Early Histamine Release and Death Due to Endotoxin (35690)

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The early hemodynamic changes associated with the syndrome referred to as Gram-negative bacteremic shock continues to be an elusive medical problem (1, 6, 10). Of particular concern has been a definition of the exact mechanims by which this form of shock is set into motion (3, 9, 11). Early cardiovascular changes have been noted which appear to follow the release of neurohumeral agents into the blood stream (1, 2, 5, 9). Vasoactive materials such as histamine, serotonin, bradykinin, and the catecholamines have all been implicated as being involved in the earlier phases of endotoxin shock (2, 3, 5, 9). Reports are conflicting, however, and no clear-cut delineation has been made as to which of these substances, if any, is involved in endotoxin shock. All things considered, however, many workers have concerned themselves with the role of histamine or a histamine-like substance in the very early stage of endotoxin shock (2, 7, 9, 10).

It is the purpose of this study, therefore, to investigate the role of histamine in early endotoxin shock and to define its relationship to the fall in arterial blood pressure and to the sometimes lethal outcome so often associated with this form of stress.

Materials and Methods. A series of 22 adult beagle dogs anesthetized with pentobarbitol sodium (30 mg/kg) was used in this study. Heart rate, EKG, respiratory rate, and blood pressure were continuously monitored on a Sanborn polygraph. In addition, a large bore polyethylene catheter was placed in the femoral artery and advanced into the abdominal aorta to allow for the rapid sampling of arterial blood. Dogs were given graded doses of *E. coli* endotoxin ranging from 0.1 to 10 mg/kg. All injections were made directly into the femoral vein of the dog. Blood samples for histamine determination were then taken at 0, 15, 30, 45, and 60 sec and at 2, 5, 10, 15, and 30 min after endotoxin. Plasma histamine determinations were made on platelet-free plasma. All samples were centrifuged at $>5000g$ (gravities-centrifuged force) for 10 min and extracted in ethanol–chloroform (30/20) mixture. Following a single alkaline wash the samples were read on a fluorometer using the method of Shore (8). All animals were followed for 72 hr or until death.

Results. The effects of each of 22 injections of *E. coli* endotoxin on plasma histamine levels at specific time intervals are shown in Table I. Doses ranged from 0.1 to 10.0 mg/kg. Those doses of endotoxin which produced death within 72 hr are also indicated. It is interesting to note that ultimate lethality is usually correlated with a sharp elevation of plasma histamine at 30–60 sec postinjection.

The response of the anesthetized dog to a sublethal (0.5 mg/kg) injection of endotoxin is such that a minimal increase in histamine, a subtle fall in platelets, and the slight decrease in blood pressure are evident (Fig. 1).

In contrast, the response of the dog to a lethal (1.0 mg/kg) injection of endotoxin is such that a marked increase in plasma histamine is noted which occurs at 30 sec and lasts for approximately 5 min (Fig. 2). A marked decrease in platelets and a precipitous fall in arterial blood pressure also takes place during this same period of time. The decrease in blood pressure occurs simultaneous and in proportion to the increase in histamine. As the histamine level decreases blood pressure returns toward control and stabilizes at a level somewhat lower than before endotoxin injection. The platelet count, however, remains low during the observation period

TABLE I. The Effect of Varying Doses of Endotoxin on Plasma Histamine Levels and Lethality.

