suggest that histamine release by dextran may likewise be modulated by cyclic AMP, but such a mechanism has not been proven.[2]

Summary. Dextran released histamine in a selective manner from suspensions of normal rat peritoneal mast cells. Some characteristics of the release reaction are described. The quantity of histamine released by dextran was reduced about 50% by the presence of isoproterenol (10^{-8} *M*), epinephrine (10^{-7} *M*) or norepinephrine (10^{-6} *M*). Propranolol

[2] Cyclic AMP determinations (V. Manganiello, M. Vaughn and J. H. Baxter, unpublished data) showed that the unfractionated peritoneal cells contained appreciable quantities of cyclic AMP, which approximately doubled after treatment of the cells for 6 min with isoproterenol (5×10^{-7} *M*) and theophylline (5×10^{-4} *M*). Similar clear increases in cyclic AMP have occurred in the nonmast cell fraction after Ficoll-gradient fractionation, but not in the mast cells; but neither have such mast cells retained their responsiveness to dextran. Other methods of cell fractionation are currently under study.

but not phenoxybenzamine abolished the isoproterenol effect. Prostaglandin E_1 (10^{-5} *M*) also partially inhibited histamine release by dextran. Theophylline and dibutyryl cyclic AMP also produced a dose-related inhibition of the release, which reached 50% effectiveness at about 2×10^{-4} *M* concentration. Effects of several miscellaneous drugs are also described. The results obtained with the principal drugs indicated that the release reaction might be modulated by cyclic AMP.

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