Observations on the Release of Histaminase After 48/80 and Polymyxin B in Sensitized and Unsensitized Rats.* (30787)

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The release of histaminase into the bloodstream during anaphylactic shock in the white rat was first reported by Code and co-workers (1), and evidence that most of it is released from the intestinal tract was presented later (2). The mechanism of the release still is not well understood. Histaminase is not found in demonstrable quantities in blood from unshocked rats. Its release is a late manifestation of anaphylaxis in rats(2). However, since the release of histamine in anaphylaxis precedes the release of histaminase(1,2) and since the enzymatic process initiated in anaphylaxis is considered to be essentially the same as that initiated by compound 48/80(3), it was thought that the administration of histamine-releasing agents such as 48/80 and polymyxin B might also cause liberation of histaminase.

Methods. White, male (and a few female) Sprague-Dawley rats, ranging from 150 to 400 g (most were between 200 and 300 g), were used. Rats were sensitized by a single intraperitoneal injection of 1.0 ml of undiluted horse serum combined in the same syringe with 0.5 ml of a saline suspension of phase I *Bordetella pertussis* vaccine (Lederle) containing approximately 30×10^9 killed *B. pertussis* organisms. These animals were used 13 to 14 days later.

Each animal was anesthetized by intraperitoneal injection of sodium pentobarbital (3.5 to 4 mg/100 g of body weight), and a no. 60 polyethylene catheter was inserted into the right atrium *via* the external jugular vein. Drugs were injected and blood samples were withdrawn through this catheter.

48/80 was administered as a 1 mg/ml solution in 0.9% NaCl, intraperitoneally in a dose of 100 μg/100 g of body weight or intravenously in a dose of 40 to 50 μg/100 g. Polymyxin B (1 mg/ml of 0.9% NaCl) was administered intraperitoneally in a dose of 2.5 mg/kg or intravenously in a dose of 0.6 to 1.0 mg/kg.

At intervals after the injection of 48/80 or polymyxin B, samples of blood were taken. Half of each sample was subjected to the histamine extraction procedure(4) as promptly as possible. The other half was incubated at 37°C for 1½ hours in a constant temperature bath and then subjected to the histamine extraction procedure. The histamine content of the extracts was assayed on the isolated atropinized guinea pig ileum. The results are expressed as amount of histamine base per milliliter of blood. The loss of histamine in the incubated specimen is expressed as a percentage of the histamine in the unincubated sample.

The time of sampling was chosen after determination, in preliminary trials, of the time the rats lived after the injection of each drug and the maximal blood histamine value attained during that time.

Blood specimens for each assay ranged from 1 to 2 ml. Difference between values for blood histamine obtained from duplicate specimens ranged from 0 to 25%; hence, only destruction of 30% or more was considered to be significant.

Results. Blood was withdrawn for histamine determination prior to injection of drug in 34 of the rats used. The mean $\pm$ SD was 0.066 ± 0.072 μg/ml.

In sensitized rats, intravenous injection of 48/80 (9 rats) or polymyxin B (10 rats) produced increased concentrations of histamine in the blood (samples taken 5 to 10 minutes after injection) but no evidence of significant release of histaminase was found (Table I). In unsensitized rats, intravenous injection of polymyxin B (10 rats) produced increased concentrations of histamine but not of histaminase; 48/80 (17 rats) induced histaminase release in a few of the animals tested. When the drugs were given by intraperitoneal

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TABLE I. Effect of 48/80 and Polymyxin B on Blood Histamine and Histaminase in Sensitized and Unsensitized Rats.

	48/80*		Polymyxin B*	
	Histamine† (μg/ml)	Histaminase‡ (No. +/total)	Histamine† (μg/ml)	Histaminase‡ (No. +/total)
Sensitized rats				
I.V. inj; sampling 5 to 10 min after inj	.71 ± .2	0/9	.58 ± .42	0/10
Unsensitized rats				
I.V. inj; sampling:				
4 to 6 min	.795 ± .45	1/7	1.2 ± .35	0/3
6 to 9 min (48/80) or 8 to 12 min (PmB)	1.08 ± .35	2/6	1.03 ± .2	0/7
11 to 14 min	1.34 ± .28	0/4	—	—
Unsensitized rats				
I.P. inj; sampling:				
10 min§	.41 ± .45	0/7	—	—
25 to 30 min	—	—	1.03 ± .42	3/10
40 min§	.83 ± .35	3/7	—	—
60 to 70 min	1.2 ± .2	0/3	—	—

* See text for methods and dosages.

