Effects of Endotoxin and Histamine on Serum Enzyme Activity (35934)

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Injections of gram-negative endotoxin or histamine into dogs produce similar physiological and hemodynamic changes which are related to the pathogenesis of shock. Endotoxin injections cause the release of endogenous histamine (1, 2) and histamine or similar acting substances have been postulated as primary stimulants in the initiation of the shock syndrome (3, 4). Studies in our laboratory demonstrated that endotoxin administration produced an elevation of tissue enzymes in the serum, and that the magnitude of serum enzyme activity was related to the severity of shock and the eventual outcome (5, 6). In this investigation the effects of endotoxin and histamine were compared with respect to serum enzyme activity and to the time of appearance of tissue enzymes in the serum.

Materials and Methods. Twenty-nine beagle dogs, 8 to 12 kg, were randomly assigned to receive endotoxin, histamine, or saline injections. The animals were anesthetized with pentobarbital sodium (30 mg/kg) and placed in dorsal recumbency. Polyethylene cannulas were inserted, as previously described (5), for injections, sampling, and monitoring blood pressure. In conducting the present research, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Commercial endotoxin (*Escherichia coli* 0111:B4-Difco Laboratories) in saline was injected intravenously (2 mg/kg of body wt) which produced 75% mortality. Histamine dihydrochloride (General Biochemicals, Inc.) was administered intravenously in doses of 1 mg/kg of body weight. The histamine was dissolved in physiological saline (25 ml) and given over a 15 min interval. An additional five dogs received only 25 ml of saline. Base line blood samples were collected following anesthetization and cannulation; repetitive samples were collected at 1, 3, 5, and 7 hr following the injection. Twenty-four and 48 hr samples were collected from all surviving animals. Within 10 min of collection, all blood samples were centrifuged for 5 min at 10,000 rpm. The sera were removed and refrigerated.

Quantitative serum enzyme analyses were performed by colorimetric procedures described in commercial kits. Lactic dehydrogenase (LDH) values are expressed in Berger and Broida units while the transaminases (SGOT and SGPT), isocitric dehydrogenase (ICDH), and creatine phosphokinase (CPK) are expressed in Sigma units. Isoenzymes were separated by agar gel electrophoresis. LDH activity was located by the method of Papadopoulos and Kintzios (7), and CPK by the method of Trainer and Gruenig (8).

Analyses of variance statistics were performed for three independent comparisons at each time period for each enzyme; endotoxin nonsurviving plus endotoxin surviving versus histamine plus controls, endotoxin nonsurviving versus endotoxin surviving, and histamine versus controls.

Results. Eight of 12 dogs injected with endotoxin died within 24 hr, while 2 of 12 dogs infused with histamine died at 20 and 35 min, respectively, after the infusion started.

Histamine (1 mg/kg) produced a fall in mean arterial blood pressure (MABP) to

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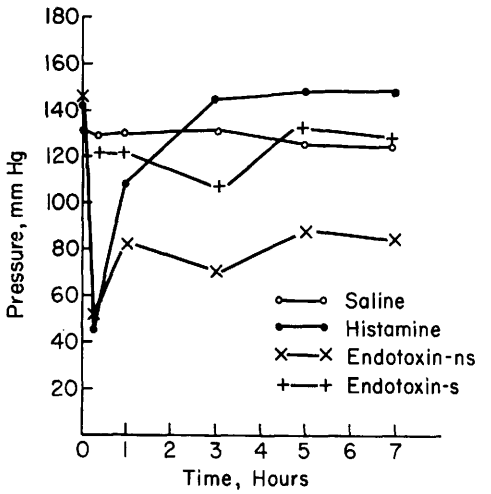

FIG. 1. Mean arterial blood pressure at times after treatment.

about 45 mm Hg (Fig. 1), and this was similar to that found with nonsurviving animals receiving endotoxin (2 mg/kg). The MABP of the histamine-treated animals returned to base line levels by the third hour following the infusion, while the nonsurviving endotoxin-treated animals remained hypotensive (<90 mm Hg) during the period of measurement. The saline treated group showed only a slight decrease in MABP during the 7 hr study.

