Effect of Endotoxin and Anaphylatoxin on Rat Mast Cells and Chopped Rat Lung[1] (37409)

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Endotoxin increases blood histamine in dogs according to some workers (1–5) but not according to others (6). Vick *et al.* (5) showed that *E. coli* endotoxin injected iv decreased blood pressure, elevated blood histamine, and decreased circulating platelets in dogs and suggested that platelets and tissue mast cells were the source of the elevated blood histamine.

Large amounts of histamine are stored in tissue mast cells (7). This histamine is released by antigen-antibody reactions (8), by certain drugs (9), and by particulate substances (*e.g.*, peptone) (10). Mast cells might be the source of that histamine released into blood by endotoxin. However, no one has determined whether endotoxin releases histamine from mast cells, although several investigators have found no morphological evidence of mast cell "degranulation" in response to endotoxins (11, 12). We, therefore, compared endotoxin and compound 48/80 on release of histamine from rat mast cells *in vitro* and rat chopped lung *in vitro*.

A spasmogen similar to anaphylatoxin was noted in some of our experiments in which rat serum was used as a source of complement. Because endotoxin might release histamine *in vivo* by an indirect mechanism, *e.g.*, formation of anaphylatoxins, and since the histamine releasing properties ascribed to anaphylatoxins have not been evaluated thoroughly (13–17), we also examined the effects of this substance on rat mast cells and rat chopped lung *in vitro*.

Methods. Mast cell preparations. Cells were collected by lavage from peritoneal and pleural cavities of male, Sprague–Dawley rats (18). Pooled cells from 3–5 rats were washed and resuspended in 10–25 ml of medium.[4] Samples of cell suspensions were incubated with endotoxin, compound 48/80, rat serum, or medium alone (see below). Mast cells, *i.e.*, those stained with toluidine blue, were counted in a hemocytometer (19). Pooled cells contained 5–10% mast cells. The mast cells contained 7.6 ± 1.0 μg histamine/10^6 cells, which is similar to that reported earlier for mixed cell suspensions (18).

Rat chopped lung. Rats were anesthesized with ether and exsanguinated. Lungs were rapidly removed, placed in cold, oxygenated medium[4] and chopped into small pieces (1–3-mm cubes). Samples of lung were blotted on filter paper, weighed (approximately 250 mg), and placed in fresh oxygenated medium for incubation with endotoxin, 48/80, rat serum anaphylatoxin, or medium alone. Lung samples contained 4.8 ± 0.6 μg histamine/g lung, which is similar to values reported by other investigators for rat lung (20).

Generation of anaphylatoxin. Rat serum, as a source of complement, was added to rat

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[4] The incubation medium consists of 150 m*M* NaCl, 2.7 m*M* KCl, 0.9 m*M* $CaCl_2$, 3.5 m*M* KH_2PO_4, 4.3 m*M* Na_2HPO_4, 5.6 m*M* dextrose, and 0.1% human serum albumin (fraction V); pH 6.8–7.0.

mast cells treated with endotoxin, and a smooth muscle spasmogen was formed that resembled an anaphylatoxin in its action on the guinea pig ileum (13, 14, 21). Serum, from venous blood of anesthesized rats, was incubated with either endotoxin or agar (1 mg/ml) at 37° for 60 min. The characteristics of these spasmogens were compared to those of "classical" anaphylatoxin (*i.e.*, that formed in rat or guinea pig sera by particulates) with respect to formation, stability, pharmacological actions, and inactivation. Human and dog sera were also tested for generation of anaphylatoxins.

Experimental design. Serous fluid cells were incubated with endotoxin (0.1–1000 μg/ml), 48/80 (1.0 μg/ml), or medium alone. Chopped lung was incubated with endotoxin (100 and 1000 μg/ml), 48/80 (50 μg/ml), or medium alone. All incubations were at 37° for 20 min. Supernatant and cell fractions of mast cells were separated by centrifugation. Supernatant and tissue fractions of chopped lung were separated by straining through gauze. Dilute HCl was added to all fractions. Lung tissue fractions were heated to 100° for 20 min. Histamine was measured by bioassay.

In order to test the histamine-releasing effect of anaphylatoxins, mast cells or chopped lung were resuspended in activated rat serum, *i.e.*, serum that had been incubated with endotoxin or agar. The existence of anaphylatoxins was verified by spasmogenic activity on guinea pig ileum.

Pyrogen tests. Endotoxin was tested for pyrogenic activity by iv injection into New Zealand white rabbits. Rectal temperature was monitored by a thermister probe and a Tele-Thermometer. Pyrogenic responses were observed at doses as low as 0.008 μg/kg.

Histamine bioassay. Histamine was estimated in each sample by assay on guinea pig ileum at 37° in an oxygenated medium containing atropine.[5] All samples were neutralized with dilute NaOH. Histamine release is expressed as amount of histamine released into the supernatant as percent of total histamine.