iv dose of endotoxin (mg/kg)	Plasma histamine levels (μg/l).								
	Control	15 sec	30 sec	45 sec	1 min	2 min	5 min	10 min	Lethality
0.1	6.4	11.3	11.1	6.8	7.8	7.9	7.1	7.0	No
0.3	4.7	4.0	4.0	17.5	28.6	19.8	15.1	8.2	Yes
0.5	5.3	4.4	5.9	5.9	6.6	7.9	5.5	5.2	No
0.5	6.4	7.0	7.1	6.9	6.4	6.0	6.2	5.9	No
0.5	5.9	4.7	4.8	4.8	4.5	5.7	6.6	6.6	No
0.75	6.9	5.8	5.2	23.0	25.1	13.3	4.3	5.9	Yes
1.0	4.7	4.1	7.8	17.8	21.4	21.0	8.1	6.8	Yes
1.0	6.8	3.9	34.2	20.2	46.6	18.4	6.5	5.3	Yes
1.0	4.2	3.5	3.5	3.8	4.3	4.2	3.8	3.8	No
1.0	4.9	4.6	4.9	4.2	4.8	4.9	4.2	4.2	No
1.0	5.9	6.1	8.8	4.7	8.8	14.3	10.8	7.8	Yes
1.0	5.4	6.4	6.7	13.2	12.2	6.0	7.0	6.0	Yes
1.0	6.4	5.8	6.4	14.5	36.1	28.9	—	—	Yes
1.0	4.4	5.8	5.3	15.4	34.7	24.5	12.5	6.3	Yes
1.0	4.7	5.6	5.5	5.2	6.0	5.3	4.9	6.2	No
1.0	4.4	4.1	11.4	12.9	17.3	10.6	7.1	6.8	Yes
1.0	5.2	5.0	6.6	5.5	5.2	5.5	5.5	5.0	No
1.0	5.0	4.0	4.0	4.1	4.1	6.8	6.2	4.1	No
3.0	4.7	4.3	17.7	19.9	48.5	38.5	12.8	7.5	Yes
3.0	6.7	5.0	5.8	17.7	18.3	11.9	5.7	5.0	Yes
4.0	5.7	10.5	13.2	18.7	18.7	16.5	9.4	6.7	Yes
10.0	6.1	6.7	7.6	14.3	27.6	13.8	6.5	5.9	Yes

with but a gradual increase observed at 10 min postendotoxin.

The average change in histamine level produced by the 13 lethal doses of endotoxin is compared with the average change in histamine produced by the nine sublethal injections of endotoxin (Fig. 3). Also shown is the standard error of the mean at each point in time. Lethal doses consistently produce elevations in histamine at 30–60 sec after endotoxin while sublethal doses do not result in a significant increase in histamine levels at any

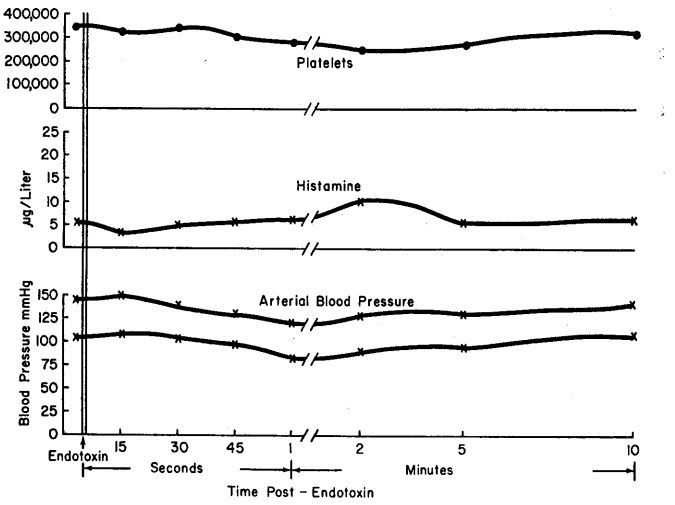

FIG. 1. The effect of 0.5 mg/kg *E. coli* endotoxin on platelets, plasma histamine levels, and arterial blood pressure.

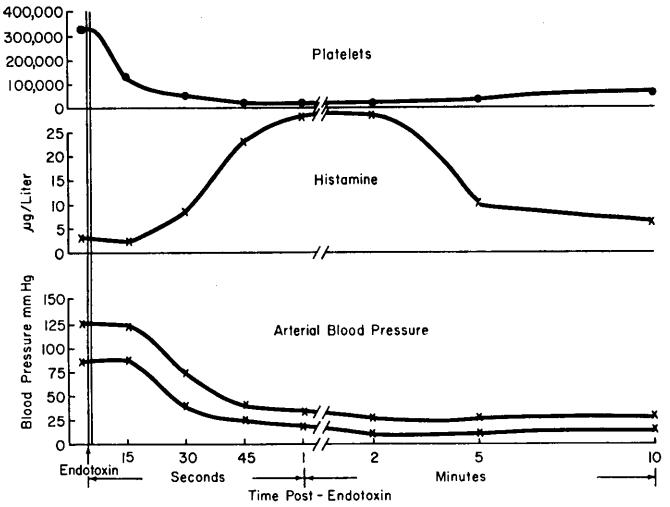

FIG. 2. The effect of 1.0 mg/kg *E. coli* endotoxin on platelets, plasma histamine levels, and arterial blood pressure.

point in time studies.