† Expressed as mean ± S.D. in terms of histamine base; normal value (34 rats) = .066 ± .072 μg/ml.

‡ No. of rats in which 30% or more of blood histamine was destroyed during incubation of the blood for 1½ hr at 37°C/total No. in each group.

§ Same animals used for both sampling periods.

injection into unsensitized rats, at about half an hour after injection one-third of the animals showed histaminase release after polymyxin B (10 rats) and 3 of 7 after 48/80. No effect on histaminase was noted at 1 hour after 48/80.

Both sexes were represented in the few rats in which evidence of histaminase activity was observed. In none of the experiments was there a relationship between the concentration of histamine in the blood and the amount of histaminase activity observed.

Discussion. The mean values for histamine concentration in blood of normal Sprague-Dawley rats has been shown to be 0.078 μg/ml(1) and 0.082 μg/ml(2), in agreement with the value found in this study. The release of histamine in unsensitized rats following administration of 48/80 or polymyxin B, reported previously(3,5-7), was confirmed in this study. The histamine concentration was not increased when sensitized rather than unsensitized animals were given these drugs (Table I).

No evidence of histaminase activity was noted in the sensitized rats given either 48/80 or polymyxin B intravenously or in the unsensitized rats given polymyxin B intravenously. However, a few animals showed a minimal amount of histamine destruction after administration of 48/80 intravenously. It seems unlikely, therefore, that histaminase release into the bloodstream is a consistent feature of the action of either 48/80 or polymyxin B given intravenously in sensitized or unsensitized rats.

Histaminase activity did occur in the blood of 3 of the 10 rats given 48/80 and in 3 of the 10 given polymyxin B intraperitoneally. It seems possible that, when the histamine-releasing agent is given by the intraperitoneal route, in some animals a higher concentration of agent reaches the gut. Since the gut has been considered to be the shock organ in the rat(8) and since it has been shown to be the chief source of histaminase in both the shocked(2) and the normal(9) rat, this could explain the finding of histaminase activity in 30% of the animals.

Högberg and Uvnäs(10) suggested that the enzymatic process initiated in anaphylactic reactions is essentially the same as that initiated by compound 48/80. It seems likely that histaminase release by 48/80 or polymyxin B occurs only when the gut is affected by the drugs. Alternatively, it may be that

histaminase release occurs only rarely and not consistently after administration of 48/80 or polymyxin B. The amounts of histamine-destroying activity identified in this study were small: in no case was destruction more than 50% in 1½ hours' incubation. This is in contrast to values of 65 to 90%, with only rare values below these, found during anaphylaxis(2).

Summary. Neither sensitized nor unsensitized rats consistently released significant amounts of histaminase into the bloodstream after intravenous administration of 48/80 or polymyxin B. Only 30% of unsensitized rats given 48/80 or polymyxin B by the intraperitoneal route showed release into the blood of histamine-destroying activity, and this in small amount. It is concluded that either (1) the intraperitoneal route allows a higher concentration of drug to reach the gut and hence release histaminase or (2) 48/80 and polymyxin B do not consistently release histaminase, in contrast to the effect of anaphylactic shock in rats.

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Enzymatic Characterization of Decapacitation Factor. (30788)

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Chang(1) has described the presence in rabbit, bull and human seminal plasma, of a substance capable of reversing capacitation. This naturally occurring anti-fertility substance has been designated "decapacitation factor" (DF). Bedford and Chang(2) described an ultracentrifugation technique for partial purification of DF. They reported that DF activity of seminal plasma was stable to heat (65°C for 30 minutes), freezing, and was non-dialyzable. Weinman and Williams (3) reported the presence of DF activity in epididymal fluid and showed that the DF pellet sedimented in the ultracentrifuge could be resuspended in buffer and passed through an ultrafilter.

This paper reports attempts to determine the chemical nature of DF by use of various hydrolytic enzymes and suggests a possible mechanism of capacitation. Portions of this work were published in a preliminary report (4).

Materials and methods. Initial studies were conducted with Pronase (Cal Biochemical Co.), a proteolytic enzyme attacking peptide bonds nonspecifically. Seminal plasma diluted 2.5-fold with calcium-free Krebs-Ringer phosphate (KRP) was treated with Pronase at a level of 10 μg/5 mg biuret reactive material (BRM) for 10 minutes at 37°C. Capacitated spermatozoa were then incubated with the Pronase-treated seminal