Agents used. Endotoxin (Bacto-Lipopolysaccharide) was obtained from three sources: (a) donated by Dr. L. B. Hinshaw, Department of Physiology, University of Oklahoma (*E. coli* Boivin 0127:B8); (b) purchased directly from Difco Laboratories, Detroit, MI (*E. coli* Boivin 0127:B8); (c) donated by Dr. P. E. Treadwell, Department of Microbiology, Emory University (*E. coli* Wesphal 0127:B8). Solutions of endotoxin were made in sterile, pyrogen-free 0.95% NaCl for pyrogen tests. All three batches of endotoxin appeared to be of similar potency based on a comparison of pyrogenic responses. Compound 48/80 was obtained from Burroughs-Wellcome Company, Research Triangle Park, NC.

Results. Direct effects of endotoxin on mast cell suspensions and chopped lung. Endotoxin (up to 1 mg/ml) did not release histamine from either mast cells or chopped lung. In contrast, 1 μg/ml of 48/80 released approximately 70% of the histamine from mast cell suspensions, and 50 μg/ml released approximately 50% of histamine from lung tissue (Fig. 1).

Indirect effects of endotoxin on mast cells. Because serum complement has been implicated in reactions with endotoxins (22–24), we added rat serum to our cell suspensions.

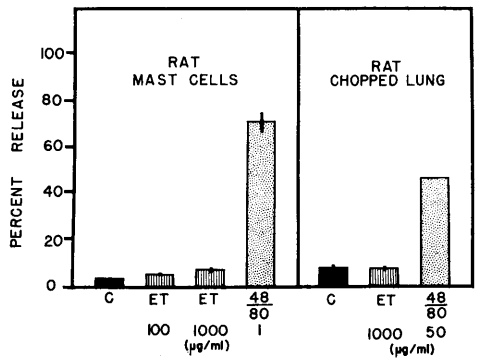

FIG. 1. Histamine release from rat mast cells and chopped lung. Cells and lung samples incubated for 20 min at 37°. Each bar shows mean (± SE) percent release of histamine in three experiments. C = medium alone; ET = endotoxin (100 and 1000 μg/ml), and 48/80 = compound 48/80 (1 or 50 μg/ml). Concentrations of ET as low as 0.1 μg/ml did not release histamine from cells.

[5] The histamine assay medium consists of 138 m*M* NaCl, 2.7 m*M* KCl, 1.8 m*M* $CaCl_2$, 2.0 m*M* $MgCl_2$, 1.2 m*M* $NaHCO_3$, 5.6 m*M* dextrose, and 1.0 μg/ml of atropine sulfate; pH 7.2–7.4.

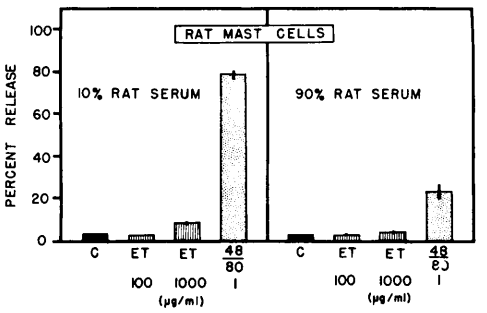

FIG. 2. Effect of rat serum on release of histamine. Rat mast cells incubated in either 10% or 90% rat serum (three experiments each). Symbols as in Fig. 1.

Endotoxin was inactive even in the presence of 10% or 90% rat serum (Fig. 2). The effectiveness of 48/80 was reduced in 90% serum, probably due to binding of the drug to the serum protein in the incubation mixture.

Generation of spasmogen by endotoxin. A smooth muscle spasmogen, generated by the combination of endotoxin or agar with rat serum, resembled anaphylatoxin in its actions on the guinea pig ileum, *i.e.,* (a) contraction blocked by antihistamine (tripelennamine) and (b) tachyphylaxis. No spasmogen was detected with endotoxin or agar with 10% rat serum nor with 90% human or dog sera.

Table I compares the characteristics of endotoxin- and agar-generated spasmogens in rat sera with those of "classical" anaphylatoxin described by other investigators (16, 25–28). There is a close resemblance in the characteristics of all three spasmogens as regards formation, pharmacological actions, stability, and inactivation. Thus, we concluded that endotoxin generated an anaphylatoxin in rat serum.

Effect of preformed anaphylatoxin on mast cells and chopped lung. Even though activated serum caused contraction of the guinea pig ileum, it did not release histamine from mast cell suspensions (Fig. 3). However, serum activated by either endotoxin or agar released 20–30% of total histamine from rat lung (Fig. 3). Compound 48/80 induced release of histamine in both preparations.

Discussion. Because histamine has been implicated in the early stages of endotoxin-induced shock in dogs (1–5), we studied the effects of endotoxin on rat mast cells, a major storage site for this amine (7). Only two papers refer to effects of endotoxin on mast cells *in vitro.* Asboe-Hansen and Glick (10) incubated isolated rat mast cells with *E. coli* endotoxin and found no evidence of disruption or loss of granules, but they

TABLE I. Comparison of the Characteristics of Anaphylatoxin Generated in Rat Serum by Endotoxin or Agar (1 mg/ml) with Those of "Classical" Anaphylatoxin.