Figure 4 shows the marked differences in histamine levels between the seven animals which expired following 1.0 mg/kg *E. coli* endotoxin and the five dogs which survive after having received this same dose. In the animals which survive minimal increases in histamine are noted while those animals which die show significant elevations at 30–60 sec postinjection. Plasma histamine levels return to control or near control levels within approximately 10 min in those animals which experience an elevation.

Discussion. Results of this study indicate that histamine is in some way involved in both the early precipitous fall in arterial blood pressure and in the final lethal outcome of *E. coli* endotoxin shock. Significant increases in plasma histamine levels were consistently observed within 30–60 sec after the intravenous injection of a lethal dose of *E. coli* endotoxin. In contrast, those animals which survived showed no elevations in plasma histamine levels at any time after having

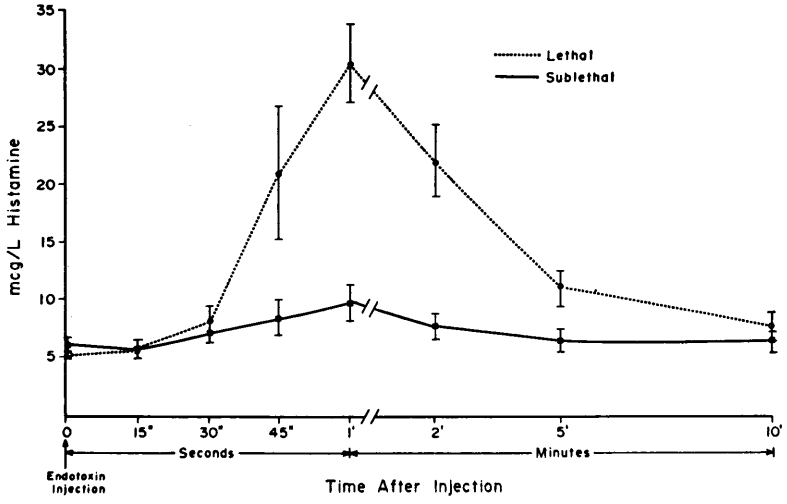

FIG. 3. Changes in mean histamine levels ± SEM produced by all lethal and all sublethal doses of *E. coli* endotoxin.

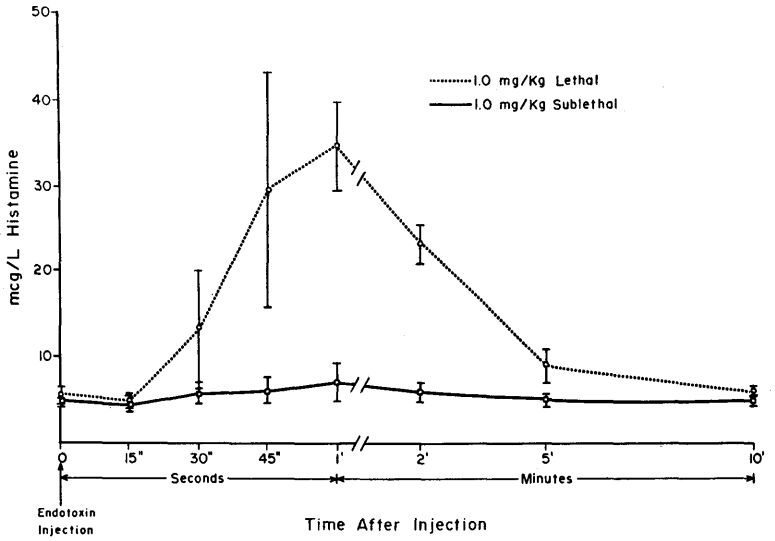

FIG. 4. The differences in histamine levels ± SEM between those animals which expired following 1.0 mg/kg *E. coli* endotoxin and those dogs which survived after having received this same dose are shown.