The release of tissue enzymes into the systemic circulation following endotoxin or histamine treatments was different both with respect to total activity and time of appearance (Tables I and II). Endotoxin treatment produced a significant ($p<.01$) elevation of serum LDH and ICDH over histamine- and saline-treated groups by the third hour and values were about 10 times histamine and saline groups by the fifth hour in nonsurviving animals (Table I). In endotoxin treated surviving animals the serum LDH activity was not significantly elevated over base line levels, but ICDH was increased four to five-fold over base line of the histamine- and saline-treated groups. Histamine, when compared with saline, produced no significant change in serum levels of either LDH or ICDH.

The distinctive LDH isozyme patterns produced by endotoxin was reported previously (6), and confirmed by these experiments. The initial rise in total serum LDH is associated with an increase in LDH isozymes two and three. (The most anodic LDH isozyme was designated as one.) Later in endotoxemia, 5 to 7 hr postinjection, LDH isozymes four and five also were elevated. Only occasionally was LDH isozyme one elevated and that was near the terminal stages. The total serum LDH was not significantly elevated by histamine, and no consistent pattern of LDH isozyme increase was noted.

Endotoxin-treated animals showed significantly ($p<.001$ for SGOT and $p<.05$ for SGPT) higher transaminase levels than histamine- and saline-treated animals during the first 7 hr (Table II). In animals which survived endotoxin treatment, the transaminase levels were five to seven times higher than base line levels at 24 hr, but were not significantly different from histamine- and saline-treated animals. The elevations in the transaminases found at 24 and 48 hr, thus appear to be related, in part, to experimental conditions.

CPK activity in the serum of endotoxin-treated animals was significantly ($p<.01$) increased by the third hour when compared with histamine- and saline-treated animals (Table II). In endotoxin-treated animals which survived, the CPK activity at 24 hr was significantly ($p<.01$) increased over that found with the histamine-treated animals. The CPK activity in histamine-treated animals was elevated over base line values but was not significantly different from saline controls. In two histamine-treated experimental animals, blood was drawn at 12 hr and the CPK activity was maximal at that time.

The initial increase in total serum CPK activity following endotoxin injection was associated with the appearance of the most anodic CPK isozyme (Fig. 2). Later, the most cathodic isozyme was also elevated. In contrast, the increase in total CPK with histamine was associated with only the cathodic CPK isozyme. The cathodic CPK isozyme is found in cardiac and other muscles, while the anodic isozyme occurs in most other tissues (9). The saline-treated animals also showed increased CPK activity at 24 hr

TABLE I. Serum Dehydrogenase Activity at Times Following Treatments.[a]

Enzyme and treatment	Time (hr)						
	0[b]	1	3	5	7	24	48
LDH							
Endotoxin (ns-8)[c]	163 ± 36[d]	371 ± 96	1384 ± 279	1764 ± 258	1554 ± 338		
Endotoxin (s-4)	198 ± 5	168 ± 15	334 ± 91	317 ± 74	341 ± 61	350 ± 74	159 ± 14
Histamine (s-10)	185 ± 20	177 ± 7	192 ± 20	185 ± 19	180 ± 25	166 ± 13	212 ± 24
Saline (s-5)	159 ± 24	196 ± 29	189 ± 36	160 ± 13	216 ± 23	273 ± 36	246 ± 41
ICDH							
Endotoxin (ns-8)	143 ± 7	618 ± 207	2110 ± 437	2117 ± 437	4532 ± 778		
Endotoxin (s-4)	178 ± 32	309 ± 38	799 ± 334	946 ± 367	1131 ± 442	1359 ± 406	213 ± 78
Histamine (s-10)	197 ± 19	183 ± 13	220 ± 15	253 ± 22	272 ± 41	272 ± 41	206 ± 39
Saline (s-5)	119 ± 10	129 ± 4	178 ± 38	180 ± 26	204 ± 34	165 ± 23	136 ± 18

[a] Experimental conditions and enzyme units are described under Materials and Methods.