	Anaphylatoxins		
	Endotoxin[a]	Agar[a]	Literature[b]
Contraction of guinea pig ileum with tachyphlaxis	yes	yes	yes
Ileum resensitizes in 30–45 min	partial	partial	yes
Contraction of guinea pig ileum blocked by antihistamines	yes	yes	yes
Formation inhibited by heating serum to 56° for 30 min	yes	yes	yes
Formation inhibited at 0°	yes	yes	yes
Not inactivated by 56°	yes	yes	yes
Inactivated by 100°	partial	partial	yes
Stable when frozen	yes	yes	yes
Inactivated by chymotrypsin[c]	partial	partial	yes

[a] "Anaphylatoxin" of our experiments.
[b] Data are from Refs. 15, 22, 23, 24, 25.
[c] Characteristic of human C_3 anaphylatoxin (14).

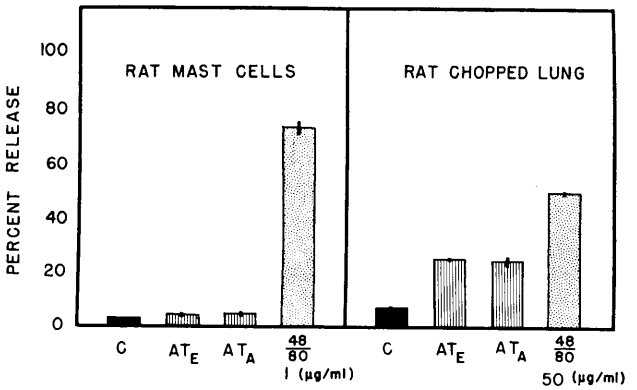

FIG. 3. Effect of anaphylatoxin and 48/80 on mast cells and chopped lung. Anaphylatoxins were generated in rat serum by incubation (37°, 60 min) with 1 mg/ml of either endotoxin (AT_E) or agar (AT_A). Symbols as in Fig. 1. Three experiments each on mast cells and lung.

did not measure histamine release. Their isolation medium (sucrose) has since been shown to reduce histamine content of mast cells (18). They also found no morphological changes with compound 48/80, a potent histamine releasing agent (29). Gustafson and Cronberg (12) found no degranulation of mesentery mast cells of rats and hamsters with *E. coli* endotoxin, but they did not assay for histamine release. We found that *E. coli* endotoxin did not release histamine from rat mast cells or rat chopped lung, but that 48/80 did.

Because the effects of endotoxin *in vivo* could be due to an antigen-antibody reaction in endotoxin-sensitized animals (23), we added rat serum as a source of complement in some experiments with mast cells. Endotoxin did not release histamine in the presence of serum. However, a smooth muscle spasmogen with the characteristics of "classical" anaphylatoxin was formed. Others have found that endotoxins generate anaphylatoxins in rat (30) or guinea pig (31) sera.

Anaphylatoxin from agar-activated rat serum or plasma has been shown to release histamine from guinea pig lung slices (15) and to degranulate guinea pig mesentery mast cells (14). Agar-activated serum of the guinea pig released histamine from perfused guinea pig lung (13). An anaphylatoxin from a purified human serum protein has been reported to release histamine from rat mast cells (17). We studied the effect of activated rat serum on mast cell suspensions and chopped lung. Rat serum activated by either endotoxin or agar did not release histamine from serous fluid rat mast cells. However, it did release 20–30% of histamine from rat lung. Dias de Silva and Lepow (15) obtained similar data using guinea pig lung slices incubated with rat serum that had been activated with agar or human C_1 esterase.

The lack of an anaphylatoxin in either human or dog serum probably is due to inactivating enzymes in these sera. Bokisch and Müller-Eberhard (32) described an enzyme in human serum which destroys both human anaphylatoxins (C_{3a} and C_{5a}).

Our experiments with rat mast cells and rat lung do not support the concept that endotoxin is a direct histamine releasing agent. However, mast cells of other species, such as dog, might release histamine in response to endotoxin. Dog mast cells have not been studied *in vitro* since they can not be obtained intact from serous fluids. The anaphylatoxins formed in agar- or endotoxin-activated rat serum had no direct effect on rat mast cell suspensions but did release histamine from rat lung. Therefore we cannot exclude the possibility that mast cell histamine is released in the early phase of endotoxin shock *in vivo*.

Conclusions. 1. Neither endotoxin, nor anaphylatoxin generated by the action of endotoxin on rat serum, released histamine from rat mast cell suspensions. 2. Preformed anaphylatoxin, but not endotoxin alone, re-

leased histamine from chopped rat lung. 3. We confirmed reports that endotoxin (and agar) causes generation of an anaphylatoxin in rat serum. 4. Although anaphylatoxin generated by either endotoxin or agar does not release histamine from rat serous fluid mast cells, it does release histamine from rat chopped lung, suggesting that formation of an anaphylatoxin may be one mechanism for release of histamine by endotoxin *in vivo*.

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