received endotoxin. It would appear in retrospect, therefore, that histamine is a good index of the lethal capabilities of endotoxin, in that no dogs died without showing an early and significant elevation in histamine. Furthermore, it may well be that histamine is in itself one of the many substances responsible for the "setting into motion" or the "triggering off" of the irreversible cardiovascular changes so often observed in this form of shock. It must be noted, however, that other neurohumeral agents such as epinephrine, norepinephrine, serotonin, and bradykinin are also released early in endotoxin shock and are undoubtedly involved in both the cardiovascular phenomenon and/or death. In these studies the elevation of plasma histamine occurred very quickly (30–60 sec) and lasted for approximately 10 min. The observation that the most pronounced increase in histamine occurred at 30–60 sec might account for the seemingly conflicting reports from other laboratories in which no elevations of histamine have been observed following endotoxin (3, 4). Blood samples obtained 15–30 min after endotoxin administration could conceivably show normal histamine levels even as the early increase in histamine has already initiated the earlier cardiovascular changes observed in endotoxin shock in the dog.

The source of histamine in the dog is mainly from disrupted platelets and mast cells (2, 7, 9). In those experiments in which endotoxin produced death, sharp decreases in the number of circulating platelets were noted along with the increase in plasma histamine. These two changes occurred simultaneous with the most commonly observed feature of early endotoxin shock: that of a precipitous fall in arterial blood pressure and a decrease in heart rate. The fall in arterial pressure in those animals which expired was in most cases a "mirror image" of the elevation of plasma histamine; that is, the greater the decline in pressure, the sharper the increase in histamine. Likewise, in those animals which did not expire the early fall in pressure and the changes in histamine and platelets were minimal.

These data support earlier work in which isolated vein strips were used to bioassay the response to endotoxin (9). In those studies histamine was indirectly implicated in the early stages of endotoxin shock. An increase in vessel tension was consistently observed within 30–60 sec following endotoxin which could be blocked by prior addition of anti-

histaminics but not by antiserotonin or antiadrenergic drugs.

It is important to note, however, that even though an early increase in histamine appears to be a good index of ultimate lethality other neurohumeral agents are undoubtedly released during all stages of endotoxin shock. Elevations of plasma bradykinin, serotonin, and the catecholamines have all been reported (3–5, 10, 11).

It seems clear from these studies that the early increase in histamine is in some way involved in both the initial cardiovascular effects of endotoxin as well as to the ultimate death of the dog. Hinshaw has shown that the early fall in arterial blood pressure and the decrease in heart rate produced by endotoxin can be mimicked by either an infusion of histamine or by the injection of 48/80, a histamine-releasing agent (2). It could well be that the final lethal outcome of canine endotoxin shock is not directly related to the observed sharp and significant increases in plasma histamine levels, yet it seems inconceivable that these changes play no role whatsoever in this form of stress.

Summary. The intravenous injection of a lethal dose of *E. coli* endotoxin into anesthetized dogs produces a precipitous fall in arterial blood pressure, a sharp increase in plasma histamine and an abrupt decrease in circulating platelets. The increase in histamine, like the fall in arterial pressure, occurs within 30–60 sec following the administration of a lethal dose of endotoxin. In contrast, sublethal doses of endotoxin produce only modest decreases in arterial blood pressure, no significant elevation in plasma histamine levels, and a slight fall in circulating platelets. In this study an increase in plasma histamine was always associated with a lethal outcome. In no instance was there death with no prior elevation in histamine. Results indicate that an early and marked increase in histamine seems to follow the injection of a lethal dose of endotoxin, and it is highly probable that this early release of histamine is in some way involved in both the cardiovascular changes and the ultimate lethality of this form of shock in the dog. The possible involvement of bradykinin, serotonin, and the catecholamines is indicated.

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