[b] Zero time (base line) following anesthesia and cannulation.

[c] In parentheses, ns = nonsurviving animals; s = surviving animals; and the numerals = the number of animals in each group.

[d] Values expressed as means ± SEM.

TABLE II. Serum Transaminase and Creatine Phosphokinase Activity at Times Following Treatments.[a]

Enzyme and treatment	Time (hr)						
	0	1	3	5	7	24	48
GOT							
Endotoxin (ns-8)	19 ± 2	78 ± 33	145 ± 23	252 ± 48	400 ± 70		
Endotoxin (s-4)	20 ± 2	34 ± 9	48 ± 13	54 ± 19	71 ± 20	155 ± 42	63 ± 15
Histamine (s-10)	15 ± 1	20 ± 1	22 ± 2	22 ± 3	22 ± 2	96 ± 18	38 ± 8
Saline (s-5)	11 ± 1	14 ± 2	15 ± 2	10 ± 1	14 ± 3	57 ± 12	34 ± 12
GPT							
Endotoxin (ns-8)	19 ± 2	33 ± 3	88 ± 36	224 ± 85	364 ± 128		
Endotoxin (s-4)	19 ± 2	31 ± 3	35 ± 6	39 ± 8	47 ± 14	74 ± 29	54 ± 13
Histamine (s-10)	17 ± 2	23 ± 2	23 ± 3	23 ± 3	22 ± 3	47 ± 7	45 ± 5
Saline (s-5)	11 ± 2	13 ± 2	13 ± 2	10 ± 2	13 ± 3	37 ± 17	35 ± 14
CPK							
Endotoxin (ns-5)	8 ± 1	17 ± 4	68 ± 26	73 ± 17	117 ± 37		
Endotoxin (s-2)	7 ± 2	6 ± 2	12 ± 3	13 ± 5	21 ± 5	539 ± 48	170 ± 37
Histamine (s-7)	14 ± 2	12 ± 2	14 ± 2	26 ± 4	61 ± 15	165 ± 42	27 ± 4
Saline (s-5)	4 ± .7	3 ± .4	4 ± 1	8 ± 2	16 ± 6	93 ± 40	30 ± 9

[a] See Table I for explanation of symbols.

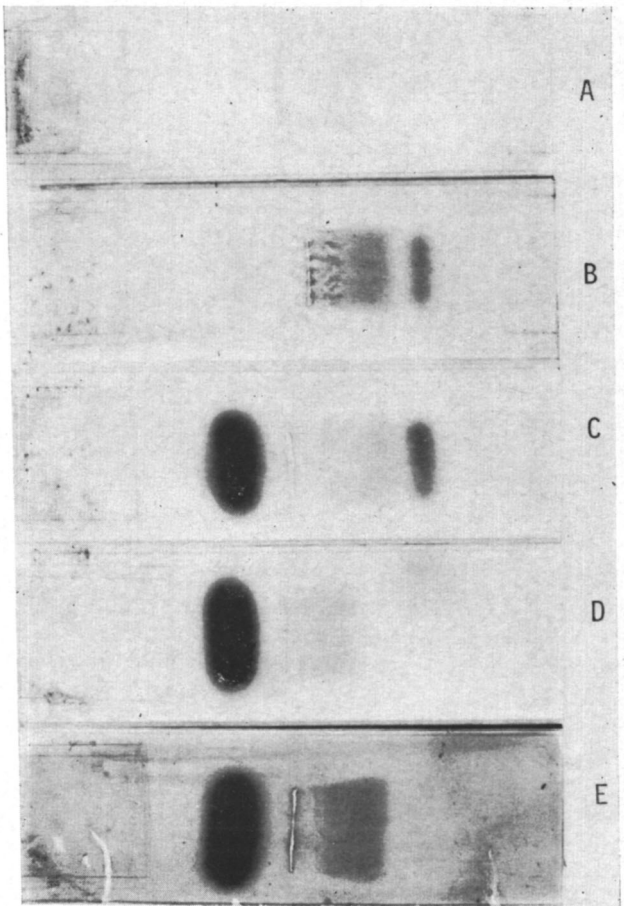

FIG. 2. Creatine phosphokinase isozymes: (A) base line; (B) 3rd hr after administering endotoxin; (C) 7th hr after administering endotoxin; (D) 7th hr after administering histamine; (E) 24th hr after administering saline. Experimental conditions are explained in Materials and Methods.

and the CPK isozyme was the cathodic one.

Discussion. Endotoxin or histamine administered iv to dogs produces many similar hemodynamic and physiologic responses. Both produce a rapid depression of MAPB (Fig. 1) and both produce similar effects on the microcirculation (1, 3). The response is immediate with histamine, but slightly delayed with endotoxin (1, 2). The slight delay in the fall of MABP observed with endotoxin was equated to the endogenous release of histamine or other vasoactive compounds (4). However, histamine depletion did not alter the vascular responses to endotoxin administration (10), while pretreatment of dogs with histamine did prevent endotoxin shock (11). Undoubtedly, the physiological interrelationships between these compounds are complex.

The pharmacological dose (1 mg/kg) of histamine chosen for these experiments was one that depressed the MABP to the same degree as endotoxin. The MABP of animals administered histamine returned to base line levels by the third hour, while nonsurviving animals administered endotoxin remained hypotensive ($<$90 mm Hg) during the 7 hr study. Endotoxin, rather than the initial precipitous fall in MABP, was responsible for the early increased levels of serum enzymes. The late increases in the transaminases and CPK found with histamine- or saline-treated

animals probably resulted from experimental conditions. However, the initial drop in blood pressure (12, 13) as well as sublethal doses of endotoxin (10), reportedly caused a rise in blood catecholamines. The catecholamines in turn have been shown to produce delayed damage to cardiac tissue (14, 15).

Based on this study, endotoxin or histamine administration does not produce similar tissue damage. Endotoxin injection produced an elevation of all serum enzymes studied by the third hour (Tables I and II). Histamine infusion had only a delayed and transient effect on the serum CPK and transaminase activity, which was not significantly different than saline controls. Endotoxin administration produced a significant elevation in total serum LDH, which was initially associated with an increase in specific LDH isozymes (6). Endotoxin also produced a significant elevation in serum ICDH, which was found even in surviving animals. Histamine infusion does not cause any elevation of these dehydrogenases in the serum. The serum CPK activity was elevated by the third hour after endotoxin injections, and the CPK isozyme was the anodic one (Fig. 2). Later in endotoxemia the cathodic CPK (muscle) isozyme was present, but without a rise in LDH isozyme one (heart). Similarly, the late rise in serum CPK activity with histamine or saline infusions was associated with the cathodic isozyme. The transaminase activity after the respective treatments paralleled that found with CPK. The increases in serum enzyme activity which occurred late in the study were not related to treatment.

Although endotoxin and histamine do not produce the same tissue damage or changes in cellular permeability, similar hemodynamic and physiologic responses are noted. Based upon these observations, several investigators have postulated a synergistic effect between these compounds (3, 4, 16), and proposed that the action of histamine is at the site of release rather than systemic. The results of these studies do not rule out this possibility.

Summary. Endotoxin and histamine injections produced similar hemodynamic and physiologic changes, but these similarities are not equated to tissue damage or changes in cellular permeability, as shown by the activity of serum enzymes. Endotoxin causes extensive tissue damage by the third hour as demonstrated by high levels of lactic dehydrogenase, isocitric dehydrogenase, the transaminases, and creatine phosphokinase, and specific tissue damage as demonstrated by isozyme determinations. Histamine produced only transient elevation in creatine phosphokinase and the transaminases, which did not differ significantly from the saline controls.

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